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REVIEW

Utility of serum galactomannan in diagnosing invasive aspergillosis among hematology patients: a meta-analysis

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ABSTRACT

Objective: In immunocompromised patients, invasive aspergillosis (IA) is a leading cause of morbidity and mortality. The serum galactomannan (GM) assay is a non-invasive test that may assist in IA diagnosis. The purpose of this meta-analysis is to determine the diagnostic accuracy of the serum GM in patients with hematological malignancies.

Materials and Methods: A search was conducted in the MEDLINE database through PubMed. After selection process and data extraction, 2x2 tables were constructed for patients with proven/probable IA and no IA, as well as for patients with proven IA and no IA. The pooled sensitivity and specificity were established using meta-analysis for the cut-off values of 0.5,1.0 and 1.5 ODI. Inter-study heterogeneity was assessed utilizing the inconsistency test (I^2). The receiver operating characteristic (ROC) curve was generated, and the area under the curve (AUC) was calculated. The data analysis was conducted using the Meta-DiSc 1.4 software.

Results: A total of 26 articles, 4502 patients and controls, together with 4761 IA episodes, were included in the meta-analysis. The total number of patients with proven and probable IA was 633 (13.3%). In the group with proven/probable IA versus no-IA, the overall pooled sensitivity and specificity were 80% and 78% (AUC: 0.892) for 0.5 ODI, 74% and 96% (AUC: 0.959) for 1.0 ODI, and 70% and 96% (AUC: 0.964) for 1.5 ODI, respectively. In the group with proven versus no-IA, the overall pooled sensitivity and specificity were 94% and 76% (AUC: 0.922) for 0.5 ODI, 86% and 96% (AUC: 0.979) for 1.0 ODI and 70% and 96% (AUC: 0.974) for 1.5 ODI, respectively.

Conclusion: Our findings indicate that the most appropriate cut-off value for Serum GM in diagnosing IA is 1.0 ODI.

Keywords: Invasive aspergillosis, Galactomannan, Diagnostic accuracy

INTRODUCTION

In immunocompromised individuals, invasive fungal diseases (IFD), particularly Aspergillus infections, are a significant cause of morbidity and mortality [1,2]. Consequently, prompt diagnosis and intervention are crucial, with culture positivity and the identification of hyphae infiltrating tissue in biopsy specimens being the most dependable methods for diagnosing invasive aspergillosis (IA); however, these techniques lack sufficient sensitivity. Furthermore, a biopsy might not be necessary because most patients have neutropenia, thrombocytopenia, bleeding risk, and other potential consequences [3]. As a result, non-invasive strategies for early detection are required. The presence of the galactomannan (GM) antigen, a wall component found in Aspergillus species, may indicate an early diagnosis [4]. GM antigen can be identified using an enzyme-linked immunosorbent

test (ELISA), with results expressed as an optical density index (ODI) [5].

The European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG) released consensus definitions in 2002 to standardize the diagnosis of IFDs. [6]. According to this report, IFDs can be classified into three categories: proven, probable, and possible. EORTC/MSG criteria were revised and updated in 2008 and 2020, resulting in a modification in the definition of the probable category, which was expanded while the scope of the possible category diminished [7,8]. One of these modifications pertains to the cut-off value of galactomannan (GM) in serum and bronchoalveolar lavage (BAL). The optimal cut-off for galactomannan antigen ODI remains debatable. The literature presents variable findings for the sensitivity and specificity of this index. We conducted a systematic-review and meta-analysis to assess the diagnostic performance of serum GM and to establish an optimal cut-off value.

METHODS

Literature Search and Article Selection Process

A search was conducted in the MEDLINE database through PubMed for the articles published in English language up to October 2014. The keywords employed for screening articles that assessed the sensitivity and specificity of the serum galactomannan antigen test were '(aspergillus pcr OR galactomannan) AND (sensitivity OR specificity)'.

Among the articles identified through the database search, publications that included adult patients with hematological cancer and/or those who underwent stem cell transplantation were selected. If a publication's population included other host factors along with hematological cancer patients, only those publications where the number of hematological cancer patients was predominant were included in the meta-analysis. Only studies employing the serum GM Platelia ELISA methodology were incorporated.

Case reports, case series, reviews, and systematic reviews were not included. In cases where multiple publications pertain to the same patient population, the publication featuring the larger sample size has been incorporated. Studies with fewer than five patients in the proven and probable IA group, as well as those without adequate data for sensitivity and specificity calculations, were excluded from the analysis.

Data Extraction

Publications that fulfilled the criteria were reviewed meticulously, and the subsequent data were extracted for each study:

1. Mean age
2. Gender distribution percentages
3. Study design (cohort, case-control, randomized controlled trial)
4. Data collection methodology (prospective, retrospective)
5. Sampling method (consecutive, random)
6. The IA diagnostic criteria employed
7. Whether the test was administered to the patient population that represented the entire risk group
8. Whether the reference standard was applied to each and every patient
9. Whether an independent, blinded process was used to evaluate the test results
10. Whether there is any bias that could alter the test outcomes, particularly incorporation bias
11. The total number of patients
12. Number of IA episodes
13. Number of proven, probable, and possible cases
14. The patients' status regarding antifungal treatment or prophylaxis during the test period
15. The minimum number of positive samples required for the test to be considered positive
16. Mean number of samples per patient
17. Prevalence of IA
18. Sensitivity and specificity for the proven or proven and probable patient group
19. The cutoff value of the galactomannan assay

Methodological Quality Assessment

The methodological quality of all publications included in the meta-analysis was assessed using the QUADAS-2 tool [9]. The articles were independently evaluated by two reviewers (A.D. and S.A.) and following individual assessment, the conflicts were analyzed and addressed through discussion. The QUADAS-2 tool comprises four domains, with the risk of bias assessed as unclear, low, or high for each domain.

The reference standard for diagnosing IA, which involves demonstrating Aspergillus hyphae in tissue biopsy, is practically challenging to apply and has not been utilized in all patients in any study. Therefore, the diagnostic criteria employed as the reference standard have been further specified. Each study employed an appropriate reference standard. (2008 EORTC/MSG, 2002 EORTC/MSG, and EORTC similar diagnostic criteria).

Statistical Analyses

The study participants were categorized into four groups as proven IA, probable IA, possible IA and no IA. The possible IA group was excluded from the analysis due to the difficulty to definitively rule out IA and the possibility of its presence. 2x2 tables were constructed for patients with proven/probable IA and no IA, as well as for patients with proven IA and no IA, to ascertain the number of true positives, false positives, false negatives, and true negatives. The pooled sensitivity and specificity were established using meta-analysis. Inter-study heterogeneity was assessed utilizing the inconsistency test (I^2). The receiver operating characteristic (ROC) curve was generated, and the area under the curve (AUC) was calculated. Subgroup analyses were performed due to significant heterogeneity. Because of the variability of cut-off values for GM, separate analyses were performed for 0.5, 1.0, and 1.5 ODI. Due to the variability in the number of samples necessary for a positive GM result between studies, separate analyses were performed for a single positive GM and for at least two consecutive positive GM. Different calculations of the same study with different cut-off values and the minimum number of samples required for the test to be considered positive were recorded as separate data. The data analysis was conducted using the Meta-DiSc 1.4 software.

RESULTS

The full selection process can be reviewed in Figure 1. A total of 26 articles were included in the meta-analysis [10-35]. A total of 4502 patients and controls, together with 4761 IA episodes, were included. The total number of patients with proven and probable IA was 633 (13.3%). Studies involving both adult and pediatric patients were not omitted from the meta-analysis; however, in a study where independent data calculation existed, only the adult patient group data were used [34]. In one study, the sensitivity and specificity of the test were additionally established for individuals from the patient group who underwent autopsy [35].

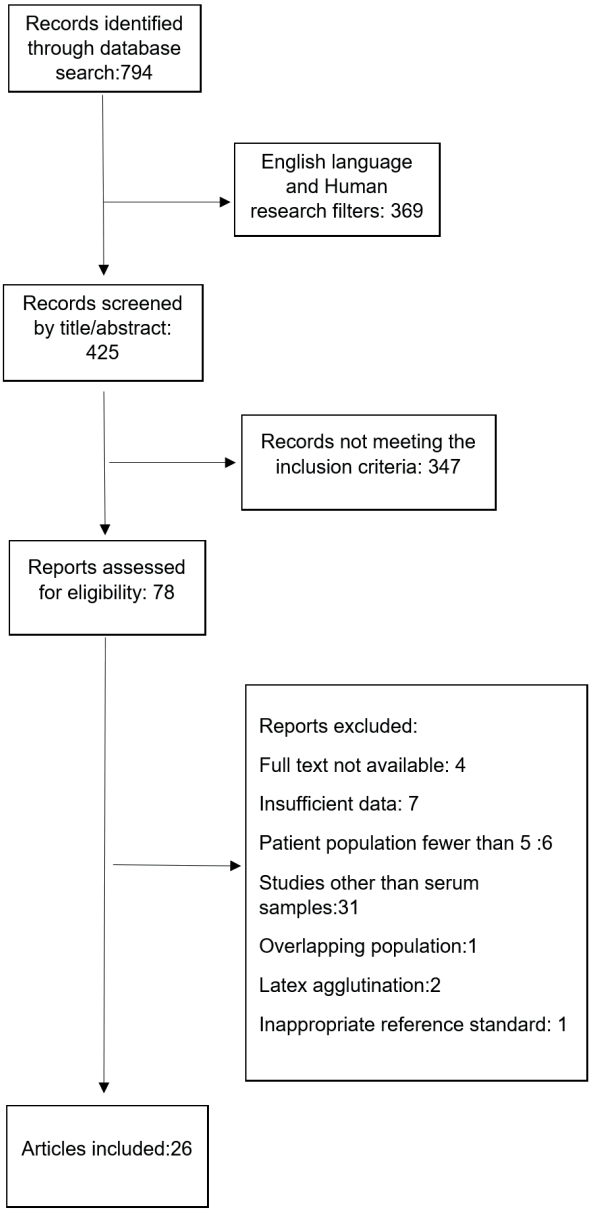


Figure 1. Article Selection Process

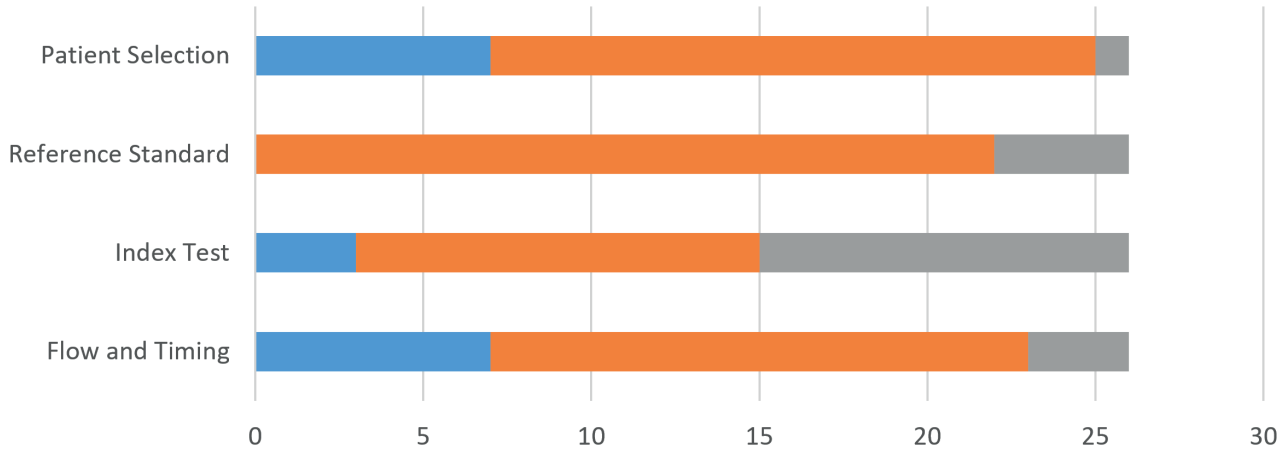
Table 1. General characteristics of the publications

Article	Year	Country	Patient Population	Mean Age	Women (%)	Study Design	Data Collecting Method	Sampling Method
Held et al. [10]	2013	Germany	Adult and Children AHSCT	?	?	Cohort	Prospective	Consecutive
Rogers et al. [11]	2013	Ireland	Adult HM	?	?	Cohort	Prospective	Consecutive
Khanna et al. [12]	2013	India	Adult/child patients with various host factors	32.2	29.6	Cohort	Prospective	Uncertain
White et al. [13]	2013	England	Adult HM	53.3	29.1	Case-contol	Retrospective	Consecutive
Hadrich et al. [14]	2012	Tunusia	Adult and Children HM	37.6	28.6	Cohort	Prospective	Consecutive
Ji et al. [15]	2011	China	Adult and Children AHSCT	30.6	37	Cohort	Retrospective	Consecutive
Tanriover et al. [16]	2010	Türkiye	Adult HM	44	32.8	Cohort	Prospective	Consecutive
Hachem et al. [17]	2009	USA	Adult and Children HM	60	27	Case-control	Prospective	Uncertain
Suarez et al. [18]	2008	France	Adult HM	?	?	Cohort	Prospective	Consecutive
Lai et al. [19]	2007	Taiwan	Adult patients with various host factors	54	50	Cohort	Prospective	Uncertain
Maertens et al. [20]	2007	Belgium	Adult HM	?	40.8	Case-control	Retrospective	Consecutive
Foy et al. [21]	2007	USA	Adult and Children AHSCT	29.5	43	Cohort	Retrospective	Consecutive
Florent et al. [22]	2006	France	Adult HM	?	?	Cohort	Prospective	Consecutive
Weisser et al. [23]	2005	Switzerland	Adult HM	48	38	Cohort	Prospective	Consecutive
Marr et al. [24]	2005	Canada	Adult and Children HM	42.3	46	Randomized and Case-control	Prospective and Retrospective	Uncertain
Pazos et al. [25]	2005	Spain	Adult HM	44	42.5	Cohort	Retrospective	Random
Maertens et al. [26]	2004	Belgium	Adult HM	49	38.7	Cohort	Prospective	Consecutive
Kawazu et al. [27]	2004	Japan	Adult HM	45	30.2	Cohort	Prospective	Consecutive
Rovira et al. [28]	2004	Spain	Adult AHSCT	37	39.1	Cohort	Prospective	Consecutive
Buchheidt et al. [29]	2004	Germany	Adult HM	46	40	Cohort	Prospective	Uncertain
Pinel et al. [30]	2003	France	Adult and Children HM	?	?	Cohort	Prospective	Uncertain
Maertens et al. [31]	2002	Belgium	Adult AHSCT	35.6	33	Cohort	Prospective	Consecutive
Ulusakarya et al. [32]	2000	France	Adult and Children HM	41	52.5	Cohort	Retrospective	Consecutive
Kami et al. [33]	2001	Japan	Adult HM	48.3	23.7	Case-control	Prospective and Retrospective	Uncertain
Sulahian et al. [34]	2001	France	Adult and Children AHSCT	?	?	Cohort	Prospective	Consecutive
Maertens et al. [35]	1999	Belgium	Adult and Children HM	44	37.6	Cohort	Prospective	Consecutive

HM: Hematologic malignancy; AHSCT: Allogeneic hematopoietic stem cell transplantation.

Table 1 outlines the general characteristics of the publications included in the meta-analysis.

Figure 2 displays the methodological quality assessment of the studies included in the meta-analysis. In just two studies, it was stated that the patients had received mold-effective antifungal treatment while the assessment of serum GM. Although certain patients received antifungal therapy in fifteen studies, the specifics of the period of pre-test treatment or the number of patients treated remain unclear. Two studies identified that patients did not receive antifungal treatment prior to the test, whereas seven studies omitted any mention of treatment. Likewise, only four studies indicated that antifungal prophylaxis effective against molds was delivered, although in eleven studies, some patients received mold-effective prophylaxis and others received non-mold-effective prophylaxis. Consequently, the impact of antifungal treatment or mold-active antifungal prophylaxis on serum GM sensitivity or specificity was unable to be assessed.



	Flow and Timing	Index Test	Reference Standard	Patient Selection
High	7	3	0	7
Low	16	12	22	18
Unclear	3	11	4	1

High Low Unclear

Figure 2. Overall quality assessment of included studies using the QUADAS-2 tool

Pooled Sensitivity and Specificity Results For Serum GM ELISA

Comparisons were conducted between the proven and probable IA group and the group including solely proven IA patients. The pooled sensitivity and specificity were calculated for cut-off values of 0.5, 1.0, and 1.5 ODI. Separate subgroup analyses were conducted for articles that deemed a single positive result significant and those that required at least two consecutive positive results for significance.

In the group with proven/probable versus no-IA and a cut-off of 0.5 ODI, the overall pooled sensitivity and specificity were 80% and 78% (AUC: 0.892), respectively. The overall sensitivity and specificity were 74% and 96% (AUC: 0.959) for 1.0 ODI and 70% and 96% (AUC: 0.964) for 1.5 ODI, respectively.

Pooled sensitivity and specificity for the proven IA group were derived from 18 studies utilizing 2x2 tables. In the group with proven versus no-IA, the overall pooled sensitivity and specificity were 94% and 76% (AUC: 0.922) for the cut-off of 0.5 ODI, 86% and 96% (AUC: 0.979) for 1.0 ODI and 70% and 96% (AUC: 0.974) for 1.5 ODI, respectively. The pooled sensitivity and specificity per cut-off value were presented in Table 2. Forest plots of sensitivity and specificity per cut-off value are presented in supplementary document.

DISCUSSION

Invasive aspergillosis is one of the leading causes of morbidity and mortality, particularly in patients with hematological malignancies or those who have undergone hematopoietic stem cell transplantation, thus early detection is critical [36]. To prevent delays in diagnosis, empirical antifungal treatment is employed; however, toxicity and high costs may restrict its utilization. Consequently, the significance of non-invasive tests for facilitating early diagnosis is increasing [37]. GM and PCR in serum and BAL fluid represent the most extensively researched methods in this context. The purpose of this meta-analysis was to establish the diagnostic accuracy of the serum GM (Platelia) test in patients at high risk for IA.

Table 2. Pooled Sensitivity and Specificity Results For Serum Galactomannan

	Cut-off value	Number of studies	AUC	SEN I^2 (%)	Pooled SEN	SPE I^2 (%)	Pooled SPE
Proven and Probable IA vs no IA	**0.5 overall**	22	0.892	84.2	0.80	96.6	0.78
	0.5 single sample	11	0.859	81.1	0.85	96.9	0.68
	0.5 two consecutive samples	11	0.921	85.1	0.72	92.8	0.89
	1.0 overall	10	0.959	82.8	0.74	92.9	0.96
	1.0 single sample	6	0.954	86.2	0.76	88.5	0.91
	1.0 two consecutive samples	4	0.973	80.4	0.71	66.3	0.99
	1.5 overall	12	0.964	78.1	0.70	72.4	0.96
	1.5 single sample	7	0.957	79.9	0.71	77.4	0.95
	1.5 two consecutive samples	5	0.978	80.3	0.70	24.3	0.97
Proven IA vs no IA	**0.5 overall**	11	0.922	0	0.94	97.7	0.76
	0.5 single sample	6	0.936	3.4	0.93	97.8	0.67
	0.5 two consecutive samples	5	0.911	0	0.96	96.5	0.89
	1.0 overall	8	0.979	80.7	0.86	93.1	0.96
	1.0 single sample	4	0.979	85.2	0.82	86.2	0.91
	1.0 two consecutive samples	4	0.990	79.6	0.89	70.4	0.99
	1.5 overall	9	0.974	74.0	0.70	60.6	0.96
	1.5 single sample	5	0.986	73.6	0.78	65.8	0.95
	1.5 two consecutive samples	4	0.974	76.1	0.64	42.7	0.97

IA: Invasive aspergillosis; AUC:Area under curve; SEN: Sensitivity; SPE: Specificity.

Table 3. Comparison of pooled analysis results with the literature

	Cut-off	Dikmeer		Leeflang		Pfeiffer	
		SEN	SPE	SEN	SPE	SEN	SPE
Proven and Probable IA vs no IA	**0.5**	0.80	0.78	0.79	0.82	0.79	0.86
	1.0	0.74	0.96	0.71	0.90	0.65	0.94
	1.5	0.70	0.96	0.62	0.95	0.48	0.95
Proven IA vs no IA	**0.5**	0.94	0.76	-	-	0.27	0.79
	1.0	0.86	0.96	-	-	0.79	0.87
	1.5	0.70	0.96	-	-	0.68	0.92

SEN: Sensitivity; SPE: Specificity

A substantial level of heterogeneity (I^2) was observed with the exception of proven IA cases at 0.5 ODI. Limiting the population of patients to individuals with hematological cancer was anticipated to decrease heterogeneity. Nevertheless, the inclusion of studies involving patients with hematological cancer alongside with other lower-risk host factors for IA such as solid organ transplantation, long-term steroid use, immunosuppressive drug use, and HIV, as well as those at the highest risk, such as allogeneic stem cell transplantation, and inclusion of both adult and pediatric populations, might have contributed to increased heterogeneity. In these studies, it was not possible to isolate data specifically for patients with hematological malignancies and the adult patient cohort, with the exception of one study that examined adult data separately; thus, subgroup analysis could not be conducted.

In patients with proven and probable IA, serum GM revealed a sensitivity of 80% and specificity of 78% at the 0.5 ODI, 74% sensitivity and 96% specificity at the 1.0 ODI, and 70% sensitivity and 96% specificity at the 1.5 ODI. Increasing the cut-off value resulted in a gradual decrease in sensitivity and a corresponding increase in specificity. The specificity remained unchanged between the 1.0 and 1.5 cut-off values. In the proven IA group, serum GM sensitivity and specificity were determined to be 94% and 76% at 0.5 ODI, 86% and 96% at 1.0 ODI, and 70% and 96% at 1.5 ODI, respectively (Table 3). As the cut-off value increased, sensitivity decreased while specificity increased, remaining

constant after the cut-off of 1.0 ODI. These findings suggested that the optimal cut-off value for serum GM in both proven IA and proven/probable IA groups were 1.0 ODI. This outcome is significant as it aligns with the EORTC/MSG recommendations revised in 2020 [8].

A meta-analysis conducted by Pfeiffer in 2006, including 27 studies, determined the sensitivity and specificity for proven and probable IA patients as follows: 79% and 86% at 0.5 ODI, 65% and 94% at 1.0 ODI, and 48% and 95% at 1.5 ODI, respectively [38] (Table 3). In patients with proven IA, the sensitivity and specificity were 27% and 79% at the 0.5 ODI, 79% and 87% at the 1.0 ODI, and 68% and 92% at the 1.5 ODI, respectively. In our study, while the results for the 0.5 ODI were comparable in the proven and probable patient group, it was noted that sensitivity had increased at the 1.0 and 1.5 ODI. The application of the EORTC/MSG 2002 criteria as the diagnostic standard for IA in Pfeiffer's meta-analysis, along with variations in patient characteristics, may have influenced the sensitivity. The 2002 EORTC/MSG diagnostic criteria define the possible IA group as encompassing a wider population than the 2008 EORTC/MSG guideline, which classify the probable IA group as covering a more narrower range of patients. The exclusion of possible IA patients from the meta-analysis and implementation of the 2008 EORTC/MSG criteria in six studies may have affected the outcomes of our study. The inclusion of patients who received solid organ transplantation in the meta-analysis by Pfeiffer might have contributed to the observed lower sensitivity. Our analysis revealed that the sensitivity of proven IA patients was greater than that reported by Pfeiffer. The disparity could be attributed to the significantly smaller number of proven IA patients in the study conducted by Pfeiffer compared to our research.

Another meta-analysis conducted by Leeflang in 2008, including 29 studies, revealed that the sensitivity and specificity for proven and probable IA patients were 79% and 82% for 0.5 ODI, 71% and 90% for 1.0 ODI, and 62% and 95% for 1.5 ODI, respectively [39] (Table 3). Seperate analysis was not conducted for patients with only proven IA group. The results align with the findings of our study.

A meta-analysis by Bukkems in 2023, encompassing studies on adult HM patients, revealed a sensitivity of 92% and a specificity of 84% for 0.5 ODI [40]. They could not provide pooled results for 1.0 and 1.5 ODI values due to the availability of data from only a single study for these cut-off values. The higher sensitivity and specificity for 0.5 ODI in comparison to our findings might be because the 2008 EORTC/MSG criteria were employed as the reference standard in all of the studies that were analyzed in the aforementioned meta-analysis.

