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Original Article

The relationship between the quality of life of patients with recurrent aphthous stomatitis with their childhood traumas and dissociative experiences

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Abstract

Aim: We aimed to evaluate childhood traumas and dissociative experiences which we think may be predisposing factors in patients with recurrent aphthous stomatitis (RAS).
Materials and Methods: The sample of the study consisted of 60 volunteer patients over the age of 18 (30 RAS patients and 30 control groups) who applied to the otolaryngology clinic. A Personal Information Form was prepared in line with main objective of the study, Childhood Traumas Questionnaire (CTQ), Dissociative Experiences Scale (DES), Hospital Anxiety and Depression Scale (HADS), Brief Symptom Inventory (BSI), and Quality of Life Scale-Short Form-36 (QLS36) were implemented on patients through face-to-face interviews.
Results: Brief symptom scale total score of RAS cases ($\bar{x}$=45.64±43.22) was compared to the control group ($\bar{x}$=37.62± 22.58), short symptom scale sub-dimension depression score ($\bar{x}$=12.17±11.21) was compared to the control group ($\bar{x}$=11.52±5.74), age traumas scale total score ($\bar{x}$=70.59±43.22) from the control group ($\bar{x}$=68.74±10.60), dissociative experiences scale total score ($\bar{x}$=14.30±7.30) from the control group ($\bar{x}$=11.02±1.24) and quality of life scale general health perception score ($\bar{x}$=52.25±12.58) was significantly higher than the control group ($\bar{x}$=51.29±11.87)($p<.05$). RAS was closely associated with psychiatric symptoms induced by traumas, somatization, anxiety and depression clinically.
Conclusion: RAS is an important disease having negative effects on the quality of life and is closely associated with depressive symptoms. This study results found a positive relationship between the childhood traumas and dissociative experiences in RAS cases. Childhood traumas and dissociative experiences can be considered as a predisposing factor in patients with RAS.

Keywords: Recurrent aphthous stomatitis, childhood traumas, dissociative experiences, hospital depression and anxiety, quality of life

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is the ulcerative lesion of the oral mucosa. The Greek word of aphtha, which means burning, inflaming and inflammation, was used by Hippocrates for the first time [1]. RAS, which is one of the most common oral ulcerative diseases in the world, affects 10-20% of the population but its incidence changes between 5-50% based on ethnic and socioeconomic groups [2]. Approximately 50% of women and 40% of men experience RAS twice or more throughout their lives [3].

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RAS starts during childhood and adolescence and is clinically seen as minor, major and herpetiform aphthae. The Minor RAS type forms more than 70% of RAS. Minor RAS is small, superficial aphthae confined to an erythematous halo and covered with grey-white pseudomembrane. These lesions heal within 10-14 days without leaving a scar. Major RAS lesions are 1-3 cm in size, painful and continue up to 6 weeks and end leaving a scar. The major type forms approximately 20% of all RAS [1]. Herpetiform type is the least common type and is characterized by multiple painful herpetiform lesions in the size of a needle. Many factors such as bacterial and viral factors, mental traumas, some unhealthy food, smoking cigarette, medicament reactions, immune system disorders, systematical disorders, hormonal imbalances, stress experiences, predispositional factors and genetic predispositions take part in the etiopathogenesis of RAS. Negative emotional factors due to stress take part as a fundamental element in the development of RAS episodes [2]. It was determined that there is a significant increase in the frequency of aphthous ulcers of students during exam periods compared to their actual life period where they do not have any stressors as an example to stressful life events [4]. It is reported that some patients with RAS benefit from antidepressant treatment [5]. RAS is a public health problem that must be underlined due to its high prevalence among society and its negative effects on the quality of life [6,7].

Objective concepts such as morbidity and mortality are addressed in the assessment of diseases. However, the patient is a whole physically, mentally and socially and must be addressed holistically [1]. As the findings show that the treatment of these cases can be completed successfully as a result of the holistic evaluation of clinical cases physically, mentally and socially, the concept of quality of life has become one of the main study subjects [8]. The RAS disease can be accompanied by some psychiatric symptoms (somatization, anxiety, depression) and the quality of life of patients can be negatively affected [9].

The number of epidemiological studies on the frequency, severity and duration of psychiatric symptoms of patients with RAS is quite limited. The experiences of childhood traumas might affect psychological functions in adulthood. Relevant studies have shown that mental traumas experienced during childhood have a major effect on the formation of dissociation [10-13]. Dissociative experiences induced by traumas cause splitting the individuals' personality structures that are closely associated with their mental and behavioral characteristics. Dissociation, as an adjustment reaction to normal dynamics of life, prevents losing the inspection and control mechanisms related to individuals' lives at a certain level. Dissociation turns into a permanent mechanism that creates the feeling of despair mentally even when the individual has physical control [14].

The uncertainty regarding the etiopathogenesis of RAS is still existing today. Therefore, the aim of this study was to examine the quality of life, childhood traumas, anxiety, depression and dissociative experiences of patients and to determine the relationship between them. Thus, a possible relationship between the variables will help us to understand the etiology of the disease and contribute to arranging new dimensions in treatment approaches.

MATERIAL AND METHOD

Study design

The sample of the study included 60 voluntary patients (30 patients with RAS and 30 in the control group) aged older than 18 years who applied to Otorhinolaryngology clinic between November 2018 and February 2019 and who were diagnosed with RAS. The patients included in the study had a complaint of RAS that continued for at least 15 days and symptomatically received drug therapy. The participants were selected using the convenience sampling method among nonstochastic sampling methods. The patients underwent otorhinolaryngology examination and the detailed anamnesis including clinical features of the patients was taken. In-mouth and oral mucosal regions were examined in detail. The patients who had an acute infection such as leucoplasia or gingivitis in addition to oral aphtha and who voluntarily agreed to participate in the study were excluded from the study. The approval of the local Scientific Study and Publishing Ethics Committee with the protocol number 2018/22-7 and the informed consent of all patients were obtained before the study was conducted.

Measures

The participants were implemented a Personal Information Form, the Hospital Anxiety and Depression Scale (HADS), Brief Symptom Inventory, Childhood Trauma Questionnaire, Dissociative Experiences Scale and Quality of Life Scale (SF36).

2.2.1. Personal Information Form: The personal information form developed by the researcher included 11 questions to determine sociodemographic characteristics of voluntary patients with RAS. Information about the patients' sex, age, marital status, the existence of chronic diseases, and previously receiving psychiatric treatment were obtained with this form.

Hospital Anxiety and Depression Scale: HADS is a commonly used scale that is filled by the patient in the hospital environment to scan the anxiety and depression symptoms. Its Turkish validity and reliability were carried out by Aydemir et al. [15]. It has 14 items and two subdimensions as HADS-A (Anxiety, 7 questions) and HADS-D (Depression, 7 questions). The cutoff point of the anxiety subdimension is 10 while the cutoff point of the depression subdimension is 7. Accordingly, those with a higher score than these are considered at risk. The Cronbach's alpha coefficient was found 0.85 for the anxiety subdimension and 0.77 for the depression subdimension in its reliability study. The internal consistency coefficient was found 0.84 for the anxiety subdimension and 0.86 for the depression subdimension in this study.

The Dissociative Experiences Scale: This self-reporting scale, which was developed by Bernstein et al. and includes 28 items, ranks dissociative experiences quantitatively. Its Turkish validity and reliability were carried out by Sar et al. [16]. It is not a diagnostic tool and is used to scan chronic dissociative disorders. The participants' grades between 0-100, and the mean of the total

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scores obtained is calculated and the result is obtained. Scores higher than 30 indicate the existence of at least one dissociative disorder. In relation to the reliability of the scale, the Cronbach's alpha coefficient was =0.91 and the test-retest correlation was r=0.78 in its Turkish validity and reliability study, and these values were found to be high. The internal consistency coefficient of the scale was determined to be 0.78 in this study.

2.2.4. Childhood Traumas Questionnaire: It was developed by Bernstein et al. and its Turkish adaptation, validity and reliability studies were carried out by Sar and Ozturk [17]. It is a self-reporting measurement tool that is useful for the retrospective and quantitative evaluation of abuse and neglect experiences before the age of 20. It has 28 items. Each item is scored between 1-5. It has five subdimensions (sexual abuse, physical abuse, emotional abuse and emotional neglect, physical neglect) and a total score of childhood traumas including all five subdimensions. All subdimension scores change between 5-25, and the total score changes between 25-125. Higher scores indicate that the individual experienced a high level of abuse and neglect. The Cronbach's alpha value of the scale was 0.93. The internal consistency coefficient of the scale was determined to be 0.82 in this study.

