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Original Article

How bruxism and temporomandibular joint disorders relate to anxiety and sleep in pregnant women?

Ayca Araci[1], Esra Top[2], Merve Cakir Kole[3], Ayse Unal[1]

[1]Alanya Alaaddin Keykubat University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Antalya, Türkiye
[2]Alanya Alaaddin Keykubat University, Graduate School of Education, Department of Anatomy, Antalya, Türkiye
[3]Alanya Alaaddin Keykubat University Training and Research Hospital, Department of Gynecology and Obstetrics, Antalya, Türkiye

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Abstract

Aim: This study aimed to investigate the relationships among TMD severity, bruxism, anxiety levels, pain perception, and sleep quality in pregnant women, offering insight into the complex biopsychosocial interactions occurring during gestation.
Materials and Methods: A retrospective cross-sectional analysis was conducted on clinical and survey data from pregnant women. Assessment tools included the Fonseca Anamnestic Index, the Pittsburgh Sleep Quality Index (PSQI), the Visual Analog Scale (VAS), and the State-Trait Anxiety Inventory (STAI).
Results: Significant associations were found between TMD severity and age, as well as between trait anxiety levels (STAI-II) and Fonseca scores. A weak but statistically significant correlation was also observed between pain levels and both state and trait anxiety. Conversely, no significant differences were found between TMD groups regarding educational status, number of pregnancies, or functional jaw movements.
Conclusion: The findings suggest that TMD symptoms during pregnancy are not solely a result of hormonal changes but are influenced by a complex interplay of psychological stress, anxiety traits, and parafunctional behaviors. Evaluating TMJ health in prenatal care settings may enhance maternal well-being, and longitudinal studies are needed to further elucidate these relationships across different stages of pregnancy.

Keywords: Temporomandibular joint dysfunction, bruxism, pregnancy, anxiety, sleep quality, parafunctional habits

INTRODUCTION

Pregnancy represents a unique period during which women undergo profound physiological, psychological, and hormonal changes. Elevated anxiety levels, hormonal fluctuations, and disruptions in sleep patterns during this time can significantly impact both maternal health and fetal development. In recent years, there has been growing scholarly interest in exploring the effects of these factors, particularly on the orofacial system [1–4].

Bruxism, characterized by habitual teeth grinding and clenching, is closely associated with temporomandibular joint dysfunction (TMJ), and both conditions can significantly impair an individual's quality of life. Numerous studies have examined the relationship between these disorders and psychological factors, particularly anxiety disorders and sleep quality. However, research focusing specifically on these conditions during pregnancy remains limited [1].

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Corresponding Author: Ayse Unal, Alanya Alaaddin Keykubat University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Antalya, Türkiye
Email: pt.aunal@gmail.com

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It has been suggested that elevated levels of hormones such as estrogen and relaxin during pregnancy affect connective tissue integrity, potentially leading to the loosening of ligaments within the temporomandibular joints. This hormonal influence may contribute to an increased susceptibility to temporomandibular dysfunctions during this period [5–7].

An epidemiological study conducted by Fichera et al. (2020) reported that 81% of pregnant participants exhibited notable symptoms of TMJ disorders, compared to 52% in the non-pregnant control group. The most frequently observed symptom was joint clicking sounds. These findings suggest that pregnancy may represent a potential risk factor for the development of TMJ disorders [1].

Pregnancy is also characterized by heightened stress and uncertainty. Women's responses to the gestational period—shaped by factors such as physical changes, lack of economic and social support, and anxieties about parenthood—can significantly influence both anxiety levels and sleep quality. Several studies have indicated that elevated stress during pregnancy is associated with reduced sleep quality, which in turn may exacerbate symptoms of temporomandibular disorders (TMD) [8,9]. Sleep disturbances not only compromise physical well-being, but also diminish psychological resilience, thereby potentially lowering an individual's pain threshold [8].

In addition to hormonal influences, elevated anxiety levels during pregnancy are also believed to contribute to an increased prevalence of bruxism. Heightened anxiety may induce involuntary tension and clenching in the masticatory muscles, potentially leading to structural damage in the temporomandibular joint and a substantial decline in sleep quality [10,11].

In this context, the interplay between physiological changes and psychological stressors during pregnancy appears to create a predisposition for functional disturbances within the temporomandibular system. Therefore, assessments that adopt a holistic approach—considering both psychological and physical health—are of critical importance.

This study examines the interrelationship between TMD, bruxism, anxiety, and sleep quality in pregnant women. It aims to determine the prevalence of TMD and explore its association with anxiety levels, addressing a gap in the literature and contributing to multidisciplinary maternal health research.

MATERIAL AND METHOD

This cross sectional analytical study is conducted through a retrospective analysis of data collected from pregnant women who visited the Obstetrics and Gynecology Outpatient Clinic of Alanya Alaaddin Keykubat University Training and Research Hospital between November 2024 and April 2025. The study involves no interventional procedures; only data obtained from archival records and assessment forms were analyzed.

Ethical Aspects of the Study: The study was approved by the Clinical Researches Ethics Committee of Alanya Alaaddin Keykubat University (Date: 16.04.2025, Decision number: 07/05). Written informed consent, prepared in accordance with the Helsinki Declaration, was obtained from the participants included in the study.

Power Analysis: The prevalence of bruxism during pregnancy was found to be 67% in the reference study. With the unknown sample size formula, the deviation margin was accepted as 10% (d=0.1) and the prevalence of Bruxism during pregnancy was accepted as p=0.67 (67%) and as a result of the calculation made at a 95% confidence level (alpha=0.05), it was calculated that at least 85 samples could be included in the study. Eighty six pregnant women were included in our study. As a result of the power analysis performed for the effect of the relationship between the anxiety levels and bruxism levels obtained from 86 participants (r=0.254), it was calculated that our study reached 78% power at a 95% confidence level (alpha=0.05) [10].

Inclusion Criteria: Healthy pregnant women, willingness to participate in the study, minimum literacy level in reading and writing, presence of teeth clenching and grinding (bruxism), limited mandibular movement.

Exclusion Criteria: Presence of intercurrent vaginal bleeding, diagnosed depression or anxiety disorders.

Criteria for Withdrawal from the Study: Medical conditions requiring intensive care or surgical intervention, pregnant individuals who do not sign the informed consent form.

Assessment Methods

The following variables were evaluated: mouth opening, temporomandibular joint dysfunction and its severity, pain intensity, sleep quality, quality of life, and anxiety levels. The assessment tools employed in the study were: Fonseca Anamnestic Index, Pittsburgh Sleep Quality Index (PSQI), Visual Analog Scale (VAS), State-Trait Anxiety Inventory (STAI).

Opening Movements: During the painless mouth opening task, the pregnant participant was instructed to open her mouth as widely as possible without experiencing any pain. The distance between the incisal edges of the upper and lower central incisors was measured using a millimetric ruler. Additionally, right and left lateral movements of the mandible were measured and recorded [11].

Fonseca Anamnestic Index: The Fonseca Anamnestic Index, consisting of 10 items, will be used to assess the presence and severity of TMD. Participants are asked to respond to each question with "Yes," "No," or "Sometimes." A "Yes" response is scored as 10 points, "Sometimes" as 5 points, and "No" as

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0 points. Each item is scored individually, and the total score determines the severity of TMD. According to the classification: A score of 0–15 indicates no TMD, 20–40 indicates mild TMD, 45–60 indicates moderate TMD, and 70–100 indicates severe TMD [12].

Pittsburgh Sleep Quality Index (PSQI): The Pittsburgh Sleep Quality Index (PSQI) is a self-report assessment tool developed by Buysse et al. in 1989, designed to evaluate sleep quality, types of sleep disturbances, and their severity over the past month. The Turkish validity and reliability study of the PSQI was conducted by Ağargün et al. in 1996.

The PSQI consists of 24 questions, 19 of which are self-reported by the participant. The remaining 5 questions are to be answered by a spouse or roommate. Of the 19 self-report items, 18 contribute to the scoring, which are grouped into seven components: subjective sleep quality (component 1), sleep latency (component 2), sleep duration (component 3), habitual sleep efficiency (component 4), sleep disturbances (component 5), use of sleeping medications (component 6), daytime dysfunction (component 7).

Each component is scored on a scale from 0 to 3. The total PSQI score is the sum of these component scores, ranging from 0 to 21. A global score of 0–4 indicates good sleep quality, while a score of 5 or higher suggests poor sleep quality [9].

Visual Analog Scale (VAS): In this study, a visual scale validated by Bryant (1993) was used to measure the intensity of pain. The scale is 100 mm in length, with one end marked as "0" and the other as "10." A score of 0 indicates "no pain at all," while a score of 10 represents "the most severe pain imaginable." Participants with chronic pain are asked to place a mark on the line that best represents the intensity of their pain. This mark is then measured in millimeters to obtain the VAS score [13].

State-Trait Anxiety Inventory (STAI): The State-Trait Anxiety Inventory (STAI) was originally developed by Spielberger and Gorsuch in 1964 to measure both state and trait anxiety levels in normal and clinical populations [14]. The Turkish adaptation and validation of the scale were conducted by Öner and Le Compte [15] between 1974 and 1977. The inventory consists of two separate subscales, each containing 20 items: The State Anxiety Scale (S-Anxiety) measures how an individual feels at a specific moment and under specific conditions. The Trait Anxiety Scale (T-Anxiety) evaluates how an individual generally feels, independent of specific circumstances. Each item is rated on a 4-point Likert scale. The scale includes both positively worded (reverse-scored) and negatively worded (direct-scored) items. For reverse-scored items (which reflect positive feelings), a response scored as 1 is converted to 4, and a response scored as 4 is converted to 1. For direct-scored items (which reflect negative feelings), higher scores (i.e., 4) indicate greater anxiety. Two separate scoring keys are used: one for direct items and another for reverse items. The total weighted score for reverse items is subtracted from the total score of direct items. A predetermined constant is then added to this result: +50 for the State Anxiety Scale, +35 for the Trait Anxiety Scale. The final score represents the individual's anxiety level. Higher scores indicate higher anxiety, while lower scores reflect lower anxiety. Possible scores range from 20 to 80.

Statistical Analysis: All statistical analyses were performed using SPSS 25.0 software. Continuous variables were defined by the mean±standard deviation, median (IQR: 25th-75th percentiles) and categorical variables were defined by number and percent. Kolmogorov Smirnov and Shapiro Wilk tests were used for determination of normal distribution. For independent groups comparisons, we used One Way Analysis of Variance test (post hoc: Tukey test) when parametric test assumptions were provided. When parametric test assumptions were not provided we used Kruskal Wallis Variance Analysis (post hoc: Mann Whitney U test with Bonferroni correction). Spearman correlation analysis was used for analyzing the relationships between continuous variables. Also, Chi-square test was used to examine differences in categorical variables. Statistical significance was determined as $p<0.05$.

RESULTS

The demographic and clinical characteristics of the participants are presented in Table 1.

An analysis based on TMD group classifications revealed that there were no statistically significant differences among the groups in relation to the following variables: educational level, number of pregnancies, current pregnancy number, number of miscarriages, gestational age (in weeks), self-reported common systemic conditions, presence of missing teeth, mouth opening, right lateral movement (cm), left lateral movement (cm), protrusion (cm), total PSQI score, VAS-rest, VAS-activity, VAS-night, STAI-I, and STAI-II scores (Table 2). A statistically significant difference was found between age and TMD severity groups. Specifically, individuals in the mild TMD group were significantly younger than those in the moderate and severe TMD groups (Table 2).

There was a weak, positive, and statistically significant correlation was found between VAS-rest scores and the Fonseca Anamnestic Index. A weak, positive, and statistically significant correlation was observed between VAS-activity and total PSQI score (Table 3). A weak, negative, and statistically significant correlation was detected between STAI-I scores and both TMD presence and Fonseca Index scores. A weak, positive, and statistically significant correlation was observed between STAI-II scores and the Fonseca Anamnestic Index (Table 3).

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Table 1. Demographic and clinical characteristics of the participants

Variables	Category	n	%
Age	Mean±SD	27.26±5.43	
	Med (IQR)	27 (23-31)	
	Min.-Max.	18-46	
Education level	No formal education	1	1.2
	Primary school	8	9.3
	Middle school	24	27.9
	High school	28	32.6
	University	24	27.9
	Postgraduate	1	1.2
Number of pregnancies	Mean±SD	2.22±1.36	
	Med (IQR)	2 (1-3)	
	Min.-Max.	1-7	
Current pregnancy number	Mean±SD	2.02±1.15	
	Med (IQR)	2 (1-3)	
	Min.-Max.	1-7	
Number of miscarriages	Mean±SD	0.34±0.71	
	Med (IQR)	0 (0-0)	
	Min.-Max.	0-3	
Gestational week	0-12. weeks	16	18.6
	13-24. weeks	27	31.4
	25-39. weeks	43	50.0
Systemic condition	Hypertension	2	14.3
	Cardiac problems	1	7.1
	Neurological problems	1	7.1
	Rheumatic disorders	5	35.7
	Diabetes	5	35.7
Presence of missing teeth	Yes	34	39.5
	No	52	60.5
TMD classification	None	38	44.2
	Mild	39	45.3
	Moderate to severe	9	10.5
Mouth opening (cm)	Mean±SD	5.25±0.99	
	Med (IQR)	5.2 (4.5-6)	
	Min.-Max.	2.5-7.5	

n: frequency; %: percentage; SD: standard deviation; Med (IQR): median (25th–75th percentiles); min–max: minimum–maximum values

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Table 1. Demographic and clinical characteristics of the participants

Variables	Category	n	%
Right lateral movement (cm)	Mean±SD	0.87±0.6	
	Med (IQR)	0.8 (0.5-1.2)	
	Min.-Max.	0-4.5	
Left lateral movement (cm)	Mean±SD	0.87±0.48	
	Med (IQR)	0.95 (0.5-1.33)	
	Min.-Max.	0-2	
Protrusion (cm)	Mean±SD	0.48±0.28	
	Med (IQR)	0.5 (0.3-0.6)	
	Min.-Max.	0-1.2	
FONSECA	Mean±SD	22.21±16.01	
	Med (IQR)	20 (10-30)	
	Min.-Max.	0-80	
PSQI total	Good	31	36.0
	Modarete	43	50.0
	Poor	12	14.0
VAS-rest	Mean±SD	2.8±2.45	
	Med (IQR)	2.5 (0-5)	
	Min.-Max.	0-10	
VAS-activity	Mean±SD	3.65±2.79	
	Med (IQR)	3 (2-5)	
	Min.-Max.	0-10	
VAS-night	Mean±SD	3.64±3.27	
	Med (IQR)	3.5 (0-7)	
	Min.-Max.	0-10	
STAI I	Mean±SD	44.55±6.85	
	Med (IQR)	44 (40-49.25)	
	Min.-Max.	29-60	
STAI II	Mean±SD	49.01±7.12	
	Med (IQR)	47.5 (44-52)	
	Min.-Max.	36-68	

n: frequency; %: percentage; SD: standard deviation; Med (IQR): median (25th–75th percentiles); min–max: minimum–maximum values

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Table 2. Mean scores obtained from the assessment scales