This study presents certain limitations. The sole screening of the MEDLINE database, the date of the database scan being outdated, and the selection of English publications may have resulted in the exclusion of relevant research. The main obstacle of IA diagnostic accuracy studies has been the challenge of utilizing the gold standard approach as a reference for all patients. Consequently, in the literature, including our research, the EORTC/MSG criteria have been employed as the reference standard. A notable source of bias worthy of discussion is incorporation bias. The EORTC/MSG criteria implement the value of GM for the diagnosis of probable IA, hence introducing the possibility of incorporation bias. This meta-analysis's strengths include the exclusion of low-risk IA patients and the comparison of patients alone with proven IA patients with no-IA patients.

CONCLUSION

This meta-analysis concluded that the serum GM test could be utilized in diagnosing IA at a 1.0 ODI cut-off value, which is consistent with current EORTC/MSG recommendations. Consequently, it is necessary to conduct additional research, preferably multiple randomized controlled trials.

Author contribution

Study conception and design: AD, MDT, and SA; data collection: AD, and SA; analysis and interpretation of results: AD, and SA; draft manuscript preparation: AD, MDT, and SA. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

Comparison of countries in the WHO European Region according to noncommunicable disease indicators by multi-criteria decision making methods

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ABSTRACT

Objective: The aim of this study is to compare the relative risk prevalence of noncommunicable diseases (NCDs) in the countries of the European Region as defined by WHO (World Health Organization) using WASPAS (Weighted Aggregated Sum Product Assessment) and MULTIMOORA (Multi-Objective Optimization by Ratio Analysis plus the full Multiplicative Form) multi-criteria decision-making (MCDM) methods.

Materials and Methods: The cross-sectional study's target population consisted of 50 countries in the WHO European Region with complete observations. The study utilizes NCDs data that the WHO publicly released. Analysis was performed using the R programming language and Microsoft Excel.

Results: Based on the CRITIC (CRiteria Importance Through Intercorrelated Corrected) weighted WASPAS analysis, it was observed that 24 European countries exhibited Q scores above the average, while 26 countries displayed Q scores below the average. Finland, Cyprus, Switzerland, Spain, Iceland, Iceland, Sweden, Slovenia, Italy, Norway, Latvia, Portugal, Luxembourg, Belgium, France, Greece, the Netherlands, Germany, Malta, Austria, Ireland, Israel, Lithuania, Israel, Lithuania and Estonia have the highest Q scores. Twenty-four countries with above-average Q scores have lower NCD prevalence than twenty-six European countries. In Türkiye, the prevalence of NCDs is above the European average. However, Switzerland, Finland, Iceland, Spain, Cyprus, Slovenia, Sweden, Portugal, Norway, and Luxembourg are among the top 10 European countries with the lowest NCD prevalence in the overall MULTIMOORA ranking. According to the overall ranking, Turkmenistan, Tajikistan, and Kyrgyzstan have the highest NCD prevalence.

Conclusions: The findings from the CRITIC based WASPAS method and the CRITIC based MULTIMOORA indicate that the prevalence of NCDs generally varies according to income level. Higher-income countries note a lower prevalence of NCDs compared to those with lower income levels. Nonetheless, the prevalence of NCDs may differ among various socioeconomic groups.

Keywords: NCDs, CRITIC, MCDM, WASPAS, MULTIMOORA

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INTRODUCTION

Examining global mortality trends, data indicate a progressive rise in the proportion of deaths related to noncommunicable diseases (NCDs), which reached 73.9% of all fatalities in 2019. In contrast, the proportion of deaths linked to communicable diseases has progressively declined, accounting for just 18.2% of total deaths by 2019. In 2020 and 2021, communicable diseases accounted for 23.0% and 28.1% of all deaths, respectively, marking a reversion to the proportions seen in 2005. As a result, the proportion of deaths caused by non-communicable diseases decreased to 70.0% in 2020 and 65.3% in 2021 [1]. Nevertheless, the advancement in the prevention and management of NCDs and their primary risk factors has been inadequate and inconsistent. Only a small number of countries are making sufficient progress towards achieving Sustainable Development Goal (SDG) goal 3.4, which requires a one third reduction in premature death from NCDs by 2030 [2].

The four main risk factors for NCDs with economic transition, fast urbanization, and 21st-century lifestyles are tobacco use, poor diet, insufficient physical exercise, and problematic alcohol consumption. Like socioeconomic determinants, these risk variables have a greater impact on low- and middle-income countries and poorer persons in all countries. In these communities, poverty exposes people to behavioural risk factors for NCDs, which may then promote the downward circle that drives families to poverty. Unless the NCD epidemic is actively challenged in the most highly impacted countries and communities, NCDs will continue to worsen and the global goal of decreasing poverty will be weakened [3].

By comparing the countries in the WHO European Region according to NCD indicators with WASPAS (Weighted Aggregated Sum Product Assessment) and MULTIMOORA (Multi-Objective Optimization by Ratio Analysis plus the full Multiplicative Form) multi-criteria decision-making (MCDM) methods, this study aims to reveal the relative risk prevalence of the countries in the region in terms of NCDs. The study employs the CRITIC (CRiteria Importance Through Intercriteria Correlation) method to determine the weights of decision criteria prior to applying the MULTIMOORA and WASPAS MCDM techniques.

MATERIALS AND METHODS

The study's population, which is cross-sectional, includes 53 European Region countries identified by WHO. However, the decision criteria used for evaluating the countries include complete observations in 50 of these countries. These countries represent the study's target population. The study utilizes NCDs data that the WHO publicly released. This dataset is accessible on the "Noncommunicable Diseases Data Portal" web page at https://ncdportal.org/ [4]. Analyses were performed using the R programming language [5] and Microsoft Excel [6].

The study identified NCD indicators as decision criteria and identified countries defined by the WHO in the European Region as decision alternatives. The CRITIC method, one of the objective weighting methods. It was compared the countries in the European Region using the WASPAS and MULTIMOORA methods from MCDM, which revealed the prevalence of NCDs in the region. Based on the study's scope, Table 1 shows the NCD decision criteria and their direction.

This study used the CRITIC method, a well-known objective weighting method, to calculate the weights of the decision criteria. An R application algorithm that had been published before and was then changed for this study's needs was used. The following R code block presents the application algorithm for the CRITIC method [7]. This critic() function can be directly executed in the R console after being pasted into the R environment. The execution of the critic() function yields results corresponding to each step of the CRITIC method's implementation.

Table 1. Decision Criteria and Direction of Decision Criteria

Category	Decision Criteria	Code	Direction of Criteria	Year
Cancer	Cancer age-standardized death rate	c1	Minimum	2019
	Percentage of cancer deaths occurring under 70 years	c2	Minimum	2019
Chronic respiratory diseases (CRDs)	CRD age-standardized death rate	c3	Minimum	2019
	Percentage of CRD deaths occurring under 70 years	c4	Minimum	2019
	Percentage of asthma deaths occurring under 70 years	c5	Minimum	2019
	Percentage of asthma deaths occurring under 30 years	c6	Minimum	2019
	Exceedance of WHO PM guidelines (by a multiple of)	c7	Minimum	2019
Cardiovascular diseases (CVDs)	CVD age-standardized death rate	c9	Minimum	2019
	Percentage of CVD deaths occurring under 70 years	c10	Minimum	2019
	Hypertension, adults aged 30–79	c11	Minimum	2019
	Diagnosed hypertension, adults aged 30–79 with hypertension	c12	Minimum	2019
	Treated hypertension, adults aged 30–79 with hypertension	c13	Maximum	2019
	Controlled hypertension, adults aged 30–79 with hypertension	c14	Maximum	2019
Diabetes	Diabetes age-standardized death rate	c15	Minimum	2019
	Percentage of diabetes deaths occurring under 70 years	c16	Minimum	2019
	Raised fasting blood glucose, adults aged 18+	c17	Minimum	2014

```
critic <- function(dm = NULL, dc = NULL, nd = NULL) {
  # Step 1: Check Input Variables and Assign Values
  dm2 <- dm
  dc1 <- ifelse(dc == "max", 1, 0) # Assign 1 if the criteria should be maximized, 0 otherwise

  # Step 2: Normalization Process
  for (r in 1:nrow(dm))
    for (c in 1:ncol(dm))
      if (dc1[c]) { # If the criteria should be maximized
        dm2[r,c] <- (dm[r,c] - min(dm[,c])) / (max(dm[,c]) - min(dm[,c])) # Normalize to [0, 1]
      } else { # If the criteria should be minimized
        dm2[r,c] <- (max(dm[,c]) - dm[r,c]) / (max(dm[,c]) - min(dm[,c])) # Normalize to [0, 1]
      }
  ndm <- dm2 # Assign the normalized decision matrix

  # Step 3: Creating the Correlation Matrix
  if (nd == TRUE) { # Use Pearson correlation
    rcm <- cor(ndm)
    rownames(rcm) <- NULL # Remove row names for cleaner output
  } else if (nd == FALSE) { # Use Spearman's rank correlation
    rcm <- cor(ndm, method = "spearman")
    rownames(rcm) <- NULL # Remove row names for cleaner output
  }

  # Step 4: Calculating Information Amount (Cj) and Weight (wj) Values
  rcm1 <- 1 - rcm # Calculate the difference between 1 and the correlation matrix
  rownames(rcm1) <- NULL # Remove row names for cleaner output
  qj <- apply(ndm, 2, sd) # Calculate the standard deviation of each column in the normalized decision matrix
  cj <- qj * apply(rcm1, 2, sum) # Calculate the information amount (Cj)
  wj <- cj / sum(cj) # Calculate the weights (wj)

  # Step 5: Return Results as a List
  return(list(
    dm = as.matrix(dm), # Original decision matrix
    ndm = as.matrix(ndm), # Normalized decision matrix
    rcm = as.matrix(rcm), # Correlation matrix
    cj = round(cj, 4),   # Information amount (Cj), rounded to 4 decimal places
    wj = round(wj, 4)    # Weights (wj), rounded to 4 decimal places
  ))
}
```

In the R programming language, the critic() function, developed to implement the CRITIC method, accepts the following arguments as input [7]:

- dm: Represents the decision matrix, where alternatives are arranged in rows and criteria in columns. This matrix contains the performance values of the alternatives being evaluated in the decision-making process.

- dc: A vector used to specify the direction of the criteria. This vector contains numerical values (1: maximization, 0: minimization) indicating whether each criterion should be maximized or minimized.
- nd: A logical value that determines the method used to calculate the correlation between criteria. A value of TRUE indicates the use of the Pearson correlation coefficient, while a value of FALSE indicates the use of Spearman's rank correlation coefficient.

The "MCDM" package [8], which can be found in the R environment, was used to analyze countries in the WHO European Region based on NCD indicators using the WASPAS and MULTIMOORA methods, which are well-known MCDM approaches. The "ggpubr" package [9], which is also available in the R environment, was used to make the graph showing the relationship between the CRITIC based WASPAS and CRITIC based MULTIMOORA methods.

It was used the energy test [10], a multivariate normality test, to determine whether the decision criteria exhibit a multivariate normal distribution. In the R programming language, "energy" package was utilized for this [11]. In the third step of the CRITIC weighting method, an energy test was performed to determine whether the decision criteria show a multivariate normal distribution. This process led to the selection of the CRITIC approach's correlation test method. If the decision criteria demonstrate a normal distribution, it will be the Pearson correlation test, a parametric correlation test; if not, it will be Spearman's rank correlation test, a nonparametric correlation test. In the multivariate normality test, the following are the null (H_0) and alternative (H_A) hypotheses:

- H_0: The decision criteria display a multivariate normal distribution.
- H_A: The decision criteria do not display a multivariate normal distribution.

Spearman's rank correlation test, one of nonparametric statistical tests, is commonly used to determine whether two rankings are statistically distinct from one another [12]. Spearman's rank correlation test was performed to assess the monotonic relationship between the score rankings generated from WASPAS and MULTIMOORA techniques, which is based on CRITIC weighting method. The nonparametric Spearman's rank correlation test, which is used to determine whether two rankings are statistically different, is also commonly used to compare MCDM rankings [13-16]. The null hypothesis (H_0) and alternative hypothesis (H_A) established in correlation tests are as follows:

- H_0: There is no monotonic relationship between the rankings of CRITIC based WASPAS method and CRITIC based MULTIMOORA method.
- H_A: There is a monotonic relationship between the rankings of CRITIC based WASPAS method and CRITIC based MULTIMOORA method.

The decision matrix used for weighting the decision criteria and comparing the countries of the European Region (N=50) according to NCD indicators is presented in Table 2.

CRITIC weighting method

One of the objective weighing approaches [17-20], the CRITIC (CRiteria Importance Through Intercorrelated Corrected) method, has the following application steps [17]:

Step 1. Making decision matrix: A decision matrix (X_{ij}) in equation (1), with decision alternatives in rows and decision criteria in columns, is created. In the decision matrix, m is the number of decision alternatives, and n is the number of decision criteria.

$$X_{ij} = \begin{bmatrix} x_{11} & x_{12} & \dots & x_{1n} \\ x_{21} & x_{22} & \dots & x_{2n} \\ \dots & \dots & \dots & \dots \\ x_{m1} & x_{m2} & \dots & x_{mn} \end{bmatrix} \text{ where } i = (1, 2, \dots, m) \text{ and } j = (1, \dots, n) \quad (1)$$

Step 2. Normalizing decision matrix: Direction of decision criteria, that is, benefit and cost criteria, is taken into account while normalizing decision matrix. In this instance, benefit criteria are determined by Equation (2), and cost criteria are determined by Equation (3).

$$r_{ij} = \frac{x_{ij} - x_j^{min}}{x_j^{max} - x_j^{min}} \quad (2)$$

$$r_{ij} = \frac{x_j^{max} - x_{ij}}{x_j^{max} - x_j^{min}} \quad (3)$$

Table 2. Decision Matrix

Country	c1	c2	c3	c4	c5	c6	c7	…	c15	c16
Albania	100.1	45.6	23.1	9.5	11.6	0.3	3.2	…	21.0	**7.4**
Armenia	126.9	54.7	21.1	23.7	34.8	0.2	6.8	…	46.7	**11.5**
Austria	108.3	33.1	19.8	16.7	27.9	3.0	2.3	…	12.7	**4.3**
Azerbaijan	84.0	72.8	28.1	40.7	47.1	0.8	4.8	…	66.9	12.3
Belarus	119.0	62.4	13.2	32.6	43.2	0.6	3.0	…	45.6	7.8
Belgium	114.1	34.4	27.4	16.8	27.0	1.0	2.3	…	18.4	4.6
Bosnia and Herzegovina	136.0	53.0	18.7	24.0	20.6	0.4	5.4	…	28.8	7.4
Bulgaria	120.1	49.5	37.7	28.1	34.2	4.4	3.4	…	31.1	7.6
Croatia	147.9	40.5	19.2	15.2	14.4	0.4	7.3	…	13.8	7.3
Cyprus	81.8	37.0	27.4	8.9	10.8	0.0	2.3	…	13.6	7.0
Czechia	128.8	38.5	20.6	26.2	24.5	1.3	2.9	…	17.0	7.5
Denmark	125.5	29.6	35.0	15.9	20.3	1.4	1.9	…	19.9	4.3
Estonia	130.5	34.8	9.4	22.8	20.0	7.3	1.2	…	24.4	7.1
Finland	95.0	30.7	11.9	17.9	10.4	0.1	1.1	…	29.6	5.3
France	123.7	35.7	16.9	13.1	25.5	2.1	2.1	…	16.5	5.9
Georgia	98.9	58.2	19.8	31.8	37.1	3.8	3.8	…	48.7	12.6
Germany	114.2	36.0	22.7	21.4	34.0	1.3	2.1	…	19.0	5.0
Greece	118.4	32.1	23.5	7.5	18.8	2.2	3.0	…	20.6	6.6
Hungary	162.4	48.9	33.3	36.7	35.0	1.7	2.8	…	27.5	7.7
Iceland	104.7	32.6	20.9	11.9	36.7	0.3	1.1	…	16.5	5.9
Ireland	114.1	36.8	32.0	12.6	23.9	3.8	1.6	…	13.4	6.2
Israel	101.4	36.5	21.0	16.5	20.4	1.3	3.9	…	20.1	6.4
Italy	105.7	28.3	19.3	7.7	18.1	1.6	2.7	…	12.1	5.8
Kazakhstan	117.0	71.1	51.0	35.9	38.6	0.7	4.9	…	50.4	11.8
Kyrgyzstan	96.0	75.3	31.8	37.5	62.9	4.4	7.1	…	75.0	10.4
Latvia	144.3	40.9	10.0	34.6	28.8	0.0	2.3	…	25.4	7.2
Lithuania	141.5	41.5	9.5	27.5	24.7	0.0	2.0	…	29.5	7.9
Luxembourg	109.0	36.5	23.4	15.9	33.7	0.3	1.8	…	18.4	5.4
Malta	103.8	36.4	19.0	12.4	21.1	1.8	2.5	…	20.8	7.7
Montenegro	123.5	56.9	8.4	21.9	33.7	1.4	3.7	…	38.4	7.1
Netherlands	125.4	34.6	26.0	17.8	17.8	1.8	2.1	…	20.7	4.3
Republic of North Macedonia	149.0	56.0	35.6	27.4	14.8	0.1	4.9	…	30.8	7.2
Norway	105.4	31.4	25.9	15.5	12.8	0.9	1.3	…	22.3	5.1
Poland	147.8	45.6	20.1	27.3	33.9	1.0	3.7	…	28.7	7.7
Portugal	116.2	34.9	22.9	8.9	17.8	1.6	1.5	…	12.0	6.8
Moldova	119.5	71.0	14.6	40.4	48.7	0.1	2.4	…	60.9	8.1
Romania	141.1	52.0	23.3	31.8	26.5	1.1	2.6	…	34.4	6.8
Russian Federation	129.2	58.0	14.7	34.9	47.4	1.6	1.7	…	42.4	7.7
…	…	…	…	…	…	…	…	…	…	…
Türkiye	138.2	56.2	32.0	22.9	22.3	1.4	4.6	…	31.1	13.6
Turkmenistan	95.5	80.9	10.3	53.0	68.1	8.2	4.9	…	79.2	12.2
Ukraine	128.0	62.2	11.9	34.3	41.4	2.3	2.6	…	54.3	7.3
United Kingdom	113.2	29.8	28.0	17.3	23.7	3.6	1.9	…	18.6	5.8
Uzbekistan	65.0	83.1	15.8	52.9	61.2	2.2	7.8	…	78.7	10.6

Step 3. Creating correlation matrix: Equation (4) calculates correlation of normalizing decision matrix. Pearson correlation coefficient is calculated if decision criteria shows normal distribution, if not, Spearman's rank correlation coefficient is computed.

$$P_{jk} = \frac{\sum_{i=1}^{m}(r_{ij-\bar{r}_j})^2(r_{ik-\bar{r}_k})^2}{\sqrt{\sum_{i=1}^{m}(r_{ij-r_j})^2 \sum_{i=1}^{m}(r_{ik-r_k})^2}} \quad (4)$$

Step 4. Information quantity calculation: Equation (5) uses C_j to represent information quantity and σ_j to represent standard deviation of decision criteria. Here, the correlation matrix is used to compute amount of information.

$$C_j = \sigma_j \times \sum_{k=1}^{m}(1 - r_{jk}) \quad (5)$$

Step 5. Calculating weights of decision criteria: Equation (6) computes weights of decision criteria (w_j) by dividing information amount (C_j) for each criterion by total information amounts of criteria.

$$w_j = \frac{C_j}{\sum_{k=1}^{m} C_k)} \quad (6)$$

WASPAS MCDM method

Weighted Sum Model (WSM) and Weighted Product Model (WPM) are combined in a unique way in the Weighted Aggregated Sum Product Assessment (WASPAS) method. The application steps in the WASPAS method are as follows [21]:

Step 1. Creation of the decision matrix: In the first step, the mxn-dimensional decision matrix X_{ij} given in equation (7) is created. In the matrix, m is the number of decision alternatives, and n is the number of decision criteria.

$$X_{ij} = \begin{bmatrix} x_{11} & x_{12} & \cdots & x_{1n} \\ x_{21} & x_{22} & \cdots & x_{2n} \\ \cdots & \cdots & \cdots & \cdots \\ x_{m1} & x_{m2} & \cdots & x_{mn} \end{bmatrix} \quad (7)$$

Step 2. Normalizing the decision matrix: In this step, the matrix X_{ij} is normalized to the cost and benefit criteria. It is used Equation (8) to normalize the decision matrix according to the cost criterion and Equation (9) to normalize it according to the benefit criterion.

$$\bar{x}_{ij} = \frac{min_i X_{ij}}{X_{ij}} \quad (8)$$

$$\bar{x}_{ij} = \frac{X_{ij}}{max_i X_{ij}} \quad (9)$$

Step 3. Calculation of the relative weights: First, we calculate the relative weight of the alternatives using the Weighted Sum Model (WSM). We use Equation (10) for this operation.

$$Q_i^{(1)} = \sum_{j=1}^{n} \bar{x}_{ij} w_j \quad (10)$$

Conversely, the Weighted Product Model (WPM) approach determines the relative weights of the alternatives by applying equation (11).

$$Q_i^{(2)} = \prod_{j=1}^{n} (\bar{x}_{ij})^{w_j} \quad (11)$$

The study used the CRITIC method to determine the weights of the decision criteria in this step.

Step 4. Determination of the oveall relative weights: To determine the overall relative weights of the alternatives, it was summed the relative weights obtained according to the WSM and WPM approaches, as shown in equation (12). The best decision alternative will be the one with the highest Q value. When the lambda (λ) value is equal to 0, the WASPAS method becomes the WPM method, and when the lambda (λ) value is equal to 1, the WASPAS method transforms into the WSM method.

$$Q_i = \lambda Q_i^{(1)} + (1-\lambda) Q_i^{(1)} \text{ where } (\lambda = 0, 0.1, \ldots, 1) \quad (12)$$

In this study, the lambda (λ) parameter was set to 0.5.

MULTIMOORA MCDM method

In 2006, Brauers and Zavadskas [22] introduced MOORA, a Multi-Objective Optimization on the basis of a Ratio Analysis, which combined Ratio System and Reference Point Approach. In 2010, Brauers and Zavadskas [23] improved MOORA to MULTIMOORA, which includes the full Multiplicative form and Dominance Theory for a final integrative ranking. Ratio System and Full Multiplicative Form belong to the first group of MCDM approaches, while Reference Point Approach belongs to

the second group. MULTIMOORA uses vector normalization and three subordinate ranking methods: Ratio System, Reference Point Approach, and Full Multiplicative Form. Each method has its advantages but has limitations, so MULTIMOORA uses multiple approaches. This section provides a detailed description of these methods to enhance understanding of the MULTIMOORA method.

The initial step in an MCDM problem construction involves creating a decision matrix and weight vector, such as MULTIMOORA, which is a decision matrix based on the ratings of m decision alternatives. In equation (13), the decision matrix is denoted as the X_{ij} matrix, where m defines the number of decision alternatives and n indicates the number of decision criteria.

$$X_{ij} = \begin{bmatrix} x_{11} & x_{12} & \dots & x_{1n} \\ x_{21} & x_{22} & \dots & x_{2n} \\ \dots & \dots & \dots & \dots \\ x_{m1} & x_{m2} & \dots & x_{mn} \end{bmatrix} \quad (13)$$

The ratings of alternatives should be normalized before being used in a MCDM model due to potential differences in dimensions [24]. MULTIMOORA uses a ratio system where each alternative's response to an objective is compared to a denominator that represents all alternatives related to that objective, chosen as the square root of the sum of squares of each alternative per objective [23]. This operation is called vector normalization [25] and is given in equation (14):

$$x_{ij}^{*} = \frac{x_{ij}}{\sqrt{\sum_{j=1}^{m} x_{ij}^{2}}} \quad (14)$$

Ratio system

Ratio System, the first part of MULTIMOORA, is a fully compensatory model that uses the arithmetic weighted aggregation operator to compensate for small normalized values of an alternative. This means that an alternative with poor performance in some criteria can be replaced by one with moderate performance in all criteria. The utility of the Ratio System is calculated by adding weighted normalized ratings for beneficial criteria and deducting them for cost criteria [26]. To calculate the utility of Ratio System, the weighted normalized ratings are added for benefit criteria and subtracted for cost criteria as in equation (15) [23,24]. w_j shows the weights of the decision criteria in equation (15).

$$y_j^{*} = \sum_{i=1}^{i=g} w_j x_{ij}^{*} - \sum_{i=g+1}^{i=n} w_j x_{ij}^{*} \quad (15)$$

y_j^{*} indicates the normalized evaluation of alternative j regarding all objectives.

i=g + 1,g + 2,…,n as the objectives to be minimized, and i=1,2,…,g as the objectives to be maximized. An ordinal ranking of the y_j reflects the final preference.