Brief Symptom Inventory: It is a five-point Likert type self-evaluation inventory developed by Derogotis to scan general psychopathological symptoms of individuals. It is a multi-dimensional symptom screening scale that was developed to detect psychological symptoms that may arise in various psychiatric and medical patients like normal samples. It was adapted to Turkish by Sahin and Durak and includes the subdimensions of anxiety, depression, negative self, somatization and hostility, and three global indexes [18]. Higher scores obtained from the subdimensions indicate the frequency of psychological symptoms of individuals. Three global indexes have different scoring methods and are named the Discomfort Seriousness Index, Symptom Total Index and Symptom Discomfort Index based on their scoring methods. The Discomfort Seriousness Index shows the level of stress. This value changes between 0 and 4. This score is obtained by diving the total subdimension score by 53. The Symptom Total Index is the total score obtained by taking all positive values except items scored as 0, as one. This score changes between 0 and 53. The Symptom Discomfort Index is obtained by dividing the total subdimension score by the total symptom score. The Cronbach's alpha coefficients of the scale for an adult sample was .87 and was .94 in this study.

The Quality of Life Scale Short Form-36: The SF-36 was developed by Rand Corporation in 1992 and its Turkish validity and reliability studies were carried out by Kocyigit et al. [19]. It has 36 items and is used to examine individuals' health status and quality of life. It has eight subdimensions as physical function, physical role difficulty, evaluation role difficulty, energy, mental health, pain, social functionality and general health perception. Each subdimension has a total score instead of one single total score for the whole scale. Each subdimension is scored between 0-100. SF-36 has a positive scoring and the quality of life-related to health increases as the score of each subdimension increases. The internal consistency between the items of the scale is 0.88, between subdimensions is 0.84 while the internal consistency coefficient between items was 0.67 and between the subdimensions was 0.70 in this study.

Statistical analysis

Data were analyzed using the SPSS 22.0 program. Descriptive statistics such as frequency distribution, mean, standard deviation were used to define the sample, and the correlation analysis was performed to determine the direction and level of the relationship between the variables. The significance level was regarded as 95% to determine the differences in the analyses.

RESULTS

The findings related to sociodemographic characteristics, brief symptom inventory, childhood traumas, dissociative experiences, quality of life (SF-36) and hospital anxiety and depression data of 30 patients with RAS were presented in this section.

Characteristics of the study groups

According to Table 1, 56.7% of the patients with RAS were female and 43.3% were male. Of the participants, 53.3% were aged between 20 and 30 while 46.7% were aged between 31 and 40. Of the participants, 60% were married and 40% were single. Of the participants, 33.3% were primary school graduates, 36.7% were high school graduates and 30% were university graduates. Among the participants, 73.4% were employed and 26.6% were unemployed. Of the participants, 80% did not receive psychiatric treatment and 90% did not receive psychological help. A total of 90% of the participants did not have a chronic disease.

Table 1. Demographic characteristics of the participants

Variables	RAS Group	Control Group
Sex		
Female, n (%)	17 (56.70)	22 (73.3)
Male n (%)	13 (43.30)	8 (26.7)
Age		
20-30 years old, n (%)	16 (53.30)	22 (73.3)
31-40 years old, n (%)	14 (46.70)	8 (26.7)
Marital Status		
Married, n (%)	18 (60.00)	10 (36.6)
Single, n (%)	12 (40.00)	20 (63.4)
Educational Level		
Primary school, n (%)	10 (33.30)	4 (13.4)
High school, n (%)	11 (36.70)	11 (36.4)
University, n (%)	9 (30.00)	15 (50.2)
Employment status		
Employed, n (%)	22 (73.40)	17 (56.7)
Unemployed, n (%)	8 (26.60)	13 (43.3)
Going to a Psychiatrist		
No, n (%)	24 (80.00)	26 (86.7)
Yes, n (%)	6 (20.00)	4 (13.3)
Chronic Disease		
No, n (%)	27 (90.00)	23 (76.7)
Yes, n (%)	3 (10.00)	7 (23.3)

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According to the comparison of the scale scores of the RAS and control groups in Table 2, the brief symptom scale total score of the RAS cases ($\bar{x}$=45.64±43.22) was significantly higher than the control group ($\bar{x}$=37.62±22.58) (t=2.36, p<.05). Brief symptom scale subscale depression score of RAS cases ($\bar{x}$=12.17±11.21) was significantly higher than the control group ($\bar{x}$=11.52±5.74) (t=2.36, p<.05). The childhood traumas scale total score of RAS cases ($\bar{x}$=70.59±43.22) was significantly higher than the control group ($\bar{x}$=68.74±10.60) (t=1.25, p<.05). Childhood traumas scale sub-dimension physical neglect score of RAS cases ($\bar{x}$=14.63±2.87) was significantly higher than the control group ($\bar{x}$=14.35±1.27) (t=0.94, p<.05). Dissociative experiences scale total score of RAS cases ($\bar{x}$=14.30±7.30) was significantly higher than the control group ($\bar{x}$=11.02±1.24) (t=1.84, p<.05). Hospital anxiety and depression scale subscale scores of RAS cases ($\bar{x}$=12.28±3.37) were significantly higher than the control group ($\bar{x}$=10.10±3.52) (t=2.54, p<.05). Hospital anxiety and depression scale sub-dimension hospital depression score of RAS cases ($\bar{x}$=9.50±2.32) was significantly higher than the control group ($\bar{x}$=8.98±4.74) (t=3.28, p<.05). Quality of life scale general health perception score of RAS cases ($\bar{x}$=52.25±12.58) was significantly higher than the control group ($\bar{x}$=51.29±11.87) (t=1.12, p<.05).

According to Table 2, the total score on the Brief Symptom Inventory was $\bar{x}$=45.64±43.22, and the highest subdimension score was on depression with $\bar{x}$=12.17±11.21. Additionally, the total score on the Childhood Traumas Questionnaire was $\bar{x}$=70.59±9.35, the highest score was obtained on the neglect subdimension with $\bar{x}$=20.78±4.07 while the lowest score was obtained on the sexual abuse subdimension with 08.40±1.18. The Dissociative Experiences Scale score was $\bar{x}$=14.30±7.30. The subdimension scores of the HADS were $\bar{x}$=12.28±3.37 in the hospital anxiety and $\bar{x}$=9.50±2.32 in the hospital depression. Lastly, the subdimension scores of the Quality of Life Scale were $\bar{x}$=60.61±18.17 in physical function, $\bar{x}$=55.99±21.62 in physical role difficulty, $\bar{x}$=47.89±24.52 in emotional role difficulty, $\bar{x}$=58.70±10.42 in energy, $\bar{x}$=42.63±11.71 in mental health, $\bar{x}$=70.91±11.28 in social functionality, $\bar{x}$=50.98±22.45 in pain and $\bar{x}$=52.25±12.58 in general health perception.