Variables	Category	TMD			p
		None (1)	Mild (2)	Moderate to severe (3)	
Age	Mean±SD	27.11±4.78	26.49±6.1	31.22±3.11	0.02* (kw=7.78) (2-3)
	Med (IQR)	27 (23-31)	26 (22-30)	32 (29-33.5)	
	Min.-Max.	18-38	18-46	25-35	
Education level	No formal education	0 (0%)	1 (2.6%)	0 (0%)	0.748 (kk=6.758)
	Primary school	2 (5.3%)	4 (10.3%)	2 (22.2%)	
	Middle school	12 (31.6%)	11 (28.2%)	1 (11.1%)	
	High school	13 (34.2%)	12 (30.8%)	3 (33.3%)	
	University	11 (28.9%)	10 (25.6%)	3 (33.3%)	
	Postgraduate	0 (0%)	1 (2.6%)	0 (0%)	
Number of pregnancies	Mean±SD	2.29±1.56	2.18±1.27	2.11±0.78	0.955 (kw=0.092)
	Med (IQR)	2 (1-3)	2 (1-3)	2 (1.5-3)	
	Min.-Max.	1-7	1-5	1-3	
Current pregnancy number	Mean±SD	2.03±1.28	2±1.1	2.11±0.78	0.768 (kw=0.527)
	Med (IQR)	2 (1-3)	2 (1-3)	2 (1.5-3)	
	Min.-Max.	1-7	1-5	1-3	
Number of miscarriages	Mean±SD	0.45±0.86	0.31±0.61	0±0	0.224 (kw=2.994)
	Med (IQR)	0 (0-1)	0 (0-0)	0 (0-0)	
	Min.-Max.	0-3	0-2	0-0	
Gestational week	0-12. weeks	5 (13.2%)	8 (20.5%)	3 (33.3%)	0.682 (kk=2.292)
	13-24. weeks	12 (31.6%)	13 (33.3%)	2 (22.2%)	
	25-39. weeks	21 (55.3%)	18 (46.2%)	4 (44.4%)	
Systemic condition	Hypertension	0 (0%)	1 (16.7%)	1 (33.3%)	0.29 (kk=9.655)
	Cardiac problems	0 (0%)	1 (16.7%)	0 (0%)	
	Neurological problems	0 (0%)	0 (0%)	1 (33.3%)	
	Rheumatic disorders	2 (40%)	2 (33.3%)	1 (33.3%)	
	Diabetes	3 (60%)	2 (33.3%)	0 (0%)	
Presence of missing teeth	Yes	11 (28.9%)	18 (46.2%)	5 (55.6%)	0.174 (kk=3.498)
	No	27 (71.1%)	21 (53.8%)	4 (44.4%)	

*p<0.05 indicates a statistically significant difference; SD: standard deviation; Med (IQR): median (25th–75th percentiles); KW: Kruskal-Wallis Variance Analysis; F: One-Way Analysis of Variance (ANOVA)

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Table 2. Mean scores obtained from the assessment scales

Variables	Category	TMD			p
		None (1)	Mild (2)	Moderate to severe (3)	
Mouth opening (cm)	Mean±SD	5.38±0.97	5.13±1.01	5.21±1.06	0.521 (F=0.657)
	Med (IQR)	5.3 (4.73-6)	5.2 (4.5-6)	5.4 (4.5-6)	
	Min.-Max.	3.7-7.5	2.5-7	3.5-7	
Right lateral movement (cm)	Mean±SD	0.88±0.72	0.88±0.5	0.81±0.56	0.805 (kw=0.434)
	Med (IQR)	0.7 (0.5-1)	1 (0.5-1.4)	0.7 (0.4-1.1)	
	Min.-Max.	0-4.5	0-2	0.1-2	
Left lateral movement (cm)	Mean±SD	0.77±0.45	0.93±0.5	1.07±0.52	0.247 (kw=2.799)
	Med (IQR)	0.65 (0.5-1)	1 (0.5-1.5)	1 (0.6-1.5)	
	Min.-Max.	0-1.7	0-2	0.4-2	
Protrusion (cm)	Mean±SD	0.45±0.26	0.48±0.29	0.6±0.31	0.543 (kw=1.22)
	Med (IQR)	0.5 (0.29-0.5)	0.5 (0.3-0.6)	0.5 (0.35-0.95)	
	Min.-Max.	0-1	0-1.2	0.2-1	
FONSECA	Mean±SD	14 (%36.8)	13 (%33.3)	4 (%44.4)	0.88 (kk=1.189)
	Med (IQR)	20 (%52.6)	19 (%48.7)	4 (%44.4)	
	Min.-Max.	4 (%10.5)	7 (%17.9)	1 (%11.1)	
PSQI total	Good	2.37±2.29	2.97±2.35	3.89±3.3	0.252 (kw=2.753)
	Modarete	2 (0-4)	3 (1-5)	4 (0.5-6)	
	Poor	0-8	0-8	0-10	
VAS-rest	Mean±SD	3.05±2.38	4.05±2.79	4.44±4.03	0.291 (kw=2.466)
	Med (IQR)	3 (0-5)	4 (2-6)	4 (0.5-9)	
	Min.-Max.	0-9	0-10	0-10	
VAS-activity	Mean±SD	3.58±3.77	3.54±2.67	4.33±3.61	0.757 (kw=0.557)
	Med (IQR)	2 (0-8)	4 (1-5)	5 (0.5-7.5)	
	Min.-Max.	0-10	0-8	0-10	
VAS-night	Mean±SD	46.11±5.1	42.77±7.3	45.67±9.81	0.088 (F=2.506)
	Med (IQR)	46 (43-50)	41 (38-48)	40 (37.5-55.5)	
	Min.-Max.	33-55	29-58	35-60	
STAI I	Mean±SD	48.37±7.18	48.77±6.67	52.78±8.39	0.235 (kw=2.895)
	Med (IQR)	46 (44-52.25)	48 (43-52)	49 (47.5-59.5)	
	Min.-Max.	38-67	36-64	45-68	

*p<0.05 indicates a statistically significant difference; SD: standard deviation; Med (IQR): median (25th–75th percentiles); KW: Kruskal-Wallis Variance Analysis; F: One-Way Analysis of Variance (ANOVA)

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Table 3. Relationships among tmd, bruxism, anxiety, and sleep quality

Variables		TMD	FONSECA	PSQI total
Age	**r**	0.107	0.061	0.194
	p	0.326	0.577	0.074
Number of pregnancies	**r**	0.018	-0.016	-0.033
	p	0.867	0.886	0.765
Current pregnancy number	**r**	0.056	0.024	-0.007
	p	0.606	0.824	0.949
Number of miscarriages	**r**	-0.147	-0.197	-0.001
	p	0.176	0.069	0.993
Gestational week	**r**	-.0126	-0.007	0.127
	p	0.249	0.949	0.243
Mouth opening (cm)	**r**	-0.083	-0.059	0.207
	p	0.449	0.587	0.056
Right lateral movement (cm)	**r**	0.012	0.052	-0.080
	p	0.913	0.632	0.465
Left lateral movement (cm)	**r**	0.180	0.175	-0.030
	p	0.097	0.108	0.783
Protrusion (cm)	**r**	0.112	0.136	-0.055
	p	0.304	0.212	0.618
VAS-rest	**r**	0.179	0.280*	0.133
	p	0.099	0.009	0.220
VAS-activity	**r**	0.163	0.186	0.369*
	p	0.135	0.086	0.000
VAS-night	**r**	0.076	0.142	0.178
	p	0.485	0.191	0.101
STAI I	**r**	-0.225*	-0.211*	-0.156
	p	0.037	0.05	0.152
STAI II	**r**	0.142	0.254*	0.102
	p	0.192	0.018	0.349
PSQI total	**r**	0.022	0.067	-
	p	0.839	0.540	-

*p<0.05 indicates a statistically significant correlation; r: Spearman correlation coefficient

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DISCUSSION

This study aimed to investigate the relationship between TMD, pain, anxiety levels, and functional status in pregnant women. The findings revealed a statistically significant difference between TMD severity and age. Specifically, the mean age of participants in the mild TMD group was significantly lower than that of individuals in the moderate-to-severe TMD group (p=0.02). This result aligns with previous literature suggesting that the severity of temporomandibular joint disorders may increase with advancing age.

However, no statistically significant differences were found between TMD and other variables such as educational level, number of pregnancies, mouth opening, lateral movements, or protrusive movements of the jaw. This finding highlights the multifactorial nature of TMD, suggesting that it is influenced not only by biomechanical factors but also by psychosocial components. TMD are known to have a higher prevalence among women, a pattern that is often attributed to a combination of hormonal, anatomical, and psychosocial factors [14]. In particular, the effects of estrogen on the temporomandibular joint have been the focus of increasing scientific attention in recent years [7].

In this context, the combination of hormonal changes, psychological stress, and disrupted sleep quality during pregnancy represents a significant set of risk factors for the development of both bruxism and TMD [15]. Although studies addressing these three factors simultaneously are limited in number, the literature increasingly emphasizes the importance of multidisciplinary approaches in understanding and managing these conditions [2,4,7,15,16]

In a systematic review and meta-analysis conducted by Minervini et al. (2023), the prevalence of TMD among pregnant women was reported as 41.8%, compared to 40.8% in non-pregnant women. The meta-analytic results suggested no significant association between pregnancy and TMD (RR=1.12; 95% CI: 0.65–1.93) [17]. In contrast, a study by Fichera et al. (2020) found that 81% of 108 pregnant women exhibited clear symptoms of TMD, while the prevalence in the control group was only 52%. The authors attributed this difference primarily to increased hormone levels during pregnancy.

Taken together, these studies suggest that the development of TMD may not be solely attributed to hormonal changes, but rather emerges from the complex interplay of multiple factors such as psychological stress, individual mental health status, and parafunctional habits [1,4].

The literature suggests that increased levels of estrogen and relaxin during pregnancy contribute to the loosening of connective tissues, which may in turn reduce joint stability. [3,7]. However, a study by Solak et al. (2020) demonstrated that hormonal changes alone are insufficient to fully explain the development of TMD. In that study, the prevalence of TMD was found to be 7.1% among pregnant women and 7.5% among non-pregnant women, with no statistically significant difference between the two groups. Additionally, no association was identified between systemic joint hypermobility and TMD. These findings suggest that pregnancy may not represent a direct risk factor for TMD; rather, individual sensitivity to hormonal changes may exacerbate TMD symptoms in certain women [3].

In our study, a weak but statistically significant positive correlation was identified between Fonseca questionnaire scores and pain levels experienced at rest (r=0.280; p=0.009). Similarly, a significant positive correlation was found between the total PSQI score and pain levels reported during physical activity (r=0.369; p<0.001). These findings indicate that TMD symptoms in pregnant women may become more pronounced both at rest and during movement, potentially leading to a decline in overall quality of life.

Sleep quality appears to play a crucial role in this dynamic. The literature suggests that sleep disturbances can impair muscle relaxation, thereby increasing pain levels and exacerbating TMD symptoms by triggering bruxism [5].

Problems such as insomnia and frequent nighttime awakenings may indirectly exacerbate temporomandibular dysfunctions by increasing the severity of bruxism [11]. However, as noted by LeResche, hormones that rise during pregnancy such as estrogen and progesterone may exert antinociceptive (pain-reducing) effects in certain individuals. This hormonal influence may contribute to interindividual variability in pain perception during pregnancy [18].

In a study conducted by Szylkiewicz (2022), it was reported that the prevalence of TMD increases during pregnancy, with symptoms of TMJ pain being more frequently observed in the first trimester. Among the 231 pregnant women included in the study, 47% reported experiencing TMD. The findings also indicated that TMJ symptoms decreased in severity as pregnancy progressed, suggesting a reduction in symptom intensity across the trimesters (p=0.02) [11].

In our study as well, a significant association was observed between TMD severity and age, with the mean age being significantly higher in the moderate-to-severe TMD group. This finding suggests that hormonal changes, along with the increased biomechanical loading that comes with age, may contribute to the intensification of TMJ symptoms.

Additionally, in Szylkiewicz's study, a high prevalence of parafunctional habits such as lip/cheek biting and teeth clenching was reported (55.8% and 40.3%, respectively), and these behaviors were considered potential triggers for TMD symptoms [11]. Similarly, in our study, indirect associations between TMD and bruxism were observed, with positive correlations identified between Fonseca scores and pain levels [3,11]. The findings from both studies indicate that TMD during pregnancy is influenced not only by hormonal factors, but also through

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interactions with behavioral (e.g., parafunctional habits) and psychological factors, such as anxiety and pain perception [3,11]. In conclusion, the recent findings in the literature, consistent with our data, emphasize that temporomandibular dysfunctions during pregnancy are associated with both physiological and behavioral processes. The variability of symptoms across trimesters further highlights the importance of addressing these conditions in the prenatal period as a significant aspect of maternal health.

Bruxism is a parafunctional habit commonly associated with stress and anxiety disorders. Research indicates that elevated anxiety levels during pregnancy may serve as a trigger for this behavior [6]. In our study, when examining the relationship between anxiety levels and TMD, a significant negative correlation was found between STAI-I (state anxiety) scores and both TMD severity and Fonseca scores ($r=-0.225$ and $r=-0.211$, respectively). This finding suggests that heightened short-term anxiety may potentially suppress the perception of symptoms or influence how individuals express their discomfort. In contrast, a significant positive correlation was observed between STAI-II (trait anxiety) scores and the Fonseca Index ($r=0.254$; $p=0.018$), indicating that long-term anxiety may exacerbate TMD symptoms. Supporting this pattern, a study conducted by Um-e-Farwa et al. (2020) in Pakistan reported that the prevalence of bruxism increased from 50.7% pre-pregnancy to 76% during pregnancy [10]. This rise suggests that heightened physiological stress during pregnancy may trigger parafunctional behaviors, potentially contributing to the development of TMD. Consistent with these findings, our study also revealed a positive correlation between Fonseca scores and pain levels, and a significant association between STAI-II scores and Fonseca scores. Collectively, these results indicate that bruxism and TMD symptoms may share common psychophysiological underpinnings.

Limitations

Several limitations of this study should be acknowledged. First, due to the cross-sectional design, it is not possible to establish causal relationships. Additionally, the assessment of TMD relied on subjective measures and was not supported by objective imaging techniques. Furthermore, variables such as anxiety and pain are inherently influenced by psychological and social factors, which may affect the consistency of self-reported data.

CONCLUSION

In conclusion, this study revealed potential associations between TMD severity and variables such as age, pain, and anxiety levels in pregnant women. The findings highlight the importance of evaluating TMJ health during the prenatal period. Future longitudinal studies are needed to further explore the dynamic effects of TMD throughout pregnancy and enhance our understanding of its progression over time.

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 committee approval for our study was obtained from the Clinical Researches Ethics Committee of Alanya Alaaddin Keykubat University (Date: 16.04.2025, Decision number: 07/05).

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Original Article

Assessment of bee-related deaths based on the scene of the incident and autopsy findings

Huseyin Cetin Ketenci

Recep Tayyip Erdoğan University, Faculty of Medicine, Department of Forensic Medicine, Rize, Türkiye

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Abstract

Aim: Although deaths resulting from bee stings are rare, they can pose a serious public health problem due to sudden systemic reactions, particularly during periods when bees are active.

Materials and Methods: This retrospective study evaluated 11 cases of deaths due to bee stings, as reported in the Eastern Black Sea Region between 2013 and 2023, for which autopsies had been performed.

Results: All of the individuals were male, with a median age of 57. The most common cause of death was anaphylaxis due to laryngeal oedema. Fatal cases frequently occurred in the spring and summer months, typically following stings to critical areas such as the head, neck and body. Multiple bee stings, underlying cardiovascular diseases and delayed medical intervention were identified as the most significant risk factors.

Conclusion: The findings suggest that bee sting-related deaths are largely preventable, and that early detection of individual sensitivity, the use of epinephrine/adrenaline autoinjectors, and the strengthening of emergency response services are of vital importance.

Keywords: Bee sting, anaphylaxis, autopsy, forensic medicine

INTRODUCTION

The relationship between humans and animals in the wild, as well as breeds of animals raised for their products, can sometimes result in injury or death, despite providing many benefits. These outcomes can result from livestock farming, hunting and unexpected human–animal contact in nature, as well as from traumatic, toxicological or allergic pathways [1–3]. Bee stings, particularly due to the allergic reactions they cause, are one of the environmental exposures encountered, generally resulting in mild clinical presentations. However, anaphylactic reactions that develop in sensitive individuals can lead to life-threatening systemic effects within a very short timeframe [4,5]. Such reactions can be fatal, especially in the presence of predisposing factors such as a known or unknown history of allergies, cardiovascular diseases or advanced age.

According to data from the World Health Organization, although deaths due to insect stings are rare, the risk increases significantly in rural areas where adequate emergency care is unavailable. Numerous forensic case studies reported in the literature show that death often results from respiratory oedema and circulatory collapse [4–7]. In this context, forensic medicine plays a critical role in determining the causes of unexpected and sudden deaths due to bee stings.

There are few autopsy-based studies on this subject in Türkiye, and very few data sets systematically evaluate variables such as the bee species, number of stings, anatomical site, time of death and accompanying diseases [1–3]. Furthermore, supporting details such as seasonal distribution, toxicological analyses and documentation of intervention attempts could facilitate a better understanding of these deaths.

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Corresponding Author: Huseyin Cetin Ketenci, Recep Tayyip Erdoğan University, Faculty of Medicine, Department of Forensic Medicine, Rize, Türkiye
Email: hcetin.ketenci@erdogan.edu.tr

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This study aims to systematically evaluate cases of people who underwent forensic autopsies following bee stings between 2013 and 2023. The findings are expected to inform public health measures, emergency intervention protocols and legal determinations of cause of death.