Reference point approach

Reference Point Approach is used for the second part of MULTIMOORA. Tchebycheff Min-Max Metric is the foundation of Reference Point Method [23]. The broad idea of Murkowski Metric, which serves as the foundation for various decision analysis techniques in the literature, including goal programming, is where the Tchebycheff Min-Max Metric got its start [18]. Reference Point Approach starts from normalized ratios (x_{ij}^{*}) and is calculated using equation (16) [23,24].

$$\min_{j} \left\{ \max_{i} \left| w_j r_i - w_j x_{ij}^{*} \right| \right\} \quad (16)$$

Full multiplicative form

Full Multiplicative Form is the third part of MULTIMOORA [23]. In equation (17), A_j represents the objectives that need to be maximized. In equation (18), B_j indicates the objectives that need to be minimized. $U_j^{'}$ denotes the overall utility of alternative j with objectives to be minimized and maximized in equation (19). In the equations, w_j shows the weight coefficients of the decision criteria [24].

$$A_j = \prod_{g=1}^{i} (x_{gi})^{w_j} \quad (17)$$

$$B_j = \prod_{k=i+1}^{n} (x_{kj})^{w_j} \quad (18)$$

$$U_j' = \frac{A_j}{B_J} \quad (19)$$

Determining overall ranking

The alternatives are ranked based on their overall significance, maximal distance to the reference point, and utility. Three different ranking lists are formed, representing the RS, RP, and FMF

approaches of the MULTIMOORA method. The final ranking is based on dominance theory, with the best-ranked alternative having the highest number of appearances [23,27].

RESULTS

Table 3 shows descriptive statistics for the WHO European Region's NCD indicators utilized as decision criteria in the study. This descriptive statistical analysis reveals notable patterns in mortality and health conditions. The finding that cancer age-standardized death rates (c1) exhibit substantial variability (Mean=117.78, Sd=21.83) suggests significant regional disparities in access to cancer care, screening programs, and environmental risk factors. The fact that a notable proportion of cancer deaths occur before the age of 70 (c2), (Mean=46.45) underscores the need for targeted prevention efforts aimed at younger populations, potentially focusing on lifestyle modifications and early detection. Cardiovascular disease (CVD) age-standardized death rates (c8) demonstrate even greater variability (Mean=236.18, Sd=155.38), potentially reflecting differences in dietary habits, physical activity levels, and access to specialized cardiac care across regions. This highlights the importance of tailored CVD prevention strategies. The substantial number of adults aged 30-79 experiencing hypertension (c10) (Mean=38.38) suggests a significant public health concern requiring widespread awareness campaigns and accessible screening programs. The challenge in achieving adequate control of hypertension (c13) (Mean=24.6), despite available treatments, suggests potential issues with medication adherence, access to follow-up care, or the need for more intensive lifestyle interventions. Finally, the significant number of diabetes-related deaths occurring before age 70 (c15) (Mean=31.04) emphasizes the importance of early diabetes diagnosis, effective disease management, and interventions targeting modifiable risk factors such as obesity and sedentary behavior.

Alternative hypothesis (H_A) is accepted (E-statistic=2.3452, N=50, R=150, $p<0.000$) since the normalized decision criteria in CRITIC weighing method do not demonstrate multivariate normal distribution by the Energy test. R in this case stands for the bootstrap replication coefficient. Because of this, when building the correlation matrix in the third phase of CRITIC method, Spearman's rank correlation method was applied.

When weighting the decision criteria in the CRITIC weighting method, the countries of the European Region with no missing observations in the decision criteria were taken into consideration. In this case, the data of fifty European Region countries were taken into account in determining the weights of the decision criteria. The weights of

Table 3. Descriptive Statistics of Decision Criteria in the WHO European Region

Description	Code	N	Mean	Sd	Min	Max	Range
Cancer age-standardized death rate	c1	50	117.78	21.83	64.98	162.44	97.46
Percentage of cancer deaths occurring under 70 years	c2	50	46.45	15.66	25.45	83.10	57.65
CRD age-standardized death rate	c3	50	22.40	9.14	8.41	51.04	42.63
Percentage of CRD deaths occurring under 70 years	c4	50	23.79	11.96	7.48	52.98	45.50
Percentage of asthma deaths occurring under 70 years	c5	50	28.73	13.74	9.85	68.11	58.26
Percentage of asthma deaths occurring under 30 years	c6	50	1.57	1.72	0.01	8.16	8.16
Exceedance of WHO PM guidelines (by a multiple of)	c7	53	3.17	1.92	1.09	10.32	9.220
CVD age-standardized death rate	c8	50	236.18	155.38	68.93	618.25	549.32
Percentage of CVD deaths occurring under 70 years	c9	50	21.40	9.88	9.07	52.04	42.97
Hypertension, adults aged 30–79	c10	51	38.38	7.79	21.90	49.20	27.30
Diagnosed hypertension, adults aged 30–79 with hypertension	c11	51	63.07	8.81	40.50	83.60	43.10
Treated hypertension, adults aged 30–79 with hypertension	c12	51	50.75	10.01	25.90	71.30	45.40
Controlled hypertension, adults aged 30–79 with hypertension	c13	51	24.60	10.76	7.40	51.80	44.40
Diabetes age-standardized death rate	c14	50	14.38	10.94	2.71	55.09	52.39
Percentage of diabetes deaths occurring under 70 years	c15	50	31.04	19.00	10.48	79.20	68.73
Raised fasting blood glucose, adults aged 18+	c16	51	7.42	2.30	4.00	13.60	9.60

Table 4. Weights of NCD Decision Criteria

Decision Criteria	Code	w_j	Rank
Diagnosed hypertension, adults aged 30–79 with hypertension	c11	0.090	1
CRD age-standardized death rate	c3	0.083	2
Percentage of asthma deaths occurring under 30 years	c6	0.081	3
Cancer age-standardized death rate	c1	0.078	4
Treated hypertension, adults aged 30–79 with hypertension	c12	0.073	5
Hypertension, adults aged 30–79	c10	0.064	6
Controlled hypertension, adults aged 30–79 with hypertension	c13	0.062	7
Diabetes age-standardized death rate	c14	0.058	8
Percentage of asthma deaths occurring under 70 years	c5	0.056	9
CVD age-standardized death rate	c8	0.055	10
Percentage of diabetes deaths occurring under 70 years	c15	0.055	11
Percentage of CRD deaths occurring under 70 years	c4	0.053	12
Percentage of cancer deaths occurring under 70 years	c2	0.051	13
Raised fasting blood glucose, adults aged 18+	c16	0.050	14
Exceedance of WHO PM guidelines (by a multiple of)	c7	0.048	15
Percentage of CVD deaths occurring under 70 years	c9	0.045	16

NCD decision criteria in CRITIC weighting method are shown in Table 4. The first three decision criteria, c11 (Diagnosed hypertension, adults aged 30-79 with hypertension) (w_j=0.090), c3 (CRD age-standardized death rate) (w_j=0.083), and c6 (Percentage of asthma deaths occurring under 30 years) (w_j=0.081), have the highest weights in the CRITIC method. Conversely, the following three decision criteria have the lowest weight values among the first three: c9 (Percentage of CVD fatalities occurring under 70 years of age) (w_j=0.045), c7 (Exceeding WHO PM limits (by a multiple of)) (w_j=0.048), and c16 (Raised fasting blood glucose, adults aged 18+) (w_j=0.050).

Table 5 shows the comparison results of CRITIC based MULTIMOORA and CRITIC based WASPAS methods for fifty countries in the European Region. The results of the application steps that come before the last step of the WASPAS and MULTIMOORA techniques are not presented due to space restrictions. According to CRITIC based WASPAS method, there are twenty-four countries in the European Region with above-average Q scores (Q=0.410) and twenty-six countries with below-average scores. The twenty-four countries with above-average scores are: Finland (Q=0.573), Cyprus (Q=0.558), Switzerland (Q=0.546), Spain (Q=0.541), Iceland (Q=0.538), Sweden (Q=0.518), Slovenia (Q=0.518), Italy (Q=0.505), Norway (Q=0.501), Latvia (Q=0.5), Portugal (Q=0.497), Luxembourg (Q=0. 484), Belgium (Q=0.479), France (Q=0.475), Greece (Q=0.461), Netherlands (Q=0.456), United Kingdom (Q=0.456), Germany (Q=0.453), Malta (Q=0.452), Austria (Q=0.45), Ireland (Q=0.44), Israel (Q=0.439), Lithuania (Q=0.438), Estonia (Q=0.413). These countries are also the top twenty-four countries with the highest Q score. In other words, the prevalence of NCDs is lower in twenty-four countries with above-average Q scores than in twenty-six countries in the European Region. On the other hand, Türkiye falls below the average Q score of the European Region (Q=0.343). In other words, Türkiye's NCD prevalence is above the average of the European Region.

On the other hand, according to overall MULTIMOORA rankings, Switzerland, Finland, Iceland, Spain, Cyprus, Slovenia, Sweden, Portugal, Norway, and Luxembourg are the top 10 countries in the European Region with the lowest NCD prevalence according to the NCD decision criteria, making them the closest to the optimal solution. The countries furthest from the optimal solution—in other words, the top 3 countries with the highest NCD prevalence according to the overall ranking—are as follows: Turkmenistan, Tajikistan, and Kyrgyzstan.

Table 5. CRITIC Based WASPAS and CRITIC Based MULTIMOORA Scores

	WASPAS		MULTIMOORA			
Alternatives	**Q**	**Rank**	**RS**	**RP**	**Multiplicative Form**	**Overall Rank**
Albania	0.409	25	-0.099	0.024	2095949275947490000000000000	31
Armenia	0.322	42	-0.114	0.014	6366030846267310000000000	41
Austria	0.450	20	-0.074	0.015	196016544241268000000000000000	19
Azerbaijan	0.282	47	-0.142	0.015	177158184076328000000000	46
Belarus	0.360	33	-0.103	0.014	29656042679830500000000000	33
Belgium	0.479	13	-0.062	0.009	10516814232180100000000000000	12
Bosnia and Herzegovina	0.347	36	-0.103	0.016	2173631228158570000000000	34
Bulgaria	0.317	43	-0.121	0.022	3328159621829150000000000	43
Croatia	0.396	28	-0.089	0.011	3663087240194790000000000000	27
Cyprus	0.558	2	-0.059	0.009	269085532845163000000000000000	5
Czechia	0.395	29	-0.080	0.007	24529284269250700000000000	20
Denmark	0.405	26	-0.083	0.014	50279393767225000000000000	25
Estonia	0.413	24	-0.103	0.036	20131678691168400000000000	32
Finland	0.573	1	-0.052	0.008	472695343994268000000000000000	2
France	0.475	14	-0.066	0.010	68277477467106100000000000	15
Georgia	0.293	45	-0.133	0.019	390748949812618000000000	45
Germany	0.453	18	-0.065	0.007	27867717719185100000000000000	13
Greece	0.461	15	-0.068	0.011	34842091849318100000000000000	16
Hungary	0.323	41	-0.108	0.012	8083591276622740000000000	38
Iceland	0.538	5	-0.051	0.008	93362979929490900000000000000	3
Ireland	0.440	21	-0.083	0.019	13978396017475600000000000000	26
Israel	0.439	22	-0.072	0.008	11862117598641700000000000000	18
Italy	0.505	8	-0.060	0.008	18191953517519600000000000000	11
Kazakhstan	0.302	44	-0.127	0.021	1536143825099690000000000	44
Kyrgyzstan	0.271	49	-0.155	0.022	64303191401821400000000	48
Latvia	0.500	10	-0.085	0.011	16322683486894500000000000000	21
Lithuania	0.438	23	-0.083	0.013	39075851160414000000000000000	22
Luxembourg	0.484	12	-0.061	0.007	20555152816878700000000000000	10
Malta	0.452	19	-0.066	0.009	18866447190736800000000000000	14
Montenegro	0.365	30	-0.098	0.009	1970245363534960000000000	30
Netherlands	0.456	16	-0.070	0.010	35529002962043100000000000000	17
Republic of North Macedonia	0.358	34	-0.112	0.015	76729304781018800000000000	40
Norway	0.501	9	-0.060	0.008	283206071242107000000000000000	9
Poland	0.363	32	-0.090	0.008	61492191077437700000000000	29
Portugal	0.497	11	-0.059	0.008	13878479798436900000000000000	8
Moldova	0.340	38	-0.114	0.014	26579723472347400000000000	42
Romania	0.363	31	-0.091	0.007	70828844674352400000000000	28
Russian Federation	0.335	39	-0.108	0.011	99121172055476300000000000	37
Serbia	0.327	40	-0.106	0.011	90624955242803900000000000	36
Slovakia	0.403	27	-0.083	0.008	52470519573145100000000000	24
Slovenia	0.518	7	-0.062	0.010	10999985975443700000000000000	6
Spain	0.541	4	-0.055	0.009	93500506874526700000000000000	4
Sweden	0.518	6	-0.057	0.011	39526824836086000000000000000	7
Switzerland	0.546	3	-0.050	0.007	83697215466423600000000000000	1
Tajikistan	0.269	50	-0.165	0.017	452934372046749000000000	49
Türkiye	0.343	37	-0.104	0.011	12091242896795600000000000	35
Turkmenistan	0.281	48	-0.178	0.040	23181889186478200000000	50
Ukraine	0.349	35	-0.110	0.012	10199740472963800000000000	39
United Kingdom	0.456	17	-0.077	0.018	25504602565688300000000000000	23
Uzbekistan	0.291	46	-0.154	0.015	60004868084669700000000	47

RS: Ratio System Approach, RP: Reference Point Approach.

Table 6. Above Average Countries according to MULTIMOORA Approaches

Ratio System (N=28)		Reference Point (N=30)		Multiplicative Form (N=8)	
Alternatives	**Scores**	**Alternatives**	**Scores**	**Alternatives**	**Multiplicative Form**
Switzerland	-0.0503	Czechia	0.0065	Finland	472695343994268000000000000000
Iceland	-0.0506	Switzerland	0.0067	Cyprus	269085532845163000000000000000
Finland	-0.0520	Germany	0.0069	Slovenia	109999859754437000000000000000
Spain	-0.0549	Luxembourg	0.0074	Spain	935005068745267000000000000000
Sweden	-0.0567	Romania	0.0074	Iceland	933629799294909000000000000000
Cyprus	-0.0586	Poland	0.0076	Switzerland	836972154664236000000000000000
Portugal	-0.0592	Italy	0.0078	Sweden	395268248360860000000000000000
Norway	-0.0598	Portugal	0.0078	Norway	283206071242107000000000000000
Italy	-0.0602	Finland	0.0079		
Luxembourg	-0.0615	Israel	0.0081		
Belgium	-0.0616	Iceland	0.0084		
Slovenia	-0.0619	Slovakia	0.0085		
Germany	-0.0649	Norway	0.0085		
Malta	-0.0655	Malta	0.0087		
France	-0.0660	Spain	0.0090		
Greece	-0.0683	Belgium	0.0092		
Netherlands	-0.0701	Cyprus	0.0092		
Israel	-0.0725	Montenegro	0.0095		
Austria	-0.0737	Netherlands	0.0095		
United Kingdom	-0.0772	Slovenia	0.0096		
Czechia	-0.0800	France	0.0103		
Slovakia	-0.0829	Serbia	0.0105		
Lithuania	-0.0831	Sweden	0.0105		
Denmark	-0.0831	Greece	0.0110		
Ireland	-0.0832	Croatia	0.0111		
Latvia	-0.0845	Russian Federation	0.0112		
Croatia	-0.0886	Latvia	0.0114		
Poland	-0.0895	Türkiye	0.0114		
		Hungary	0.0121		
		Ukraine	0.0125		

In the CRITIC based MULTIMOORA method, the countries above the average score of the European Region according to the approaches in the MULTIMOORA method are given in Table 6. According to the Ratio System approach, one of the MULTIMOORA approaches, the number of countries above the European Region average (Average Score=-0.091) is twenty-eight. These countries also have the lowest NCD prevalence. According to the Reference Point approach, which is one of the MULTIMOORA approaches, 30 countries are above the European Region average (Average Score=0.013). According to this approach, Türkiye is the country with above average. Finally, according to Multiplicative Form, one of the MULTIMOORA approaches, the number of countries above the European Region average (Average Sco re=260322513614281000000000000000) is eight, and these countries are as follows: Finland, Cyprus, Slovenia, Spain, Iceland, Switzerland, Sweden, and Norway.

Based on the results of the analysis, it was found that there is a strong positive relationship between the rankings obtained from the CRITIC based WASPAS and CRITIC based MULTIMOORA ($r_s(48)=0.970$,

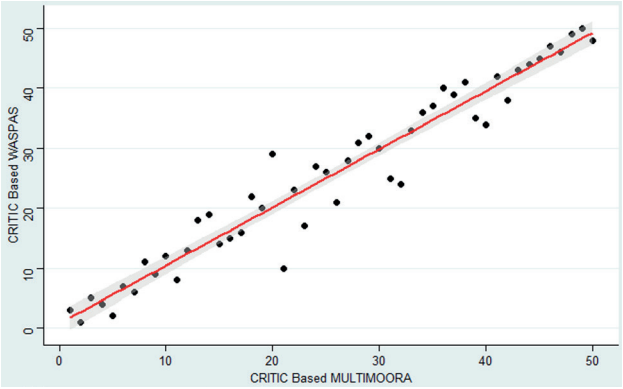

Figure 1. Correlation between CRITIC Based WASPAS and CRITIC Based MULTIMOORA Methods

p<0.05, N=50). In CRITIC based MULTIMOORA, overall rankings were used in the correlation test. As a result, the alternative hypothesis (HA) was accepted. The correlation between the two weighting methods is given in Figure 1.

DISCUSSION

NCDs remain the primary cause of mortality globally, with a growing need for treatment and escalating healthcare expenses [28]. Government officials are required to fulfill their commitment to decrease premature death from non-non-communicable diseases by one third by 2030 by means of preventive measures and medical care, as well as to advance mental health. Failure to make substantial expenditures would result in an annual mortality rate of 15 million individuals from non-communicable diseases (NCDs), with around 800,000 succumbing to suicide. By adopting the WHO "best buys" for non-communicable diseases (NCDs), it is possible to save 17 million strokes and heart attacks by 2030, stimulate $350 billion in economic development, and achieve a minimum return of $7 by 2030 [29]. This study assessed the relative risk prevalence of NCDs in fifty countries within the WHO European Region. Comparative analysis was conducted by examining NCD indicators using the WASPAS and MULTIMOORA MCDM approaches.

The CRITIC weighting method assigns the highest weights to the first three decision criteria: "Diagnosed hypertension, adults aged 30-79 with hypertension", "CRD age-standardized death rate", and "Percentage of asthma deaths occurring under 30 years". Conversely, the three decision criteria with the lowest weights among the first three are "Percentage of cardiovascular disease (CVD) deaths occurring under 70 years of age", "Exceeding WHO guidelines for particulate matter (PM) by a multiple of)", and "Raised fasting blood glucose, adults aged 18+".

The study has demonstrated a strong positive correlation between the rankings derived from the CRITIC based WASPAS and the CRITIC based MULTIMOORA methods.

The CRITIC based WASPAS method reveals that 24 European countries have above-average Q scores, while 26 have below-average scores. The top 24 countries with the highest Q score are Finland, Cyprus, Switzerland, Spain, Iceland, Sweden, Slovenia, Italy, Norway, Latvia, Portugal, Luxembourg, Belgium, France, Greece, Netherlands, United Kingdom, Germany, Malta, Austria, Ireland, Israel, Lithuania, and Estonia. In summary, NCDs are less prevalent in twenty-four countries that have above-average Q scores compared to twenty-six countries in the European Region. In general, the common characteristic of these 24 countries is that they have higher income levels than other countries [24]. On the other hand, the prevalence of NCDs in Türkiye surpasses the average prevalence in the European Region. Türkiye falls into the middle-income group [30], and income distribution among social groups is unequal [31].

Nevertheless, according to the CRITIC based MULTIMOORA overall rankings, Switzerland, Finland, Iceland, Spain, Cyprus, Slovenia, Sweden, Portugal, Norway, and Luxembourg rank among the top 10 countries in the European Region with the lowest prevalence of NCDs. On the other hand, according to the overall ranking, the top three countries with the highest prevalence of NCDs are Turkmenistan, Tajikistan and Kyrgyzstan. According to World Bank data for 2022, Turkmenistan is in the upper middle-income group, while Tajikistan and Kyrgyzstan are in the lower middle-income group [30]. It is known that countries' income levels are linked to the prevalence of NCDs. We also know that the distribution of NCDs is unequal in low- and middle-income countries and among socioeconomic groups [31]. Robust evidence from 283 studies indicates a positive correlation between low income, low socioeconomic status, and NCDs [32].

In summary, according to the CRITIC based WASPAS method and the CRITIC based MULTIMOORA results, NCD prevalence generally varies by income level. It is observed that the prevalence of NCDs is lower in countries with higher income levels than in countries with lower income levels. However, NCD prevalence may also vary across socioeconomic groups.

Author contribution

Study conception and design: TB; data collection: TB; analysis and interpretation of results: TB; draft manuscript preparation: TB. The author reviewed the results and approved the final version of the manuscript.

Ethical approval

Since the data used in the study are publicly published by WHO, ethics committee approval is not required.

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acta medica

ORIGINAL ARTICLE

Fibrosis- 4 index and survival in early breast cancer patients

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ABSTRACT

Background: The objective of this study is to assess the correlation between survival outcomes and fibrosis-4 (FIB-4) index in patients with non-metastatic breast cancer treated with anthracyclines

Methods: This study was conducted on individuals with non-metastatic breast cancer who were treated with at least one dose of anthracycline from 2018 to 2023. The FIB-4 index was calculated based on the following parameters: age, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) serum levels, and platelet count for each patient before anthracycline treatment.

Results: A total of 208 patients were included in the study. Patients below 35 years of age (n=28) and those above 65 years of age (n=11) were excluded from the study as the FIB-4 index is less reliable in these age groups. Patients were then divided into two subgroups, low and high, according to the pre-defined cut-off value of 1.45, which is obtained from the primary reference. In univariate analysis, hemoglobin (p=0.03), FIB-4 index (p=0.02), and diagnosis at stage (p=0.01) were statistically related to overall survival (OS). In multivariate analysis, patients with higher FIB-4 index (HR: 4.36, 95% CI 1.38-13.78 p=0.012), anemia (HR: 3.32, 95% CI 1.32-8.34, p=0.011), and stage 3 (HR: 4.53, 95% CI 1.22-16.76, p=0.024) had decreased OS. An additional aim was to evaluate the association between anthracycline-induced cardiotoxicity and the FIB-4 index. Our study showed no relationship (p=0.738).

Conclusions: The FIB-4 index, a marker easily obtained through routine biochemistry testing at low cost, could serve as an independent predictor of OS patients with non-metastatic breast cancer treated with anthracyclines. Routine lab tests performed for cancer patients may help clinicians identify high-risk patients in whom closer follow-up or protective measures should be considered.

Keywords: breast cancer, fibrosis-4 index, anthracyclines, survival

INTRODUCTION

Breast cancer is the most common tumour in women worldwide, accounting for approximately 40% of all cancer cases. Nearly 2.5 million people are diagnosed with breast cancer each year [1]. The incidence of breast cancer is increasing, especially in high-income countries. This may be due to a sedentary lifestyle, such as less physical activity, and an unhealthy diet [2]. Fortunately, 70-80% of patients are diagnosed at a non-metastatic stage, which is likely to be curable [3,4].