Table 2. Comparison of RAS and control group scale scores

		RAS Group		Control Group			
Scale	**Dimension**	**$\bar{x}$**	**sd**	**$\bar{x}$**	**sd**	**t**	**p**
Brief Symptom Inventory	Total	45.64	43.22	37.62	22.58	2.36	.04
	Anxiety	9.90	11.27	8.21	4.36	1.01	.03
	Depression	12.17	11.21	11.52	5.74	0.91	.04
	Negative Self	9.39	11.36	8.58	4.95	0.97	.04
	Somatization	6.79	5.72	6.32	3.42	1.08	.03
	Hostility	7.40	6.87	7.10	3.75	2.01	.05
Childhood Traumas Questionnaire	Total	70.59	9.35	68.74	10.60	1.25	.02
	Emotional Abuse	10.52	3.01	10.01	3.82	0.83	.04
	Physical Abuse	11.27	3.92	11.23	3.58	1.27	.05
	Physical Neglect	14.63	2.87	14.35	1.27	0.94	.04
	Emotional Neglect	20.78	4.07	19.29	3.57	1.85	.05
	Sexual Abuse	8.40	1.18	8.01	2.45	1.74	.04
Dissociative Experiences	Total Score	14.30	7.30	11.02	1.24	1.84	.04
Hospital Anxiety and Depression Scale	Hospital Anxiety	12.28	3.37	10.10	3.52	2.54	.05
	Hospital Depression	9.50	2.32	8.98	4.74	3.28	.04
The Quality of Life Scale	Physical Function	60.61	18.17	62.52	7.85	2.75	.05
	Physical Role Difficulty	55.99	21.62	54.65	13.25	3.85	.04
	Emotional Role Difficulty	47.89	24.52	46.74	12.68	0.87	.03
	Energy/Liveliness/Vitality	58.70	10.42	57.21	15.25	1.45	.02
	Mental Health	42.63	11.71	41.31	10.85	2.98	.05
	Social Functionality	70.91	11.28	68.87	10.36	2.58	.04
	Pain	50.98	22.45	59.23	21.34	2.36	.03
	General Health Perception	52.25	12.58	51.29	11.87	1.12	.05

According to Table 3, no significant correlation was found between the RAS gruop quality of life subdimensions and brief symptoms, childhood traumas, dissociative experiences and hospital anxiety and depression total scores and subdimensions (p>.05). However, a negative and significant correlation was found between the physical functions subdimension of the quality of life and brief symptom total score (r=-.43*) and anxiety (r=-.53**), negative self (r=-.37*), somatization (r=-.54**), discomfort seriousness index (r=-.43*) and symptom total index (r=-.40*) (p<.05). Additionally, a positive significant correlation was found between the pain subdimension of the quality of life and somatization (r=.38*) and dissociative experiences (r=.42*) while a negative correlation was found with physical abuse (r=-.45*) (p<.05).

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Table 3. Correlation analysis findings of the RAS group

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	1																									
2	.96**	1																								
3	.98**	.92**	1																							
4	.94**	.88**	.90**	1																						
5	.77**	.74**	.74**	.57**	1																					
6	.93**	.84**	.89**	.88**	.65**	1																				
7	1.00**	.96**	.98**	.94**	.77**	.93**	1																			
8	.72**	.68**	.75**	.60**	.66**	.64**	.72**	1																		
9	.42*	.38*	.34	.46*	.27	.49**	.42*	-.22	1																	
10	-.17	-.19	-.18	-.14	-.04	-.17	-.16	-.08	-.14	1																
11	-.11	-.19	-.09	-.11	.09	-.13	-.11	-.01	-.09	.71**	1															
12	-.09	-.12	-.07	-.11	.05	-.13	-.09	-.02	-.16	.51**	.49**	1														
13	-.07	-.03	-.11	-.05	-.11	-.05	-.07	-.05	-.05	.68**	.18	-.10	1													
14	-.06	-.05	-.08	-.003	-.11	-.07	-.06	-.06	.01	.42*	-.10	-.23	.58**	1												
15	-.23	-.24	-.28	-.26	-.03	-.16	-.23	-.17	-.12	.43*	.52**	.22	.26	-.41*	1											
16	.60**	.62**	.58**	.56**	.39*	.57**	.60**	.43*	.28	-.12	-.30	-.33	.30	.13	-.13	1										
17	-.69**	-.63**	-.64**	-.70**	-.41*	-.75**	-.69**	-.44*	-.54*	.12	.05	.16	-.07	.13	.02	-.56*	1									
18	.04	.06	-.03	.10	.06	-.01	.04	.15	.04	-.13	-.15	-.13	-.02	.02	-.13	.08	-.02	1								
19	-.26	-.27	-.24	-.33	-.17	-.14	-.27	-.08	-.07	-.03	-.15	.20	-.13	.03	-.10	-.20	.09	.11	1							
20	-.43*	-.53**	-.33	-.37*	-.54**	-.23	-.43*	-.40*	-.04	.10	.05	.04	.06	.11	.03	-.18	.22	-.17	.32	1						
21	-.23	-.25	-.18	-.23	-.10	-.25	-.23	-.07	-.29	.16	-.001	.02	.16	.33	-.11	-.36	.23	-.20	.33	.09	1					
22	-.12	-.07	-.07	-.22	.03	-.17	-.12	.03	-.34	.13	-.09	.15	.14	.15	.03	-.31	.30	-.12	.41*	.10	.66**	1				
23	-.10	-.09	-.10	-.07	-.26	-.01	-.10	-.05	.03	-.29	-.36	.14	-.36	-.07	-.34	-.13	.04	.11	.74**	.17	.04	.06	1			
24	-.24	-.23	-.21	-.25	-.14	-.23	-.24	.01	-.18	.09	.08	.36*	-.19	-.06	-.03	-.23	-.01	.12	.74**	-.01	.10	.21	.55**	1		
25	-.09	.01	-.21	-.10	.03	-.10	-.09	-.20	.29	.09	-.08	.21	.08	.01	-.002	.02	.04	.30	.24	-.12	-.21	.05	.12	.14	1	
26	.25	.24	.21	.09	.38*	.36	.25	.21	.21	-.33	-.22	-.45*	.001	-.12	.11	.42*	-.19	-.003	-.11	-.15	-.26	-.24	-.24	-.38*	.05	1

1: Brief Symptom (Total); 2:Anxiety; 3:Depression; 4:Negative Self; 5:Somatization; 6: Hostility; 7: Discomfort Seriousness Index; 8: Symptom Total Index; 9: Symptom Discomfort Index; 10: Childhood Traumas (Total); 11: Emotional Abuse; 12: Physical Abuse; 13: Physical Neglect; 14: Emotional Neglect; 15: Sexual Abuse; 16: Dissociative Experiences (Total); 17: Hospital Anxiety; 18: Hospital Depression; 19: Physical Function; 20: Physical Role Difficulty; 21: Emotional Role Difficulty; 22: Energy/Liveliness/Vitality; 23: Mental Health; 24: Social Functionality; 25: Pain; 26: General Health Perception

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DISCUSSION

RAS is an important disease characterized by recurrent ulcerations and oral mucosa and its etiology is not known certainly. The findings obtained from the analyses of the study, which was conducted to examine the relationships between the quality of life, psychopathological symptoms, childhood traumas and dissociative experiences, were discussed in line with the literature. It was found in this study that most of the participants were female, aged between 20 and 30, married, high school graduates and employed. A similar study has reported that RAS is seen in both sexes but it is more common among women [20]. Similar to the literature, more than half of the patients with RAS were women in this study.

The mean scores on the Brief Symptom Inventory, Hospital Depression and Anxiety Scale, Childhood Trauma Questionnaire, and Dissociative Experiences Scales, which were used to determine the mental states of the patients in this study, were high. Most patients did not receive psychiatric treatment and psychological support before. Since the participants' score on the depression subdimension of the Hospital Depression and Anxiety Scale indicated the existence of at least one depressive disorder, the patients need to receive professional support to eliminate depressive symptoms. The most common mental disorders in physical diseases are anxiety and depression, and they often co-exist [21]. A study conducted with 34 patients with Behçet's disease and 43 healthy individuals determined that the depression and anxiety scores of the patients were high [22]. A study, which was conducted to determine the relationship between recurrent aphthous stomatitis with oral mucosal lesions similar to Behcet's disease and psychiatric comorbidity and depression, determined that most patients experienced depressive disorders at least once [23]. A study by Gavi et al. conducted with 110 patients with RAS revealed that there was a high-level correlation between anxiety and depression symptoms and RAS symptoms [24]. Another study conducted with 50 individuals of whom 25 were patients with RAS and 25 were in the control group found that the patients with RAS had more psychiatric symptoms than the control group [25]. A study, which included patients with RAS, determined that hospital depression was higher compared to non-clinical cases, and there was a positive correlation between the diagnosis of RAS and psychological changes [26]. A study conducted with 50 patients with RAS and 25 individuals in the control group revealed that stressful experiences are triggering for the formation of chronic ulcers and the patients with RAS had more anxiety and depression symptoms than the control group [27]. The findings of this study indicate that RAS has a positive correlation with depression and anxiety in parallel with the relevant literature.