MATERIAL AND METHOD

A total of 11 autopsy cases reported to have resulted from bee stings in the Eastern Black Sea Region between 2013 and 2023 were examined retrospectively . All cases were identified through the official forensic reporting system, and only those with complete case histories were included in the study. All autopsies were performed by forensic pathologists and subjected to multidisciplinary evaluation, including toxicological analysis.

The data were transferred to a standardised form and evaluated based on the following parameters: age; gender; nationality; year of death; location of incident; location of death; season; anatomical site of bee sting; number of stinging bees; bee species; whether cardiopulmonary resuscitation (CPR) and intubation were performed; underlying diseases; autopsy findings; toxicological analysis results; and forensic medical diagnosis. Cases with missing or conflicting information were excluded from the analysis. Diagnostic classification was performed by evaluating autopsy findings alongside toxicology and incident location information. In all cases, the cause of death was reported as 'anaphylactic reaction', and the deaths were confirmed as being related to bee stings through toxicological analysis. Differantial diagnosis criteria such as acute cardiac events, traumatic death and toxic substance exposure excluded.

The inclusion criteria for the study were defined as follows: the cause of death must have been directly or indirectly related to a bee sting; the autopsy must have been completed, with pathological evaluations performed; and the file must have contained basic demographic information, details of the preclinical process, toxicological analysis and histopathological findings. The dataset was created in Microsoft Excel and certain parameters were standardised. These parameters included gender and age; date and time of incident and death; the location of the sting (anatomical localisation); the number of stings; the reported species of bee (if known); the location of the incident; the duration of the intervention; the presence of a known allergy or comorbidity; the results of the toxicological analysis; the autopsy findings (e.g. pulmonary oedema, laryngeal oedema and congestion); and the cause of death (e.g. direct anaphylaxis, cardiac arrest or respiratory failure). The data were analysed using descriptive statistical methods. Qualitative variables were summarised using frequency and percentage, while quantitative variables were summarised using mean ± standard deviation and median (minimum–maximum). Graphs and tables were created using Microsoft Excel.

This study was accepted at the Education and Scientific Research Commission meeting of the Forensic Medicine Institution in response to the study proposal titled 'Evaluation of Deaths Due to Bee Stings Based on Autopsy Findings', as evidenced by the letter dated 30/04/2024 and numbered 21589509/2024/140. Ethically, the study does not require committee approval as it consists solely of data obtained for forensic purposes and does not contain any personally identifiable information. Nevertheless, the study was conducted in accordance with the principles of the Declaration of Helsinki and relevant national legislation.

RESULTS

This study retrospectively evaluated a total of 11 cases of death due to bee stings. All of the individuals . All cases were male individuals, ranging in age from 35 to 86 years old, with a median age of 57years. Figure 1 shows the age distribution of the cases. 90.9% of the cases (n=10) were citizens of the Republic of Türkiye, while 9.1% (n=1) were foreign nationals. When the distribution by year was examined, it was seen that the highest number of deaths occurred in 2023 (n=3). The other deaths were distributed across 2018 (n=2), 2017 (n=2), 2016, 2015, and 2013.

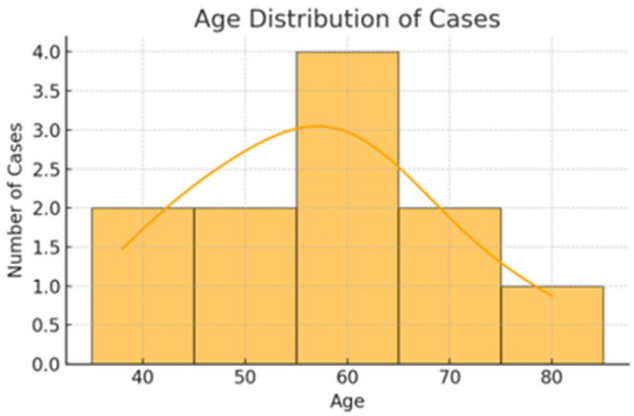

Figure 1. Most cases involve individuals over the age of 40, with the highest frequency occurring in the 50–70 age group

The 'home garden' (n=4) was identified as the most common location for fatal bee stings. Other locations included tea fields (n=2), the area around vehicles/the hive transport area (n=1), a construction site (n=1), a picnic area (n=1), a wooded area (n=1) and an unknown location (n=1). When the location of death was evaluated, it was found that all cases occurred in a hospital setting. This suggests that, despite being transported to hospital during emergency medical intervention, the interventions were insufficient and severe anaphylactic reactions developed.

Examining the seasonal distribution of cases revealed that four cases (36.3%) occurred in spring, four (36.3%) in summer, and three (27.2%) in autumn. No bee sting-related deaths were recorded during the winter months. This distribution is parallels to the seasonal characteristics of bee activity [4–6]. Figure 2 shows that the highest number of deaths occurred in spring and summer.

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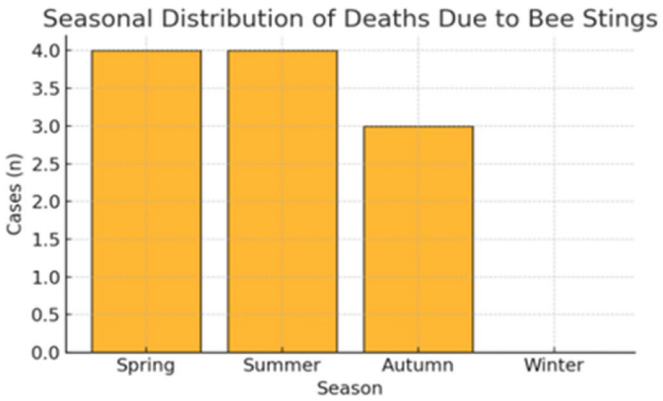

Figure 2. Seasonal distribution of bee sting-related deaths

The area most frequently stung by bees was identified as the 'face' (n=3). This was followed by 'the body in general' (n=3), 'the toe' (n=2), 'the leg' (n=1), 'the arm' (n=1) and 'an unknown area' (n=1). Figure 3 provides a statistical visual representation of the number of body areas stung.

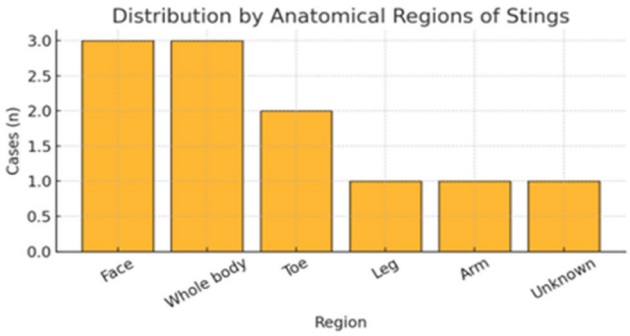

Figure 3. Distribution of stings according to anatomical site

In terms of the number of bees that stung, multiple stings were reported in 72.7% of cases (n=8). Death occurred due to a single bee sting in only three cases (Figure 4). All cases reported that the bee that stung them was a honeybee.

Figure 4. Autopsy photographs reveal a solitary lesion accompanied by evident erythema, oedema, and needle marks on the skin's surface

CPR (cardiopulmonary resuscitation) or intubation was performed in nine cases, indicating that pre-hospital or in-hospital emergency intervention was sought. Toxicological analyses revealed no evidence of ethanol, drug or substance use in any of the cases. Figure 5 shows that deaths due to multiple bee stings were significantly more common than those due to a single sting.

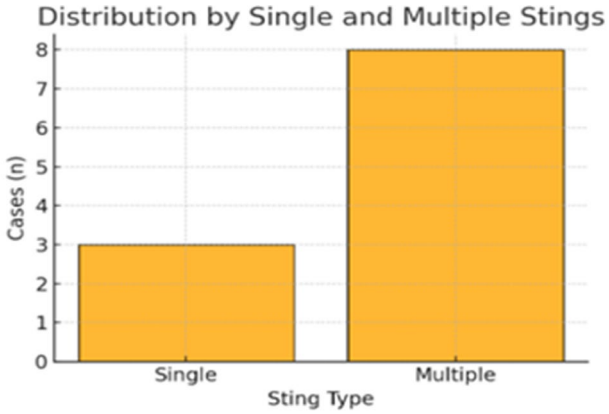

Figure 5. Distribution of cases according to single and multiple bee stings

In terms of underlying diseases, cardiovascular diseases were present in seven cases. No clear information was available in three cases and diabetes mellitus was present in one case. These findings suggest that existing comorbidities may exacerbate the clinical severity of anaphylactic reactions.

Autopsy findings were similar in all cases, with marked laryngeal oedema noted in all. This finding supports the diagnosis that the deaths were directly related to anaphylaxis. In the forensic evaluation, all cases were reported as 'anaphylaxis'. Figure 6 shows the advanced degree of laryngeal oedema based on autopsy findings. Significant oedema and hyperaemia are evident in the epiglottis and surrounding tissues, as well as in the tracheal mucosa. These findings support the diagnosis of anaphylactic shock as the cause of death.

Figure 6. Selected macroscopic findings from autopsies. Laryngeal and upper airway oedema was observed in most cases included in the study. The photograph shows typical laryngeal oedema and mucosal hyperaemia. There is an advanced degree of laryngeal oedema. Significant oedema in the epiglottis and surrounding tissues, as well as hyperaemia in the tracheal mucosa, can be seen. These findings support anaphylactic shock as the cause of death

DISCUSSION

Honey production is an important source of livelihood throughout Türkiye. The Eastern Black Sea Region is also an area where beekeeping is widely practised, with various types

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of honey produced from the region's endemic flora, mostly by amateur beekeepers. This study retrospectively examined forensic autopsy and toxicological data from 11 cases of death due to bee stings between 2013 and 2023. The findings show that, as reported in the international literature, deaths due to bee stings in Türkiye mostly occur as a result of sudden systemic reactions [1,2]. Bee stings, particularly from honey bees (Apis mellifera) and wasps (Vespula species), can lead to anaphylactic shock and death within a short period of time in susceptible individuals [8,9].

In most of the cases included in our study, deaths occurred in spring and summer. This is consistent with the seasonal distribution commonly reported in the literature. The increase in the bee population, particularly between June and September, combined with the increased frequency of people being outdoors, significantly increases the risk of exposure. The seasonal trend is more pronounced for individuals living in rural areas or in close contact with nature (Figure 7). This situation is consistent with our study, where the majority of cases were exposed to bee stings in rural areas, outdoors [10].

Figure 7. An example of amateur beekeeping activities in the Eastern Black Sea Region. The combs built on the region's unique flora reflect the natural environment where bee stings are common

In most cases, the stung area was the head-neck or torso, and it is known that stings in these areas cause more severe systemic effects [11]. The dense vascularization in these anatomical regions and the faster passage of toxins into the circulation may explain this result. Furthermore, in stings around the throat, tongue, or windpipe, rapid development of edema can obstruct the airway, leading to sudden death. Indeed, some cases report death occurring very quickly, with the individual dying at the scene.

Another noteworthy finding in the study is the presence of multiple bee stings. It is known that toxic effects increase with the number of bee stings [12]. Serious toxic reactions and multiple organ failure can develop in non-allergic individuals, especially in cases of multiple bee stings. This condition is defined in the literature as “toxic envenomation” and can result in death in some cases without anaphylaxis. In our study, rapid systemic responses resulting in death were observed in several cases known to have been stung by ≥10 bees. However, individual factors such as underlying health conditions, as well as the total amount of venom to which the individual was exposed, may also be decisive in this process [8].

When autopsy findings are evaluated, most cases show significant oedema of the larynx and epiglottis, hyperaemia of the inner surface of the trachea, lung congestion and widespread oedema are evident in most cases. These pathological changes are consistent with the typical morphological features of anaphylactic shock [13]. While these findings are often non-specific, when considered alongside the clinical history and site of injection, it is possible to establish a strong correlation with the cause of death. Similarly, the presence of widespread oedema in the lungs indicates circulatory failure and hypoxia, resulting in a systemic inflammatory response.

In toxicological analyses, no significant alcohol or drug interactions were detected in the cases included in our study. However, the literature indicates that anaphylaxis management may be challenging, particularly in individuals taking antihypertensive, beta-blocker, or ACE inhibitor drugs. It is thought that these types of drugs may increase the risk of mortality by rendering patients unresponsive to epinephrine treatment. Therefore, it may be useful to inquire about the history of prescription drug use in future studies [10,11].

Compared to cases reported in the literature, our study's data reveal that bee sting-related deaths also account for a significant proportion of sudden deaths caused by allergies and toxicity in our country. Furthermore, the fact that most of these deaths occur in rural areas without access to emergency medical intervention highlights the importance of life-saving tools such as first aid and epinephrine auto-injectors. Community-based studies in Türkiye investigating issues such as access to these tools, their frequency of use and health literacy could help to prevent these deaths.

CONCLUSION

In conclusion, the majority of bee sting-related deaths could be prevented by taking some simple measures. Critical measures include identifying individuals at risk, conducting preliminary allergy tests, mandating the carrying of auto-injectors containing epinephrine/adrenaline, and training healthcare personnel, particularly those working in rural areas [12]. Our study is important as it is one of the few case series in forensic medicine to draw attention to this issue. In the future, this knowledge gap could be filled by studies with larger sample sizes and more rigorous designs.

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

Ethically, the study does not require committee approval as it consists solely of data obtained for forensic purposes and does not contain any personally identifiable information. Nevertheless, the study was conducted in accordance with the principles of the Declaration of Helsinki and relevant national legislation.

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Original Article

A legal and clinical analysis of Constitutional Court decisions on heel prick testing in Türkiye

Huseyin Cagri Sahin[1], Muhammet Ali Oruc[1,2]

[1]*Council of Forensic Medicine, İstanbul, Türkiye*
[2]*Samsun University, Faculty of Medicine, Department of Family Medicine, Samsun, Türkiye*

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Abstract

Aim: Newborn screening programs are a critical public health intervention in preventing permanent morbidity and mortality through the early diagnosis of metabolic and genetic diseases. Although heel prick testing is a mandatory screening practice supported by legal regulations in Türkiye, some parents' refusal to consent to this procedure has led to legal and ethical debates. The aim of this study is to systematically examine the Constitutional Court (TCC) individual application decisions regarding heel prick blood collection and to assess how the Court balances parental autonomy with the best interests of the child.
Materials and Methods: In this study, four decisions retrieved by searching for the keyword "heel prick" in the TCC Decisions Database were examined using qualitative document analysis.
Results: The study revealed that the TCC generally found heel prick testing to be proportionate, citing its clear legal basis, legitimate public health purpose, and minimally invasive nature as justification. However, in cases where the medical necessity for repeated heel prick testing was not sufficiently justified, the Court ruled that there had been a violation of rights. The Constitutional Court's assessments are consistent with the principles of legality, necessity, and proportionality accepted in international human rights law and point to the importance of the processes of information, recording, and medical justification for healthcare professionals.
Conclusion: This study presents important findings that strengthen the legal framework of newborn screening programs in Türkiye, concretize the balance between parental consent and the best interests of the child, and guide clinical practice.

Keywords: Heel prick, newborn screening, parental refusal, best interests of the child, medical-legal analysis

INTRODUCTION

Newborn screening programs play a critical role in preventing permanent morbidity and mortality by enabling the early detection of many metabolic and genetic diseases. In Türkiye, newborn screening using heel blood covers serious diseases such as Phenylketonuria, Congenital Hypothyroidism, Biotinidase Deficiency, Cystic Fibrosis, and Spinal Muscular Atrophy (SMA) and is considered a mandatory public health intervention for the sake of public health [1]. Similarly, newborn screening is a fundamental component of public health policies worldwide and is implemented through "mandatory" or "presumptive consent" practices in the United States, the United Kingdom, and European countries [2,3].

However, some parents' refusal to consent to heel prick blood sampling due to religious beliefs, alternative medicine tendencies, or concerns about medical intervention raises the tension between the best interests of the child and the personal autonomy of the parent. In the international literature, the balance between the

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Corresponding Author: Muhammet Ali Oruc, Samsun University, Faculty of Medicine, Department of Family Medicine, Samsun, Türkiye /Council of Forensic Medicine, İstanbul, Türkiye
Email: muhammetalioruc@gmail.com

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parent's refusal of medical intervention and the state's duty to protect the child is a controversial issue in health law [4].