Breast cancer is a heterogeneous disease, with both diagnostic and prognostic features exhibiting significant variability [5]. For instance, key molecular features such as HER2 status [6], hormone receptor status [7], and BRCA mutations all impact treatment decisions. It is important to note that patients who present with similar molecular features may display different clinical outcomes. As a consequence, treatment and prognosis are not exclusively guided by molecular features, but also by other clinical

factors, which is why it is important to define new prognostic factors [8].

The fibrosis-4 (FIB-4) index is a non-invasive tool to assess liver fibrosis [9]. Noninvasive tests like simple laboratory tests are becoming important to predict the histology of the liver and the prognosis of many diseases [10]. For instance, an increased FIB4 index is associated with mortality in cardiovascular diseases and rheumatoid arthritis [11,12]. Furthermore, the FIB-4 index has been demonstrated to be related to hepatocellular carcinoma and gastric cancer [13,14]. However, the prognostic impact of using the FIB4 index for patients with breast cancer is not well understood yet. Therefore, we hypothesized that the FIB4 index is associated with an adverse prognosis in patients with non-metastatic breast cancer treated with anthracycline.

This study aims to define the correlation between survival outcomes and the FIB-4 index in patients with non-metastatic breast cancer treated with anthracycline.

MATERIALS AND METHODS

Patients

We conducted a retrospective study of patients with non-metastatic breast cancer who received at least 1 cycle of anthracycline-based chemotherapy in Hacettepe University Hospital between 2018 and 2023. Patients below 35 (n=28) and above 65 (n=11) were excluded as these groups' FIB-4 index is less reliable. Patients who did not have at least one of the FIB4 index components (aspartate aminotransferase (AST), alanine aminotransferase (ALT), and platelet count) were excluded from this study (n=14).

Definition of FIB-4 index

The FIB-4 index was calculated based on the following parameters: age, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) serum levels, and platelet count. The FIB-4 index was calculated for each patient before anthracycline treatment using the following formula:

$$\text{FIB4 Index} = \frac{\text{Age(years)} \times [\text{Aspartate Aminotransferase(AST)(IU}/L)]}{\text{Platelet Count}\left(10^9/L\right) \times \sqrt{\text{Alanine Aminotransferase(ALT)(IU}/L)}}$$

The pre-defined cut-off value was used to categorize patients as having either a high or low FIB-4 index [9].

Definition of anthracycline cardiotoxicity

Cardiotoxicity was defined as the presence of systolic dysfunction according to LVEF criteria of the European Society of Cardiology (ESC) or diastolic dysfunction according to the criteria of the American Society of Echocardiography (ASE) [15,16].

Data collection

We gathered comprehensive data on each patient from their medical records, including information on their age, gender, medical history, other health conditions they had, the specific treatment they were receiving, and results from their baseline laboratory tests, such as complete blood count, albumin, creatinine, and liver function tests. This information was collected before any chemotherapy treatment was administered.

Statistical analysis

Statistical analyses were performed using SPSS V.24. All data are expressed as either median (IQR) for continuous variables or the number of patients (percentage) for categorical variables. Groups were compared using the chi-square test for categorical variables and Mann–Whitney U test or the Kruskal-Wallis test for quantitative variables. The overall survival (OS) time was defined as the period from treatment initiation to the last follow-up and/or death. Survival analyses were conducted using Kaplan–Meier analyses, and comparisons of survival times between prognostic subgroups were done using the log-rank test. The significant predictors of OS were evaluated by multivariate analysis using Cox's proportional hazards model. Variables showing associations at a significance level of α=0.25 in univariable analysis were selected for inclusion in the multivariable model. Covariates were also selected according to the results of previous research. A type-1 error level of <5% was used to infer statistical significance.

RESULTS

Baseline characteristics

This study included 208 patients. The median follow-up was 46 (IQR=25-61) months. The median age was 49 (IQR=43- 55), and 206 (99%) patients were women. Demographic and clinicopathologic characteristics of the study population are presented in Table 1.

Table 1. Baseline Characteristics of breast cancer patients treated with anthracycline therapy

	All patients (n=208)	Low FIB-4 index (n=189)	High FIB4- index (n=19)
Median Age (IQR)	49 (43-55)	48 (43-54)	57 (54-60)
Sex			
Male	2 (1.0%)	2 (1.1%)	0
Female	206 (99.0%)	187 (98.9%)	19 (100%)
Anthracycline dosage (mg/m²) (IQR)	235 (230-240)	235 (229-239)	235 (228-240)
Histologic Subtype			
Ductal Carcinoma	168 (80.8%)	152 (80.4 %)	16 (84.2%)
Lobular Carcinoma	18 (8.7%)	16 (8.5 %)	2 (10.5%)
Mix	20 (9.6%)	19 (10.1 %)	1 (5.3%)
Missing Data	2 (1.0%)	2 (1.1%)	0
TNM Stage			
Stage 2	88 (42.3 %)	83 (43.9 %)	5 (26.3 %)
Stage 3	118 (56.7 %)	104 (55.0 %)	14 (73.7 %)
Missing Data	2 (1.0 %)	2 (1.1 %)	0
Hormone Receptor Status			
Positive	151 (72.6%)	140 (74.%1)	11 (57.9%)
Negative	55 (26.4%)	47 (24.9%)	8 (42.1%)
Missing Data	2 (1.0%)	2 (1.1%)	0
HER2 status			
Positive	96 (46.2%)	89 (47.1%)	7 (36.8%)
Negative	110 (52.9%)	98 (51.8%)	12 (63.2%)
Missing Data	2 (1.0%)	2 (1.1%)	0
Hemoglobin (g/dl)			
<12 g/dL	34 (16.3%)	33 (17.5%)	1 (5.3%)
≥ 12 g/ dL	174 (83.7%)	156 (82.5%)	18 (94.7%)
Cardiovascular disease			
Yes	43 (20.7%)	38 (20.1%)	5 (26.3%)
No	165 (79.3%)	151 (79.9%)	14 (73.7%)
Cardiotoxicity			
Yes	181 (87.0%)	164 (86.8%)	17 (89.5%)
No	27 (13.0%)	25 (13.2%)	2 (10.5%)
Body Mass Index (kg/m²)			
< 25	58 (27.9%)	55 (29.1%)	3 (15.8%)
≥ 25	150 (72.1%)	134 (70.9%)	16 (84.2%)
Median Platelet Count (10³/L) (IQR)	283 (241-329)	285 (242-329)	262 (235-337)
Median ALT (IU/L) (IQR)	17 (13-25)	17 (13-25)	17 (11-24)
Median AST (IU/L) (IQR	19 (17-24)	20 (17-24)	19 (17-21)

FIB-4: FIBrosis-4, IQR: Interquartile range, HER2: Human epidermal growth factor receptor 2, ALT: Alanine transaminase, AST: Aspartate aminotransferase

Association of FIB-4 index with patient outcomes

In univariate analysis, hemoglobin (p=0.03), FIB-4 index (p=0.02), and TNM stage (p=0.01) were statistically related to overall survival. In multivariate analysis, patients with higher FIB-4 index (p=0.012 HR: 4.36 95% CI 1.38-13.78), anemia (p=0.011 HR: 3.32 95% CI 1.32-8.34), and stage 3 (p=0.024 HR:4.53 95% CI 1.22-16.76) had decreased overall survival (OS) (Table 2 and Figure 1)

FIB-4 index and anthracycline-induced cardiotoxicity

We discovered no connection between the FIB-4 index and cardiotoxicity associated with anthracycline. (p=0.738)

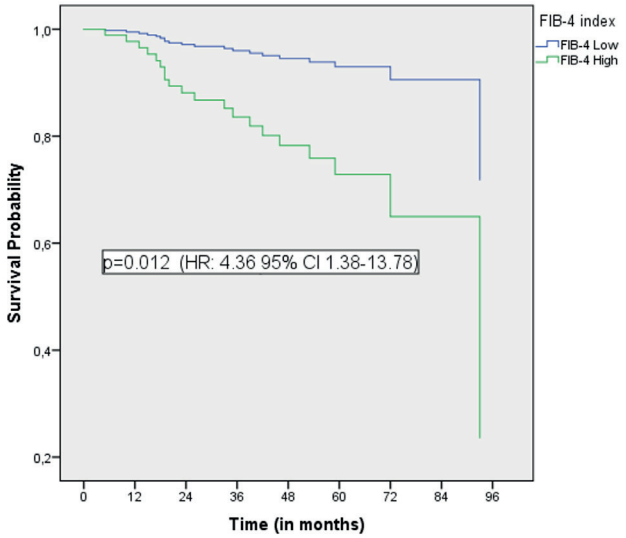

Figure 1. Survival outcomes in patients with non-metastatic breast cancer treated with anthracycline

Table 2. Univariate and multivariate Cox regression analysis of covariates associated with overall survival

	Overall Survival	
	Univariate analysis p-value	**Multivariate analysis-p (HR, 95 %CI)**
Median Age (IQR)	0.21	0.29
Anthracycline dosage (mg/m²) (IQR)	0.18	0.92
Hemoglobin (g/dl) <12 g/dL ≥ 12 g/ dL	*0.03*	*0.011* *(HR: 3.32 95%CI 1.32-8.34)*
Cardiovascular disease Yes No	0.58	(-)
Body Mass Index (kg/m²) < 25 ≥ 25	0.22	0.43
FIB-4 index Low (< 1.45) High (≥ 1.45)	*0.02*	*0.012* *(HR: 4.36 95% CI 1.38-13.78*
Cardiotoxicity Yes No	0.70	*(-)*
TNM Stage at diagnosis Stage 2 Stage 3	*0.010*	*0.024* *(HR: 4.53 95% CI 1.22-16.76)*

DISCUSSION

This study demonstrates a significant association between the FIB-4 index and survival in non-metastatic breast cancer patients treated with anthracycline. This study showed that the FIB-4 index and hemoglobin level were independent indicators of better OS.

We found no relationship between the FIB-4 index and anthracycline-related cardiotoxicity. (p=0.738). Similarly, no relationship was detected between anthracycline dose and the overall survival of patients (p=0.92). This might be due to the characteristics of the study population, which did not include other cancers (such as lymphoma and sarcoma). Furthermore, the anthracycline dose we used (Median dose: 235mg/m^2) might be lower to detect cardiovascular morbidity. However, further prospective trials are needed to demonstrate the relationship between FIB-4 index, survival outcomes, and anthracycline-induced cardiotoxicity.

It is known that the FIB-4 index can be used as an inflammatory marker and assist in defining patients with higher risk. For example, it was shown that the FIB-4 index can predict mortality in patients with rheumatologic disease [12,17]. Although the inflammatory process is important in cancer patients, the relationship between FIB-4 score and breast cancer has not been widely assessed. On the other hand, Xu et al. [13] showed that patients with gastric cancer and higher FIB-4 index had shorter OS (HR: 4.65; 95% CI 1.07-4.29; P = 0.031). Association was identified between elevated levels of the FIB-4 index and colorectal cancer patients in terms of decreased recurrence-free survival (RFS) and overall survival (OS) [18]. Similarly, an elevated FIB-4 score was related to the development of hepatocellular cancer in patients with pre-existing liver disease [19-21].

The FIB-4 index may be a helpful method for identifying high-risk patients who will be treated with anthracyclines. Nevertheless, the FIB-4 index may not be a reliable indicator for patients below 35 or above 65 [22]. Consequently, the interpretation of the FIB-4 index must be considered with caution. One of its advantages is that the FIB-4 index can be easily calculated without additional intervention. Most patients already undergo baseline laboratory tests, making the process easy for patients and clinicians [23]. Moreover, the FIB-4 index provides information before chemotherapy is initiated. Overall, the FIB-4 index is a useful method to define prognosis.

Our study has some limitations. Firstly, our study is retrospective and our patient number is relatively small. Secondly, we only included patients with breast cancer. Therefore, the interpretation of the results on all cancers needs to be taken cautiously. Furthermore, other inflammatory parameters, such as pro-inflammatory cytokines, immunoglobulins, complement proteins, and CRP, were not included in the study as they are not routinely measured at our institution. Lastly, no association was found between cumulative doxorubicin dose and the risk of cardiotoxicity, which could be due to the relatively lower doses used in most patients. Despite these limitations, we showed that there is a significant relationship between the FIB-4 index and overall survival in patients with non-metastatic breast cancer treated with anthracycline.

In conclusion, this study indicates that the FIB-4 index can be easily calculated before treatment with laboratory tests and is linked to the overall survival of non-metastatic breast cancer patients.

Author contribution

Study conception and design: OB, MT and TKŞ; data collection: OB and NG; analysis and interpretation of results: OB and SA ; draft manuscript preparation: OB and LK . All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Hacettepe University Clinical Research Ethics Committee (Protocol no. 2024/13-15-30.07.2024).

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ORIGINAL ARTICLE

The relationship between perfectionism, depressive symptom severity, rumination and burnout in physicians

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ABSTRACT

Objective: A large number of studies indicate that clinicians are at risk for burnout. However, literature on the relationship between burnout and personal traits is limited. Perfectionism may play a role in the development of burnout by increasing ruminative thoughts and depressive symptoms. This study aimed to investigate the relationship between burnout and perfectionism, rumination and depressive symptoms.

Materials and Methods: This study included 317 physicians who completed online surveys between May 2024 and September 2024. The questionnaires included sociodemographic and work environment characteristics, as well as the Maslach Burnout Inventory, Frost Multidimensional Perfectionism Scale, the Patient Health Questionnaire-9, and the Ruminative Response Scale - Short Form self-report scales. Work-related ruminative thoughts and ruminative thoughts related to other life domains were assessed separately.

Results: The results of our study showed that being unmarried, having a history of mobbing, taking the primary responsibility for household chores, being a resident, and working night shifts were associated with burnout. According to the results of regression analysis, emotional exhaustion and depersonalization were predicted by depressive symptom severity and work-related rumination, whereas perfectionism scores were significantly predicted personal accomplishment. The likelihood of being in the suicide risk group was associated with low personal accomplishment in addition to depressive symptom severity. Mediator analysis revealed that the relationship between perfectionism and burnout was fully mediated by depressive symptom severity and rumination.

Conclusion: Perfectionism leads to an increase in burnout through ruminative thoughts and an increase in depressive symptoms. Although small sample size of the study, our results have an important potential to guide interventions for perfectionism in physicians to reduce burnout and associated suicide risk.

Keywords: Burnout, perfectionism, depression, rumination

INTRODUCTION

Burnout syndrome is a long-term, negative emotional state consisting of emotional exhaustion, physical fatigue, and cognitive wear [1]. The currently accepted definition of burnout consists of three dimensions: (i) emotional exhaustion (EE), which is associated with a decrease in the emotional resources of individuals and manifested by weakness, fatigue and exhaustion, (ii) depersonalization (DP), which refers to negative feelings and behaviors towards colleagues or

individuals served, and (iii) a decrease in the sense of personal accomplishment (PA), which is characterized by seeing oneself as inadequate and professionally unsuccessful due to these two dimensions [2]. Life events that cause chronic stress and demanding work conditions create vulnerability to burnout [1]. Physicians are exposed to numerous work-related stressors. In fact, compared to other workers, physicians have higher rates of burnout [3]. At the same time, burnout levels in physicians were found to vary across different medical specialties [4]. This diversity is thought to arise from factors such as extended working hours, sleep deprivation, night shifts, and exposure to violence and mobbing [4,5]. Identifying potential work-related causes of burnout and changing working conditions could help implement the necessary measures within preventive approaches.

Burnout leads to decreased professional effectiveness [6]. In addition, burnout in physicians is associated with psychiatric disorders such as major depressive disorder (MDD) and anxiety disorders [7]. In particular, MDD shares significant clinical similarities with burnout [8]. Several studies have demonstrated the relationship between burnout, especially emotional exhaustion, and MDD [4,9]. Decreased energy, anhedonia, feelings of inadequacy, and suicidal thoughts are common to both burnout and MDD [9]. However, factor analysis studies showing depression and burnout do not share the same items indicate that they are distinct conditions [8,10]. On the other hand, a longitudinal study using latent class analysis, a person-centered approach, has shown that depressive symptoms and burnout symptoms cluster together and follow similar trajectories [11]. Therefore, some authors believe that burnout corresponds to work-related depression [11]. According to another perspective, depression is considered as one of the advanced stages of burnout [12]. At the same time, subthreshold depressive symptoms secondary to chronic stress may contribute to burnout and negatively affect functioning [9]. While the distinction between depression and burnout continues to be debated, burnout is considered a useful concept for recognizing and preventing depressive symptoms caused by chronic stress in the workplace.

Burnout does not result in depression in everyone despite the close relationship between depression and burnout. When tracing the development of burn-out in physicians, individual factors need to be taken into account in addition to work-related factors. Because, burnout is not only related to increased workload, but also to the difficulty in maintaining a balance between work demands and psychological resources [13]. Individual psychological factors such as high levels of perfectionism can disrupt this balance by causing chronic stress. Thus, high personal expectations, concern over mistakes and high self-criticism may play a mediating role in the development of burnout. Maladaptive perfectionism, characterized by fear of making mistakes and high self-criticism, was found to be higher in medical students compared to students in other programs [14]. Another study showed a positive correlation between both adaptive and maladaptive perfectionism and depressive symptom severity in medical students [15]. On the other hand, only one study was found investigating the relationship between burnout and perfectionism in physicians. The study revealed that high self-critical perfectionism predicted both high EE and DP scores [16].

Another personal factor that may pose a risk for burnout is rumination. Studies evaluating psychotherapists, intensive care physicians, and nurses have found that rumination is associated with burnout, particularly emotional exhaustion [17,18]. However, no study has investigated the mediating role of rumination in the development of burnout among physicians. The main hypothesis of our study is that rumination will play a mediating role in the development of depressive symptoms and burnout in the presence of high perfectionism.

There has been an increase in physician suicides in our country in the last 10 years [19]. Among the most important reasons for physician suicides are difficult training process, fear of stigmatization that creates difficulty in accessing treatment, MDD and burnout syndrome [9]. In a large sample study, suicidal ideation was found to be associated with burnout independent of depression [20]. In our country, there is no study investigating the relationship between suicidal risk and burnout in physicians. Another aim of this study is to investigate the relationship between suicidality and burnout.

MATERIALS AND METHODS

Participants

The study is a cross-sectional survey study conducted online between May 2024 and September 2024. The information form and questionnaires were distributed to the target population using the Google Forms platform. The informational text accompanying the survey assured participants that the collected data would be used solely for research purposes and highlighted their right to withdraw from the study at any time. After reading the brief information form, participants who agreed to participate proceeded to fill out the questionnaires. Duplicate participation was prevented by the system.

The questionnaires were distributed to a total of 3998 physicians (WhatsApp groups: 1496, email groups: 2502) through social media platforms where clinicians were members. Out of the 340 physicians who read the brief information text, 11 did not consent to participate, and 12 had incomplete sociodemographic information (see Figure 1). Consequently, data of 317 participants were included in the analysis.

This study was approved by Başkent University Institutional Review Board (Date/Number: 12.19.2023/KA23/424). The study protocol is consistent with the principles of the Declaration of Helsinki.

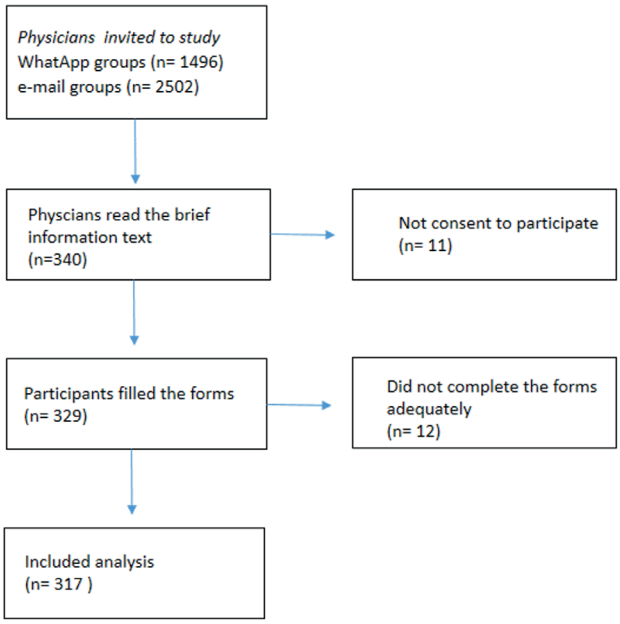

Figure 1. Flow Chart of the Participants

Instruments

Sociodemographic data and work-related features

In the study sociodemographic and work-related characteristics were collected from participants, including variables such as age, gender, marital status, history of psychiatric illness, current psychiatric treatment status, person primarily responsible for household chores (self or partner/parent or equally shared), presence of at least one child requiring care (childcare status), years in the profession (since the beginning of residency training), number of patients examined per day (daily patient load), history of mobbing or violence by patients or their relatives, and the frequency of night shifts (NSs) or on-call shifts (OCSs). Participants were also asked to rate their level of financial satisfaction on a scale from 1 to 10. Furthermore, information regarding suicidal ideation, planning, or attempts within the last week was obtained.

Maslach Burnout Inventory (MBI)

The Maslach Burnout Inventory (MBI), developed by Maslach and Jackson in 1981, is a 22-item scale consisting of three subdimensions: emotional exhaustion (9 items), depersonalization (5 items), and personal accomplishment (8 items). The Turkish validity and reliability study of the scale was conducted by Ergin et al. in 1992 [21]. In the original version of the scale, a 7-point scale was used for each item. However, in the Turkish adaptation, a 5-point scale is employed, ranging from "never" to "always". The internal consistency coefficients of the Turkish version were calculated as 0.83 for emotional exhaustion (MBI-EE), 0.65 for depersonalization (MBI-DP), and 0.72 for personal accomplishment (MBI-PA). Unlike the other two subscales, low scores on the MBI-PA indicate a high level of burnout.

Ruminative Reponse Scale - Short Form (RRS-SF)

The short form of the Ruminative Response Style Questionnaire (RRS), which assesses ruminative thoughts, consists of 10 items rated on a 4-point scale ranging from 1 to 4. The Turkish version of the RRS-SF has been shown to be valid and reliable [22]. Since nonwork-related negative life events may influence the severity of depressive symptoms and burnout scores, and because we are interested

in whether work-related and non-work-related rumination have different effects on burnout, our study assessed work-related rumination (RRS-Work) and rumination related to other areas of life (RRS-Other) separately.

The Patient Health Questionnaire-9 (PHQ-9)

PHQ-9 is a self-report scale assessed the severity of depressive symptoms. It consists of 9 items that evaluate the frequency of symptoms over the past two weeks, with each item rated on a 4-point scale ranged between 0 and 3 [23]. It is considered major depressive disorder if at least one of the first two items and at least 5 of all items have a 'positive' response (2 or 3 points from the first 8 items and a score higher than 1 from the last item). The Turkish adaptation and validity study of the scale was conducted by Corapcioglu et al. [23].

Frost's Multidimensional Perfectionism Scale (FMPS)

Frost et al. developed a scale comprising six subscales: concern over mistakes (FMPS-CM), doubts about actions (FMPS-DA), parental expectations (FMPS-PE), parental criticism (FMPS-PC), order/organization (FMPS-Ord), and personal standards (FMPS-PS) [24]. The scale consists 35 five-point likert-type items. Organisation subscale should not be included in the total scoring due to its weak correlation with the other subscales [24]. The Turkish validty and reliability study of the scale was conducted by Kağan [25]. In the Turkish form, the internal consistency coefficients of the subscales ranged from 0.64 to 0.94, while the total scale was 0.91.

Statistical analysis

All analyses were performed with R version 4.1.2 [26]. Skewness and kurtosis were used to test the conformity of the variables to normal distribution (values between -1 and +1 were considered acceptable), and Levene's test was used to assess the homogeneity of variances [27]. Skewed data were presented with median and minimum-maximum values, while normally distributed data were presented with mean and standard deviation. Comparisons between groups were made with independent sample t-test for continuous data (burnout, depression and rumination scores) and chi-squared test for categorical data. One-way ANOVA (Tukey test for post-hoc comparisons) or Welch ANOVA (Games-Howell test for post-hoc comparisons) was used for comparisons of scale scores of more than two groups according to the homogeneity of variance between groups. Since 9 comparisons were made between the groups regarding burnout scores, Bonferroni corrected significance level was applied to adjust for multiple comparisons ($p<0.005$). Cohen's d, partial eta-squared and omega-squared values were calculated for t-test for effect sizes, one-way ANOVA and Welch ANOVA, respectively. In cases where variances were not homogeneous, Hedges' g effect size was reported for the t-test. The following conventional thresholds were used to interpret effect sizes: for Cohen's d and Hedges' g, small 0.2, medium 0.5, large 0.8; for partial eta squared and omega squared, small 0.01, medium 0.06, large 0.14 [28].