RAS negatively affects the quality of life at a certain level since it cannot be completely treated, has a chronic course, and has not one single ideal treatment method. The SF-36 is the most commonly used scale to determine the quality of life of patients with RAS, which is a multisystemic disease. It was found that the patients' scores on the physical function, physical role difficulty, emotional role difficulty, mental health, pain and general health perception subdimensions of the Quality of Life Scale were lower compared to the cutoff points. Cardoso et al. conducted a study on 22 patients with RAS and 22 individuals in the control group and revealed that the quality of life of patients with RAS was lower than the control group [28]. Another study conducted with 53 patients with RAS determined that RAS significantly decreased the quality of life of individuals by negatively affecting their oral health [29]. The study by Eren et al. conducted with 54 patients with Behcet's disease determined that the scores on physical function, physical role difficulty, general health and social function subdimensions of the quality of life were low [30]. Another study on the quality of life of patients with Behcet's disease and RAS found low values on all of the subdimensions of the SF-36 [31]. Dhopte et al. state that the diagnosis of RAS is a fundamental determinant factor for the quality of life and emphasize that practices for improving the living standards of these patients will contribute to the positive development of the treatment process [27].

Another finding obtained in this study is that there is a significant correlation between the patients' quality of life subdimension scores and brief symptom, childhood traumas, dissociative experiences, hospital anxiety and depression total scores. The variables do not affect the quality of life of the patients together. However, a positive correlation was found between the pain subdimension of the Quality of Life Scale and the somatization subdimension of the Brief Symptom Inventory and dissociative experiences. According to this finding, the somatization symptoms increase as the patients' pain increases. Studies conducted with a clinic sampling revealed that patients with dissociative disorder experienced somatization disorder more often and severe, and the consultation ratios and hospitalization due to medical reasons were high and determined that the severity of somatization in these patients increased as the severity of dissociative experiences increased [32, 33].

No study on the childhood traumas and dissociative experiences of patients with RAS was found in the literature. This situation makes this study more important. It is known that individuals experience conditions like depression, anxiety and hostility because of experiencing physical abuse in childhood, and its permanent or long-lasting effects continue throughout their life [34]. The number of studies that examine childhood traumas on the formation of most psychiatric disorders has been increasing in recent years and the relationships between childhood traumas and different psychiatric disorders (dissociative disorders, somatic symptom disorders, sleep disorders, personality disorders, obsessive-compulsive, anxiety and depression) have been determined [35, 36].

RAS is an important disease that must be paid attention to and examined since it has negative effects on the quality of life and is closely associated with some psychiatric symptoms (somatization, anxiety, depression) [37]. It is stated that stress-originated experiences like anxiety and depression are significantly associated with the quality of life in patients with RAS instead of personality structures and defense mechanisms [29]. Stress experiences may also lead to the onset of new RAS episodes in patients with a history of RAS. In general, it is seen that psychological stressors

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have a stronger relationship with RAS episodes compared to physical stress factors [38]. The emergence of cumulative scientific facts about the relationship between RAS and mental disorders emphasizes the importance of the need for evaluating these cases with a multidisciplinary perspective.

CONCLUSION

RAS is an important disease having negative effects on the quality of life and is closely associated with depressive symptoms. According to the results of this study, most of the patients with RAS show depressive symptoms. This study results found a positive relationship between the childhood traumas and dissociative experiences in RAS cases. Childhood traumas and dissociative experiences can be considered as a predisposing factor in patients with RAS. In order to reveal the relationship between RAS and childhood traumas, dissociative experiences and depression more comprehensively, studies with larger samples should be conducted. Patients with RAS should be investigated in terms of childhood trauma and treatment should be approached multidisciplinary. Mental evaluation should be an essential part of the examination of patients with RAS The cooperation between otorhinolaryngology and psychiatry-psychology will have significant effects on patients' treatments and quality of life.

Conflict of interests

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

Financial Disclosure

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Ethical approval

Approval was obtained from the Scientific Research and Publication Ethics Committee of İnönü University on 04.12.2018 with the decision number 2018/22-7.

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Case Report

Determination of ink aging by two different methods

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Abstract

Ink age determination; based on the detection of changes in the structure of the ink on the document over time. These changes are degradation of dyes, evaporation of solvents and polymerization of resins. The time for these changes to ocur varies according to the structure of the ink and the environment in which the document stored. In this study, the age of the document was determined by analyzing the dyes and solvents in the structure of the ink that document was sent by the court in 2017. The phenoxyethanol was which found in the structure of the ink was detected by thermal desorption –gas chromatography-mass spectrometer while the determination of the dyes (crystal violet, methyl violet, tetramethyl pararosanil and Victoria blue) were determined by high pressure liquid chromatography-ultraviolet detector. Dye and relative solvent ratios were calculated and compared in age determination. It was discovered that the signature was produced after the writings by examining the ratio of CV/MV for the signature and personal name. Additionally, the presence of contamination from a different document was detected through phenoxyethanol analysis. The age of the document was determined using the V%-time curve, revealing that the signature was created between 3-7 months from the date of analysis.

Keywords: Ink aging, questioned document, case report, GC-MS

INTRODUCTION

Forensic document experts works on the detection of document forgery. Experts analyze the document by dividing it into three parts. These are paper, writing instruments and ink. Ink age is determined according to changes in the ink structure over time by analytical methods[1]. There are many studies from past to present regarding ink age determination. Most of these studies based on at detecting either the degradation of colorants or the evaporation of their solvents by chromatographic or spectroscopic methods [2-13]. The reason for using ratio in studies on age determination is to eliminate changes in the amount of ink caused by ink print. In this study, the age of suspicious writings was determined by analyzing both the colorants with high pressure liquid chromatography-ultraviolet detector (HPLC-UV) and the phenoxyethanol with thermal desorption –gas chromatography-mass spectrometer (TD-GC/MS). In this study, both dye ratio(Crystal Violet peak area/ Methyl Violet peak area) and the relative solvent ratio (V %) were used.

CASE

Creditor A.D. and Debtor H.U. issue a promissory note in 10/10/2014. The promissory note also bears the signature of the debtor. According to Debtor H.U, Creditor A.D. changed the amount section on the signed promissory note and increased the amount that Debtor H.U. should pay. In 2017, when it came to us, the court requested a report on whether the ink structures of the writing, numbers and signatures on the deed are the same, whether

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there is an age difference between them, and whether they are issued on a new date and shown as old. Samples taken from name and signature shown in Figure 1.

Figure 1. Samples taken from name, numbers and signature in promissory note (Note: No.1 and 2 were taken for dye analysis. No.3 and 4 were taken for solvent analysis)

MATERIAL AND METHOD

Analytical Methods

To perform the HPLC analysis for crystal violet (CV), methyl violet (MV), tetramethyl pararosanil (TPR), and Victoria blue (VB), the Thermo Scientific Degasser System SCM 1000, Pump Spectra System P1000, Autosampler Spectra System AS3000, and UV detector 1000 system were utilized. The Phenomenex Onyx C18 Monolithic Column (100×4.6 mm) was used. For the analysis of phenoxyethanol, the Unity Thermal Desorber Agilent HP 6890N GC 5975B MS (Agilent Technologies, Palo Alto, CA) was used. The column used was a DBVR-X (60m×0.25 mm; thickness 1.4 µm), and a Tenax tube with activated carbon removal was used. Method validation and experimental steps are described elsewhere [3].

Sample Preparation

To prepare samples for analyzing dye content, two samples were taken from the suspicious writings using a 1.2 mm punch. These samples were mixed with 200 µL of methanol and vigorously mixed for 5 minutes using a vortex. The resulting mixture was then subjected to analysis using HPLC.

To analyze phenoxyethanol content, samples were taken from the signature, text, and non-ink parts of the paper using a 5 mm punch. This was done because solvents tend to diffuse both horizontally and vertically from the moment ink is transferred to paper. Placing a newly written paper on top of a promissory note can cause solvent migration and contamination. The samples were then analyzed using TD-GC/MS.

RESULTS and DISCUSSION

HPLC and TD-GC/MS are commonly used together for ink ageing analysis because they are complementary analytical techniques that allow for the detection and quantification of a wide range of ink components. HPLC is used for the separation and identification of dyes and pigments in ink samples, while TD-GC/MS is used for the identification and quantification of volatile organic compounds (VOCs) in the same sample. By using both techniques together, it is possible to analyze both non-volatile and volatile components in the ink sample, providing a more complete picture of its composition. TD-GC-MS is used for documents requiring short-term age determination, while HPLC is used for documents requiring long-term age determination. Phenoxyethanol analysis by TD-GC-MS is used to determine the time of suspicious writings for 0-24 months old documents. If phenoxyethanol analysis cannot be used to determine the time of suspicious writings, i.e. if the allegation in the document is older than 24 months and it is thought that there is a difference of at least 4 years between the creation times of suspicious writings, dyestuff analysis by HPLC is used.