Article 3 of the United Nations Convention on the Rights of the Child emphasizes that "the best interests of the child shall be a primary consideration" in all proceedings; however, it also states that the religious and moral preferences of the parents must be respected [5].

The Constitution of the Republic of Türkiye strikes a balance between respect for the individual's physical integrity and family life (Articles 17 and 20) and the state's duty to protect children from all dangers (Article 41) [6]. Within this constitutional framework, disputes arising from parental refusal of medical interventions have increasingly been brought before the Constitutional Court through individual applications.

In cases involving refusal of heel prick blood sampling, the Constitutional Court (TCC) examines whether the intervention satisfies the principles of legality, necessity, and proportionality. Decisions concerning heel prick testing have contributed to the development of constitutional jurisprudence at the intersection of health law and children's rights.

MATERIAL AND METHOD

This study was designed as a qualitative document analysis examining individual application decisions of the TCC concerning newborn heel prick blood sampling.

Data Source and Search Strategy

The data for the study were obtained from the TCC's publicly accessible Decisions Information Bank (https://kararlarbilgibankasi.anayasa.gov.tr/). The search covered the period from January 1, 2012, to December 31, 2024. The following keyword was used during the search: "heel blood" Secondary keywords such as "newborn screening," "blood sample," and "health measure" were also tried to the extent permitted by the system's search engine; however, the only keyword that yielded results specifically for heel blood was "heel blood."

Inclusion Criteria All decisions meeting the following criteria were included in the study: 1. The decision directly relates to the collection of newborn heel blood. 2. The applicants are parents and claim to have refused consent for the intervention. 3. The decision was issued within the scope of an individual application. 4. The full text of the decision is accessible.

Exclusion Criteria The following decisions are excluded from the study:

1. Decisions that only involve vaccine refusal and are not related to heel prick testing.
2. Decisions related to health measures that are not related to newborn screening.

Data Analysis and Coding Process The analysis was conducted manually without the use of any qualitative data analysis software.

Document analysis and thematic content analysis methods were used together in the study.

1. The decisions were first read independently by two researchers.
2. Each decision was coded according to the following variables: o Type of intervention (first heel prick / repeat heel prick / vaccination + heel prick) o Reasoning behind the first-instance court decision o The Constitutional Court's assessments of "legality," "necessity," and "proportionality" o Analysis regarding the best interests of the child and parental consent o Outcome (violation / no violation / dismissal of the application)
3. The codes were compared; points of disagreement were discussed and consensus was reached.
4. Four main themes were identified from the data in the final stage: o The best interests of the child o Medical necessity criterion o Principle of legality o Proportionality and necessity analysis

Ethical Evaluation: This study does not require ethical committee approval as it is based solely on the analysis of publicly available court decisions and does not contain any personal data. TCC decisions are anonymized, publicly accessible official documents. No personal information was processed or interfered with during the study.

Limitations of the Study

- The limited number of TCC decisions on the subject (n=4) narrows the scope of the analysis.
- The decisions only reflect the Turkish context and do not include examples from international courts.
- Although there are similar cases among the decisions, the content of each is different; therefore, the possibility of generalization is limited.

RESULTS

Within the scope of this study, four individual application decisions of the TCC were examined as a result of a search using the keyword "heel prick blood." The decisions were evaluated in terms of content, legal reasoning, and outcome; the criteria used to establish the balance between parental consent and the best interests of the child were analyzed thematically.

In all four decisions, the applicants were parents who did not consent to heel prick blood sampling from their newborn children, and all applications were based on allegations of violation of family privacy, parental rights, and bodily integrity.

In three of the decisions, health measures were applied by the courts in accordance with Article 5 of the Child Protection Law No. 5395; one decision was dismissed on the grounds that the intervention did not take place and therefore the victim status ceased to exist.

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Decision 1: Application in Which Vaccination and Heel Prick Testing Were Assessed Together In this decision, the parents objected to both vaccination and heel prick blood sampling. The court of first instance ruled in favor of the health measure, citing the best interests of the child. The TCC stated that the condition of "legality" was not met with regard to vaccination, on the grounds that there was no clear, predictable legal regulation concerning general mandatory vaccinations, and ruled that there had been a violation of rights. In contrast, regarding the heel prick test, it was determined that the Ministry of Health had clear regulations, that the intervention aimed to protect the child's right to life and health, and that it was proportionate; therefore, it was concluded that Article 17 of the Constitution had not been violated.

Decision 2: Withdrawal of the Application Following the Removal of the Health Measure In this application, the parents did not consent to the heel prick test; the court of first instance ordered the health measure. However, before the decision was enforced, the court subsequently lifted the health measure based on case law requiring consent for medical intervention. The TCC ruled that the application was dismissed, stating that the applicants had lost their status as "victims" because the intervention had never taken place and the measure had been lifted. In this decision, the Court did not deem it necessary to assess the medical necessity or proportionality of the intervention.

Decision 3: Violation of Rights in Repeated Heel Prick Blood Sampling The applicants refused the intervention, stating that heel blood samples had been taken from their babies in the hospital after birth and that therefore a second sample at the family health center was not medically necessary. The court ruled that the health measure was necessary on the grounds that the parents' refusal was contrary to the best interests of the child. The TCC ruled that the first-instance court failed to demonstrate the medical necessity of repeated sampling and therefore the intervention did not meet the criteria of proportionality and necessity, thus constituting a violation of rights. This decision is significant in that, although the TCC generally considers heel prick testing to be legitimate, it emphasizes that the state's authority to intervene must be limited when there is no medical necessity.

Decision 4: Heel Prick Blood Sampling Considered a Proportionate Intervention in the Best Interests of the Child In this decision, the parents again did not consent to heel prick blood sampling; the court issued a health measure decision to protect the child's right to health. The TCC ruled that there was no violation of rights, stating that heel prick blood sampling has a legal basis, a legitimate purpose, does not pose any unnecessary risk to the child, and is necessary for public health.

Thematic Assessment When the decisions are examined thematically, the following four main themes emerge:

Theme 1: The Best Interests of the Child as a Continuous Priority Principle In all its decisions, the TCC has centered the obligation to protect the health of the child and emphasized that the state has a certain degree of authority to intervene in this regard.

Theme 2: The Medical Necessity Criterion is Particularly Decisive in Repeated Cases The Court has deemed it mandatory to provide concrete justification for medical necessity in cases where heel blood must be taken a second time.

Theme 3: The Principle of Legality is Problematic in Vaccination Applications but Strong for Heel Prick Blood While Decision 1 cited the inadequacy of regulations on mandatory vaccination, it noted that heel prick testing is clearly regulated by relevant legislation.

Theme 4: Proportionality and Necessity Analysis is Conducted Separately for Each Case Instead of a general principle, the TCC assessed each application based on the specific characteristics of the case.

The table below summarizes the main features of the four decisions (Table 1).

Table 1. Key features of the decisions

Decision no.	Type of intervention court	Rationale constitutional court	Constitutional court assessment	Result
1	Vaccination + Heel prick blood test	Health measure in the best interests of the child	No legality regarding vaccination; legitimate purpose and proportionality ensured regarding heel prick	Partial violation / no violation
2	Heel prick	Health measure, but subsequently withdrawn	No intervention took place, no victimization	Application dismissed
3	Repeated heel prick	Claim that a new sample is necessary	Medical necessity argument insufficient – proportionality not ensured	Violation of rights
4	Heel prick blood	Best interests of the child	Heel prick blood test is necessary, proportionate, and legitimate	No violation

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DISCUSSION

This study examined individual application decisions regarding heel prick blood sampling in newborns in Türkiye and assessed how the TCC balanced parental consent with the best interests of the child within the framework of constitutional rights and public health obligations. The findings indicate that the TCC generally considers heel prick blood sampling to be a legitimate medical intervention when it has a clear legal basis, pursues a legitimate aim related to the protection of the child's health, and complies with the principles of medical necessity and proportionality [7].

An analysis of the Court's decisions reveals that parental autonomy is not treated as an absolute right. Instead, the best interests of the child are evaluated alongside public health considerations. First-time heel prick sampling is consistently regarded as a minimally invasive and preventive intervention that does not violate bodily integrity. However, the Court adopts a more restrictive approach in cases of repeated sampling, requiring concrete medical justification to satisfy proportionality requirements.

Newborn screening programs are integral components of public health systems worldwide and are essential for preventing irreversible harm [2,3]. The Court's recognition of heel prick testing as a legitimate intervention aligns with international practices and guidelines. This approach is also supported by comprehensive policy analyses from the United States, which emphasize the public health value and sustainability of mandatory newborn screening programs [8].

The TCC's approach is consistent with the jurisprudence of the European Court of Human Rights (ECHR), which permits interference with bodily integrity only when it is lawful, necessary, and proportionate [7]. In this context, the Court's emphasis on medical justification in repeated interventions reflects both ethical standards and clinical best practices [2,9].

Within this international legal and public health framework, the Turkish newborn screening system demonstrates similar preventive characteristics and regulatory foundations.

Similarly in Türkiye, the Ministry of Health's National Newborn Screening Program has clear and binding regulations [1]. Therefore, heel prick blood sampling is seen to serve the purpose of protecting the child's right to life and health and is necessary for public health.

Parental consent is one of the fundamental legal elements of medical interventions; however, it may be limited in certain circumstances based on the best interests of the child and public health. In the international literature, the boundary between parental refusal of medical intervention and state intervention is generally addressed through the "harm principle" [4].

Therefore, the TCC emphasis on medical necessity is also appropriate in terms of clinical practice. This approach is consistent with screening guidelines in the UK and the US, as interventions are only recommended to be repeated when necessary [2,3].

As seen in Decision 1, the lack of a clear legal basis for vaccination led to a violation on the grounds that the principle of legality was not met. However, the same decision deemed the relevant regulation and national screening program sufficient for heel prick testing. This demonstrates that the Constitutional Court considers transparent, accessible, and predictable legal regulations to be essential for medical interventions to be legitimate.

From a practical standpoint, these decisions underscore the responsibilities of healthcare professionals to ensure transparent communication, accurate documentation, and evidence-based justification when performing or repeating heel prick sampling. Effective communication has been shown to reduce parental refusal rates [10].

Future studies may be expanded to include heel prick refusal data from Family Health Centers and hospitals, parental opinions, and physician practices.

In conclusion, the decisions of the TCC clearly demonstrate that the best interests of the child are central to newborn screening practices, but that every medical intervention must be consistent with the principles of concrete medical necessity, legal basis, and proportionality.

The heel prick test has been confirmed as a mandatory and legitimate health service; however, a sensitive approach has been adopted towards protecting individual rights in cases of repeated interventions where the medical justification is insufficiently specified. This situation imposes significant responsibilities on health authorities and clinicians, both legally and ethically.

Strengths and Limitations

This study is the first Turkish study to systematically examine all TCC decisions related to heel prick blood refusal. However, the limited number of decisions and the fact that it only covers national case law limit the generalizability of the findings.

CONCLUSION

The TCC jurisprudence confirms that heel prick blood sampling is a legitimate and mandatory public health intervention in Türkiye, grounded in the best interests of the child. However, the Court also emphasizes that medical interventions must comply with the principles of legality, necessity, and proportionality. In particular, repeated sampling requires clear medical justification. These findings provide important guidance for healthcare professionals and contribute to the ongoing legal and ethical discourse on newborn screening practices.

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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
This study does not require ethical committee approval as it is based solely on the analysis of publicly available court decisions and does not contain any personal data.

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Original Article

Investigation of carbon monoxide-poisoning related deaths

Abuzer Gulderen[1], Murat Kamalak[1], Sertac Dalgic[2], Tuba Sahinoglu Gunes[3]

[1]Gaziantep Forensic Medicine Group Directorate, Gaziantep, Türkiye
[2]Akhisar Forensic Medicine Branch Directorate, Manisa, Türkiye
[3]Independent Researcher Forensic Medicine Specialist, Tokat, Türkiye

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Abstract

Aim: Carbon monoxide (CO) is a colorless and odorless gas. This non-irritating gas is produced by the combustion of carbon-containing compounds in the presence of insufficient oxygen. Because it is non-irritating, exposure is often detected late, which can lead to severe injury or death. This study aimed to discuss the mortality rates due to CO poisoning in our country, examining the factors and etiology of CO poisoning through the example of Gaziantep province, and to raise awareness about preventable accidents.
Materials and Methods: Postmortem examinations—including external examination, autopsy, toxicological, and histopathological analyses—and forensic investigations conducted in Gaziantep over a three-year period between 2022 and 2024 were included to identify deaths caused by carbon monoxide poisoning. Cases were evaluated based on age, gender, month, season, origin, time to death, CO source, and carboxyhemoglobin (COHb) levels.
Results: It was determined that 41 of 3274 forensic cases died due to carbon monoxide poisoning. Among the cases, 31.7% were female and 68.3% male. The highest recorded COHb level was 78.6%, and the lowest was 17%. The majority of poisonings were attributed to gas originating from stoves.
Conclusion: The findings of this study were generally consistent with the existing literature. No cases of suicidal CO poisoning were identified in the region; all deaths were determined to result from preventable accidents. Implementing necessary precautions is essential to reduce such fatalities. Taking the necessary precautions, conducting annual maintenance of stoves and heaters, and installing CO alarms in homes will reduce fatalities caused by CO poisoning.

Keywords: Forensic medicine, forensic death, carbon monoxide poisoning

INTRODUCTION

Carbon monoxide (CO) is a colorless and odorless gas. This non-irritating gas is produced by the combustion of carbon-containing compounds in the presence of insufficient oxygen [1]. Due to its lack of irritating properties, CO exposure is often detected late, leading to serious harm and potentially fatal outcomes [2,3].

The density of CO is 0.97, making it slightly lighter than ambient air. It is present in trace amounts in normal atmospheric air, with higher concentrations found in enclosed spaces and urban centers [4,5]. While the CO level is approximately 2% in non-smokers, it can reach up to 10% in smokers [5-7]. CO is commonly generated by incomplete combustion of heating devices fueled by coal, wood, and gas, such as stoves, barbecues, braziers, automobile exhausts, and water heaters. Furthermore, if the heating device lacks a chimney or if the chimney is obstructed, environmental CO levels will progressively increase, resulting in greater inhalation exposure [8-11].

CO poisoning remains a significant global health issue. In developed countries, CO poisoning frequently occurs in the context of fire-related accidents and suicides, whereas in developing or underdeveloped countries, such as ours, accidental poisonings related to stoves and heaters are more prevalent during the winter months [8,12,13].

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Corresponding Author: Abuzer Gulderen, Gaziantep Forensic Medicine Group Directorate, Gaziantep, Türkiye
Email: md.abuzergulderen@gmail.com

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Inhalation or ingestion of methylene chloride (dichloromethane), an industrial paint remover, can lead to endogenous CO production through hepatic metabolism even without exposure to exogenous CO gas, resulting in elevated internal CO levels and may cause toxicity even in the absence of CO in the ambient air [14].

When exposed to CO gas, CO competes with oxygen and binds to hemoglobin, resulting in hypoxia. The affinity of CO for hemoglobin is approximately 250 times greater than that of oxygen, which impedes the release of oxygen bound to hemoglobin into the tissues, thereby causing tissue-level hypoxia. CO also binds to myoglobin, leading to symptoms of muscle weakness and fatigue. By disrupting oxygen transport and exchange within tissues, CO causes damage and cell death, particularly in organs that are highly sensitive to oxygen deprivation [9,15,16].

Because the initial symptoms of CO poisoning are nonspecific, it is often difficult for individuals to recognize that they have been poisoned. Symptoms associated with CO poisoning are nonspecific and may be mistaken for viral infections; these include dizziness, nausea, vomiting, cough, numbness, and weakness. Binding to myoglobin induces profound weakness, so even when individuals realize they have been poisoned and attempt to reach a fresh environment, fatalities frequently occur due to collapse near windows or doors. In CO toxicity, where the heart and brain are particularly sensitive to hypoxia, symptoms such as impaired consciousness, confusion, and coma are observed. Among survivors of poisoning, neurological and cardiac sequelae may persist, including cardiomegaly, myocardial infarction, arrhythmias, coma, seizures, behavioral disorders, cognitive impairments, fecal and urinary incontinence, ataxia, muscle rigidity, memory deficits, and personality changes [15,16].