The variables years of experience, financial satisfaction, number of night shifts, and number of call shifts were not normally distributed. These variables were to be included in the regression analyses along with the scale scores. Therefore, logarithmic transformation was applied to all variables included in the regression analyses so that the variables were not on different scales. Pearson correlation coefficients and partial correlation analyses were used to assess the relationships between normally distributed scale scores. For variables that were not normally distributed, Spearman's correlation analysis was used.

Multiple linear regression analyses were conducted with work related conditions, perfectionism scores, depressive symptom severity, and rumination scores as predictors and burnout dimensions as dependent variables. Assumptions required for multiple linear regression were tested prior to analyses. All variables were log-transformed, and normal distribution was achieved. Scatter plots examining the distribution of residuals showed random dispersion around zero with no detectable pattern [27]. Correlations between dependent and independent variables were significant. Multicollinearity checks indicated that none of the correlation coefficients between independent variables exceeded 0.8. In addition, all variance inflation factor (VIF) values were below 5, and Durbin-Watson statistics ranged between 1 and 3 across all models [28]. Thus, all assumptions of the models were considered to be met.

In addition, logistic regression analysis was conducted to assess whether burnout and depressive symptom severity were associated with a higher risk of suicide. The backward conditional technique was used in the regression analyses.

Depressive symptom severity and work-related rumination - but not rumination about other life events - predicted burnout dimensions, whereas no burnout dimension predicted depressive symptom severity. The mediating role of depressive symptom severity and rumination in the relationship between perfectionism and burnout was assessed by serial mediation analysis (using the lavaan package). Prior to the mediation analysis, the common factor (ComPer) representing the subscale scores was calculated using Principal Component Analysis (PCA) instead of the total scale score to represent the shared variance among the subscales (using *psych package*). The correlations between the subscale scores ranged from -0.356 to 0.621 (all $p< 0.001$). Since the factors were not independent, oblimin rotation was applied. The Organization subscale, which is not recommended to be included in the total scale score due to its low factor loading in the literature, was not included in the factor analysis [24]. Similarly, another common-factor (ComBurn) was calculated for MBI. In the serial mediation model, ComPer was used as the independent variable (IV), ComBurn as the dependent variable (DV), while RRS-Work served as the first mediator (M1) and PHQ-9 total score as the second mediator (M2). To analyze indirect effects, we applied bootstrapping with 5000 samples. Indirect effects were considered significant if the bootstrap-derived 95% CI interval did not include zero.

RESULTS

A total of 317 participants (184 females, age=33.35±8.28 years) were included in the analyses. According to the PHQ-9 scale, 27.76% (n=88) of the participants met the diagnosis of major depressive disorder. Sociodemographic data of the participants are given in Table 1.

Differences in Burnout, Depression and Rumination Scores Between Groups

In between-group comparisons, having at least one child in care and being married were associated

Table 1. Sociodemographic and Clinical Characteristics of Participants

	Mean	SD
Age	33.35	8.28
Financial satisfaction	5.65	1.9
Number of working hours per week	46.69	6.07
	Median	**Min-Max**
Years of experiencea	4	0-43
Daily patient load	30	0-200
Number of NSs/month	3	0-15
	n	**%**
Gender		
Female	184	58.04
Male	133	41.96
Marital status		
Married	170	53.63
Single	147	46.37
Childcare status		
Yes	99	31.23
No	218	68.77
Housework responsibility		
Primarily self	132	41.64
Primarily partner/parent	74	23.34
Equally shared	111	35.02
Academic title		
Resident	180	56.78
Specialist	95	29.97
Academician	42	13.25
Shift status		
Night-call shift	216	68.14
On-call call shift	53	16.72
No shifts	48	15.14
Mobbing and Violence		
Physical violence exposed by patient relatives or patients in the last year	30	9.46
Mobbing in the last year	90	28.39
History of psychiatric illness	134	42.27
Distribution of psychiatric ilnesses		
MDD	71	22.39
Anxiety disorder	59	18.61
ADHD	13	4.1
OCD	6	1.89
Bipolar disorder	3	0.94
PTSD	4	1.26
Adjustment disorder	2	0.63
Suicide risk		
Suicidal thoughts in the last week	55	17.35
Suicide plan or attempt in the last week	8	2.52
	Mean	**SD**
Burnouıt Scores		
MBI-EE	30.22	7.61
MBI-DP	12.72	4.3
MBI-PA	30.37	4.55
PHQ-9 Total	10.55	6.15
Perfectinonism scores		
FMPS-CM	21.02	6.36
FMPS-DA	11.16	3.77
FMPS-PE	13.78	5.13
FMPS-PC	9	3.71
FMPS-PS	22.89	5.33
FMPS-Ord	22.64	4.46
Rumination scores		
RRS-Work	20.71	6.35
RRS-Other	21.57	6.33

[a] From the beginning of the residency.

NSs: Night shifts, MDD: Major depressive disorder, ADHD: Attention deficit and hyperactivity disorder, OCD: Obsessive compulsive disorder, PTSD: Posttraumatic stress disorder, MBI-EE: Emotional exhaustion subscale of Maslach burnout inventory, MBI-DP: Depersonalization subscale of MBI, MBI-PA: Personal accomplishment subscale of MBI, PHQ-9: Patient Health Questionnaire-9, FMPS-CM: Concern over mistakes subscale of Frost multidimensional perfectionism scale, FMPS-DA: Doubts about actions subscale of FMPS, FMPS-PE: Parental expectations subscale of FMPS, FMPS-PC: Parental criticism subscale of FMPS, FMPS-PS: Personal standarts subscale of FMPS, FMPS-Ord: Ordering/organization subscale of FMPS, RRS: Ruminative Responses Scale

with lower burnout scores (lower MBI-EE and MBI-DP and higher MBI-PA) (see Table 2). Physicians who had been exposed to mobbing in the past year had higher levels of burnout (high MBI-EE and MBI-DP and low MBI-PA) than those who had not, whereas exposure to violence in the past year was not significantly associated with burnout.

Physicians primarily responsible for household chores exhibited higher levels of burnout compared to those who shared household responsibilities equally with their partners or parents. Academicians reported the lowest levels of MBI-DP and the highest levels of MBI-PA, whereas residents exhibited the opposite pattern, with the highest levels of MBI-DP and the lowest levels of MBI-PA. Emotional exhaustion was also found to be higher in resident physicians compared to academicians.

No significant differences in any scale scores were observed between physicians who did not take on-call shifts and those who did. However, physicians working night shifts reported significantly higher MBI-EE and lower MBI-PA scores compared to both those on on-call shifts (see Table 3). Moreover, MBI-EE scores were significantly higher among physicians working night shifts compared to those who did not take any shifts.

Table 2. Comparisons of Burnout, Depression and Rumination Scores Between Groups in Terms of Gender, Marital Status, Exposed Violence and Mobbing

	Gender				**Marital status**			
	Female (n= 184)	**Male (n=133)**			**Single (n= 147)**	**Married (n= 170)**		
	Mean±SD	**Mean±SD**	**Statistics**	**p**	**Mean±SD**	**Mean±SD**	**Statistics**	**p**
MBI-EE	30.66±7.2	29.61±8.12	*t(315)*=1.189 *d*=0.138	0.235	31.8±7.56	28.85±7.4	*t(315)*=-3.506 *d*=0.395	<0.001*
MBI-DP	12.58±4.32	12.92±4.28	*t(315)*=-0.713 *d*=0.081	0.477	13.77±4.23	11.82±4.17	*t(315)*=-4.124 *d*=0.465	<0.001*
MBI-PA	30.12±4.46	30.71±4.67	*t(315)*=-1.126 *d*=0.129	0.261	29.31±4.72	31.28±4.2	*t(315)*=3.884 *d*=-0.441	<0.001*
	Exposed violence in the last year				**Exposed mobbing in the last year**			
	Yes (n=30)	**No (n= 287)**			**Yes (n= 90)**	**No (n=227)**		
	Mean±SD	**Mean±SD**	**Statistics**	**p**	**Mean±SD**	**Mean±SD**	**Statistics**	**p**
MBI-EE	30.47±7.49	30.21±7.66	*t(315)*=-0.18 *g*=0.034	0.858	32.91±6.95	28.95±7.46	*t(315)*=-4.471 *g*=0.54	<0.001*
MBI-DP	12.57±4.48	12.71±4.29	*t(315)*=0.166 *g*=-0.033	0.869	13.9±4.15	12.15±4.21	*t(315)*=-3.363 *g*=0.416	0.001*
MBI-PA	30.27±4.77	30.33±4.52	*t(315)*=0.069 *g*=-0.014	0.945	29.33±4.3	30.8±4.61	*t(315)*=2.675 *g*=-0.323	-
	Childcare Status				**Current psychistric treatment**			
	Yes (n=99)	**No (n=218)**			**Yes (n=90)**	**No (n=227)**		
	Mean±SD	**Mean±SD**	**Statistics**	**p**	**Mean±SD**	**Mean±SD**	**Statistics**	**p**
MBI-EE	27.64 (7.44)	31.39 (7.4)	*t(315)*=4.168 *d*=-0.506	<0.001*	29.99±6.73	30.31±7.94	*t(315)*=0.362 *d*=-0.042	0.718
MBI-DP	11.25 (4.3)	13.39 (4.14)	*t(315)*=4.148 *d*=-0.51	<0.001*	12.68±3.7	12.74±4.53	*t(315)*=0.127 *d*=-0.014	0.899
MBI-PA	31.73 (4.33)	29.75 (4.52)	*t(315)*=-3.721 *d*=0.443	<0.001*	29.5±4.97	30.71±4.33	*t(315)*=2.022 *d*=-0.267	0.045

MBI-EE: Emotional exhaustion subscale of Maslach burnout inventory, MBI-DP: Depersonalization subscale of MBI, MBI-PA: Personal accomplishment subscale of MBI

t: Independent Samples t-Test, d=Cohen's d coefficient, g=Hedges' g coefficient

Significant ANOVA results ($p < 0.005$) are indicated by *

Table 3. Comparisons of Burnout, Depression and Rumination Scores in Terms of Home Housework Responsibility, Academic Title and On Shift Status

	Housework responsibility					
	PS (n=132)	PP (n=74)	ES (n=111)			
	Mean±SD	Mean±SD	Mean±SD	Statistics	*p*	Post-hoc
MBI-EE	31.56±7.24	29.28±7.66	29.24±7.82	F(2.314)=3.584 η2=0.022	0.029	-
MBI-DP	13.47±4.23	12.31±4.71	12.09±3.99	F(2.314)=3.596 η2=0.022	0.029	-
MBI-PA	29.24±4.64	31.11±4.49	31.21±4.33	F(2.314)=7.174 η2=0.044	0.001*	ES> PS=PP
	Academic title					
	Resident (n=180)	Specialist (n=95)	Academician (n=42)			
	Mean±SD	Mean±SD	Mean±SD	Statistics	*p*	Post-hoc
MBI-EE	31.1±7.11	29.86±8.75	27.24±6.03	F(2.314)=6.49 *ω2*= 0.033	0.002*	Res>Ac
MBI-DP	13.65±4.02	12.21±4.44	9.91±3.78	F(2.314)=15.11 *ω2*=0.081	<0.001*	Res>Spec>Ac
MBI-PA	29.27±4.31	31.52±4.18	32.45±5.09	F(2.314)=13.66 *ω2*=0.074	<0.001*	Ac>Spec>Res
	Shift status					
	No shifts (n=48)	On-call call shifts (n=53)	Night-call shifts (n=216)			
	Mean±SD	Mean±SD	Mean±SD	Statistics	*p*	Post-hoc
MBI-EE	26.94±7.34	28.07±6.91	31.47±7.52	F(2.314)=10.048 *ω2*=0.054	<0.001*	NS>OS=NoS
MBI-DP	12.15±4.11	11.36±4.22	13.18±4.29	F(2.314)=4.441 *ω2*=0.021	0.013	-
MBI-PA	30.71±4.6	32.68±3.92	29.72±4.49	F(2.314)=9.646 *ω2*=0.052	<0.001*	NS>OS

MBI-EE: Emotional exhaustion subscale of Maslach burnout inventory, MBI-DP: Depersonalization subscale of MBI, MBI-PA: Personal accomplishment subscale of MBI, PS: Primarily self, PP: Primarily partner or parent, ES: Equally shared, Res: Resident, Ac: Academician, Spec: Specialist, NS: Night-call shifts, OS: On-call call shifts, NoS: No shifts

F: F statistic of ANOVA, η2: partial eta-squared, *ω2=omega squared*

Significant ANOVA results ($p < 0.005$) are indicated by *

When burnout, depression, rumination and perfectionism scores were compared between physicians with and without suicidal ideation-plan-attempt (*suicide risk*), the increased suicide risk group had higher MBI-EE and lower MBI-PA scores. In addition, depression, rumination, FMPS-CM, FMPS-PE, FMPS-PC and FMPS-DA scores were higher in the suicide risk group (see Table S1). Perfectionism dimensions except FMPS-PE and FMPS-Ord, all burnout and rumination scores, and depressive symptom severity were significantly higher in those who met the diagnosis of depression according to the PHQ-9. Participants in the depression group were more likely to be in the suicide risk group (see Table S1).

Correlations Between Work-Related and Clinical Variables

Number of night shifts and number of working hours per week were associated with an increase in MBI-EE, MBI-DP, depression, and rumination scores and a decrease in MBI-PA. Age and financial satisfaction level were associated with a decrease in burnout, rumination, and depressive symptom severity. There were positive correlations between the number of patients examined per day and MBI-EE and MBI-DP (see Table S2).

Significant correlations were found between burnout, depressive symptom severity, and perfectionism. MBI-EE, MBI-DP, PHQ-9, and rumination scores were positively correlated with

each other and with most perfectionism scores. Negative correlations were found with the MBI-PA, as expected (see Table S3).

Linear Regression Models of Burn-out and Depressive Symptom Severity

Four different regression models were constructed to evaluate the associations between all three dimensions of burnout and PHQ-9 total score (as dependent variables), and perfectionism, rumination scores, age, academic title (academicians vs. specialists/residents), number of NCSs, number of working hours per week, housework responsibility (equally shared vs. primarily self/partner or parent), caregiving for a child, number of patients examined per day, financial satisfaction, and history of mobbing in the last year (see Table 4). The models established for MBI-EE, MBI-DP, and MBI-PA were all significant [adjusted R^2=0.494, F(3,313)=100.702, p<0.001; adjusted R^2=0.289, F(4,312)=31.248, p<0.001; and adjusted R^2=0.269, F(7,309)=16.062, p<0.001, respectively].

Table 4. Results of Linear Regression Analyses

Variables	Unstandardized coefficients		Standardized coefficient			95%CI for B	
	B	SE	ß	t	*p*	Lower	Upper
MBI-EE							
(Constant)	1.076	0.058		18.619	<0.001***	0.962	**1.190**
PHQ-9 total	0.175	0.021	0.420	8.190	<0.001***	0.133	**0.217**
RRS-Work	0.255	0.045	0.287	5.623	<0.001***	0.166	**0.344**
Financial satisfaction	-0.144	0.031	-0.195	-4.718	<0.001***	-0.204	**-0.084**
MBI-DP							
(Constant)	0.637	0.096		6.606	<0.001***	0.447	**0.827**
PHQ-9 total	0.150	0.035	0.265	4.342	<0.001***	0.082	**0.218**
RRS-Work	0.251	0.073	0.207	3.424	0.001**	0.107	**0.395**
Age	-0.003	0.001	-0.149	-2.665	0.008**	-0.005	**-0.001**
Being an academician	-0.070	0.025	-0.151	-2.753	0.006**	-0.020	**-0.120**
MBI-PA							
(Constant)	1.414	0.051		27.636	<0.001***	1.313	**1.514**
PHQ-9 total	-0.037	0.015	-0.155	-2.533	0.012*	-0.066	**-0.008**
FMPS-CM	-0.150	0.036	-0.313	-4.195	<0.001***	-0.221	**-0.080**
FMPS-PS	0.214	0.041	0.345	5.279	<0.001***	0.134	**0.294**
FMPS-DA	-0.071	0.027	-0.165	-2.617	0.009**	-0.124	**-0.018**
Age	0.001	<0.001	0.158	3.104	0.002**	<0.001	**0.002**
Sharing of houseworks	0.015	0.007	0.107	2.131	0.034*	0.001	**0.029**
Financial satisfaction	0.058	0.022	0.135	2.663	0.008**	0.015	**0.101**
PHQ-9							
(Constant)	-0.432	0.199		-2.171	0.031*	-0.824	**-0.040**
RRS-Work	0.866	0.116	0.405	7.485	<0.001***	0.639	**1.094**
FMPS-CM	0.576	0.130	0.290	4.439	<0.001***	0.321	**0.831**
FMPS-PS	-0.295	0.144	-0.114	-2.042	0.042*	-0.578	**-0.011**
FMPS-PC	-0.139	0.081	-0.089	-1.722	0.086	-0.298	**0.020**
FMPS-DA	0.266	0.101	0.150	2.642	0.009**	0.068	**0.465**
Age	-0.003	0.001	-0.082	-1.866	0.063	-0.006	**<0.001**
Sharing of houseworks	-0.056	0.025	-0.096	-2.216	0.027*	-0.106	**-0.006**
Financial satisfaction	**-0.182**	**0.077**	**-0.103**	**-2.358**	**0.019***	**-0.334**	**-0.030**

PHQ-9: Patient Healt Questionnaire-9, RRS: Ruminative Responses Scale, FMPS-CM: Concern over mistakes subscale of Frost multidimensional perfectionism scale, FMPS-DA: Doubts about actions subscale of FMPS, FMPS-PE: Parental expectations subscale of FMPS, FMPS-PC: Parental criticism subscale of FMPS, FMPS-PS: Personal standarts subscale of FMPS.

Model for MBI-EE: *adjusted* R^2=0.494, $F(3,313)=100.702$, $p<0.001$

Model for MBI-DP: *adjusted* R^2=0.289, $F(4,312)=31.248$, $p<0.001$

Model for MBI-PA: *adjusted* R^2=0.269, $F(7,309)=16.062$, $p<0.001$

Model for PHQ-9: *adjusted* R^2=0.460, $F(8,308)=32.434$, $p<0.001$

*p<0.05, **p<0.01, ***p<0.001

MBI-EE scores were positively predicted by depressive symptom severity, RRS-Work, and working night shifts, whereas financial satisfaction was negatively predicted MBI-EE. MBI-DP was positively associated with depressive symptom severity and RRS-Work scores, but negatively associated with being an academician.

Depressive symptom severity and maladaptive perfectionism negatively influenced MBI-PA, whereas adaptive perfectionis and shared household responsibilities were associated with increased MBI-PA. Depressive symptom severity was positively predicted by FMPS-CM, FMPS-DA, and RRS-Work, whereas negative associations were observed with age, shared household responsibilities, high FMPS-PS, and financial satisfaction [R^2=0.460, F(8,308)=32.434, p<0.001].

The Relationship Between Burnout, Depressive Symptoms, and Suicide Risk

According to the logistic regression analysis conducted to evaluate whether the relationship between suicide risk and burnout is independent of depressive symptoms, the PHQ-9 total score (Wald=37.345, p<0.001) and low MBI-PA (Wald=4.612, p=0.032) were significant predictors of being in the suicide risk group (Chi-square=60.747, p<0.001, Log likelihood=253.75, Nagelkerke R^2=0.283). The results of the logistic regression analysis are presented in Table 5.

Effects of depressive symptoms and rumination on the relationship between perfectionism and burnout

Before conducting the mediation analysis, common factors for burnout and perfectionism were calculated using principal component analysis (PCA). The derived common factor for burnout (ComBurn) explained 48.2% of the total variance. For adaptive perfectionism, the calculated common factor based on the PS and Ord subscales explained 36% of the total variance, which was considered insufficient. Consistent with the literature, the Ord subscale showed inadequate correlations with other subscales. Conversely, the PS subscale, another form of adaptive perfectionism, showed positive and significant correlations with other

Table 5. Logistic Regression Model Predicting Suicide Risk from Depression and Burnout Scores

	ß	SE	Wald	OR	95% CI for OR	p
Constant	0.891	1.468	0.369	2.438		
MBI-EE	-0.051	0.031	2.672	0.95	0.893-1.01	0.102
MBI-DP	-0.083	0.048	2.95	0.921	0.837-1.012	0.086
MBI-PA	-0.082	0.038	4.612	0.921	0.855-0.993	0.032*
PHQ-9	0.226	0.037	37.345	1.254	1.171-1.353	<0.001**

ß= Unstandardized coefficient, SE= standard error, OR = odds ratio, CI= confidence interval

MBI-EE: Emotional exhaustion subscale of Maslach burnout inventory, MBI-DP: Depersonalization subscale of MBI, MBI-PA: Personal accomplishment subscale of MBI, PHQ-9: Patient Health Questionnaire-9

*p<0.05, **p<0.001

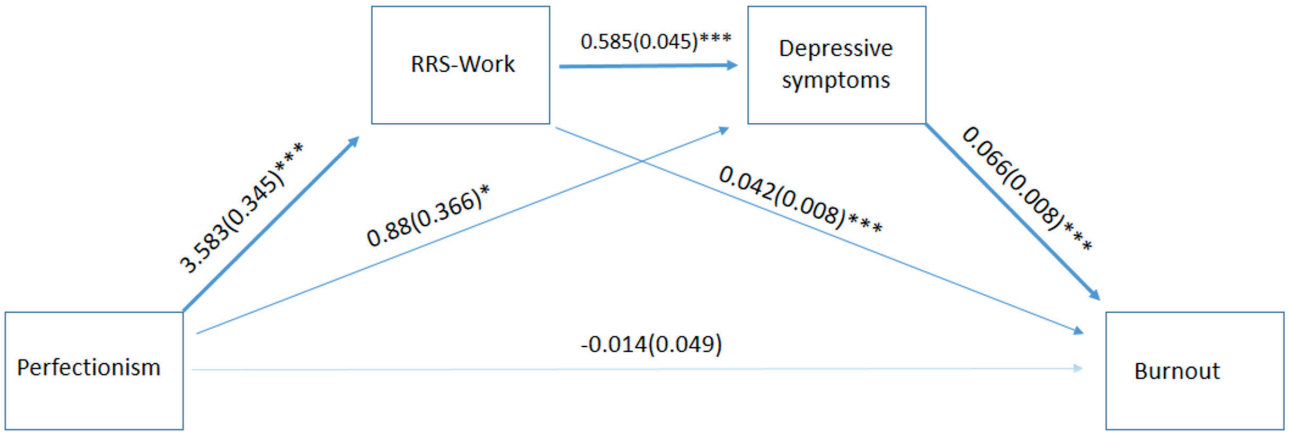

Figure 2. Effects of depressive symptoms and rumination on the relationship between perfectionism and burnout

The numbers above the lines represent the standardized beta coefficient and standard error: beta coefficient (standard error)

RRS-Work: - Work related score of Ruminative Responses Scale

*p<0.05, **p<0.01, ***p<0.001

Table 6. The Role of Depressive Symptoms and Work Related Rumination in the Relationship Between Perfectionism and Burnout

	ß	SE	z	p	LLCL	ULCL
Direct effects						
ComPer → RRS-W	3.583	0.345	10.387	<0.001	2.907	4.249
ComPer → PHQ-9	0.88	0.366	2.403	0.016	0.184	1.607
RRS-W → PHQ-9	0.585	0.045	12.944	<0.001	0.444	0.675
RRS-W → ComBurn	0.042	0.008	4.986	<0.001	0.025	0.058
PHQ-9 → ComBurn	0.066	0.008	8.055	<0.001	0.05	0.082
ComPer → ComBurn	-0.014	0.049	-0.283	0.777	-0.013	0.082
Indirect effects						
ComPer → RRS-W → ComBurn	0.15	0.032	4.77	<0.001	0.091	0.217
ComPer → PHQ-9 → ComBurn	0.058	0.026	2.276	0.023	0.013	0.113
RRS-W → PHQ-9 → ComBurn	0.039	0.006	6.894	<0.001	0.028	0.051
Total Indirect effect	0.138	0.024	5.648	<0.001	0.096	0.191
Total effect	0.124	0.055	2.276	0.023	0.019	0.234

ComPer: Common-factor for perfectionism, RRS-W: Work related score of Ruminative Response Scale; PHQ-9: Patient Health Questionnaire-9; ComBurn: Common-factor for burnout, SE: Standard error; LLCL: Lower limit of the 95 % confidence interval; ULCL: Upper limit of the 95 % CI.

subscales. The factor loading for the recalculated common factor excluding the Ord items was 0.668. Thus, perfectionism was treated as a unified construct. The common factor calculated for Perfectionism (ComPer) explained 52.2% of the total variance. For ComPer, the factor loadings of the subscales ranged from 0.651 to 0.803, whereas for ComBurn, the factor loadings for MBI-EE, MBI-DP, and MBI-PA were 0.807, 0.777, and -0.437, respectively. While RRS-Work positively predicted MBI-EE, MBI-DP, and PHQ-9 total scores, RRS-Other had no significant predictive effect. Therefore, only RRS-Work was included as a first mediator in the mediation analysis.