Analysis of Dyes

Crystal violet and methyl violet dyestuffs were detected in the inks taken from both signature and writing samples. Chromatograms were given in Figure 2 and 3. Results were given in Table 1.

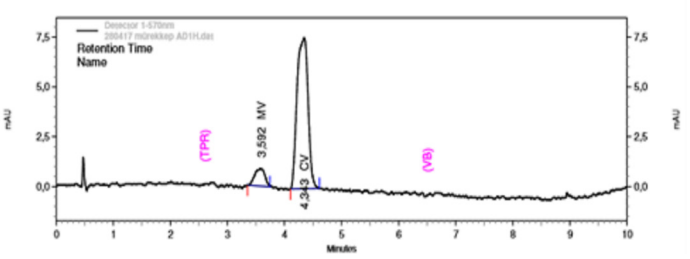

Figure 2. Analysis result of the sample taken from the personel name

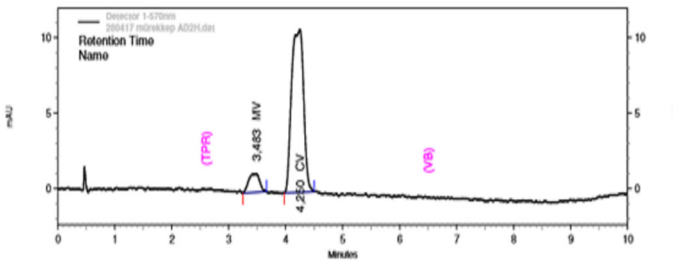

Figure 3. Analysis chromatogram of the sample taken from signature

Table 1. Analysis results of personal name and signature

Sample	TPR Peak area	MV Peak area	CV Peak area	Total area	CV Peak area/ MV Peak area
Personal name	-	10880	101549	112429	9.3
Signature	-	16459	158390	174849	9.6

When the CV peak area/MV peak area ratios of two suspicious texts on the same document are compared, three situations will emerge.

These :

Ratio A < Ratio B: A is written before B

Ratio A = Ratio B: Both articles were written at the same time

Ratio A> Ratio B: A is written after B

When the obtained rates were evaluated, it was determined that there was no significant difference and no interpretation could be made. In order for there to be a significant difference between the rates, there must be at least 4 years between the writing time of the suspicious articles.

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Analysis of Phenoxyethanol

The relative phenoxyethanol ratios (V%) of the analyzed samples were calculated. Results were given in Table 2.

Table 2. The relative phenoxyethanol ratios

Sample	90°C peak Area	200°C Peak Area	%V
2.000.000 £	835	10933	7
Signature	2310	4791	33

$\%V = M_{90°C} / (M_{90°C} + M_{200°C})$

$M_{90°C}$ = Phenoxyethanol Peak Area (Thermal Desorption 90°C)

$M_{200°C}$ = Phenoxyethanol Peak Area (Thermal Desorption 200°C)

In order to determine whether there is phenoxyethanol contamination in the document from another document, a sample was taken from the non-ink part of the suspect document and analyzed in TD-GC/MS as mentioned in the analytical methods section. We used V%-time(month) curve for V% values obtained from suspicious articles. V%-time curve was used from our previous study as the age curve. When we compare the V% values we obtained from suspicious articles with the curve, the signature was made within 3-7 months from the date of analysis but 2.000.000£ was not evaluated because the V% value was below 10. Since the curve starts to flatten below 10 in the V%-time(month) curve, there is no comment for this value and below.

CONCLUSION

In conclusion, the analysis of the suspicious promissory note aimed to determine whether the writing on it was done in 2014 or on a later date. Two analytical methods, dye analysis and phenoxyethanol analysis, were utilized to provide evidence for the court. Samples were taken from the ink and non-ink parts of the document and analyzed using HPLC and TD-GC/MS, respectively.The phenoxyethanol analysis also provided evidence of contamination from another document. The V%-time curve was used to determine the age of the document, and it was found that the signature was made within 3-7 months from the date of analysis. Therefore, the document could not have been issued in 2014 as alleged.

Consequently, the use of analytical methods in forensic science plays a critical role in providing evidence in legal cases. The combination of different techniques allows for a comprehensive evaluation of the samples and provides strong evidence that can be used in court. In this particular case, the use of HPLC and TD-GC/MS allowed for the determination of the age of the document and provided evidence that the document was not written in 2014 but at a later date. This study demonstrates the importance of analytical methods in forensic science and their potential to contribute to the resolution of legal cases.

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

Financial Disclosure
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Ethics committee approval is not required.

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Review Article

Identification methods in mass disasters

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Abstract

Different numbers of people are affected in mass disasters. Identification of the disaster victims is a very important issue. For an accurate identification process, good and fast management of this process is of great importance. Identification of the victims includes investigating the disaster field, autopsy and following that post mortem data collection, ante-mortem data collection, and comparison of collected data. If these steps can be applied properly, identification of victims will be completed successfully. There are different techniques for victim identification in mass disasters and these methods can be divided into two categories as primary identification methods and secondary identification methods. Primary identification methods are more reliable than secondary identification methods. Fingerprint analysis, DNA analysis, medical records of the victims & physical examination and dental examination are primary identification methods. Visual identification and determination of personal belongings of the disaster victims are secondary identification methods. In the light of these methods, the identification process will be completed with an accurate evaluation.

Keywords: Disaster victim identification, fingerprint, DNA, dental examination

INTRODUCTION

Classification of disasters

A sudden event or events that cause significant damage or loss of life and disrupt the normal functioning of a community or society is called a disaster.

Disaster victim identification (DVI) is the process of identifying victims of a mass fatality incident, such as a natural disaster, accident or terrorist attack, after they have died. The aim of DVI is to determine the identity of each victim so that their families can be notified and their remains can be handled appropriately. This process involves various scientific techniques and methods, including forensic odontology, DNA analysis, fingerprinting, and anthropological analysis. Identification of victims following a mass death involves collecting and analyzing official, physical, and personal information for each deceased individual. It is essential to complete this identification process as quickly as possible to minimize the psychological impact on surviving families and expedite the recovery process. [1]. Regardless of the scale of the event, the primary goal of entities involved in DVI is to accurately identify victims using any viable means available. Although various countries have their own established standards and protocols for conducting DVI, in 1984, the International Criminal Police Organization (INTERPOL) published a comprehensive DVI guide to promote universal practices and ensure proper identification techniques are utilized [2].

Disasters can occur in many ways, either naturally, such as earthquakes, hurricanes, floods, forest fires, and volcanic eruptions,

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or man-made, such as terrorist attacks, industrial accidents, and transportation accidents [3].

We can classify disasters under two main headings;

●Natural disasters: Disasters caused by natural events such as earthquakes, hurricanes, floods, forest fires and tsunamis are included in this group.

●Technological disasters: Disasters caused by man-made technology and systems are included in this group. Examples of such disasters are industrial accidents, transportation accidents, nuclear accidents and explosions.

Disasters also can be classified into two groups known as "open disasters" and "closed disasters" based on the assumption that the event causes numerous specific and non-specific victims. In an open disaster we know the victims names but in closed disasters we don't. Earthquakes or tsunamis can be an example for open disasters. But a hotel fire or an airplane crash can be classified as a closed disaster [4].

They are also disasters that are caused by the combination of several factors like armed conflict or political instability. Such disasters are called complex emergencies [5].

There are several methods used for disaster victim identification (DVI), depending on the circumstances of the disaster and the available resources. Some common methods are visual identification, dental identification, fingerprint identification, DNA analysis, anthropological analysis, personal effects and belongings, medical records and implants [6,7].

In most cases, a combination of these methods is used for identification. Teeth, fingerprints and DNA are used as primary identifiers in DVI studies, as they remain intact for a long time and can be measured by scientific methods [8]. It's important to know that disaster victim identification can be a complex process, and it requires careful documentation, coordination, and communication among different agencies.