Approximately 30.000 people worldwide die annually due to accidental CO poisoning. Although exact figures are not available for our country, hundreds of deaths from CO toxicity occur each year. While some survivors achieve full recovery, others may suffer permanent neurological damage. In our country, the majority of fatalities are generally attributable to preventable accidents [8,17,18].

This study aims to examine the mortality rates related to CO poisoning in our country, using Gaziantep as a representative sample, and to explore the contributing factors and etiology of CO poisoning. Additionally, it seeks to raise awareness regarding preventable accidents through a review of the literature.

MATERIAL AND METHOD

Forensic deaths that occurred in Gaziantep province between the beginning of 2022 and the end of 2024 and were examined/autopsied at the Gaziantep Forensic Medicine Autopsy Branch were retrospectively reviewed, and deaths due to CO poisoning were included in the study. A total of 3.836 forensic deaths occurred in our branch during the 3-year period, of which 562 were excluded because they were related to the February 6 earthquake. Thus, 3.274 forensic deaths were retrospectively analyzed. Data were obtained from the autopsy archive, histopathological and toxicological report results, and the Gaziantep Forensic Medicine Group Presidency Morgue Specialization Department reports after obtaining ethical approval from the Council of Forensic Medicine. This approval was granted by the Council of Forensic Medicine Education and Scientific Research Board with decision number 21589509/22025/352 dated 18.03.2015.

The cases were evaluated in terms of age, gender, month, year, season, COHb level, duration of hospital stay, source of carbon monoxide, place of residence, cohabitation status, and cardiac findings. Due to the insufficient number of cases for statistical comparison, subgroups were compared by calculating percentages.

RESULTS

Our study examines deaths due to carbon monoxide poisoning in Gaziantep Province over a three-year period from January 2022 to December 2024. During this time, a total of 3.836 autopsies/corpse examinations were conducted in the province, of which 562 were excluded from evaluation as they were related to earthquake fatalities. It was determined that 41 (1.25%) of the 3.274 non-earthquake deaths were attributable to CO poisoning.

Thirteen cases (31.7%) were female, and 28 cases (68.3%) were male. The cases were analyzed according to age groups, with infants aged 0–1 year categorized separately. Other age groups were classified by decades. The youngest case was 1 year old, and the oldest was 98 years old. The highest number of deaths occurred in the seventh decade of life. The lowest number of deaths was observed in the second and third decades. No deaths were recorded in the 0–1 year age group, and four deaths occurred in the first decade. The gender distribution of the cases is presented in Figure 1, and the distribution by age groups is shown in Figure 2.

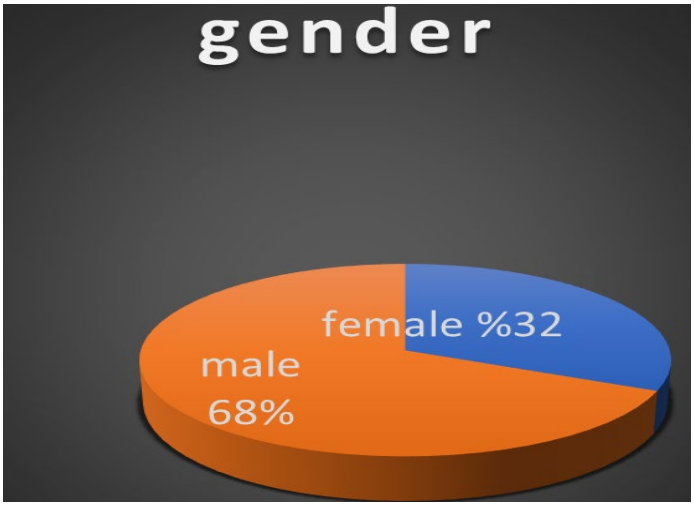

Figure 1. Distribution of cases by gender

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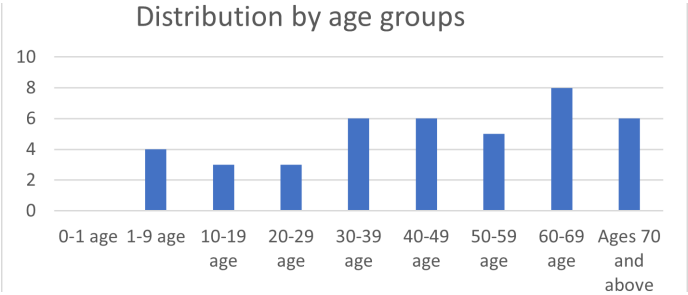

Figure 2. Distribution of cases by age groups

When the death cases were analyzed according to the months in which they occurred, the highest number of deaths was observed in February, with 9 cases (21.9%). No deaths due to CO poisoning were recorded in May, July, August, and September. When the death cases were evaluated by season, the majority occurred in winter, with 24 cases (58.5%). It was noted that 3 deaths occurred during the summer season, all resulting from CO poisoning caused by fire. The distribution of cases by month is presented in Figure 3.

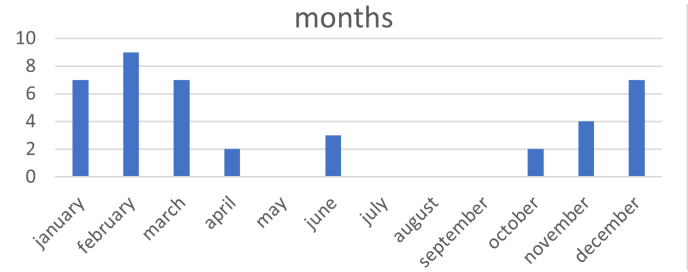

Figure 3. Distribution of cases by month

In the analysis conducted by year, it was found that the highest number of deaths occurred in 2022, with 23 cases (56.09%) reported that year. Ten deaths occurred in 2023, and eight deaths were recorded in 2024.

When evaluating CO levels, the lowest COHb level among fatal poisonings was 17%, and the highest was 78.6%. Most deaths occurred at COHb levels of 60% and above. While 27 cases were found deceased at the scene, the longest hospital stay due to CO poisoning was four months. The majority of deaths in men were observed at COHb levels of 60% and above, whereas in women, the most frequent deaths occurred at COHb levels between 50% and 59%. The distribution of COHb levels by gender is presented in Table 1. The distribution of COHb levels by age groups is shown in Table 2.

In 32 cases, the source of CO was the stove, while in 6 cases, poisoning was attributed to CO gas originating from a fire. It was identified that 3 cases were poisoned due to gas leaking from a water heater. Among the cases, 21 individuals resided in the city center, and 20 lived in districts and villages. The distribution according to the CO source is presented in Figure 4.

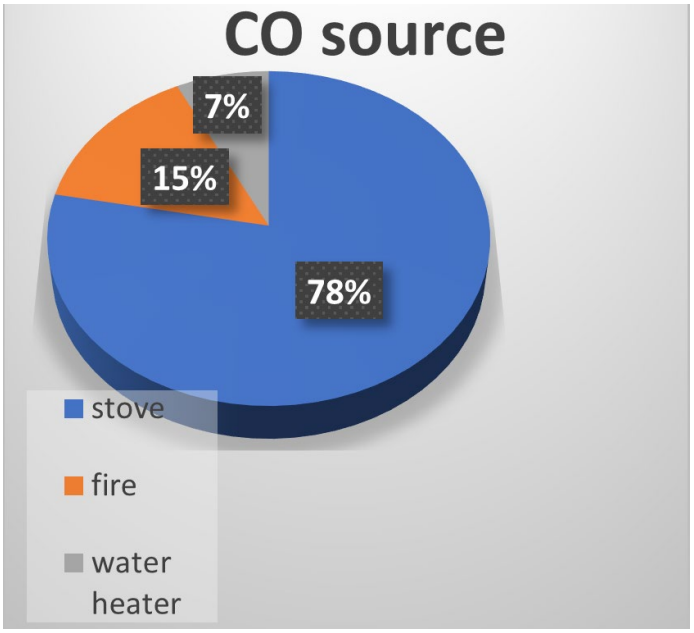

Figure 4. Distribution by CO source

It was determined that 11 of the 41 cases underwent autopsy, while 30 were investigated for cause of death. Among the autopsied cases, one exhibited bridging, one had moderate coronary artery occlusion, one had severe coronary artery occlusion, and the remaining cases showed mild coronary artery occlusion. The CO level in the case with severe occlusion was measured at 37.5%.

Table 1. Distribution of COHb levels of cases by gender

Gender	COHb levels													
	<20%		20-30%		30-40%		40-50%		50-59%		≥60%		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Female	1	2.43	2	4.87	2	4.87	3	7.31	4	9.75	1	2.43	13	31.7
Male	3	7.31	4	9.75	4	9.75	2	4.87	4	9.75	11	26.83	28	68.3
Total	4	9.75	6	14.63	6	14.63	5	12.19	8	19.51	12	29.26	41	100

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Table 2. Distribution of COHb levels of cases by age groups

Age groups	COHb levels													
	<20%		20-30%		30-40%		40-50%		50-59%		≥60%		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
1-9	1	2.43	0	0	0	0	2	4.87	1	2.43	0	2.43	4	9.75
10-19	0	0	1	2.43	0	0	0	0	0	0	2	4.87	3	7.31
20-29	0	0	1	2.43	0	0	0	0	1	2.43	1	2.43	3	7.31
30-39	1	2.43	1	2.43	0	0	0	0	1	2.43	3	7.31	6	14.63
40-49	1	2.43	0	0	0	0	2	4.87	1	2.43	2	4.87	6	14.63
50-59	0	0	1	2.43	2	4.87	1	2.43	0	0	1	2.43	5	12.51
60-69	0	0	2	4.87	2	4.87	0	0	2	4.87	2	4.87	8	19.51
70 and above	1	2.43	0	0	2	4.87	0	0	2	4.87	1	2.43	6	14.63
Total	4	9.75	6	14.63	6	14.63	5	12.19	8	19.51	12	29.26	41	100

DISCUSSION

Humans have been burning carbon derivatives to generate heat since ancient times, aiming to satisfy heating needs in enclosed spaces. CO, which is present in trace amounts in normal air, increases in concentration within closed environments. When carbon derivatives are combusted in such settings and combustion is incomplete, the level of CO rises rapidly. Symptoms of CO poisoning begin to manifest when the COHb level in the blood reaches 10% [5,19,20]. The earliest historical reference to CO is attributed to Aristotle in antiquity, who stated, "Coal fumes cause severe headaches and death." In the 1600s, a Swedish scientist documented bright red livor mortis (postmortem discoloration) on bodies due to inhalation of carbon gas, and clinical CO poisoning was first reported by the French physician Dominique-Benoît Harmant in 1775 [21].

Deaths resulting from accidental CO poisoning are preventable; however, a considerable proportion of accidental fatalities continue to be caused by CO poisoning [8]. In our province, 1.25% of forensic deaths over the three-year study period were attributed to CO poisoning. A study conducted in Eskişehir reported this rate as 1.43%, while in Ankara, it was 3.54%. Although regional differences were observed depending on the types of fuels used for heating, these findings generally align with existing literature [8,22].

When cases were evaluated by gender, the male-to-female ratio in our study was approximately 2:1. Studies have demonstrated that although women and men are exposed to CO at nearly the same rate, mortality in men is about twice as high. This difference has been attributed to faster elimination and shorter half-life of COHb in women compared to men. Consistently, in our study, deaths were twice as frequent in men [5,23].

When the distribution by age groups was analyzed, the highest mortality was observed in the 60–69-year age group, accounting for approximately 20% of deaths, with the youngest fatality being 1 year old. The increased mortality in the older age group is thought to be related to elderly individuals living alone in rural areas or slums, where stoves are used for heating. These individuals tend to light stoves late at night, leading to deaths caused by incomplete combustion of coal. While the average age of death reported in the literature is around 30–40 years, our study found that fatalities occurred more frequently in older age groups [24].

In our study, the highest number of deaths occurred during the winter season, particularly in February, which aligns with existing literature. Factors contributing to this seasonal increase include the widespread use of stoves in winter months, prolonged time spent indoors, lack of regular chimney maintenance, and the reduced efficiency of chimneys in evacuating smoke due to prevailing southern winds that are more common in winter [8,25].

When examined on an annual basis, 23 cases were recorded in 2022, decreasing to 10 in 2023 and 8 in 2024. Literature reports indicate a general decline in the number of deaths over time [12]. The significant reduction between 2022 and the following year may be attributed to the earthquake on February 6, 2023, which forced people to live in more crowded and outdoor environments during the winter months. The lowest COHb level resulting in death was 17%, and the highest was 78.6%. Although various rates have been reported in studies, an average COHb level of 50% or higher has commonly been observed [8,22,26].

In our study, the most frequent fatal cases occurred at COHb levels of 60% and above. Specifically, deaths in women were most frequent at COHb levels of 50-59%, whereas in men, deaths were most common at levels of 60% and above.

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When COHb levels were analyzed according to age groups, it was found that 10 cases occurred in individuals up to 39 years of age, while 31 cases were seen in those aged 40 and above. The most frequent deaths occurred at COHb levels of 60% and above in individuals up to 49 years of age, whereas no significant difference in distribution according to COHb levels was observed in those aged 50 and above. The occurrence of death at lower COHb levels in elderly individuals aligns with existing literature, which recognizes chronic diseases as factors that predispose to fatality at lower COHb concentrations [10].

In our study, deaths due to CO poisoning were accidental in origin, with stoves identified as the most common source (81%). Six cases involved smoke inhalation poisoning resulting from building fires without any visible signs of burning. Half of the fire-related poisonings occurred during the summer months. Studies conducted in Ankara and Bursa also reported stoves as the most frequent source [10,11].

It was determined that one autopsied case had severely occluded coronary arteries, and the COHb level in this case was 37.5%. Morbidity was identified as a contributing factor facilitating death, consistent with the literature [1,10].

CONCLUSION

Although CO poisoning has long been recognized, it remains a significant cause of preventable accidental deaths. Such fatalities are frequently encountered by forensic medicine specialists, particularly during the winter months. These poisonings can be reduced through specific preventive measures. According to US Environmental Protection Agency recommendations the most critical precautions include installing CO alarms near sleeping areas, conducting annual inspections of heating systems and devices, avoiding the use of combustion appliances without proper ventilation, never burning fuel indoors except in devices designed for safe use such as stoves or ovens, refraining from using chimneyless devices like barbecues and water heaters in enclosed spaces, recognizing and responding to the possible symptoms of CO poisoning, and prioritizing the use of safer heating systems, especially in households with elderly individuals [27].

Considering that the most frequent deaths in our study occurred during the winter months and were caused by stoves, it is important to perform annual maintenance of chimneys and stoves and to install CO alarms in homes.

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 committee approval for our study was obtained from the Council of Forensic Medicine. This approval was granted by the Council of Forensic Medicine Education and Scientific Research Board with the decision numbered 21589509/22025/352 dated 18.03.2015.

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Original Article

The relationship between temporomandibular dysfunction, sleep quality, perceived stress, and upper crossed syndrome in young adults: A cross-sectional study

Ayca Araci[1], Zeynep Sena Selcuk[1], Huseyin Tas[1], Seher Ulker[1], Ismet Asli Topcuoglu[2], Ayse Unal[1]

1Alanya Alaaddin Keykubat University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Antalya, Türkiye
2Alanya Alaaddin Keykubat University, Faculty of Medicine,Department of Physical Medicine and Rehabilitation, Antalya, Türkiye

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Abstract

Aim: This study aims to examine the relationship between temporomandibular dysfunction (TMD), sleep quality, perceived stress level, and postural alignment in young adults.
Materials and Methods: A total of 168 university students aged 18-30 were included in the study. New York Posture Analysis Scale (NPA) for posture, the Fonseca Anamnestic Index (FAI) for TMD, the Pittsburgh Sleep Quality Index (PSQI) for sleep quality, and the Perceived Stress Scale (PSS) for stress level were used. Statistical analyses were conducted using parametric and nonparametric tests and multiple regression analysis.
Results: 63.1% of the participants were female, and the mean age was 20.66±2.22 years. According to the regression analysis, only orthodontic treatment history was found to have a significant effect on posture ($p=0.032$). No statistically significant correlation was found between gender, age, grade, missing teeth, chronic disease, FAI, PSQI, or PSS scores, and posture ($p>0.05$). Furthermore, a weak negative correlation was found between FAI and PSS ($p<0.001$).
Conclusion: Our study revealed that orthodontic treatment history is associated with postural disorders. TMD, sleep quality, and perceived stress level were found to have no direct effect on posture. The findings suggest that postural disorders should be evaluated from a multifactorial, biopsychosocial perspective.