There were significant positive direct effects of ComPer on RRS-Work and PHQ-9 total score, RRS-Work on PHQ-9 total score and ComBurn and PHQ-9 on ComBurn (see Table 6 and Figure 2)

The total indirect effect of ComPer on ComBurn mediated by depressive symptoms and rumination was statistically significant. The direct effect of ComPer on ComBurn was not significant. There were significant indirect effects of ComPer on ComBurn mediated by RRS-Work and mediated by PHQ-9.

DISCUSSION

In this study, the relationships between burnout, perfectionism, depressive symptoms and rumination in physicians, as well as the mediating role of depression and rumination in the relationship between perfectionism and burnout were evaluated. The findings of our study indicated that being unmarried, having a history of mobbing, taking the primary responsibility for household chores, being a resident, and working night shifts were associated with burnout. According to the results of regression analysis, emotional exhaustion and depersonalization were predicted by depressive symptom severity and rumination, while significant relationships were found between personal accomplishment and perfectionism scores. The likelihood of being in the suicide risk group was determined by personal accomplishment in addition to depressive symptom severity. Mediation analysis revealed that the relationship between perfectionism and burnout was fully mediated separately and jointly by depressive symptom severity and rumination.

Our study revealed that low financial satisfaction, younger age, high daily patient load, number of working hours per week and number of night shifts, as well as being exposed to mobbing in the last 1 year were related to increased emotional exhaustion and depersonalization. Being married and having children to care for were shown to be protective in terms of all burnout subscores and sharing housework responsibility was related to increased personal accomplishment. Our findings are consistent with the literature [5,29]. Financial satisfaction is closer to psychological qualities

rather than objective economic indicators [30]. Low financial satisfaction may affect the enjoyment of life and lead to difficulties in coping with work-related stressors. In fact, financial satisfaction negatively predicted emotional exhaustion and positively predicted personal accomplishment. In parallel with our findings, in a large sample study, burnout severity was found to be lower in those with higher financial satisfaction. [30].

High daily patient load, the number of hours worked per week and the number of night shifts are known to have both psychologically and physically exhausting effects like sleep deprivation and attention deficits [4,5,12,31]. At the same time, spending extended periods in the hospital may reduce the time available for professional development and decrease the appreciation received relative to the effort exerted, leading to a diminished sense of personal accomplishment. Devoting a significant portion of time to work may also reduce the sense of autonomy in life and limit time for social activities, potentially resulting in increased loneliness and decreased job satisfaction [30]. Moreover, being single and not sharing household responsibilities—factors potentially associated with loneliness and inadequate social support —were found in our study to be related to increased burnout, rumination, depression, and suicide risk. Our findings align with the literature suggesting that being married and having children are protective against burnout [30,32]. Sharing household responsibilities can also be interpreted as a sign of increased social interaction, in addition to reducing the physical workload. To our knowledge, no studies in the literature have specifically evaluated the impact of sharing household responsibilities on burnout.

Another factor shaping working conditions is the sense of psychological and physical safety. Mobbing can disrupt the sense of psychological security. In our study, individuals who experienced mobbing in the past year exhibited higher scores for burnout, depressive symptom severity, and rumination. Consistent with our findings, mobbing has been associated with increased emotional exhaustion and depersonalization [5,33]. The lack of a significant relationship between exposure to violence and burnout in our study may suggest that mobbing has more long-term and profound psychological effects than violence. However, factors such as duration and severity of violence were not assessed in our study. In contrast, a study conducted in our country that comprehensively assessed violence (n=310) found that physical or verbal violence was associated with emotional exhaustion and depersonalization [34]. The literature on the relationship between exposure to violence and burnout is limited and further studies are needed.

In our study, in line with the literature, residents had higher emotional exhaustion, depersonalization, and lower personal accomplishment scores than academicians [4,5]. Specialists were between residents and academics in terms of depersonalization and personal accomplishment scores. In addition, age and academic title were negatively predictive of emotional exhaustion and depersonalization. Differences between academic title groups may indicate the impact of roles and expectations in the professional hierarchy on burnout. Lower personal accomplishment scores in residents compared to specialists and academicians may be related to inexperience and lower sense of control. The fact that there was no difference between residents and specialists in terms of emotional exhaustion, but that the sense of personal accomplishment was higher and depersonalization was lower in specialists, may be a sign that ways of coping with emotional exhaustion have developed over time. In the literature, a negative correlation between age and burnout has been found in parallel with our findings [4,5,9].

In the correlation analyses, an increase in depressive symptom severity and rumination scores was associated with a decrease in the personal accomplishment and an increase in emotional exhaustion and depersonalization. In addition, depressive symptom severity significantly predicted burnout, while work related rumination score predicted emotional exhaustion and depersonalization in multiple linear regression analyses. In support of our findings, it is known that there is a positive correlation between burnout level and severity of depressive symptoms and rumination [8,11,17,18]. Rumination is a way of thinking triggered by uncertainty or unresolvable situations [35]. Factors such as coping with problems that arise in health care processes and worrying about making mistakes, along with fatigue, can lead to the development of rumination. Rumination is

thought to increase sensitivity to negative cues and contribute to the onset or persistence of depressed mood [36]. Increased sensitivity to negative situations may lead to emotional exhaustion and depersonalization through decreased enjoyment of the health care process. Thus, depressive symptom severity and work related rumination together positively predicted depersonalization and emotional exhaustion scores. The fact that work related rumination score and depressive symptom severity predicted burnout scores supports the hypothesis that burnout is a subthreshold or clinical manifestation of depression secondary to work-related stress [11,37]. There is a need for more studies investigating the effect of rumination on burnout. In addition, determining the characteristics of the work environment that create a triggering atmosphere for rumination and interventions to address them may be a protective approach to preventing the development of burnout in physicians.

The only study investigating the relationship between perfectionism and burnout in physicians found that self-critical perfectionism predicted high emotional exhaustion and depersonalization [16]. In the study, three dimensions of perfectionism were investigated with the Big Three Perfectionism Scale. In our study, perfectionism scores were found to be associated with personal accomplishment. High personal standards, which represents a dimension of adaptive perfectionism, was found to positively predict the sense of personal accomplishment. In contrast, concern over mistakes and doubts about actions, components of maladaptive perfectionism, were linked to a reduction in personal accomplishment. A balance between personal standards, including goal-setting and attentiveness, and maladaptive perfectionism dimensions, such as fear of making mistakes and doubting one's actions, which reduce self-confidence and sense of competence, seems to be associated with an increased sense of personal accomplishment. Future research may provide insights for the development of individualized approaches to the prevention and treatment of burnout.

The mediation analysis we conducted to present a holistic picture revealed that perfectionism increases burnout through rumination and depressive symptoms. Additionally, rumination exacerbates depressive symptoms, thereby contributing to higher burnout levels. Similarly, perfectionism was shown to independently increase depressive symptoms through rumination, leading to an increase in burnout. These results were consistent with the other findings of the study. Our findings suggest that interventions targeting rumination and perfectionist beliefs may be beneficial in preventing burnout. Such intervention efforts could help reduce depression and suicidal tendencies among physicians.

Burnout has been shown to be associated with increased suicide risk in the literature [9,20,38]. In our study, in parallel with the literature, emotional exhaustion, depersonalization, Depressive symptom severity, rumination and maladaptive perfectionism scores were higher and personal accomplishment scores were lower in the suicide risk group. Perfectionism can also be a source of suicidal thoughts. It has been shown that self-stigma is higher and help-seeking is lower in individuals with high perfectionism [39,40]. Physicians experiencing burnout may avoid seeking professional help for their psychiatric complaints and try to cope with depressive symptoms. Therefore, perfectionism may be a condition that prevents help-seeking behaviors in physicians. This may lead to increased rumination, exacerbation of depressive symptoms and burnout, and the emergence of suicidal thoughts. Our study also demonstrated that a higher depressive symptom severity and lower personal accomplishment scores increased the likelihood of being in the suicidal risk group. A dynamic process (including maladaptive perfectionism) whose final common pathway is a decrease in the sense of low personal achievement may lead to an increase in feelings of inadequacy and worthlessness and hopelessness. There is no study investigating the role of perfectionism and rumination in the relationship between burnout and suicide. Follow-up studies with large samples investigating the relationship between suicide risk, burnout and self-stigma may be instructive in illuminating the path to suicide in physicians.

Our study is the first to examine the role of perfectionism in the development of physician burnout, taking into account indirect effects. Examining the role of mediators such as depressive symptoms and rumination are among the strengths of the study. However, this study has several

limitations. First, the relatively small sample size limits the generalizability of the results and further analyses to analyze more complex relationships. Second, factors not assessed in our study, such as obsessive-compulsive and other personality traits, autistic traits, anxious temperament, self-esteem, coping mechanisms, resilience, and perceived social support, may have influenced the relationship between perfectionism and burnout. Future studies with larger samples that examine both vulnerability factors for burnout and positive coping strategies from a broader perspective may provide clearer inferences about the underlying mechanisms of burnout. Third, depressive symptoms were assessed using a self-report scale. Fourth, our study is cross-sectional. Follow-up studies may provide a clearer understanding of the causal relationships between burnout and individual psychological characteristics.

Author contribution

Study conception and design: EM, BKY; data collection: EM, BKY; analysis and interpretation of results: EM, BKY; draft manuscript preparation: AA, EM, BKY. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Baskent University Institutional Review Board (Protocol no. KA23/424/12.19.2023).

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Conflict of interest

The authors declare that there is no conflict of interest.

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Table S1. Burnout, Depression, Rumination and Perfectionism Scores in Suicide Risk and Depression Groups

	Suicide Risk			
	Yes (n=63)	No (n=254)		
	Mean±SD	Mean±SD	Statistics	p
MBI-EE	32.76±7.35	29.59±7.55	*t(315)*=-3.053 *d*=0.423	0.003**
MBI-DP	13.48±4.71	12.54±4.18	*t(315)*=-1.449 *d*=0.219	0.151
MBI-PA	28.75±4.3	30.77±4.53	*t(315)*=3.303 *d*=-0.451	0.001**
PHQ-9	15.49±6.01	9.33±5.55	*t(315)*=-7.393 *d*=1.092	<0.001***
RRS-Other	25.92±6.75	20.49±5.75	*t(315)*=-5.878 *d*=0.912	<0.001***
RRS-W	24.13±7.31	19.86±5.8	*t(315)*=-4.309 *d*=0.697	<0.001***
FMPS-CM	24.3±5.8	20.2±6.24	*t(315)*=-4.946 *d*=0.666	<0.001***
FMPS-PS	23.92±6.23	22.65±5.06	*t(315)*=-1.507 *d*=0.24	0.135
FMPS-PE	14.95±5.08	13.5±5.11	*t(315)*=-2.034 *d*=0.285	0.045
FMPS-PC	10.52±3.7	8.62±3.62	*t(315)*=-3.667 *d*=0.523	<0.001***
FMPS-DA	12.73±4.01	10.76±3.61	*t(315)*=-3.549 *d*=0.532	<0.001***
FMPS-Ord	22.4±5.26	22.7±4.24	*t(315)*=0.425 *d*=-0.068	0.672
	Major Depressive Disorder			
	Yes (n=88)	No (n=229)		
	Mean±SD	Mean±SD	Statistics	p
MBI-EE	36.49±6.2	27.81±6.67	t(315)=-10.925 *d*=1.326	<0.001***
MBI-DP	15.43±4.25	11.68±3.85	t(315)=-7.215 *d*=0.946	<0.001***
MBI-PA	28.86±3.89	30.94±4.66	t(315)=4.026 *d*=-0.466	<0.001***
PHQ-9	18.44±3.8	7.52±3.69	t(315)=-23.101 *d*=2.936	<0.001***
RRS-Other	26.92±6.28	19.51±5.02	t(315)=-9.91 *d*=1.372	<0.001***
RRS-W	26.5±6.11	18.48±4.87	t(315)=-11.04 *d*=1.53	<0.001***
FMPS-CM	24.83±5.65	19.55±6.01	t(315)=-7.322 *d*=0.893	<0.001***
FMPS-PS	25.11±5.73	22.05±4.91	t(315)=-4.43 *d*=0.595	<0.001***
FMPS-PE	14.62±5.35	13.46±5.01	t(315)=-1.762 *d*=0.227	0.08
FMPS-PC	10.12±4.23	8.57±3.4	t(315)=-3.093 *d*=0.427	0.002**
FMPS-DA	13.06±3.91	10.42±3.46	t(315)=-5.545 *d*=0.734	<0.001***
FMPS-Ord	23.24±4.68	22.41±4.36	t(315)=-1.438 *d*=0.186	0.152
Suicide risk n (%)				
Yes	35 (55.6)	28 (44.4)	$\chi 2$=30.2981 *V*= 0.309	<0.001
No	53 (20.9)	201 (79.1)		

MBI-EE: Emotional exhaustion subscale of Maslach burnout inventory, MBI-DP: Depersonalization subscale of MBI, MBI-PA: Personal accomplishment subscale of MBI, PHQ-9: Patient Health Questionnaire-9, FMPS-CM: Concern over mistakes subscale of Frost multidimensional perfectionism scale, FMPS-DA: Doubts about actions subscale of FMPS, FMPS-PE: Parental expectations subscale of FMPS, FMPS-PC: Parental criticism subscale of FMPS, FMPS-PS: Personal standarts subscale of FMPS, FMPS-Ord: Order/organization subscale of FMPS, RRS: Ruminative Responses Scale.

*p<0.05,**p<0.01, ***p<0.001

Table S2. Correlations between work-related variables and burnout, depressive symptoms and rumination

	Age	Financial satisfaction	Years of experience	Daily Examined Patients	Number of NSs	Number of OCSs	Working Hours
MBI-EE	r=-0.199 p<0.001***	r=-0.326 p<0.001***	r=-0.15 p=0.008**	r=0.207 p<0.001***	r=0.267 p<0.001***	r=-0.096 p=0.089	r=0.26 p<0.001***
MBI-DP	r=-0.296 p<0.001***	r=-0.164 p=0.003**	r=-0.278 p<0.001***	r=0.121 p=0.031*	r=0.223 p<0.001***	r=-0.199 p<0.001***	r=0.242 p<0.001***
MBI-PA	r=0.215 p<0.001***	r=0.182 p=0.001**	r=0.223 p<0.001***	r=-0.04 p=0.479	r=-0.202 p<0.001***	r=0.188 p=0.001	r=-0.181 p=0.001**
PHQ-9	r=-0.214 p<0.001***	r=-0.198 p<0.001***	r=-0.145 p=0.01*	r=0.058 p=0.3	r=0.212 p<0.001***	r=-0.079 p=0.161	r=0.206 p<0.001***
RRS-Other	r=-0.176 p=0.002**	r=-0.117 p=0.037*	r=-0.125 p=0.026*	r=0 p=0.995	r=0.111 p=0.049*	r=-0.07 p=0.216	r=0.102 p<0.001***
RRS-Work	r=-0.163 p=0.004**	r=-0.173 p=0.002**	r=-0.109 p=0.053	r=0.109 p=0.054	r=0.202 p<0.001***	r=-0.084 p=0.137	r=0.204 p<0.001***

MBI-EE: Emotional exhaustion subscale of Maslach burnout inventory, MBI-DP: Depersonalization subscale of MBI, MBI-PA: Personal accomplishment subscale of MBI, PHQ-9: Patient Health Questionnaire-9, RRS: Ruminative Responses Scale.

*p<0.05,**p<0.01, ***p<0.001

Table S3. Correlations between burnout, depressive symptom severity, rumination and perfectionism

	MBI-EE	MBI-DP	MBI-PA
PHQ-9 Total	r=0.638 p<0.001***	r=0.45 p<0.001***	r=-0.365 p<0.001***
RRS-Other	r=0.449 p<0.001***	r=0.367 p<0.001***	r=-0.327 p<0.001***
RRS-Work	r=0.584 p<0.001***	r=0.423 p<0.001***	r=-0.324 p<0.001***
FMPS-CM	r=0.357 p<0.001***	r=0.274 p<0.001***	r=-0.315 p<0.001***
FMPS-PS	r=0.204 p<0.001***	r=0.178 p=0.001**	r=-0.032 p=0.569
FMPS-PE	r=0.194 p=0.001	r=0.126 p=0.025*	r=-0.076 p=0.177
FMPS-PC	r=0.24 p<0.001***	r=0.2 p<0.001***	r=-0.172 p=0.002**
FMPS-DA	r=0.342 p<0.001***	r=0.288 p<0.001***	r=-0.295 p<0.001***
FMPS-Ord	r=0.11 p=0.049*	r=0.045 p=0.424	r=0.007 p=0.897

MBI-EE: Emotional exhaustion subscale of Maslach burnout inventory, MBI-DP: Depersonalization subscale of MBI, MBI-PA: Personal accomplishment subscale of MBI, PHQ-9: Patient Health Questionnaire-9, FMPS-CM: Concern over mistakes subscale of Frost multidimensional perfectionism scale, FMPS-DA: Doubts about actions subscale of FMPS, FMPS-PE: Parental expectations subscale of FMPS, FMPS-PC: Parental criticism subscale of FMPS, FMPS-PS: Personal standarts subscale of FMPS, FMPS-Ord: Order/organization subscale of FMPS, RRS: Ruminative Responses Scale

*p<0.05,**p<0.01, ***p<0.001

acta medica

ORIGINAL ARTICLE

Comparison of two main fragmentation methods of amyloid beta fibrils for establishing an Alzheimer disease model in cell culture

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ABSTRACT

Aim: Amyloid beta fibrils have been shown to play a role in plaque formation and aggregation in Alzheimer's disease. Obtaining these fibrils using two main methods and applying them to Alzheimer's modelling is crucial in understanding the pathology of the disease at the molecular level and identify in therapeutic targets. The aim of our study is to determine the optimum sonication parameters using probe and ultrasonic bath sonication laboratory methods and to demonstrate Alzheimer's disease modelling at the cellular level.

Methods: Lyophilized human peptide amyloid beta$_{1-42}$ fibrils (fAβ_{1-42}) were subjected to probe sonication for 1 minute with 1, 3 and 5 second on/off pulse applications at varying ambient temperatures (room temperature, ice and ice surrounded by dry ice [ISDI]) for 20, 40 and 60 cycles, respectively. Then, ultrasonic bath sonication was performed in 10 °C water for 1 hour. The length of the fragmented fibrils was quantified by transmission electron microscopy (TEM). fAβ_{1-42} at different concentrations was applied to SH-SY5Y cell line. The non-toxic dose and time of fAβ_{1-42} application were analysed using the WST-1 assay. Intracellular and extracellular fibrils were visualized with immunofluorescence (IF) labelling.

Results: Although, fragmentation was observed under all conditions, it was observed that fibrillar lengths decreased as the on/off pulse times increased, regardless of the number of cycles with ice and dry ice. Additionally, decreasing the temperature increased fibrillar fragmentation.

Conclusion: We anticipate that our study will contribute to the literature by developing an effective and economical sonication method for fibrillar fragmentation with two main laboratory methods and obtaining fAβ_{1-42} that can be used in cells at optimum concentration.

Keywords: Amyloid beta, Alzheimer disease, sonication method, fragmentation, SH-SY5Y

INTRODUCTION

Amyloidosis is a broad term that encompasses various protein misfolding diseases, all characterized by the accumulation of soluble precursor proteins into insoluble aggregates outside the cell. It is known that this fibrillar structure, which is unbranched and has a diameter of approximately 10 nm, generally has non-parallel peptides that form a beta-pleated structure [1]. In the pathology of Alzheimer's disease (AD), amyloid beta (Aβ) peptides, produced through the breakdown of amyloid precursor protein (APP), play a significant role as they aggregate to form amyloid plaques [2]. Although the main cause of AD - characterized by dementia, memory problems and learning difficulties due to the neuron damage - [3], is not yet fully understood, it has been stated that Aβ plaques

can accumulate outside the nerve cells and disrupt the signalling transmission between them, leading to functional losses such as memory impairment as a result of brain damage [4]. Furthermore, this accumulation has been suggested to trigger immunity by causing neuroinflammation [5].

The significance of Aβ oligomer internalization in the pathogenesis of AD has been demonstrated in numerous studies. One study provided evidence that the uptake of oligomers via pinocytosis increases neurotoxicity, while a reduction in toxicity induction is observed in the absence of intracellular uptake [6]. Additionally, research has shown that inhibiting endocytosis prevents neuronal damage, suggesting that intracellular amyloid beta$_{1-42}$ (Aβ$_{1-42}$) plays a more critical role in neurodegeneration than extracellular aggregates [7]. Another study indicated that intracellular Aβ impairs intracellular trafficking and organelle movement, potentially contributing to synaptic damage [8]. Furthermore, Aβ oligomer internalization has been shown to potentiate tau pathology, leading to increased synaptic toxicity [9]. Consequently, preventing Aβ oligomer internalization has been emphasized as a potential therapeutic target in AD research.

The APP, a type 1 transmembrane structure, is encoded by the *APP* gene located on human chromosome 21 [10-12]. This protein, which is particularly expressed in the central nervous system and has a role in various physiological processes, consists of 639-770 amino acids [13]. The processing of APP into smaller fragments, including the ~4 kDa Aβ peptide [14], is carried out by three key secretases: α-secretase, β-secretase, and γ-secretase [15]. In amyloidogenic and non-amyloidogenic pathways involving these secretases, the soluble APP domain is generated through the combined action of α- and γ- secretase [15], while the insoluble Aβ$_{1-42}$ form is produced via the cooperation of β-secretase and γ-secretase [16]. During plaque formation, Aβ$_{1-40}$ and Aβ$_{1-42}$ forms are the main components. With the increase in the Aβ$_{1-42}$/Aβ$_{1-40}$ ratio in favour of Aβ$_{1-42}$, this structure, promotes protein misfolding, forms protein aggregates and plaques [2], causing toxicity for cells. These plaques stimulate immune system cells and cause inflammation, which further contributing to damage to neurons [16].

In addition to the frequent use of animal models to study human diseases, their evolutionary differences may affect the accuracy of scientific findings [17]. *In vitro* methods offer significant advantages for investigating tau and amyloid pathology, analysing biochemical products and organelles, testing therapeutic agents, and understanding AD, as they better represent the natural microenvironment of the pathology [18]. Both *in vitro* and *in vivo* studies have demonstrated that high molecular weight oligomers, known as protofibrils, not only increase amyloid plaque aggregation but also contribute to neuronal degeneration and cognitive impairment [19]. It has been reported that utilizing these methods is beneficial for gaining a deeper understanding of the disease mechanism and exploring potential solutions. Specifically, they provide valuable insights into the structural intermediates and fibrils formed during the aggregation process, starting from high-yield recombinant Aβ peptide and its oligomeric building blocks [20].