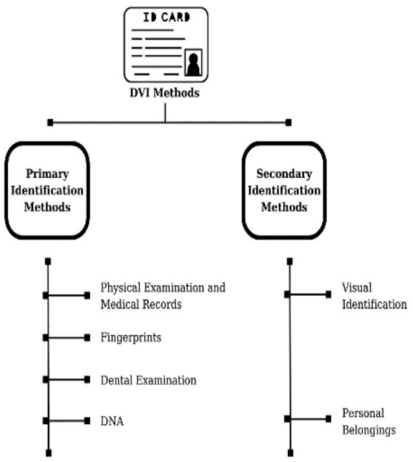

Figure 1. Summary of disaster victim identification (DVI) methods

Disaster Victim Identification Methods

Creation of proper guidelines for disaster victim identification is very important. According to information given by Interpol, the first guideline for DVI was produced in 1984 and updated every five years [9].

Primary Identification Methods

Physical Examination and Medical Records

The accurate identification of individuals involved in a mass fatality incident can be difficult, depending on the quality and availability of information about the victims [10]. Interpol has categorized this information into two types: circumstantial and physical evidence. Circumstantial evidence includes personal belongings such as clothing, watches, keys. However, it should never be relied upon alone to identify the individuals [11].

On the other hand, physical evidence obtained through external or internal examination of the individual. General external examination can reveal important data for identification, such as body anomalies, tattoos, scars, and wounds. Autopsy findings, such as pacemakers and prostheses, can also provide valuable information [12]. Similarly, internal examination can provide evidence of previous surgical procedures, natural disease, or prosthetics, which may be specific to the individual. Interpol has established standard operating procedures for gathering this physical information due to its significance in accurate identification.

Fingerprint Examination

Fingerprint examinations are one of the most reliable methods used for identification for many years. The structure consisting of raised linear lines on the fingertips and forming folds by coming together in different variations in each person is called the papilla line. Fingerprints are formed by the contamination of the biological fluid secreted by the body and accumulated on the papilla lines [13].

Fingerprints, which begin to form in the mother's womb and have personal shapes, have the characteristics of being dissimilar, unchanging and classifiable. There is no absolute overlap in the papilla lines in two people or in one person's fingers. Even identical twins have different fingerprints. This is due to the irregular formation of the skin on the palms of the hands and soles of the feet during the embryonic period. The uniqueness arises thanks to the large number of cracks and the different formation of papilla lines. Papilla lines form after the fourth week of the embryo and they remain present after death. They reappear at the same rate in injuries that occur on the surface. In this way, they are used as an identification method with high discrimination power.

The pattern of fingerprints corresponds to the unique arrangement of friction ridges. These friction ridges are classified into three primary types - loops, whorls, and arches - each characterized by

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distinct variations in their shape and the relationship between the ridges. As seen in Figure 2, the three basic fingerprint patterns are as follows: (a) the arch, which is the simplest of all configurations; (b) the loop, where ridges flow towards the margin of the digit - if the loop opens towards the ulnar margin, it is known as an ulnar loop, while if it opens towards the radial margin, it is a radial loop; and (c) the whorl, which is the most complex of the three patterns [14].

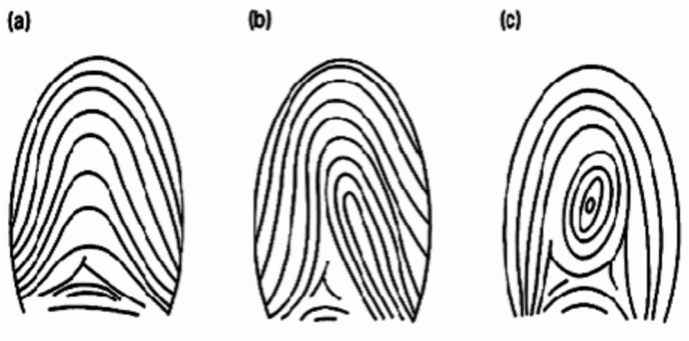

Figure 2. Major types of fingerprint patterns a) arch, b) loop, and c) whorl patterns [15]

Dental Examination

Dental identification is a crucial part of the disaster victim identification process. The vast array of dental characteristics available to us is an abundant source of information [16]. From the number of teeth present to their distinctive variations and history of dental procedures, each aspect provides unique insights into a person's identity. This method has been shown to be highly effective in cases where human remains are unidentifiable, such as when they are skeletal, decomposed, burned, or only partial.Identification with teeth is especially useful in situations where there has been a high number of casualties. If the features of the victim are recognizable, identification should be based on facial features or fingerprints. Estimating age in certain samples can be easier due to tooth development in children and adolescents or age-related morphological changes in adults. Dental age can be estimated through various methods, such as analyzing age-related morphological or radiological parameters. To ensure accurate recording of all dental characteristics, it is recommended to use both color photography and radiography.

For better access to the dentition, it is recommended to use non-destructive techniques. Jaws should not be removed by dental experts as a general rule, unless a more specific examination is necessary.

The 2004 Indian Ocean earthquake and tsunami was a major open disaster for the Thai government. The Japanese government initially classified the disaster as open too. However, after conducting over 3,000 inquiries and reviewing a significant amount of information, the Japanese government reclassified the event as a closed disaster due to their comprehensive knowledge of all Japanese citizens and nearly all Japanese victims in the disaster was accomplished through dental findings [17].

The data was divided into two categories: post-mortem (PM) data, collected during forensic medicine autopsies at the disaster site, and ante-mortem (AM) data, collected in the victim's native country. The dental information for each victim was entered into a computer system and compared to identify matches. If a match was established, it was reviewed and confirmed by forensic odontologists and Thai authorities, and an official death certificate was issued. The matching of the AM and PM files was a crucial step in verifying the identity of the victims [18].

Also estimating the dental age post-mortem allows forensic odontologists to narrow down their search for matching ante-mortem files within a specific age range among the potential candidates for identification from the missing persons list. Furthermore, lip prints (morphological differences in the wrinkles of the red lip) are also distinctive and employed in personal identification [19].

DNA Analysis

DNA is one of the best reliable identification methods. In mass disasters, diverse factors may be effective in the identification process. Intermixing of victim remains, decomposition or deterioration of body integrity are some of these factors and due to them, anthropological analysis and physical characteristics may not be sufficient for identification. Due to these factors, DNA profiling became the gold standard [20].

In identification processes, it is important that samples maintain their durability. Because in major disasters, identification can take a very long time and the time taken until the samples are analyzed may vary. To illustrate, in the World Trade Center attack, more than 2700 people lost their lives on September 11th 2001 [21].

With people from many different fields working together, approximately 1500 victims were identified through June 2003. Even though the process seems like two years, the majority of these identifications were performed within the 8-12 months after the attack [21]. According to this information, it can be said that it is important that the samples are kept in good condition.

In disaster victim identification, different parameters may affect DNA identification goals [22]. Number of victims involved in the disaster, places where bodies are found, extent and mechanism of body fragmentation are some of these factors. Degradation rate of DNA may be affected by many factors depending on the environment. Lastly, availability of DNA reference samples is important for identification of victims.

The shorter the time until the DNA analysis, the better the damage from external influences can be managed. In order to achieve that Rapid DNA Identification systems are developed. In a study, this system was evaluated in two different scenarios (human bodies stored in a morgue/cooler and placed above ground) that may be faced in disasters. In addition, Rapid DNA Identification system was assessed in different types of tissues. The research

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group concluded that all samples types from refrigerated remains showed excellent DNA identifications for 3-month period while in the other group; tooth and bone samples showed excellent DNA identifications for 1-year and buccal swabs showed excellent DNA identifications up to 11 days exposure [23].

It is very important to manage the situation well in mass disasters. Preserving the bodies of victims in good conditions is one of the most important steps in terms of the quality of the analyzes to be made. In a study in which the identification process of the victims of the tsunami in 2004 was handled in Thailand, Indonesia, and Sri Lanka, it is stated that rapid decomposition of bodies caused serious problems for the identification process after 24-48 hours. In addition, they stated that identification of victims was achieved by dental and fingerprint data in Thailand. Identification by DNA was applied for the few number of victims [24]. Depending on the type of disaster, body integrity may not be preserved in mass disasters. While 86.2% of the samples studied in the collapse of a mine tailing dam in Brazil were body parts, only 13.8% were bodies that had preserved their integrity. In the disaster that killed 270 victims, 603 biological materials were studied and 259 of these victims could be identified [25].