Keywords: Temporomandibular dysfunction, sleep quality, stress, upper crossed syndrome, university students

INTRODUCTION

The human body consists of complex chain systems that enable multidirectional movements. According to Janda, one of these chains, the articular chain, is the product of the biomechanical interactions of different joints during a specific movement pattern and is divided into two substructures: the postural chain and the kinetic chain. The postural chain refers to the positional relationships between joints in an upright position of the body, while the kinetic chain refers to the functional flow of movement through open and closed chain movements [1]. The Janda approach defines these chain concepts as a fundamental framework for the evaluation of muscle imbalances and motor control disorders [2]. Frank, Kobesová & Kolář (2013) emphasized that the functional integration of chain systems is critical for postural stability [3]; Kobesová & Kolář (2014) explained these structures with a three-layered motor control model that includes spinal, subcortical, and cortical levels [4]. Postural disorders are not limited to musculoskeletal problems but can also be associated with neurological and psychological systems.

Temporomandibular dysfunction (TMD) is a complex clinical condition characterized by pain and dysfunction of the masticatory muscles, temporomandibular joint (TMJ), and related structures.

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Corresponding Author: Ayse Unal, Alanya Alaaddin Keykubat University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Antalya, Türkiye
Email: pt.aunal@gmail.com

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Electromyographic studies show that TMD alters the mandibular resting position, and abnormal activity of the masticatory muscles may increase the load on the TMJ [5,6]. The relationship between postural disorders and TMD gains clinical importance, especially when the biomechanical connections between the cervicocranial region and the mandibular structure are taken into consideration. In the study by Uritani et al. (2014), it was stated that the head position of individuals with TMD was more anterior compared to healthy individuals, and that this difference was both statistically and clinically significant [1]. Minervini et al. (2023) revealed a significant relationship between TMD and posture at the neuromuscular level, emphasizing that this interaction may also be shaped by biopsychosocial factors [5,7].

The literature emphasizes that TMD may be associated not only with postural parameters but also with psychophysiological factors such as sleep quality and perceived stress. A study conducted on university students found significant relationships between sleep quality and head-neck posture, demonstrating that postural control mechanisms may be linked to sleep quality [8]. Sleep disorders manifest with symptoms such as difficulty initiating sleep, nighttime awakenings, or sleep fragmentation, and are often associated with psychological problems such as anxiety, depression, and low self-esteem. This may lead to disruption of the circadian rhythm and dysregulation of the neuroendocrine system, resulting in negative effects on muscle tone and postural control [9,10].

This multidimensional interaction can play a decisive role not only at the biomechanical level but also on an individual's biopsychosocial functioning. The pain, muscle tension, sleep disturbance, and increased stress accompanying TMD can limit an individual's participation in daily activities, leading to functional impairment and disability.

In addition to their clinical significance, postural alignment and temporomandibular function play a crucial role in forensic assessments. Posture, craniofacial alignment, and temporomandibular joint characteristics are important parameters in forensic identification, trauma analysis, and the interpretation of musculoskeletal injuries. Furthermore, stress and sleep disturbances, common in individuals exposed to trauma, violence, or legal stress, can affect muscle tone and posture, thus impacting forensic assessments. In this context, health status should be assessed not only by the presence of the disease but also by the individual's level of physical, psychological, and social participation. The coexistence of postural control, muscle function, and psychophysiological balance disorders provides important clues that can be considered early indicators of disability in young individuals.

However, multidimensional analyses that evaluate these variables simultaneously, particularly those conducted on young individuals, are limited in the literature. Therefore, the aim of our study is to examine the relationship between the level of postural impairment and temporomandibular dysfunction, sleep quality, perceived stress level, and postural alignment in young individuals and to reveal the potential effects of these variables on individuals' functional participation and disability levels.

MATERIAL AND METHOD

This is a cross-sectional study examining the relationships between postural level, temporomandibular dysfunction, perceived stress, and sleep quality in young adults. The study was conducted with volunteer students studying at Alanya Alaaddin Keykubat University.

Ethical Approval: The study was approved by the Clinical Research Ethics Committee of the Faculty of Medicine, Alanya Alaaddin Keykubat University (Date: 25/09/2024; Decision No. 21-03). The research process was conducted in accordance with the Declaration of Helsinki. Participants were informed about the study and their written consent was obtained. Assessments were conducted by the researcher through face-to-face interviews.

Power Analysis: The effect size obtained in the reference study was found to be of a weak level (ρ=0.183) [8]. Based on the assumption that a higher effect size could be achieved, a power analysis was conducted. With a confidence level of 95% and a power of 90%, it was determined that with a sample size of 168 participants, the effect size would be ρ=0.35.

Inclusion Criteria: Being between 18 and 30 years old, being a university student, not having had orthopedic, neurological, or dental surgery in the last 3 months, voluntary participation in the study.

Exclusion Criteria: Having had previous trauma or surgery to the TMJ region, being diagnosed with a neurological or systemic disease, being pregnant, filling out the questionnaires incompletely or incorrectly

Data Collection Tools

The study used the New York Posture Analysis Scale (NPAS) for posture analysis, the Fonseca Anamnestic Index (FAI) to determine the presence of TMD, the Pittsburgh Sleep Quality Index (PSQI) to determine sleep quality, and the Perceived Stress Index (PSI) to assess perceived stress.

Posture Analysis: The New York Posture Analysis (NPA) monitors postural changes that may occur in 13 different parts of the body, including the head, neck, shoulder, back, waist, hip, and ankle. Based on the observation results, if the individual has good posture, they are given five (5) points; if their posture is moderately impaired, they are given three (3) points; and if there is a severe impairment, they are given one (1) point. The total score obtained from the test ranges from 13 to 65. Standard evaluation criteria developed for this test are defined as "very good" if the total score is ≥45, "good" if it is 40-44, "moderate" if it is 30-39, "poor" if it is 20-29, and "poor" if it is ≤19 [11].

Fonseca Anamnestic Index (FAI): The FAI was developed to identify TMD based on signs and symptoms. This index consists of 10 items and has three response options: "yes" (10 points), "sometimes" (5 points), and "no" (0 points). The result is determined by the sum of the scores for all items and is classified as follows: no signs and symptoms of TMD (0-15 points), mild TMD (20-45 points), moderate TMD (50-65 points), and severe TMD (70-100 points) [12,13].

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Pittsburg Sleep Quality Index (PSQI): It consists of seven components: subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbance, use of sleep medications, and daytime dysfunction [14]. The Turkish version of the PSQI was used in this study [15]. The score for each subgroup ranges from 0 to 3. The sum of these component scores provides an overall score for subjective sleep quality (range: 0–21). Sleep quality is considered "good" for those with a total score of ≤5, and "poor" for those with a total score of >5 [14].

Perceived Stress Scale (PSS): The PSS consists of 14 items and is designed to measure the perceived stress level in specific situations in a person's life [16]. PSS scores range from 0 to 56. A high total score indicates a high level of perceived stress [17].

Statistical Analysis

Statistical analyses were performed using SPSS for Windows, version 22 software. The conformity of the variables to a normal distribution was examined using visual (histogram and probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Demographic data and the results of the parameters to be evaluated were presented as mean (Mean) ± standard deviation (SD) when the parameters were normally distributed, and using the median and interquartile range when the parameters were not normally distributed. Parameters affecting postural alignment were examined using multiple linear regression analysis, and the relationships between the parameters were examined using the Pearson test when the data were normally distributed and the Spearman test when they were not normally distributed. The significance level for statistical analyses was accepted as 0.05.

RESULTS

A total of 168 participants aged 18-30 (mean age= 20.66±2.22 years) were included in the study. 63.1% of the participants were female (n=106) and 36.9% (n=62) were male. Demographic characteristics are detailed in Table 1.

Table 1. Demographic and clinical characteristics of participants

Variables	Category	n	%
Gender	**Female**	106	63.1
	Male	62	36.9
Age (year)	**Mean ± SD**	20.66 ± 2.22	
	Med (IQR)	20 (20-21)	
	Min-Max	18-28	
Grade	**1**	68	40.5
	2	52	31.0
	3	31	18.5
	4	17	10.1
Have you received orthodontic treatment?	**Yes**	42	25.0
	No	126	75.0
Is there a missing tooth?	**Yes**	43	25.6
	No	125	74.4
Do you have a chronic illness?	**Yes**	18	10.7
	No	150	89.3
FAI total score	**Mean ± SD**	23.91 ± 3.9	
	Med (IQR)	25 (22-27)	
	Min-Max	12-30	
PSQI	**Mean ± SD**	11.85 ± 2.77	
	Med (IQR)	12 (9.25-14)	
	Min-Max	6-19	
PSS	**Mean ± SD**	28.94 ± 8.79	
	Med (IQR)	30 (25-34)	
	Min-Max	0-52	
NPA	**Mean ± SD**	53.52 ± 5.42	
	Med (IQR)	53 (49.25-57)	
	Min-Max	37-65	

FAI: Fonseca Anamnestic Index, PSQI: Pittsburgh Sleep Index, PSS: Perceived Stress Scale, NPA: New York Posture Analysis, SD: Standard Deviation; Med (IQR): Median (25th-75th percentiles); Min-Max: Minimum-maximum values

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Twenty-five percent (n=42) of the participants had a history of orthodontic treatment, 25.6% (n=43) had missing teeth, and 10.7% (n=18) had a chronic health problem. Clinical data revealed a mean Fonseca score of 23.91 ± 3.9, a mean Pittsburgh Sleep Quality Index (PSQI) of 11.85 ± 2.77, and a mean Perceived Stress Scale (PSS) of 28.94 ± 8.79. The mean New York Posture Analysis Test score was 53.52 ± 5.42. Multiple linear regression analysis was performed to determine the factors affecting NPA scores. Among the independent variables, only history of orthodontic treatment was found to be a statistically significant factor (β=0.165; p=0.032). Participants who had received orthodontic treatment had higher NPA scores, which was associated with greater postural deterioration. No statistically significant correlation was found between the NPA scores and other variables (gender, age, grade, missing teeth, presence of chronic disease, FAI, PSQI, and PSS) (p>0.05) (Table 2). A weak, positive, statistically significant correlation was found between the FAI and PSS scores (r=0.287; p=0.0001). No statistically significant correlation was found between the NPA and other variables (FAI, PSQI, and PSS) (p>0.05). Furthermore, no significant correlation was found between the PSQI and PSS (p=0.335) (Table 3).

Table 2. Multiple linear regression analysis of factors affecting postural alignment

Dependent variable: New York posture analysis				
Independent Variables	**Std. Beta**	**t**	**p**	**95% C.I. Lower–Upper**
Gender	0.065	0.839	0.403	-0.985–2.44
Grade	0.022	0.279	0.781	-0.713–0.948
Age	0.070	0.907	0.366	-0.202–0.546
Have you received orthodontic treatment?	0.165	2.160	0.032*	0.177–3.950
Is there a missing tooth?	0.044	0.569	0.570	-1.349–2.442
Do you have a chronic illness?	0.105	1.364	0.174	-0.822–4.502
FAI	0.040	0.514	0.608	-0.157–0.268
PSQI	-0.129	-1.678	0.095	-0.551–0.045
PSS	0.093	1.205	0.230	-0.037–0.151

*p<0.05 statistically significant effect; Std. Beta: Standardized Beta Coefficient; 95% C.I: 95% Confidence Interval FAI: Fonseca Anamnestic Index, PSQI: Pittsburgh Sleep Index, PSS: Perceived Stress Scale

Table 3. Correlations between fonseca score, sleep quality, perceived stress, and posture score

Variables		**FAI**	**PSQI**	**PSS**	**NPA**
FAI	**r**	1.000	-0.087	0.287*	0.013
	p		0.264	0.0001	0.868
PSQI	**r**		1.000	0.075	-0.097
	p			0.335	0.211
PSS	**r**			1.000	0.130
	p				0.094
NPA	**r**				1.000
	p				

*p<0.05 indicates a statistically significant correlation; r: Spearman correlation coefficient FAI: Fonseca Anamnestic Index, PSQI: Pittsburgh Sleep Index, PSS: Perceived Stress Scale, NPA: New York Posture Analysis

DISCUSSION

Our study investigating the relationship between TMD, sleep quality, perceived stress level, and postural alignment in young adults found that postural disorders were more prevalent in those receiving orthodontic treatment. However, TMD, sleep quality, and perceived stress level were not associated with postural alignment. Postural alignment and TMD are frequently assessed in forensic cases involving trauma, physical assault, occupational injuries, and disability assessments. Therefore, these results should be interpreted not only clinically but also within a forensic context.

Karahan (2024) reported that poor sleep quality can negatively affect postural control, especially under eyes-closed conditions [18]. However, Stemplewski et al. (2023) stated that sleep deprivation did not significantly impair postural stability, emphasizing that the relationship between sleep and posture

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may be influenced by individual factors [19]. Dolina (2025) demonstrated that the effects of TMD subtypes on posture and balance may differ, and this may explain the diversity of findings in our study [20].

In our study, no statistically significant relationship was found between gender, age, grade level, tooth deficiency, presence of chronic disease, FAI, PSQI and PSS and NPA scores. This suggests that these factors do not have a significant effect on posture. According to a TMD risk model developed for university students and including factors such as stress, oral habits, and malocclusion, stress has been reported as an important factor in determining the risk of TMD [10]. Studies conducted in Turkiye show that approximately 50–60% of university students have poor sleep quality and high stress levels, which can have negative effects on physical and musculoskeletal health [21,22]. While Karahan (2024) reported that sleep quality, especially in eye-closed conditions, can affect postural control [18], Badau et al. (2024) found significant relationships between sleep quality and head and neck posture [8]. However, Özsoy et al. (2022) found that these parameters had no significant direct effect on posture, supporting some conflicting results in the literature [23]. Therefore, these relationships are complex, and a unidimensional assessment may be insufficient. Our study found a weak but statistically significant correlation between FAI and PSS scores, suggesting that higher stress levels are associated with increased TMD severity. However, the low correlation strength suggests that stress alone is not a primary determinant of TMD. These findings suggest that stress acts as a contributing factor rather than a determining factor, supporting the multifactorial and biopsychosocial nature of TMD.

The most important feature of our study that will contribute to the literature is the conclusion that supports the fact that postural disorders are more common, especially in those who receive orthodontic treatment. Orthodontic interventions can influence craniofacial structure and musculoskeletal alignment, potentially affecting forensic identification, facial reconstruction, and interpretation of antemortem records. Therefore, dental and orthodontic history should be carefully considered during forensic assessments. In the study conducted by Zieliński et al. (2018), where they examined the relationship between TMD, sleep quality, and postural alignment in young adults, a similar conclusion was reached with our study, revealing that individuals receiving orthodontic treatment had higher rates of postural disorders and that there was no direct relationship between TMD and sleep quality [7]. Gault (2008) emphasized in his studies that orthodontic interventions can affect the postural system [24]. This prediction was supported by the finding of Parrini et al. (2018) that clear aligner treatment can create changes in pelvic tilt, kyphosis, and lordosis angles [25]. In addition, Klostermann et al. (2021) reported that overjet correction with early orthodontic treatment led to significant improvement in pelvic torsion, demonstrating that these postural changes can be observed as a result of treatment [26]. More broadly, a systematic review by Różańska-Perlińska et al. (2024) strengthens the consistency in the literature by revealing significant relationships between malocclusion and posture, balance, head posture, and gait parameters [27].

CONCLUSION

Considering all these results and the literature, our results contribute to the literature because there is no consensus in the literature and because this study examines both physical and emotional factors simultaneously. In future studies, a multidisciplinary approach to assessing physical and emotional factors such as temporomandibular dysfunction, stress, and sleep quality, which may be associated with postural disorders, based on a biopsychosocial model, is crucial for defining the problem. Following the analysis process, multifaceted planning of therapeutic approaches will increase treatment success. Furthermore, it should be noted that these factors can have long-term impacts on individuals' functional capacity, participation in daily activities, and quality of life, thus potentially contributing to disability. In this regard, postural disorders and TMD are believed to play a decisive role not only in musculoskeletal health but also in an individual's level of functionality and participation. Therefore, conducting future research within a holistic approach that includes disability will contribute to both clinical practice and public health policies.