In addition to the fact that the sonication method has been shown to promote Aβ aggregation [21], it has also been observed that proteins associated with the disease or not associated with the disease form amyloid during sonication [22]. Changes in the covalent bond structure of proteins, along with factors such as high temperature, elevated pH levels, or alcohol addition, can disrupt protein stabilization and promote amyloid aggregation *in vitro*. Sonication contributes to amyloid pathology, which stimulates immunity in the living organism and leads to disease, by disrupting protein stabilization [21]. Transmission electron microscopy (TEM) can be employed to assess the length and diameter of fibrils obtained post-sonication, verifying through imaging whether small fibrils of homogeneous size are produced [23]. However, evidence suggests that molecular conformation and aggregation potential have a greater impact on neurotoxicity than size characteristics [24].

In this study, fibril fragmentation was achieved using both probe and ultrasonic bath sonication methods. Probe sonication involved 20, 40, and 60 cycles under varying pulse durations (1, 3, and 5 seconds on/off) and ambient temperatures (room temperature (RT), ice, and ice surrounded by dry ice [ISDI]). In terms of probe sonication, a pulse duration of 1 second on/off is most commonly used, while RT is the preferred ambient temperature [25,26]. To evaluate a range of pulse durations, we chose 1, 3, and 5 seconds on/off. Although RT is the

most preferred ambient, some studies use ice for sonication [24,27], so we included both conditions for comparison. Additionally, we surrounded the ice with dry ice to further cool the environment, preventing re-fibrillation due to heating and assessing whether more effective fragmentation could be achieved. Moreover, [24] reported the sonication of fibrils for 20 cycles, whereas [28] indicated that fibrils were sonicated for 60 cycles. Therefore, we applied 20, 40 and 60 cycles to determine the optimal sonication cycle. The primary aim was to evaluate the effects of 20, 40, and 60 cycles of probe sonication on fibril fragmentation while maintaining constant pulse durations and ambient temperatures. Additionally, fibrils were subjected to bath sonication for 1 hour at 10 °C, as described by Creed et al. [29], aiming to compare the effects of probe sonication and ultrasonic bath sonication on $A\beta_{1-42}$ fragmentation. Following TEM verification, the immunofluorescence technique was employed to determine whether fibrils associated with AD pathology could be observed in the presence of cells. Thereby, an AD cell model was subsequently established.

MATERIALS AND METHODS

Preparation of Amyloid Beta$_{1-42}$ Fibrils (fAβ_{1-42})

$A\beta_{1-42}$ human peptide (lyophilized-1 mg) were purchased from Peptiteam, Middle East Technical University, Ankara, Turkiye.

$A\beta_{1-42}$ is reported to spontaneously form amyloid fibrils upon incubation of $A\beta_{1-42}$ solution at 37 °C at a concentration that is much higher than the physiological concentration of its in biological fluids [30]. Additionally, it is widely known oligomeric $A\beta_{1-42}$ intermediates, which are denoted fibrillar oligomers, enter the fibril-forming pathway to form fibrils [31]. In order to form fAβ_{1-42}, $A\beta_{1-42}$ peptide was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and dried overnight under laminar flow cabinet. The peptide microfilm was then dissolved in sterile phosphate buffer salin (PBS) (200 µM, pH 7.4). The unaggregated peptide was incubated at 37 °C for 24 h, and then gently mixed to promote aggregation. Next, fAβ_{1-42} was aliquoted and stored -20 °C until it will be used [32] (Table 1).

Sonication of fAβ_{1-42}

Sonications were performed with a probe sonicator and an ultrasonic bath sonicator. Sonication steps were conducted in a biosafety level-2 cabinet in order to prevent exposure to fibrils that may become aerosolized during probe sonication (Figure 1).

Probe Sonication

1. Attach a 3 mm diameter probe to the converter, and set the general sonicator parameters (30% amplitude; the time to 0:01:00). The amplitude value of 30% [33] and a duration of 1 minute were the most commonly used in previous studies [25,26] was also selected for our study. Pulse duration, sonication cycles and ambient temperature were set as stated in Table 2.

2. Thaw fibrils at room temperature (RT) ($24^0C \pm 2$).

3. To achieve a final concentration (20 µM), stock solution of fAβ_{1-42} was diluted with sterile-filtered deionized water (dH_2O) or PBS to 200 µl volume for TEM imaging or cell culture experiments, respectively.

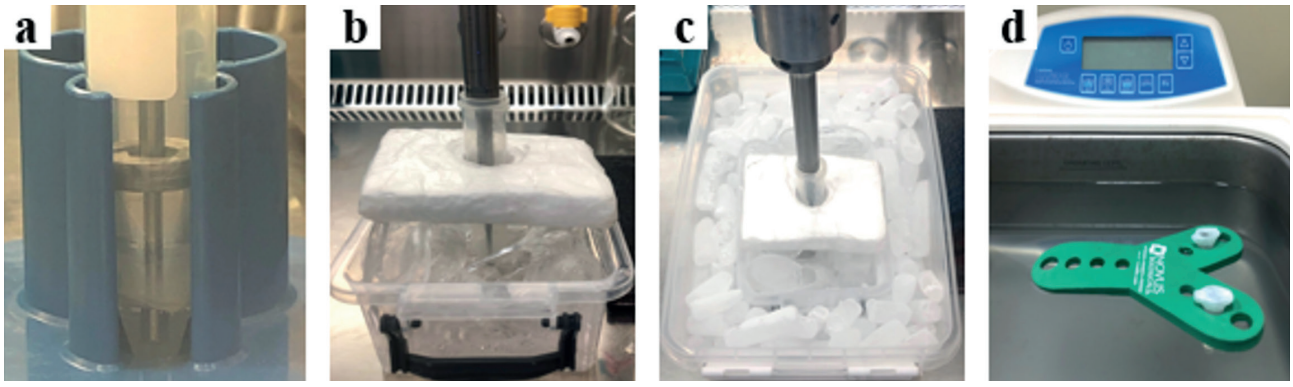


Figure 1. Sonication Images. Probe sonication at RT (a), in ice (b), in ISDI (c), ultrasonic bath sonication (d).

Table 1. Materials used in all experiments

Name	Company	Catalog Number
Antibodies		
Beta Amyloid 1-42 Polyclonal Antibody	Bioss	BS-0107R
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)	Abcam	ab150077
Cell Line		
Human Neuroblastoma Cells (SH-SY5Y)	ATCC	CRL-2266
Equipments		
Steril Conical Centrifuge Tubes, 15 ml	Greiner Bio-One, Cellstar®	
Steril Conical Centrifuge Tubes, 50 ml	Greiner Bio-One, Cellstar®	
Steril Microcentrifuge Tube, 1.5 ml	Greiner Bio-One, Cellstar®	
Parafilm	Parafilm M	PM-996
Stopper	Made in our laboratory	
Lockable Tweezer		
Formvar/Carbon Coated Copper Grids	Ted Pella Inc.	01801
Grids Box	Ted Pella Inc.	
Cell Culture Flask, 75 cm^2	Greiner Bio-One, Cellstar®	658175
Cell Culture Chamber Slide	SPL Life Sciences	30108
Devices		
Centrifuge Device	Hitachi	Himac CT6E
Probe Sonicator	Sonics Vibra-Cell™	VCX 750
Probe	Sonics Vibra-Cell™	630–0422 (microtip)
Utrasonic Bath Sonicator	Branson	CPX5800H
Transmission Electron Microscope	FEI	Tecnai G^2 Spirit BioTwin model
Microplate Reader	Heales	MB-580 Elisa Reader
Fluorescent Microscope	Leica	DM 2000 LED
Proteins		
Human Amyloid Beta1-42	Purchased from Peptiteam, Ankara, Turkiye	
Reagents		
1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP)	Sigma-Aldrich	105228-5G
Uranyl Acetate	Eletron Microscopy Sciences	22400
Dulbecco's Modified Eagle Medium (DMEM)	Capricorn Scientific	DMEM-HA
L-Glutamine	Capricorn Scientific	GLN-B
Penicillin–Streptomycin	Capricorn Scientific	PS-B
Fetal Bovine Serum	Capricorn Scientific	FBS-HI-22A
Trypsine-EDTA	Capricorn Scientific	TRY-3B
WST-1	Roche	Cellpro-Ro-11 644 807 001
Phosphate-Buffer Salin	Capricorn Scientific	PBS-1A
Normal Goat Serum	Capricorn Scientific	GOA-1B
EPI-IHC-Antibody Diluent	EpiplasT	D220520-100 ml
UltraCruz® Aqueous Mounting Medium with DAPI	Santa Cruz Biotechnology	sc-24941-10 ml

4. The probe was cleaned using 70% ethanol and sterile distilled water. Additionally, the probe was sterilized with UV light for 15 minutes.

5. In order to minimize the loss of fibrils, a parafilm-wrapped stopper with a hole was placed inside the sterile conical centrifuge tube (15 ml).

6. Place the probe tip in the middle of the sample.

a. It is important that the probe tip does not touch either the tube or the stopper in order to prevent energy loss.

b. In order to prevent shifting during sonications in ice (-20^{0}C ± 4) and in ISDI (-80^{0}C ± 10), a white foam holder was placed around the sterile conical centrifuge tube.

Table 2. Probe and ultrasonic bath sonication protocols for fibrils

Sonication Technique	Sonication Features		
	Cycles	Pulse (on/off)	Temperature
Probe	20 / 40 / 60	1 second / 1 second	-Room temperature (240C ± 2)
		3 second / 3 second	-Ice (-200C ± 4)
		5 second / 5 second	-Ice surrounded by dry ice (-800C ± 10)
Ultrasonic Bath	1 hour		10 °C (water temperature)

7. Move the probe up and down during each pulse to ensure that all fibrils are sonicated.

8. To prevent the tube from overheating, wait one minute between each 1-minute sonication cycle.

9. In order to collect liquid that splashes onto walls, remove the probe from the sample and briefly centrifuge it at 2000 g for 1 second.

10. Following that, the entire volume was transferred to a sterilized microcentrifuge tube.

11. To clean the probe, wipe it with lab tissue dampened with 1% SDS, 70% ethanol, and distilled water, respectively. Then, dry the probe with a lab tissue.

12. Detach the probe from the converter and store.

13. After cleaning the cabinet with 1% SDS, wipe it down with 70% ethanol.

a. The 1% SDS solution was used to dissociate fibrils and clean surfaces and equipments.

Ultrasonic Bath Sonication

1. The temperature of the distilled water was set to 10°C in the bath tank.

2. The entire sample volume (200 µl) was immersed in water using a holder.

3. Sonication was performed for one hour.

Transmission Electron Microscopy for $fA\beta_{1-42}$ Visualization

The bench surface was covered with a parafilm. Copper grids (200 mesh, Ted Pella Inc.), coated with formvar and carbon, were washed twice on the drops of deionized water (dH_2O) for one minute each time. After washing, the grids were gently blotted with filter paper. The grids were then floated on a drop of $fA\beta_{1-42}$ sample for 3 minutes and blotted again with filter paper. Next, the grids were negatively stained by applying two 1% (w/v) uranyl acetate drops for one minute each and filtered to remove excess uranyl acetate. Afterward, was removed by wicking with filter paper. Finally, the grids were stored in a grid box until imaging. The TEM images were taken by the Central Laboratory at Middle East Technical University, Ankara, Turkiye, using a Tecnai G^2 Spirit BioTwin TEM (FEI) at 65,000× magnification. The grids were examined at a magnification of 65000 using a TEM (Tecnai G^2 Spirit BioTwin, FEI). The microscope was equipped with a LaB6 gun operated at an acceleration voltage of 80 kV. Fibril lengths were measured using ImageJ 1.53k software for semi-quantitative analysis [34,35].

Cell Culture

Human neuroblastoma cells (SH-SY5Y) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM)-high glucose supplemented with 10% foetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin/streptomycin. Cells were maintained in T75 flasks at 37 °C in a 5% CO_2 atmosphere. Cells from passage 2 were used in the experiments. For assays, cells were plated on 96-well plates or 8-well chamber slides and grown to 70–80% confluence [36].

Assessment of $A\beta_{1-42}$ Administration by WST-1 Assay

Cell viability following $A\beta_{1-42}$ treatment was assessed using the WST-1 assay (Roche, Cat no 11 644 807 001) to determine the optimal concentration of Aβ and treatment time for inducing Aβ pathology. SH-SY5Y cells were seeded at 4×10^3 cells/well in 96-well plates with 200 µL growth medium [37].

After 24 hours, the cells were treated with different concentrations of $A\beta_{1-42}$. Samples of $A\beta_{1-42}$ was diluted with the growth medium to prepare serial concentrations (1 µM, 5 µM, 10 µM, 20 µM; [38,39]). At hours 24 and 48 after $A\beta_{1-42}$ treatment, 10 µL of WST-1 solution was added to the each well and incubated for 4 hours. Absorbance was measured at 450–600 nm using a microplate reader (Heales, MB-580 Elisa Reader, India), and cell viability was calculated. Each concentration and time point was tested in triplicate [37].

Immunofluorescence Staining for SH-SY5Y Cells

For immunofluorescence, SH-SY5Y cells were cultured on 8-well chamber slides. After fixation with 100% methanol for 5 minutes, cells were washed with PBS. Serum blocking was performed using 1:50 goat serum for 1 hour. The primary antibody (bs-0107R, $A\beta_{1-42}$ Polyclonal Antibody, Bioss, USA, 1:200) was incubated with cells at room temperature for 2h. After washing with PBS, cells were incubated with seconder antibody (ab150077, Abcam, USA, 1:1000) in the dark and RT conditions for 1h. Nuclei were stained with DAPI (H-2000, Vector Laboratories, USA). The slides were analyzed using a fluorescent microscope. In 400X magnification, micrographs were captured and analyzed.

Statistical Analysis

TEM data were expressed as mean ± standard error of the mean (SEM) and analyzed using GraphPad Prism Version 7.0 (GraphPad Software Inc., San Diego, CA). The normality of the experimental data was assessed using the Shapiro–Wilk test. For comparisons between multiple groups, one-way ANOVA followed by Dunnett's multiple comparisons test was applied for normally distributed data, while the Kruskal-Wallis test followed by Dunn's multiple comparisons test was used for non-normally distributed data. For comparison of two groups (ultrasonic bath analysis), the Mann-Whitney test was employed. A p-value < 0.05 was considered statistically significant.

RESULTS

Transmission Electron Microscopy for $fA\beta_{1-42}$ Visualization

In our study, we aimed to expose Aβ fibrils to different sonication conditions (20, 40 and 60 cycles with probe sonication and ultrasonic bath) and various ambient temperatures (RT, in ice, and in ISDI) to fragment the fibrils to the required for cellular uptake and AD model formation. To assess the efficacy of these methods, we measured the fibril length using TEM and calculated the mean length.

When evaluating probe sonication parameters, a statistically significant reduction in fibril length was observed in all groups compared to the control. Notably, the extension of the pulse time led to successful fibril fragmentation, irrespective of the ambient temperature and number of cycles. Among these results, fibrils were significantly broken in all on/off pulse groups compared to the control group in the groups with 20, 40 and 60 cycles (Table 2). As the on/off pulse duration increased from 1 second to 3 and 5 seconds, fibril fragment lengths decreased (Table 3, Figure 2, 3, 4 and 5).

Fibrils must be shorter than 100 nm to internalize into cells and induce intracellular neuropathology [40,41]. Accordingly, among the probe sonication methods tested, the average length measurements of fibrils after the following treatments were all below 100 nm: 20 cycles with 5 second on/off pulse at ice (mean length: 86.97±4.653 nm), 40 cycles with 5 second on/off pulse at RT, and 40 cycles with 5 second on/off pulse in ISDI (mean lengths: 89.93±5.988 nm and 96.79±9.662 nm, respectively); 60 cycles with 5 second on/off pulse at RT, and 60 cycles with 5 second on/off pulse in ISDI (mean lengths: 96.64±3.09 nm and 98.93±7.298 nm, respectively). The average fibril length obtained from ultrasonic bath sonication (mean length: 92.62±2.755 nm) also fell below 100 nm (Table 3, Figure 2, 3, 4 and 5). These results demonstrate that decreasing the temperature and extending the on/off pulse duration in probe sonication significantly enhance fibril fragmentation.

Table 3. Mean of fibrillar lengths of all groups

20 CYCLES – 100 nm Room Temperature (RT)				
	Control	**1 sec on/off**	**3 sec on/off**	**5 sec on/off**
Mean	386.8	259.6	208.9	183.5
SEM	19.66	5.871	11.04	8.808
Ice				
	Control	**1 sec on/off**	**3 sec on/off**	**5 sec on/off**
Mean	386.8	215.7	198.9	186.92
SEM	19.66	11.69	12.17	4.653
Ice surrounded by dry ice				
	Control	**1 sec on/off**	**3 sec on/off**	**5 sec on/off**
Mean	386.8	202.1	177.4	141.7
SEM	19.66	8.857	13.66	7.63
40 CYCLES – 100 nm RT				
	Control	**1 sec on/off**	**3 sec on/off**	**5 sec on/off**
Mean	386.8	221.9	193.4	89.93
SEM	19.66	9.571	9.759	5.988
Ice				
	Control	**1 sec on/off**	**3 sec on/off**	**5 sec on/off**
Mean	386.8	226.7	200.5	136.2
SEM	19.66	6.503	15.89	10.18
Ice surrounded by dry ice				
	Control	**1 sec on/off**	**3 sec on/off**	**5 sec on/off**
Mean	386.8	221.2	153.5	96.79
SEM	19.66	11.52	9.52	9.662
60 CYCLES – 100 nm RT				
	Control	**1 sec on/off**	**3 sec on/off**	**5 sec on/off**
Mean	386.8	215.6	170.3	96.64
SEM	19.66	13.5	13.51	3.09
Ice				
	Control	**1 sec on/off**	**3 sec on/off**	**5 sec on/off**
Mean	386.8	198	160.6	148.8
SEM	19.66	5.766	12.25	8.645
Ice surrounded by dry ice				
	Control	**1 sec on/off**	**3 sec on/off**	**5 sec on/off**
Mean	386.8	119.4	149.2	98.93
SEM	19.66	9.292	13.26	7.298
ULTRASONIC BATH – 100 nm				
	Control		**Bath**	
Mean	386.8		92.62	
SEM	19.66		2.755	

All values are nanometers (SEM: standard error of the mean).

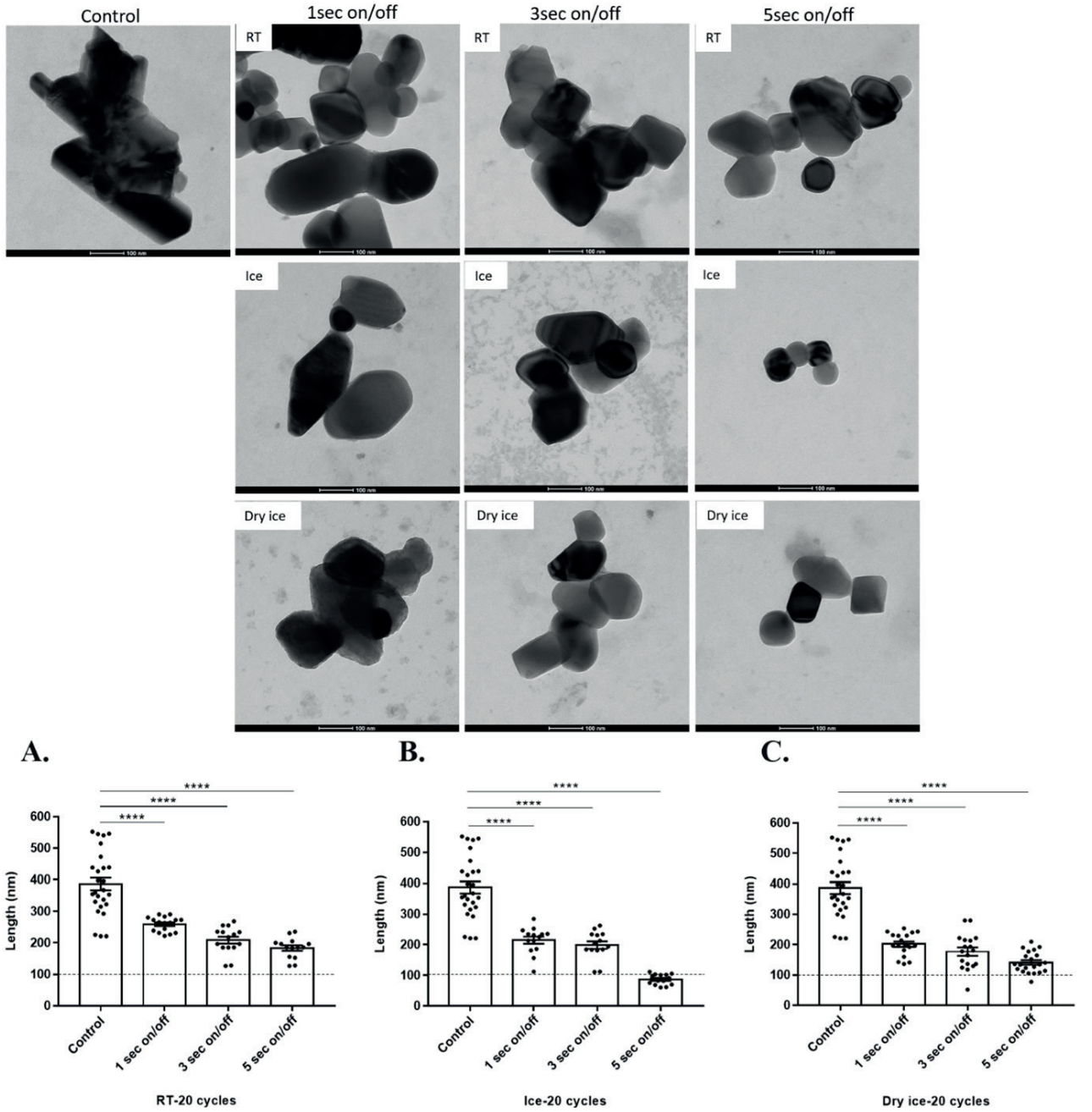


Figure 2. Changes in fAβ1-42 length, following 20 cycles probe sonication in different temperature and pulse duration.

The fibrils were significantly broken in all pulse groups compared to control group (A) at RT (****p=0.0001; one-way ANOVA followed by post hoc Dunnett's multiple comparisons test), (B) in ice (****p=0.0001; one-way ANOVA followed by post hoc Dunnett's multiple comparisons test), and (C) in ISDI (****p=0.0001; one-way ANOVA followed by post hoc Dunnett's multiple comparisons test, scale bars are 100 nm).