We have previously stated that different identification methods can be used depending on the type of disaster and the time of analysis. In this case, while the identification was made with fingerprints in the first weeks following the disaster, depending on decomposition of body parts, the DNA method was preferred [25].

In the context of disaster victim identification (DVI), short tandem repeats (STRs) are usually used in DNA testing. These biomarkers are found in 2-7 nucleotide base length and they are inherited in a proper way to classical Mendel characteristics [26]. For the DVI process; DNA is isolated and copied by PCR. Then, fragment analysis is done and STR comparison is conducted with reference samples. Usually, autosomal STRs are analyzed but in some cases gonosomal STRs (X-STR, Y-STR) may be investigated additionally.

Another type of biomarkers that may be useful in DVI applications are single nucleotide polymorphisms (SNPs). They are single base changes that occur in the DNA sequence. These changes are base changes, insertions, and deletions. SNPs can be used in forensic sciences to determine paternity and lineage, or to determine the phenotypic characteristics of an individual. SNPs that are not as polymorphic as STRs need to be included more in number in order to provide equivalent information with STRs on discrimination power [27]. In the concept of DVI, SNPs can be used as supportive biomarkers in some cases.

STR typing was conducted in the World Trade Center human identification project and the success rate of DNA profiling was 75% of the examined cases. Merging of soft tissue transfer and other remnants, there may be some problems. According to the project data, they concluded that if only a soft tissue test is applied, there may be problems in DNA testing. Therefore, it was stated that it is important for anthropologists to take part in studies and identify inconsistencies [28].

DNA degradation is one of the important issues in DNA testing. In mass disasters, DNA degradation may be observed at different rates depending on distinct conditions. In order to solve this problem mini primer sets are implemented for reducing STR size. Yudianto and Setiawan conducted a study that investigates mini primers for three different loci at two different temperatures. According to study; as temperature rises, DNA content decreases. Out of these three loci, only one of them was detected at highest temperature [29]. If the number of such loci can be tested for different parameters, they can become quite useful in DVI studies.

In disaster victim identification DNA testing is usually performed by using STRs. However, some cases may require additional biomarkers. To illustrate, a boat with more than 500 migrants sank near Lampedusa in 2013 and most of the identification was done by using 16 autosomal STRs. Yet, in some cases, gonosomal and lineage (Y-chromosome and mtDNA) markers were used as additional markers [30].

Depending on the place where the disaster occurred, degradation rate and quality of DNA may be affected. Unlike traditional methods like STR typing, other methods may be useful. Mitochondrial DNA (mtDNA) analysis is one of the methods that can be used in such cases.

Mitochondria have a unique division cycle and a circular DNA of 16,569 base pairs [31]. Mitochondrial DNA (mtDNA) analysis is one of the analyzes used in forensic sciences since 1996 [32], and hypervariable regions called HVRI and HVRII, which have a highly polymorphic structure in the non-coding D loop region of mtDNA, are analyzed [31].

MtDNA is passed on from mother to child. Therefore, all maternal line relatives share the same mtDNA. When there is a limited amount of biological samples, mtDNA is a very useful biomarker. Because mtDNA is found in greater numbers (with a high copy number) than other types of DNA. Individual identification capability of mtDNA is limited due to the lack of recombination, but it is an advantageous method for confirming maternal lineage and it may be used in disaster victim identification (DVI) triage [33].

In an evaluation made in 2021, It has been stated that the identification of the victims of the 2011 Great East Japan Earthquake is still ongoing. Victims are identified by mtDNA when identification is not available by using nuclear DNA. Identification by mtDNA is a useful technique but it is time-consuming, expensive and requires well-trained analysts [34].

In order to detect genetic relatedness between victims of disasters with relatives, different techniques may be used and Machine Learning (ML) algorithms are one of the novel techniques. In a study, ML was applied in order to predict genetic relatedness using

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human mtDNA hypervariable region I sequences and according to the study, ML can be employed as a complementary tool [35]. In identification analysis; Compared to bone tissue and teeth, muscle tissue samples and bone marrow swabs are preferred because these tissues are faster and easier to process [36].

The transfer of muscle tissue to the FTA card from an incision made in the body to be identified can be used for sampling. It is stated that this method is an effective and economical method in identification studies in mass disasters. In addition, a similar method was applied for tsunami victims in 2004 [37].

Human remains preferred in identification studies; soft tissues (skeletal muscle, organ tissues and skin) and blood. Hard tissues (bone and teeth) are the preferred specimens if the corpse is decomposed or if environmental factors exist that would cause the DNA in soft tissue to deteriorate. Samples that can be collected for identification from corpses that have no signs of decay and that have preserved their integrity; blood sample and oral swab sample. The blood sample can be transferred on an FTA card or collected as a swab. However, if a sample is to be taken from a corpse with disrupted body integrity, taking into account that there is no evidence of decay, blood or approximately 1 g of deep red muscle tissue can be taken as a sample [13].

Within the scope of identification of disaster victims, DNA tests can be performed from many different biological materials. If the event is very recent and blood, tooth or bone samples cannot be collected, heart muscle is the most suitable biological material for DNA testing. The analysis can be continued by collecting the heart muscle, which is also very rich in mitochondrial DNA.

Secondary Identification Methods

While primary methods provide an accurate identification, the results of secondary methods are not certain. However, they can be used as supportive methods. Visual identification is one of the secondary identification methods. According to the type and the occurrence form of disaster, visual identification may be used. If there is no deformation on the body, this method can be preferred as a support. But in some disasters, victims can be found beyond recognition. In such cases, it can be very difficult to identify people visually. People often recognize their relatives visually, but in some cases, they may not be able to identify even their closest relatives because they are psychologically affected by the disaster. Due to the psychological trauma they are in at that moment, there may be a problem in identifying the victim. In psychology, this is explained by people's urge not to learn the truth or to reject it [13].

Personal belonging of victims is the other secondary identification method. They can be very important in locating victims in disaster areas. For example, in the event of an earthquake, it is very important to get an idea of who the person is thanks to the belongings next to a person who was removed from the rubble, or it is very important to have an identity on the person.

To give another example, jewelries or physical identification of documents may be used in DVI processes [20]. In some cases, uniforms or similar professional clothes on people will shorten the identification process considerably. Religious clothes or some symbols of religious belief are also important data that will affect the identification process. In addition, reference samples can be collected from the person's belongings to be used in DNA analysis. For example, a person's comb or toothbrush can be good sources of DNA. In addition, used glass-like items (for instance, mugs) or underwear are also sources of medium quality DNA. Although they are weaker sources than others, items such as wrist watches, jewelry or clothes of the person to be identified can also be used as DNA sources [13].

CONCLUSION

Disasters can affect different numbers of people. Identification of victims is much more difficult when the number of affected people is high. Disasters can actualize open air or interior. In general terms, there are four different steps in the DVI process. Depending on the disaster place, methods for identification can change. Field study about disaster areas is one of the most important parts of identification. It is very important that the field analysis is done correctly and that the victims are reached without breaking the integrity. After field work, autopsy and collection of post mortem data takes place. During this process, necessary information for identification can be obtained. Different biological materials and physical determinants are examined. Depending on disaster type, the extent of bodily damage may vary. Therefore, fingerprints cannot be obtained. If fingerprints are in good condition, they are very reliable for identification. In addition, distinctive physical features of people can be used for identification. Third identification method is dental examination. Teeth are one of the most reliable identification methods. The other reliable identification method is DNA analysis. Comparison of DNA samples from victims with family members is a very robust identification method. All of these four mentioned methods are primary identification methods. Therefore, they are very reliable. In addition to them, there are secondary identification methods. Visual identification and personal belongings are in this category.

These methods can be used as auxiliary methods but they are not as reliable as primary identification methods. After autopsy, ante-mortem medical records of a victim are collected in order to use for comparison. Relatives or personal belongings of victims can be used as comparison material for identification. Later, ante-mortem medical records of a victim are compared with their post-mortem findings. As a result, identification of the victim is completed.

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

Financial Disclosure
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Ethical approval
Ethics committee approval is not required.