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 committee approval for our study was obtained from the Clinical Researches Ethics Committee of Alanya Alaaddin Keykubat University (Date: 25.09.2024, Decision number: 21-03).

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Review Article

The role of micrornas in forensic genetics and a comparative efficiency analysis of isolation methods from body fluids

Fatma Ebru Bekiroglu, Nazli Holumen, Emel Hulya Yukseloglu

İstanbul University-Cerrahpaşa, Institute of Forensic Sciences and Legal Medicine, İstanbul, Türkiye

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Abstract

This review aims to evaluate the role of microRNAs (miRNAs) as molecular biomarkers in forensic genetics and to comparatively analyze the efficiency of different miRNA isolation methods from various body fluids. A systematic literature review was conducted following PRISMA guidelines. Publications published between 2010 and 2025 were screened in PubMed, Scopus, and Google Scholar databases using the keywords "microRNA", "forensic genetics", "RNA isolation", and "body fluids". Fifty-three peer-reviewed studies met the inclusion criteria and were evaluated in terms of yield, purity, cost, and applicability of the isolation method. The comparative analysis included phenol–chloroform, column-based, magnetic bead-based, and hybrid protocols. The reviewed studies revealed that while phenol–chloroform methods provided the highest RNA yield, column-based and hybrid systems ensured superior purity and reproducibility. Magnetic bead–based techniques were advantageous for automation and reduced contamination risk. The optimal isolation method varied depending on the biological fluid type. miRNAs exhibit high stability and diagnostic potential in forensic applications such as body fluid identification, age estimation, and post-mortem interval determination. Selecting isolation methods optimized for specific biological matrices enhances analytical reliability and facilitates the integration of miRNA-based analyses into routine forensic workflows.

Keywords: microRNA, forensic genetics, RNA isolation, comparative analysis

INTRODUCTION

The need for reliable molecular biomarkers in forensic genetics has increased as the limits of conventional Short Tandem Repeat (STR) analyses become more apparent. In this context, microRNAs (miRNAs) have emerged as valuable alternatives due to their stability, tissue-specific expression, and regulatory roles in gene expression [1].

miRNAs are small non-coding RNAs, about 18–25 nucleotides long, and remain stable in various cellular and extracellular environments, a feature that supports their forensic usefulness [2]. Their multistep biogenesis, from primary to mature forms, further contributes to their robustness and analytical value [3].

Because miRNA expression differs across fluids such as blood, saliva, and semen, they provide practical advantages for body fluid identification and for interpreting mixed or degraded biological traces [4,5]. These properties also support their growing use in applications such as age estimation and evaluating environmental influences on evidence.

This review summarizes the biomarker potential of miRNAs and compares commonly used isolation techniques—phenol–chloroform extraction, column-based methods, magnetic bead systems, and hybrid approaches—based on yield, purity, processing time, and cost [6]. Appropriate method selection is essential to obtain reliable results and ensure the validity of forensic interpretations [5].

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Corresponding Author: Fatma Ebru Bekiroglu, İstanbul University-Cerrahpaşa, Institute of Forensic Sciences and Legal Medicine, İstanbul, Türkiye
Email: fatmaebru.bekiroglu@ksbu.edu.tr

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Data Sources and Search Strategy

This review followed PRISMA recommendations for systematic literature searches. Publications from 2010 to 2025 were screened in PubMed, Scopus, and Google Scholar using the keywords "microRNA," "forensic genetics," "RNA isolation," and "body fluids." Only peer-reviewed articles in English were considered.

The search identified 168 records. After removing duplicates and screening titles and abstracts, 60 full texts were examined. A total of 53 studies met all criteria and were included. The overall selection steps are summarized in Figure 1.

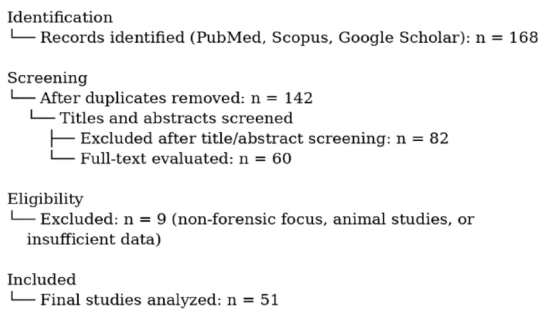

Figure 1. PRISMA flow summary; The figure outlines the literature selection process, from database search to final inclusion, based on PRISMA 2020 standards

Inclusion and Exclusion Criteria

Inclusion criteria:

- Studies published between 2010–2025 addressing microRNA isolation in forensic, biomedical, or diagnostic contexts.
- Research comparing yield, purity, or reproducibility of miRNA extraction methods.
- Experimental or review papers involving body fluids such as blood, semen, saliva, milk, or plasma.
- Exclusion criteria:
- Non-English papers, conference abstracts, or non–peer-reviewed preprints.
- Studies limited to animal samples, cell lines, or unrelated biomedical topics.
- Papers without quantitative measures such as RNA yield or purity ratios [5,7].

Data Extraction and Analysis

For each study, the sample type, extraction technique, commercial kit, and performance indicators (yield, A260/280, A260/230) were recorded. Statistical comparisons were performed using one-way ANOVA ($p<0.05$). Findings were summarized narratively and grouped by isolation method and sample type [8]. Additional comparative tables are provided in Tables 1-5.

Applications of miRNAs in Forensic Genetics

miRNAs have gained attention in forensic genetics due to their stability, tissue-specific expression, and regulatory roles in gene expression [9]. Rocchi et al. (2021) reported that these molecules support several forensic applications, including body fluid identification, age estimation, and post-mortem interval (PMI) evaluation [10].

Identification of Body Fluid Sources

Distinct miRNA expression patterns across body fluids provide a practical framework for determining the origin of biological traces. For instance, miR-205 is strongly associated with saliva, whereas miR-10b is predominantly detected in semen, making them useful fluid-specific markers [1,4].

Arat and Kaya Akyüzlü (2024) emphasized that miRNAs offer valuable discriminatory power, particularly in mixed or degraded samples frequently encountered in forensic casework [11]. Wang et al. (2013) showed that a panel of four miRNAs can distinguish body fluids with an accuracy of up to 95% [12,13]. Similarly, an in silico study by Çevik and Güzel Tanoğlu (2025) confirmed the high discriminatory potential of saliva-related miRNAs [14].

Together, these findings demonstrate that miRNA profiles form a reliable molecular basis for body fluid identification in forensic investigations.

Individual Identification and Age Estimation

Although miRNAs are not yet specific enough for direct individual identification, several age-related miRNAs—such as miR-34a, miR-181a, and miR-21—exhibit expression patterns that correlate with biological aging [15,16]. These changes can be incorporated into regression-based models to estimate an individual's age range with reasonable accuracy [17].

While still developing, this molecular approach may serve as a useful complement to traditional anthropological or skeletal assessments, especially when physical remains are limited or degraded.

Estimation of Post-Mortem Interval (PMI)

The post-mortem stability of miRNAs has generated interest in their use as molecular indicators of PMI. Several studies report time-dependent expression changes in cardiac miRNAs, including miR-1 and miR-133a, supporting their potential as molecular timing markers [8].

Pasaribu et al. (2023) reviewed findings from 18 studies and showed that miRNAs from the liver, skeletal muscle, and peripheral blood display measurable shifts in expression as PMI progresses [18]. Compared with morphological or entomological techniques, these molecular patterns may offer more consistent estimates, particularly when environmental conditions limit traditional approaches. Overall, current evidence suggests that miRNA-based profiling could strengthen PMI estimation in forensic practice.

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miRNA Isolation from Various Body Fluids

Because miRNAs are present in low quantities and are prone to nuclease degradation, the efficiency of the isolation step is critical in forensic workflows. Reported extraction strategies vary widely in yield, purity, and susceptibility to contamination. The most widely used methods include phenol–chloroform extraction, column-based purification, magnetic bead systems, and hybrid protocols that combine multiple steps [2,19].

General Protocols

Selecting an appropriate isolation method depends on the biological matrix—such as blood, saliva, or semen—as well as sample volume and planned downstream analyses. This choice is particularly important in forensic settings, where samples may be limited, degraded, or contain PCR inhibitors. Reducing processing steps and maximizing RNA recovery increase the likelihood of obtaining usable results.

miRNA quality is usually evaluated through RNA concentration (ng/µL), purity ratios [A260/A280 and A260/A230), and assessments of RNA integrity performed by electrophoresis or microfluidic systems. These metrics guide both method selection and the reliability of subsequent analyses.

Detailed Evaluation of Phenol-Chloroform-Based Methods

Phenol–chloroform extraction separates RNA through organic phase partitioning and has long been valued for its ability to recover high yields, including low-abundance miRNAs. Despite this advantage, the method involves multiple manual steps and relies on volatile, hazardous chemicals, which limit its suitability for routine forensic laboratories. Its hands-on complexity and operator-dependent variability also increase the risk of contamination and inconsistent results.

Conventional Phenol-Chloroform Extraction: This classical approach uses acidic phenol and chloroform to separate RNA into the aqueous phase, followed by ethanol or isopropanol precipitation to recover the RNA. Although it can produce high-quality RNA, the procedure requires repeated centrifugation and careful handling of unstable reagents, making it impractical for high-throughput forensic workflows [20].

TRIzol-Based Extraction: TRIzol combines phenol and guanidinium thiocyanate, enabling cell lysis and RNase inhibition in a single step. It is effective across a wide range of sample types; however, the multiple pipetting steps and phase transitions increase the chance of RNA loss, especially in limited forensic samples. Modified TRIzol protocols have shown improved performance in lipid-rich or viscous matrices such as plasma and brain tissue [21,22].

QIAzol-Based Extraction: QIAzol, a phenol-containing reagent similar to TRIzol, is designed for samples with high lipid or viscosity levels. Studies on human milk and neural tissues report improved yields where standard methods underperform. Nonetheless, purity ratios (A260/A280 and A260/A230) vary considerably between matrices, highlighting the need for sample-specific optimization—an essential consideration in forensic genetics where reproducibility is critical [4].

Proteinase K-Assisted Extraction: Proteinase K digestion helps break down proteins and improves RNA release, particularly in protein-dense fluids like semen. When combined with organic extraction, this pre-treatment significantly increases RNA recovery. Precise temperature and incubation control are essential, as suboptimal conditions can reduce RNA integrity, affecting downstream analytical reliability [23,24].

Column-Based miRNA Isolation Methods

Silica membrane–based column systems are widely used for miRNA extraction because they offer standardized workflows, ease of use, and reproducible results. RNA binds to the silica matrix under chaotropic conditions and is subsequently washed and eluted, producing clean nucleic acids suitable for downstream analyses. These methods are particularly useful for low-volume forensic samples, where limiting RNA loss is critical [25].

Li and Kowdley (2012) showed that column-based methods provide strong reproducibility and maintain RNA integrity in low-biomass materials such as serum. Kits such as miRNeasy (Qiagen) and mirVana (Ambion) remain common choices in both clinical diagnostics and forensic settings due to their reliable performance [26].

Evaluation of Major Column-Based Kits

miRNeasy (Qiagen): This kit isolates both small and large RNA through silica membrane binding. Its high purity and sensitivity, especially in serum and plasma, make it a frequent choice for forensic applications [13].

mirVana (Ambion): mirVana begins with an organic solvent–based lysis step before RNA binding to silica membranes. Although it provides high-quality RNA, the multiple centrifugation steps add complexity and extend handling time, which may increase variability in forensic workflows [27].

Promega ReliaPrep™ System: ReliaPrep offers a rapid, user-friendly protocol and performs well in complex matrices such as human milk. However, some studies report lower overall yields, which can be a limitation when dealing with low-template forensic samples [15].

Total Exosome RNA & Protein Isolation Kit (Thermo Fisher): This kit is designed for simultaneous isolation of RNA and proteins from exosomes in body fluids. It performs particularly well for exosomal miRNAs—an emerging category of forensic biomarkers. However, its requirement for adequate sample volume can be problematic in degraded or limited forensic specimens [5,28].

PureLink™ miRNA Isolation Kit (Invitrogen): PureLink targets small RNA recovery from complex matrices, including plasma and human milk. It consistently produces high-purity RNA

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based on A260/280 and A260/230 ratios, although overall RNA yield can vary depending on sample type and input quantity [6].

NucleoSpin® miRNA Kit (Macherey-Nagel): NucleoSpin enables parallel isolation of total and small RNA from diverse sample sources and is appreciated for its affordability and ease of use. Nonetheless, some studies report reduced yields in dilute fluids like saliva or plasma, which may limit its forensic utility in trace samples [7].

Magnetic Bead-Based Isolation Methods

Magnetic bead–based extraction systems have gained traction as an efficient and automation-friendly option for miRNA isolation. These methods use magnetizable particles coated with nucleic acid–binding surfaces, allowing RNA to bind selectively while contaminants are washed away under a magnetic field. The result is a rapid, clean isolation process that works well even when sample volumes are small—an important advantage in forensic casework.

Their key strengths include short processing times, reduced manual handling, minimal contamination risk, and strong compatibility with automated platforms. These features improve workflow reproducibility and biosafety, making bead-based systems increasingly valuable for downstream applications such as RT-qPCR and next-generation sequencing in both forensic and clinical settings [16].

Chen et al. (2020) evaluated a two-step magnetic bead protocol and reported extraction efficiencies approaching 91% for exosomal miRNAs in plasma, demonstrating the method's strong performance in complex matrices [29]. These findings support the use of magnetic bead systems as a practical and reliable alternative to more labor-intensive manual extraction methods, particularly when automation is needed.

Hybrid Extraction Methods

Hybrid strategies combine components of conventional and modern extraction techniques to improve both yield and purity. These methods are especially useful in forensic work, where samples may be degraded, scarce, or contain inhibitors.

TRIzol + miRNeasy Combination: This approach begins with TRIzol lysis, followed by silica column purification using the miRNeasy system. The pairing of TRIzol's strong lysis efficiency with the high-purity output of column methods produces consistently higher-quality RNA, particularly in challenging materials such as serum and human milk [8].

Liquid–Liquid Extraction + Solid-Phase Cleanup: In this workflow, the aqueous phase from phenol–chloroform extraction is further purified on a silica column. It performs well in protein-rich fluids such as seminal plasma. Additives like Proteinase K, glycogen, or linear acrylamide can improve yields, although studies indicate that exosome pre-isolation does not provide significant added benefit for low-abundance forensic samples [30].

TRIzol Plus Liquid–Liquid Extraction Method: Developed by Zununi Vahed et al. (2016), this protocol begins with TRIzol lysis, removes large RNAs via potassium acetate, and selectively precipitates small RNAs—including miRNAs—using lithium chloride. It has shown strong performance in FFPE samples and viscous fluids and is noted for being cost-effective and adaptable for laboratories that do not rely on commercial kits [31].

Other Methods

Several alternative miRNA extraction strategies have been described for difficult or highly contaminated biological matrices, although these approaches are not yet standard in forensic workflows. Many of these methods focus on modifying reagent composition or adjusting extraction steps to improve recovery from samples with low RNA content or high levels of inhibitors.

Non-Standard Chemical Protocols: Procedures such as lithium chloride precipitation, PEG-assisted extraction, and glass fiber–based filtration have been applied successfully to challenging fluids including bile, milk, and fecal material [32]. Because these matrices often contain substantial debris or enzymatic inhibitors, they typically require pre-processing steps—such as high-speed centrifugation, filtration, or differential ultracentrifugation—to reduce contaminants before the actual extraction begins [33].

Exosomal RNA Isolation Approaches: Extraction of miRNAs from extracellular vesicles, particularly exosomes, has been performed using workflows such as differential ultracentrifugation, size-exclusion chromatography, or microfluidic separation. These methods can provide highly enriched RNA fractions but also come with drawbacks, including long processing times, specialized equipment requirements, and increased operational complexity [34].

Use of DNA Extraction Protocols for Small RNA Recovery: Interestingly, several studies have noted that some DNA extraction kits—both silica column and magnetic bead formats—retain small RNAs during purification. This allows the recovery of miRNAs and genomic DNA from the same sample, a significant advantage when working with limited or irreplaceable forensic material [35].

Extracellular RNA Isolation from Biofluids: In serum and plasma, techniques such as precipitation, membrane filtration, affinity-based purification, and ultracentrifugation have been used to isolate extracellular miRNAs. Commercial systems like ExoQuick and miRCURY often yield satisfactory recovery, whereas ultracentrifugation tends to produce the highest-purity RNA fractions [36,37].

Next-Generation Isolation Platforms: Emerging technologies—including microfluidic devices and antibody-coated magnetic systems—are being evaluated for their potential to enrich low-abundance miRNAs with greater selectivity and throughput. These platforms may support future forensic workflows that require high sensitivity and scalable processing [6].

A summary of the commercial isolation kits referenced in this review is provided in Table 1.

Table 1. Summarizes the types of isolation kits used, classification of the methods, their suitability for experimental purposes, and the primary evaluation criteria

Reference source	Commercial kit name	Biological sample type	Forensic diagnostic utility	Matrix composition	Advantages	Limitations
Chen et al. (2020) [29]	2MBB	Plasma	Magnetic bead-based method	Magnetic bead platform	High purity; specific RNA binding; suitable for automation	Long protocol duration
Li and Kowdley (2012) [26]	miRNeasy	Serum	Column-based method	Silica membrane column	High reproducibility; recovery of high-quality RNA	Reduced RNA yield in certain samples; may require high input volume
Li and Kowdley (2012) [26]	mirVana	Serum	Column-based method	Silica membrane column	High reproducibility; recovery of high-quality RNA	Multi-step protocol may be time-consuming and increase user-dependent variability
Li and Kowdley (2012) [26]; Rocchi et al. (2021) [10]	Promega ReliaPrep™	Plasma, serum, breast milk	Silica column + Proteinase K	Hybrid method	High yield; strong lysis capacity; compatible with complex matrices such as breast milk	Risk of protein contamination; requires multiple processing steps
Xu et al. (2022) [6]; Rocchi et al. (2021) [10]	PureLink miRNA	Plasma, tissue	Silica membrane column	Column-based method	Easy to implement; suitable for high-throughput applications	Low RNA yield reported in certain sample types
Rocchi et al. (2021) [10]; Glynn ve O'Leary (2018) [38]	NucleoSpin	Serum, plasma	Silica column	Column-based method	Cost-effective; simple protocol	Reduced RNA yield in aqueous or dilute samples
Rekker et al. (2014) [36]	ExoQuick	Plasma, cell culture supernatant	PEG-based precipitation	Polymer-based precipitation method	Membrane-supported selective isolation; high RNA purity	Expensive; long protocol duration
El-Khoury et al. (2016) [42]; Rekker et al. (2014) [36]	miRCURY Exosome Kit	Blood, cell culture, organ tissue	Membrane filtration + precipitation	Hybrid method (membrane filtration + precipitation)	High yield; compatible with vacuum-based workflows	Contains phenol; toxic and time-consuming
Roy et al. (2020) [22]; Zununi Vahed et al. (2016) [31]; Glynn et al. (2020) [17]	TRIzol	Blood, brain, liver	Phenol–chloroform	Organic extraction method	Efficient lysis; prevents DNA contamination	Phenol toxicity requires careful handling; laborious
Rocchi et al. (2021) [10]; Xu et al. (2022) [6]	QIAzol	Serum, plasma	Phenol–chloroform derivative	Organic extraction method	High purity RNA from complex fluids such as plasma	Lower yield compared to TRIzol
Eldh et al. (2012) [28]	Total Exosome RNA & Protein Isolation Kit	Plasma	Exosome purification with organic solvents	Exosome precipitation + organic extraction	Isolates exosome-specific RNA with high purity	May require protocol customization for different matrices

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Comparison of miRNA Isolation Methods from Different Body Fluids

Different extraction protocols vary substantially in their RNA yield, purity, and stability, largely because each biological fluid has its own biochemical profile. Kit chemistry and workflow complexity further influence performance. This section summarizes comparative findings from the literature and highlights method preferences for specific specimen types.

Evaluation of RNA Purity, Concentration, and Degradation Rates

Multiple studies have assessed isolation efficiency using metrics such as total RNA concentration, A260/280 and A260/230 ratios, and replicate consistency.

An early multicenter comparison by Eldh et al. (2012) found that the miRCURY protocol produced the highest RNA concentrations, whereas RNeasy and TRIzol + Cleanup achieved a more balanced outcome between yield and purity (Table 2) [28].

Zununi Vahed et al. (2016) reported that a potassium acetate + 2.5M LiCl workflow yielded the best overall purity for urine samples. Although extraction with 0.4M LiCl produced higher RNA amounts, purity was markedly lower—limiting its applicability in downstream analyses (Table 1) [31].

O'Leary and Glynn (2018) compared three commercial kits across several body fluids. Their data indicated that miRNeasy generated the highest RNA yield overall, while a modified mirVana protocol performed best with saliva. Each experiment included ten technical replicates (Table 2) [38].

Table 2. Comparative Analysis of Total RNA Yield and Purity Across Seven Isolation Methods [28, 38]

Method	RNA yield (ng/10^6 cells)	A260/280 ratio	A260/230 ratio	Technical replicates (n)	Comment
Trizol®	24.1±8.3	2.1	1.8	3–4	Limited recovery of small RNAs
Trizol® + Cleanup	41.3±6.6	2.2	2.1	3–4	Improved yield and purity
RNeasy®	82.8±27.1	2.3	2.6	3–4	Among the highest purity values
Modified RNeasy®	75.7±27.6	1.9	1.2	3–4	Lower purity observed
miRNeasy	13.0±7.7	1.9	1.5	3–4	Overall RNA yield was low
miRCURY™	107.7±25.7	2.0	2.0	3–4	Highest total RNA yield
mirVana™	33.1±17.7	1.9	1.8	3–4	High yield of small RNA species

Spectrophotometric and fluorometric assessments continue to guide forensic decision-making. TRIzol protocols often produce strong yields but frequently show reduced A260/230 ratios due to residual organic contaminants [39]. Column-based systems typically yield cleaner RNA but at the expense of lower total recovery, a pattern echoed across several comparative studies [40].

Williams et al. (2015) emphasized that, particularly in forensic contexts where sample quantity is limited, the isolation method chosen can directly alter quantitative outcomes and downstream interpretation [41]. El-Khoury et al. (2016) further noted that miRCURY delivers high purity but may underperform with small input volumes due to column saturation, whereas miRNeasy captures more small RNA species but with slightly reduced purity [42].

Finally, even standardized protocols show noticeable inter-laboratory variation, reinforcing the need for harmonized isolation procedures in both forensic and clinical molecular settings [43].

Experimental Design

Comparative studies evaluating miRNA isolation methods depend heavily on using a consistent experimental setup. Most investigations process the same biological material—often serum—side-by-side with several extraction protocols and then compare RNA yield, purity ratios, and RT-PCR performance. Typically, 3–5 technical replicates are included, and statistical differences are assessed using independent t-tests or one-way ANOVA [44]. The use of internal reference miRNAs is also essential, as it reduces technical variation and supports reliable normalization [25].

In the experimental data reviewed here, differences in RNA concentration and purity across methods were analyzed using one-way ANOVA, with statistical significance defined as $p<0.05$.

Selection of the Most Efficient Isolation Method According to Body Fluid Type

Comparative findings from the included studies highlight that miRNA isolation efficiency is strongly dependent on the biochemical characteristics of each fluid.

Mercadal et al. (2020) examined six EV isolation techniques for semen. Their results showed that the miRCURY 1500×g protocol produced the highest RNA yield, while ultracentrifugation remained the benchmark for purity (Table 3) [45].

For human milk, Ahlberg et al. (2021) tested five column-based kits. Promega ReliaPrep™ generated the highest RNA concentrations, whereas the Sigma-Aldrich mirPremier™ kit achieved superior purity metrics (Table 4) [20].

Urbizu et al. (2023) evaluated saliva collection systems combined with column-based kits. The NucleoSpin + Oragene pairing yielded the most RNA, while miRNeasy produced samples with the lowest levels of DNA contamination (Table 5) [40].

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Table 3. Among the compared miRNA isolation protocols from urine specimens, the KCH_3COOH + LiCl 2.5M method demonstrated superior performance in terms of yield and purity [31, 45]

Method	RNA Yield (ng/µL)	A260/280 ratio	A260/230 ratio	Technical replicates (N)	Comment
KCH_3COOH + LiCl 2.5M + Ethanol	231±5.9	1.84	3.85	3	Best purity and yield
LiCl 0.4M + Ethanol	1382±3.4	1.71	0.70	3	Highest yield but poor purity
LiCl 8M	657±21	1.43	0.32	3	Low yield and low purity
PEG 4000	~25.7 Ct	-	-	3	Low RNA yield based on Ct value
PEG 6000	~28.1 Ct	-	-	3	Lowest PCR efficiency

Table 4. miRNA isolation efficiency from distinct body fluids was compared using three commercial kits, with the number of technical replicates specified [20, 38]

Body Fluid	Method	Mean RNA yield (ng/µL)	Technical replicates (n)	Comment
Venous blood	miRNeasy	29.3±6.4	10	Highest yield
Venous blood	miRVana	4.5±1.5	10	Lowest yield
Venous blood	Modified miRVana	14.8±18.2	10	High variation
Menstrual blood	miRNeasy	232.2±188.6	10	High yield, variable
Menstrual blood	miRVana	56.4±53.4	10	Moderate yield
Menstrual blood	Modified miRVana	53.6±61.5	10	High inconsistency
Semen	miRNeasy	83.8±101.5	10	Large variation
Semen	miRVana	13.6±10.3	10	Low yield
Semen	Modified miRVana	16.8±9.8	10	More consistent
Saliva	miRNeasy	65.7±50.3	10	Good yield
Saliva	miRVana	5.9±4.7	10	Very low yield
Saliva	Modified miRVana	67.5±47.3	10	Best saliva RNA yield
Vaginal material	miRNeasy	331.6±226.0	10	Highest overall yield
Vaginal material	miRVana	111.9±68.5	10	Moderate yield
Vaginal material	Modified miRVana	110.3±58.8	10	Comparable outcome

Table 5. Comparison of six different EV isolation protocols applied to semen samples in terms of RNA yield [40, 45]

Isolation Method	RNA yield (ng/µL)	A260/280 ratio	A260/230 ratio	Technical replicates (n)	Comment
Ultracentrifugation (UC)	22.39±7.79	1.78±0.17	0.60±0.40	5	Reference method, medium quality
ExoGAG 1500×g	8.17±2.55	1.76±0.05	0.13±0.05	2	Low purity
ExoGAG 3500×g	12.29±8.60	1.79±0.08	0.47±0.40	4	Acceptable quality
ExoQuick Ultra A 3000×g	4.27±0.94	1.53±0.04	0.39±0.18	2	Lowest yield
miRCURY Cell/Urine/ Cerebrospinal Fluid (CSF) 1500×g	31.03±2.94	1.63±0.11	0.40±0.26	4	Highest yield, low purity
miRCURY Cell/Urine/CSF 10,000×g	3.65±3.04	1.17±0.01	0.10±0.01	2	Very poor quality

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Given the biological diversity of fluids—differences in lipid content, protein density, and enzyme activity—method selection must be matrix-specific. TRIzol LS performs well with lipid-rich matrices such as milk, whereas column-based systems (mirVana, miRNeasy) are widely favored for plasma [46]. In protein-dense fluids like semen, adding a Proteinase K pretreatment step has been shown to improve recovery [47].

Glynn and O'Leary (2018) further demonstrated that each biological fluid exhibits characteristic miRNA signatures, reinforcing the need for extraction methods tailored to the specimen type [38].

Table 6. Comparison of five column-based kits used for RNA isolation from human milk in terms of yield and purity (20).

Kit	Total RNA (ng/µL)	miRNA (ng/µL)	A260/280 ratio	A260/230 ratio	Technical replicates (n)	Comment
Promega ReliaPrep™	28.30 (7.68–52.65)	12.75 (3.35–56.65)	1.94	0.52	10	Highest quality and yield
Zymo Quick-RNA MicroPrep	20.30 (8.47–28.45)	10.60 (3.72–34.70)	1.35	0.32	10	Low A260/280 ratio
Norgen Total RNA	23.10 (10.70–34.05)	8.40 (3.99–45.35)	1.48	0.24	8	Moderate yield, low purity
Norgen Single Cell	6.20 (0.02–6.89)	1.92 (1.02–22.92)	1.53	0.14	8	Lowest yield
Sigma-Aldrich mirPremier™	14.16 (3.97–38.25)	3.99 (0.85–37.95)	2.20	1.48	10	Best purity, low yield

Table 7. RNA yield and DNA contamination levels obtained from saliva samples using different sample collectors and column-based kit combinations [40]

Combination	Total RNA (ng)	Yield (ng/µL)	A260/280 (%)	DNA Contamination
Oragene + miRNeasy	2636	4.39	60	No
Oragene + miRVana	2365	4.73	60	Yes
Oragene + NucleoSpin	5003	5.56	60	Yes
50 mL Tube + miRNeasy	575	2.00	80	No
50 mL Tube + miRVana	2914	5.83	80	Yes
50 mL Tube + NucleoSpin	825	2.28	80	Yes
Salimetrics + miRNeasy	402	2.00	50	No
Salimetrics + miRVana	1311	2.82	50	Yes
Salimetrics + NucleoSpin	340	1.22	50	Yes

Comparative Analysis

Comparative findings across the literature show that no single protocol consistently outperforms others for miRNA isolation. The most effective method depends on the biological matrix and the demands of the downstream analysis. Column-based systems generally provide higher purity and straightforward operation, whereas TRIzol-based protocols are known for producing greater RNA yields. Magnetic bead–based methods offer reduced contamination risk and work efficiently with automated platforms. Hybrid approaches, which combine organic extraction with silica purification, often achieve a useful balance between purity and yield and are particularly effective for complex or inhibitor-rich samples [47,35].

Advances in downstream analytical tools—especially digital PCR and high-sensitivity microarrays—further improve the reliability of miRNA detection after isolation, allowing accurate measurements even from low-template forensic samples [48].

This review compared the main miRNA isolation strategies used in forensic genetics and evaluated their strengths across different biological matrices. Although phenol–chloroform protocols consistently produce high RNA yields, their dependence on hazardous chemicals, operator skill, and extended processing steps reduces their suitability for routine forensic workflows [44,46].

Column-based systems, on the other hand, provide more reproducible purity and are easier to standardize between laboratories. Their primary drawbacks are the higher cost and reliance on commercial reagents, which may limit feasibility in low-resource forensic units [25]. Magnetic bead–based techniques represent an appealing alternative because they

minimize contamination and can be integrated with automated systems, although differences in bead chemistry and the need for specialized equipment remain significant challenges [35].

Hybrid protocols—particularly combinations of TRIzol extraction followed by silica-based purification—showed the most balanced performance, yielding both high purity and high RNA recovery [47]. However, the heterogeneity of study designs, measurement platforms, and sample storage conditions highlights the ongoing need for harmonized validation standards.

Beyond isolation efficiency, the forensic value of miRNAs also lies in their tissue-specific expression patterns, which support the identification of biological traces even when samples are degraded [49]. Despite this potential, the absence of internationally recognized guidelines for miRNA extraction, quantification, and result interpretation continues to restrict their acceptance as forensic evidence [50].

Limitations and Future Directions

This review has several limitations.

First, the included studies used different protocols, instruments, and analytical criteria, which can affect comparability and overall reproducibility.

Second, publication bias is possible, as many studies report optimized or successful outcomes.

Third, restricting the search to English-language publications may have excluded relevant regional or local research.

Finally, although isolation efficiency was compared across studies, a quantitative meta-analysis could not be performed due to inconsistent reporting of standardized metrics.

Future work should focus on:

1. Developing internationally accepted guidelines for forensic miRNA isolation and validation.
2. Incorporating automation and next-generation sequencing into forensic workflows.
3. Conducting comparative studies using low-template, aged, or environmentally compromised samples.
4. Evaluating chain-of-custody, judicial admissibility, and quality assurance frameworks for RNA-based evidence.

CONCLUSION

miRNAs represent stable and tissue-specific molecular markers with considerable promise for forensic applications, including body fluid identification, age estimation, and post-mortem interval analysis [45]. Findings from this review show that no single isolation protocol is universally optimal; the most effective approach depends on the biological matrix and the requirements of downstream analyses. Establishing standardized extraction procedures and robust quality-control measures will be essential for integrating miRNA-based assays into routine forensic practice. With such developments, miRNA analysis has the potential to transition from an experimental technique to a reliable evidentiary tool that strengthens the interface between molecular biology and forensic medicine [51].

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

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