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Review Article

Detection of alcohol use: Guidance of direct biomarker phosphatidylethanol

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Abstract

Acknowledging the fact that alcohol is an important source of fatalities in traffic, the amount of alcohol consumed and the exact time of the consumption could enlighten forensic cases and guide the justice system correctly. However, determining the alcohol use is a difficult problem due to alcohol metabolism in individuals and parameters such as sex, age, amount of alcohol in the drink, satiety, should be taken into account which can be challenging for amount of alcohol interpretation. Considering that blood alcohol concentration (BAC) may not be reliable, alternative metabolic products of alcohol has arisen after alcohol consumption. One of the most interesting alcohol biomarker phosphatidylethanol (PEth) has caught attention due to its long half-life and not being affected from sex, liver diseases or age in addition to that it is only synthesized under the presence of ethanol. PEth is synthesized in cell-membranes and not being a single molecule, its homologues should be considered when determining the amount of alcohol intake. Although the homologues of PEth could be isolated from whole blood, less invasive dried blood spots (DBS) also provides reliable information. The analysis of PEth is performed in LC-MS/MS which is highly sensitive and specific. For forensic applications, direct alcohol biomarker PEth may be useful for distinguishing the alcohol use and helpful for justice system. This review focuses on studies about PEth biomarker, its applications and limitations conducted from 2010 to 2019.

Keywords: Direct biomarker PEth, LC-MS/MS, alcohol

INTRODUCTION

According to the report published from OECD, alcohol-related traffic accidents in Europe is approximately 12.9% [1]. Alcohol is an easily accessible legal drug and used in social environments. According to World Health Organization, in 2016, 3 million people died worldwide due to alcohol-based reasons [2]. Giving the importance of alcohol in terms of forensic sciences, determining the amount of alcohol consumed become essential. Although it is hard to detect the ethanol in biological matrices after 10-12 hours after consumption [3], other biomarkers produced in the body might be more convenient for detection of alcohol. There are indirect and direct alcohol biomarkers to identify alcohol consuming for helping the justice. Among indirect biomarkers aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), mean corpuscular volume (MCV) and carbohydrate deficient transferrin (CDT%) provide information with indirect response of body after the ethanol consumption [4]. Indirect biomarkers generally used for supportive evidence of alcohol use due to their low sensitivity and specificity [4]. To obtain reliable information for alcohol use, direct biomarkers which are ethyl glucuronide (EtG), ethly sulfate (EtS), fatty acid ethyl esters (FAEE) and phosphatidylethanol (PEth), are relied upon due to their power of being ethanol metabolites [5]. However, there are some limitations in every biomarker. For instance, EtG and EtS are the most common used biomarkers for clinical studies and forensic laboratories, although their detection time in both urine and serum is short [3]. FAEEs are generally used

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for chronic alcohol determination from hair [4] and alcohol misuse of the mother from meconium [6].

PEth as a Biomarker

PEth is the name of different phospholipids found in the outer layer of the cell membranes. Not representing a single molecule, PEth is synthesized from one of the basic homologues forming the outer cell membrane phosphatidylcholine (PC). PC is an interesting substance that is affected by the nutrients intake and when water is present in the cell, with the help of phospholipase D (PLD) enzyme, it is converted to phosphatidic acid (PA) and choline. However, if there is an alcohol intake, PLD enzyme has more affinity to ethanol than water, PC forms PEth and choline which makes the reaction highly specific [3,4,7,8].

PEth homologues can be detected in various parts of the body including brain, liver, and kidney. Although it is found in some parts of the body, antemortem forensic cases require different matrices for detection of PEth. The fact that PEth is successfully isolated from erythrocytes in whole blood, it is routinely analyzed in some countries for determining alcohol misuse. In whole blood, the most abundant homologue of PEth is 16:0/18:1. This homologue made up 37% of all PEth and it is formed by PC 16:0/18:1, which the left side represents the number of carbon atoms and the right side shows the number of double bonds in the homologues. Among forty homologues, other PEthsbe considered while deciding the amount of alcohol intake are 16:0/18:2, 16:0/20:4, 18:1/18:1from high percentage to low, respectively [7-9].

As being discovered at 1983 in rats and seen as a potential alcohol biomarker at 1997 [7], scientists have studied PEth homologues and their applicability for approximately 40 years. PEth is a powerful source for deciding chronic alcohol use and abstinence. In addition, it can guide to differentiate between moderate and low alcohol consumption. There has been studies for PEth and promising results have been found (Table 1).

Table 1. The summary of PEth studies

Biomarkers Studied	Study Matrices	Study Groups	Study Design	Results	Reference
MCV **ALT** **AST** **GGT** **CDT** **FAEE** **EtG** **EtS** **PEth**	Serum Whole Blood Hair Urine	Driving Under the Influence (DUI) participants	Examining the indirect and direct biomarkers, studying AUDIT, TLFB, DRINC, TRI as psychometric evaluation, The duration time of the study:8 months	PEth is the most strong indicator to correlate with BAC, other biomarkers and psychometric tests	[10]
PEth (16:0/18:1) **CDT** **GGT**	Whole blood	Healthy social-drinkers	Studying volunteers with 3 weeks of abstinence and requesting them to consume alcohol for 5 consecutive days to reach BAC with 1 g/kg by blood collection of 20 days	PEth can be detected as long as ethanol is present in the body and there is a concentration and half-time difference between alcoholics PEth values versus social drinkers, which is alcoholics have higher PEth values and shorter half-lives	[11]
PEth (16:0/18:1) **(16:0/18:2)** **BAC**	Whole blood	DUI cases	Determining cut-off values for PEth 16:0/18:1 and 16:0/18:2 by examining BAC levels of DUI cases with prolonged excessive drinking	Cases with BAC ≥ 1.6% levels accepted in the category of excessive drinking and the cut-off levels determined as PEth (16:0/18:1) ≥ 700 ng/mL and PEth (16:0/18:2) ≥ 300 ng/mL	[12]
PEth (16:0/18:1) **CDT** **GGT** **AST** **ALT**	Whole blood	Alcohol-dependent patients in a clinical trial to reduce alcohol consumption	Patients level of alcohol biomarkers examined and self-report of AUDIT including AUDIT-C tests	Although PEth is superior to CDT, both of which are the biomarkers correlate with self-report; however, no difference between sexes compared to PEth half-lives	[13]
PEth (16:0/18:1) **(16:0/18:2)** **EtG**	Whole blood Urine Hair	16 healthy volunteers	Single-dose of alcohol prepared to reach 1 g/kg BAC levels for two	PEth 16:0/18:2 was detected at a lower concentration than 16:0/18:1 and the latter	[16]

AUDIT: Alcohol Use Disorders Identification Test, TLFB: Alcohol Timeline Followback Method Assessment, DRINC: Drinkers Inventory of Consequences, TRI: Temptation and Restraint Inventor

CONCLUSION

In conclusion, phosphatidylethanol (PEth) has emerged as a promising alcohol biomarker due to its high specificity and sensitivity for detecting recent heavy alcohol consumption. PEth is formed in the body when alcohol is metabolized, and its levels in blood and other bodily fluids can be used to determine the extent and duration of alcohol consumption. Its reliability is based on the fact that PEth is produced only in the presence of ethanol and is not influenced by other factors such as food intake or non-alcoholic beverages.

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In the light of the results obtained from the previously mentioned articles, studies on PEth in 2020 and beyond show us that PEth shows promise as a biomarker [17-22].

PEth is advantageous over traditional alcohol biomarkers such as blood alcohol concentration (BAC) and liver function tests (LFTs) because it is less susceptible to short-term fluctuations and can detect alcohol use up to several weeks after consumption. Additionally, PEth testing is non-invasive and can be performed using a simple blood test, making it a more convenient option for both patients and healthcare providers. PEth has been shown to be useful in a variety of settings, including clinical practice, forensic toxicology, and alcohol research. Although PEth has limitations such as inter-individual variability and the need for specialized analytical equipment, its advantages make it a valuable tool for assessing alcohol consumption in both clinical and research settings.

Further research is needed to establish standardized cutoff levels for PEth and to determine its usefulness in detecting different patterns of alcohol consumption, such as binge drinking or chronic heavy drinking. Nevertheless, PEth is a promising alcohol biomarker that has the potential to improve the accuracy of alcohol assessment and to inform treatment decisions for individuals with alcohol use disorders.

Conflict of interests

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

Financial Disclosure

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Ethical approval

Ethics committee approval is not required.

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