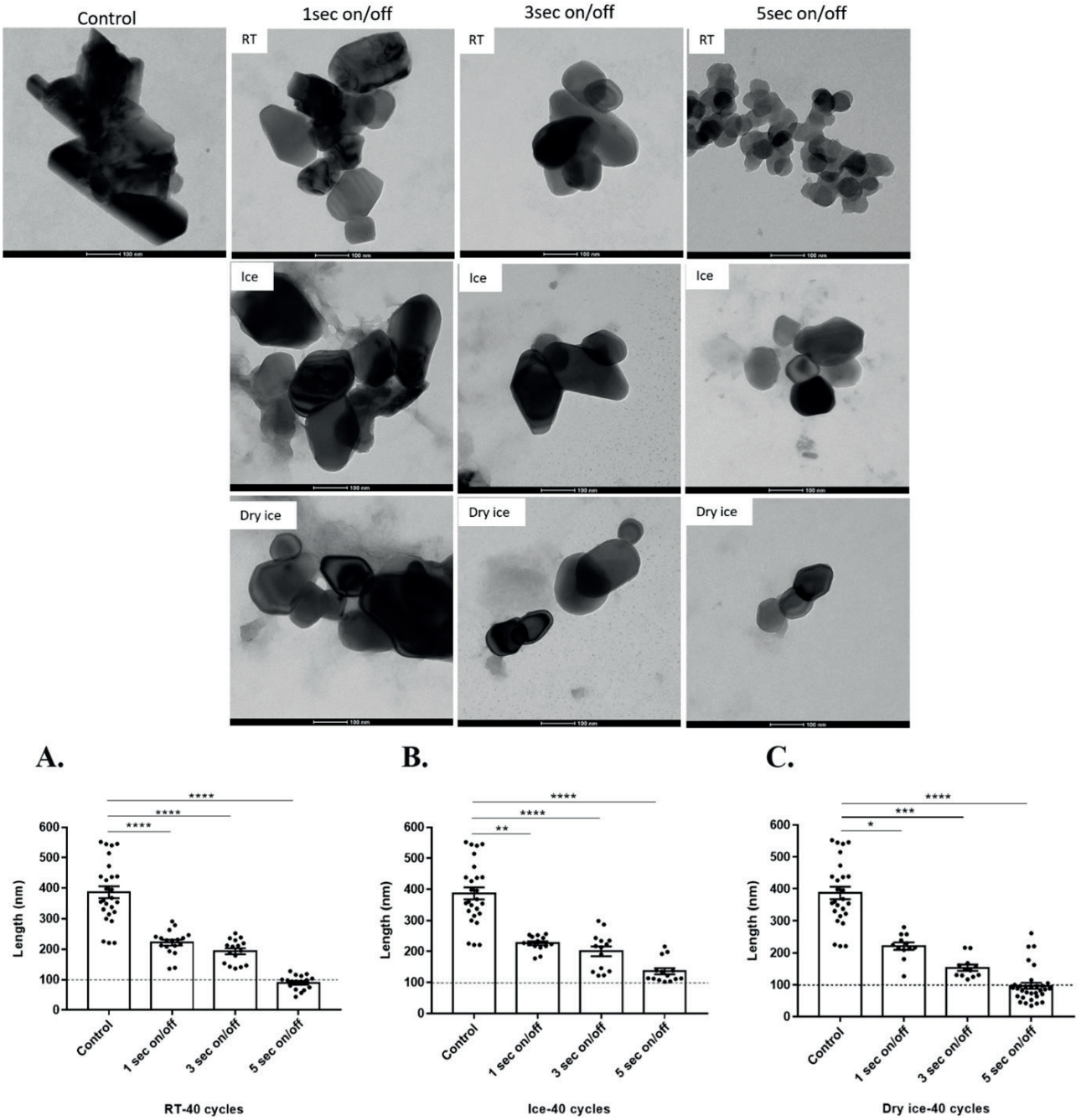


Figure 3. Changes in fAβ_{1-42} length, following 40 cycles sonication in different temperature and pulse duration.

The fibrils were significantly broken in all pulse groups compared to control group (A) at RT (****p=0.0001; one-way ANOVA followed by post hoc Dunnett's multiple comparisons test), (B) in ice (**p=0.0023; ****p<0.0001, Kruskal Wallis followed by post hoc Dunn's multiple comparisons test), and (C) in ISDI. (*p=0.0424, ***p=0.0002, ****p<0.0001; Kruskal Wallis followed by post hoc Dunn's multiple comparisons test, scale bars are 100 nm).

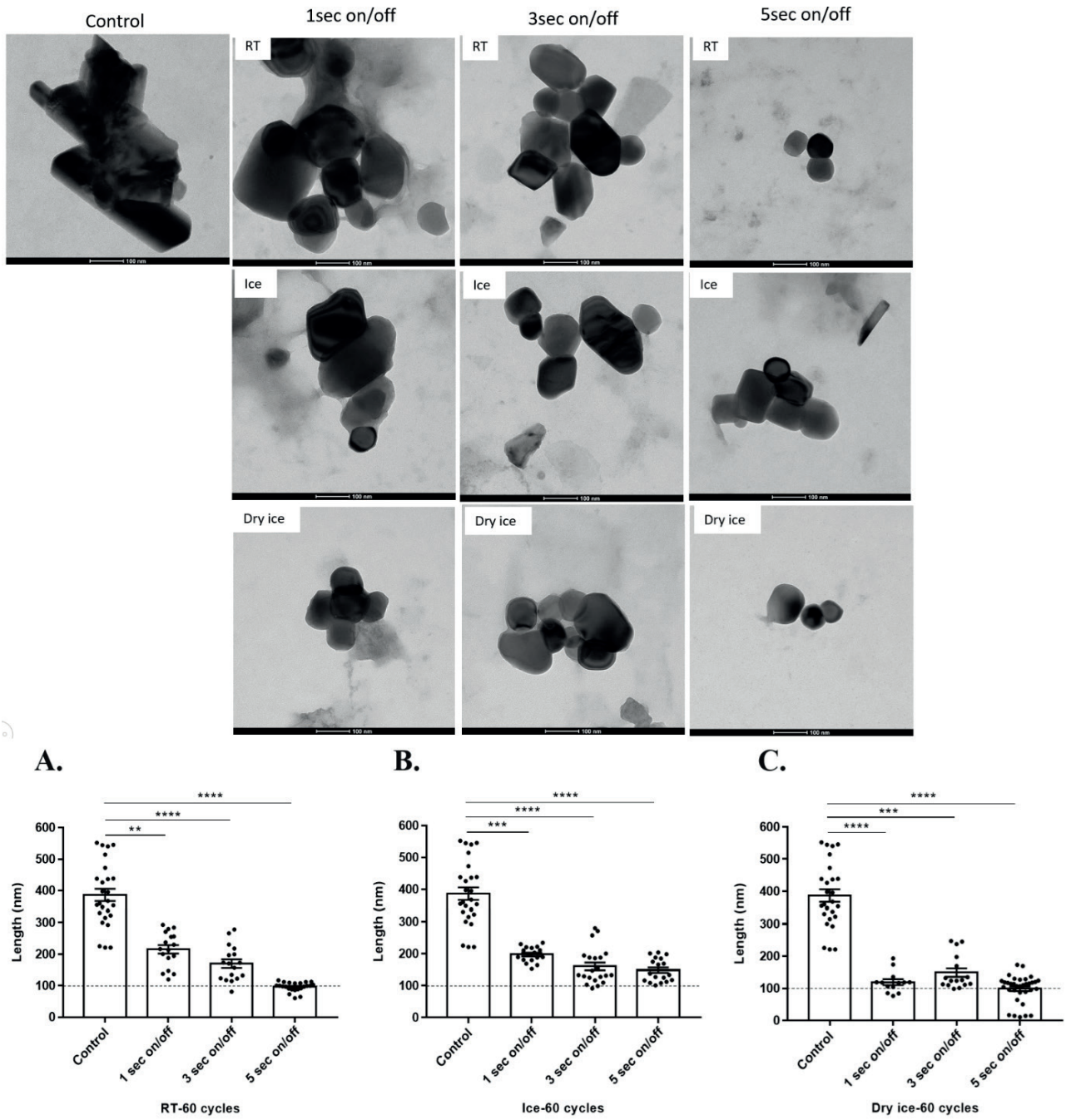


Figure 4. Changes in fAβ_{1-42} length, following 60 cycles sonication in different temperature and pulse duration.

The fibrils were significantly broken in all pulse groups compared to control group (A) at RT (**p=0.0058, ****p<0.0001; Kruskal Wallis followed by post hoc Dunn's multiple comparisons test), (B) in ice (***p=0.0005; ****p<0.0001, Kruskal Wallis followed by post hoc Dunn's multiple comparisons test), and (C) in ISDI. (***p=0.0002, ****p<0.0001; Kruskal Wallis followed by post hoc Dunn's multiple comparisons test, scale bars are 100 nm).

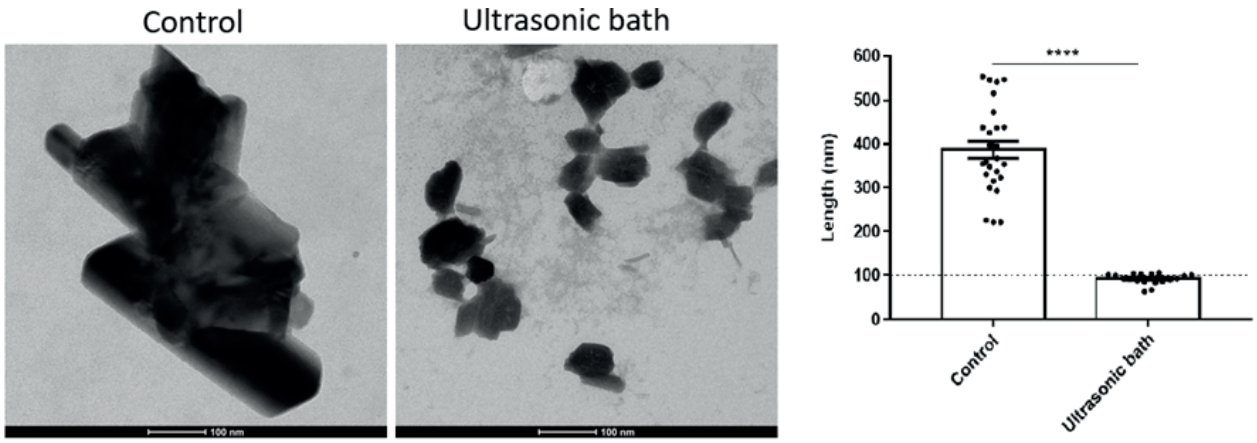
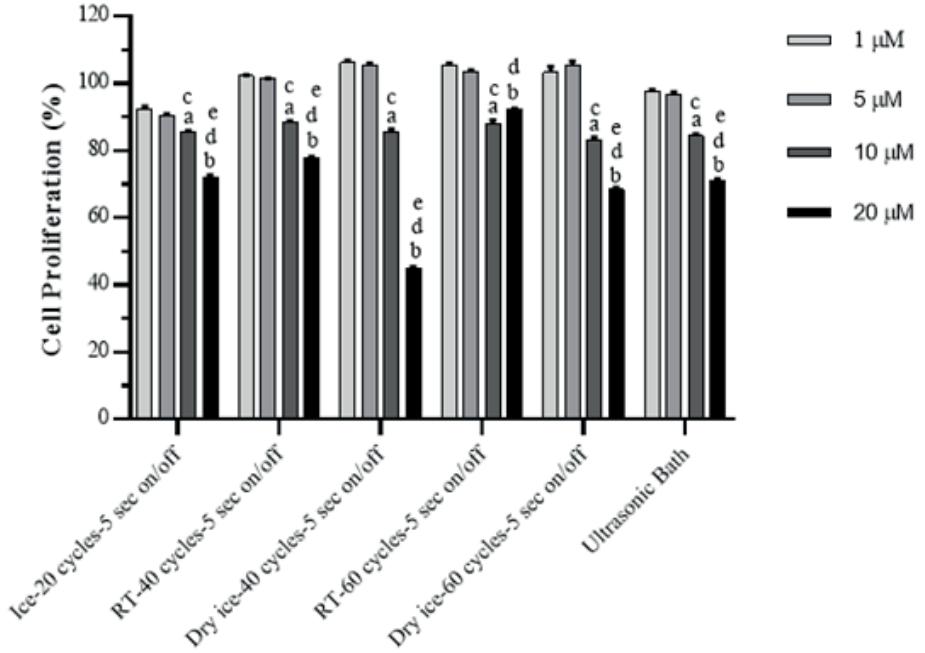


Figure 5. Changes in $fA\beta_{1-42}$ length, following ultrasonic bath sonication.

The fibrils were significantly broken in ultrasonic bath group compared to control group (****p<0.0001; Mann Whitney test, scale bars are 100 nm).

A



B

	1 µM		5 µM		10 µM		20 µM	
Sonication	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
1	92.430	0.840	90.368	0.570	85.732	0.344	72.077	0.681
2	102.353	0.164	101.527	0.132	88.575	0.320	77.756	0.478
3	106.137	0.784	105.383	0.617	85.534	0.927	45.115	0.364
4	105.316	0.781	103.673	0.359	88.164	1.020	92.487	0.118
5	103.311	1.795	105.476	1.184	83.211	0.847	68.549	0.541
6	97.825	0.462	96.707	0.736	84.545	0.525	70.973	0.594

Figure 6. A: The percentage of cell proliferation after administration with different concentrations of selected sonication species at 48 hours. **B:** Sonication species: 1: Ice-20 cycles-5 second on/off; 2: RT-40 cycles-5 second on/off; 3: Dry-ice-40 cycles-5 second on/off; 4: RT-60 cycles-5 second on/off; 5: Dry ice-60 cycles-5 second on/off; 6: Ultrasonic Bath.

Data are presented as percentages of the control group. Data was given as mean ± SEM of three replicates of experiments. Different letters show statistically significant differences between concentrations in all groups (a p<0.0001 1 µM vs 10 µM; b p<0.0001 1 µM vs 20 µM; c p<0.05 5 µM vs 10 µM; d p<0.0001 5 µM vs 20 µM; e p<0.0001 10 µM vs 20 µM; two-way ANOVA followed by post hoc Tukey).

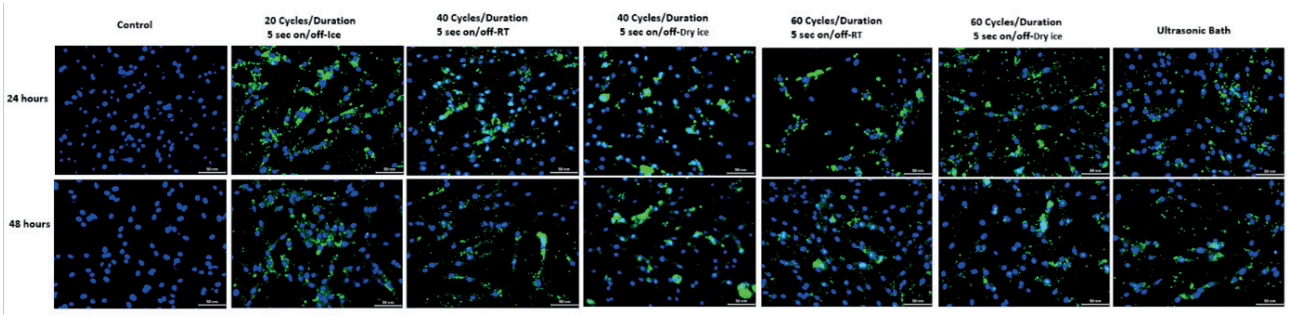


Figure 7. Immunofluorescence staining of all groups.

$A\beta_{1-42}$ accumulations were seen on both intracellular and extracellular areas. As indicated in the reference papers [54,55], images were captured at x40 magnification (Blue: nuclei, green: $A\beta_{1-42}$ protein; scale bars are 50 nm).

Assessment of $A\beta_{1-42}$ Administration by WST-1 Assay

To determine the optimal non-toxic concentration of Aβ fibrils for in vitro application, we employed the colorimetric WST-1 assay. Fibrils with an average length below 100 nm (produced by ice-20 cycles-5 second on/off; RT-40 cycles-5 second on/off; dry-ice-40 cycles-5 second on/off; RT-60 cycles-5 second on/off; dry-ice-60 cycles-5 second on/off; and ultrasonic bath methods) were administered to SH-SY5Y cells at 1, 5, 10, and 20 µM concentrations. Based on the results, 5 µM was identified as the highest non-toxic dose (Figure 6).

Immunofluorescence Staining for SH-SY5Y Cells

To visualize the accumulation of broken Aβ fibrils in SH-SY5Y cells, indirect immunofluorescence was performed. Cells were incubated with fibrils at a 5 µM concentration for 24 and 48 hours. Both intracellular and extracellular accumulation of Aβ fibrils was observed in each group, with a notable increase in accumulation at 48 hours compared to 24 hours (Figure 7).

DISCUSSION

Aβ accumulation is a hallmark of neuroinflammation and cell death in AD [42]. This accumulation disrupts neuronal signalling and contributes to the progression of AD pathology [4]. Understanding the behaviour of Aβ in both *in vivo* and *in vitro* models is critical for identifying potential therapeutic targets [43]. In our study, we compared different sonication methods to find the most effective procedure for fragmenting Aβ fibrils for AD model creation. Our findings indicate that varying the pulse durations (1, 3, and 5 seconds) during sonication significantly contributes to fibril fragmentation. Moreover, performing sonication at lower temperatures (ice or ISDI) enhanced fibril breakdown. Sonication, both with probe and ultrasonic bath methods, has been shown to be effective for producing sufficiently fragmented Aβ fibrils that can be used for AD [21,44].

The aggregation tendency of the fragments obtained by preserving the structural properties of the protein through the sonication method applied to mature fibrils is important for the formation of a realistic model [24]. It has been stated that acoustic cavitations occur with both probe and ultrasonic bath sonication, which are performed to provide a homogeneous distribution and fragmentation within the fibrils. It has been stated that an increase in the duration and power of the mentioned sonication methods reduces the stability of the liquids containing nanoparticles and that there is an optimum point for thermal conductivity [45]. One of the aims of our study was to evaluate the effect of 1-minute sonication performed for 20, 40 or 60 cycles. To prevent protein denaturation during sonication, a 1-minute rest period was applied between each 1-minute sonication cycle. After sonication, TEM imaging was used to observe amyloid fibril morphology and for measuring the length of the fibrils [46]. When the decrease of the fibril lengths in the repeat groups were compared with the control group, it was revealed that the extension of the sonication time was statistically significant, independent of the ambient temperature and the number of cycles. These data are also consistent with previous studies [23]. It was also observed that the lengths of the formed fibril fragments decreased as the on/off times increased, regardless of the number of cycles. The duration of the pulses exerted an effect on the fragmentation of amyloid fibrils. This suggests that different pulse durations may cause changes in the fragmentation of fibrils. Consequently, this study aimed to investigate the effect of different pulse durations

(1, 3 and 5 seconds on/off) on the fragmentation of fibrils. According to TEM images, the findings show that the on/off pulses increasing from 1 second to 3 and 5 seconds, regardless of the number of pulses (20-40 and 60 repetitions), decreased the lengths of fibrils. The working principle of sonication is to create acoustic cavitation and create intense zones of shear with extreme pressure and heat. While it has been stated that Aβ formation can be triggered in these regions at ambient temperatures [44], another study has also reported that accumulation increases with temperature at below the thermal denaturation point (60 ^{0}C) in sonication-induced aggregation and then decreases when the temperature is further increased [21]. In our study, experiments were conducted at RT and in different ice environments. It was observed that fibrils broke in all environments during sonication. The average fibril length showed a proportional decrease in accordance with the decrease in ambient temperature. According to our findings, decreasing the temperature and extending the on/off time increase the breaking success of Aβ fibrils.

The use of *in vitro* assays is important for drug screening because it reduces the use of animals and provides advantages in initial risk screening [47]. Analyses are possible with various cells in which Aβ accumulation can be observed in precursor or mature form [48,49]. Besides, these fibrils were observed in both intracellular and extracellular areas of SH-SY5Y cells and resulted in a time-dependent increase in total Aβ aggregation.

An immunofluorescence analysis of total amyloid density showed that lyophilized human peptide $A\beta_{1-42}$ fibrils were formed by SH-SY5Y cells after 24 and 48 hours of incubation. When reviewing the literature, it has been seen that in similar studies on drug targets in AD models created with the same cell group, the incubation period is generally applied as 24 and 48 hours [36,50-52]. Following this, in these studies intra and extracellular $A\beta_{1-42}$ accumulation was visualized by immunofluorescence. Our results successfully established the pathological image of fragmented fibrils in both spaces produced by different sonication procedures following incubations.

We propose that the sonication methods and the variable parameters utilized in this study may serve as an optimization framework to enhance the diversity of methodologies in the literature for developing AD models. Furthermore, we anticipate that this model, designed for drug targeting applications, will provide a valuable contribution to the existing body of knowledge.

Strengths

Our study provides a comprehensive examination of the sonication parameters that can be used to generate fragmented $fA\beta_{1-42}$ for the creation of an AD cell model. It presents, for the first time, a comparison between probe and ultrasonic bath sonication methods and investigates the impact of different parameters in probe sonication. Moreover, our study makes a significant contribution to the development of a reproducible and cost-effective sonication protocol for $fA\beta_{1-42}$ under diverse laboratory conditions.

Limitations

Although the durations of ultrasonic bath sonication varied in previous studies [23,27], we selected a single duration based on our specifications of ultrasonic bath sonicator. Additionally, while different forms of $A\beta_{1-42}$ are generated under varying incubation times, this study specifically focused on the sonication of $A\beta_{1-42}$ fibrils under a single incubation time, however, different incubation durations could potentially affect the fibril structure [24,25,53].

Author contribution

Study conception and design: EBZ, HY and SZ; data collection: EBZ, HA and SZ; analysis and interpretation of results: EBZ, HA and SZ; draft manuscript preparation: EBZ, HY, HA, CBT and SZ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Hacettepe University Local Ethics Committee for Animal Experiments (Protocol no. 2023/03-06, April 11, 2023). This methods article represents the first phase of the broader research project.

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

Evaluation of outcomes of replantation, acute repair, and revision amputation for digital amputations: A 5-year retrospective study*

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ABSTRACT

Objective: This study aims to objectively assess postoperative hand function by categorizing patients into three groups: replantation, acute stump repair, and revision amputation after unsuccessful replantation. Additionally, functional evaluation questionnaires were used to assess patient satisfaction among these groups.

Materials and Methods: A total of 150 patients were included in the study, 50 patients in each group. Patients' age, gender, comorbidities, dominant hand, the level of amputation, injured fingers and the mechanism of trauma were recorded. Afterwards, the patients were administered the EQ-5D-5L quality of life scale, the Quick DASH test, the Cold Intolerance and the Semmes–Weinstein monofilament test.

Results: A total of 167 finger amputations in 150 patients, 82% of whom were male and 18% were female. Their ages ranged from 19 to 92, and the mean age was 45.5. The mean score in patients who underwent acute repair was higher than in patients who underwent replantation and revision amputation in the EQ-5D-5L scale, the score of the replantation group was minimally lower than in the other two groups for the Quick DASH scoring, sensory results were minimally decreased in the replantation group compared to the revision amputation and acute repair groups. Cold intolerance was reported in 39% of replantation cases compared to 30.3% in revision amputation and 20% in acute repair ($p<0.05$).

Conclusion: Digital amputations were most frequently seen in male patients, in the 3rd finger and at the level of the distal interphalangeal joint. According to the EQ-5D-5L scale, Semmes-Weinstein monofilament test and cold intolerance assessment, the results were worse in the replantation group compared to the other two groups, and better in the Quick DASH score. The advantages and disadvantages of possible treatment options should be explained to the patient and their expectations should be taken into consideration in choosing the treatment for finger amputations.

Keywords: digit, amputation, replantation, reconstruction

INTRODUCTION

Trauma-related hand injuries involving digital amputations constitute a significant percentage of emergency department visits [1]. Restoration of the hand functions of these patients is crucial for enabling them to perform daily life activities optimally. The success of replantation varies depending on the etiology of trauma, but the primary goal in digital reconstruction is to restore both aesthetic and functional integrity. Since the first successful microsurgical replantation over 50 years ago, advances in microsurgical techniques have established replantation as the preferred

treatment for digit amputations [2]. Factors affecting replantation success including trauma etiology, level of amputation, time elapsed since injury and the preservation conditions of the amputated part [3]. Additional factors such as chronic illnesses, medication use, and smoking habits can also affect postoperative outcomes [4].

Successful replantation rates have been reported up to 90% in the literature. However, during follow up period after replantation, vascular complications may necessitate revision amputation despite all efforts. In cases where replantation is not feasible due to improper preservation or absence of the amputated digit, alternative repair options should be considered to maintain digit integrity and length [3,5]. In such cases, acute stump repair, later-stage graft or flap reconstruction may be chosen based on patient expectations and needs [6].

All surgical interventions aim to prevent workforce loss while restoring functional and aesthetic components of the digit [5,6]. This study aims to objectively evaluate postoperative hand function in patients by categorizing them into three groups: replantation, acute stump repair, and revision amputation following unsuccessful replantation. Additionally, functional evaluation questionnaires were used to assess patient satisfaction among these groups.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of Ankara Training and Research Hospital (Decision No: E-24-33). Patients who presented with traumatic digital amputations to our clinic and completed at least a six- month follow up between January 2019 and January 2024 were retrospectively analyzed. Patients younger than 18 years, those who did not compliant to scheduled clinical examinations, and those with additional injuries were excluded. The remaining patients were categorized into three groups: Replantation (Figure 1), Acute stump repair (Figure 2) and Revision amputation (Figure 3), with 50 patients in each group (150 total). Records regarding demographic data including age and gender, dominant hand involvement, comorbidities, as well as amputation level, affected digits, and trauma mechanisms were collected.

Patients completed the EQ-5D-5L quality of life questionnaire [7] and the Quick DASH test [8] which evaluates upper extremity function after injury.

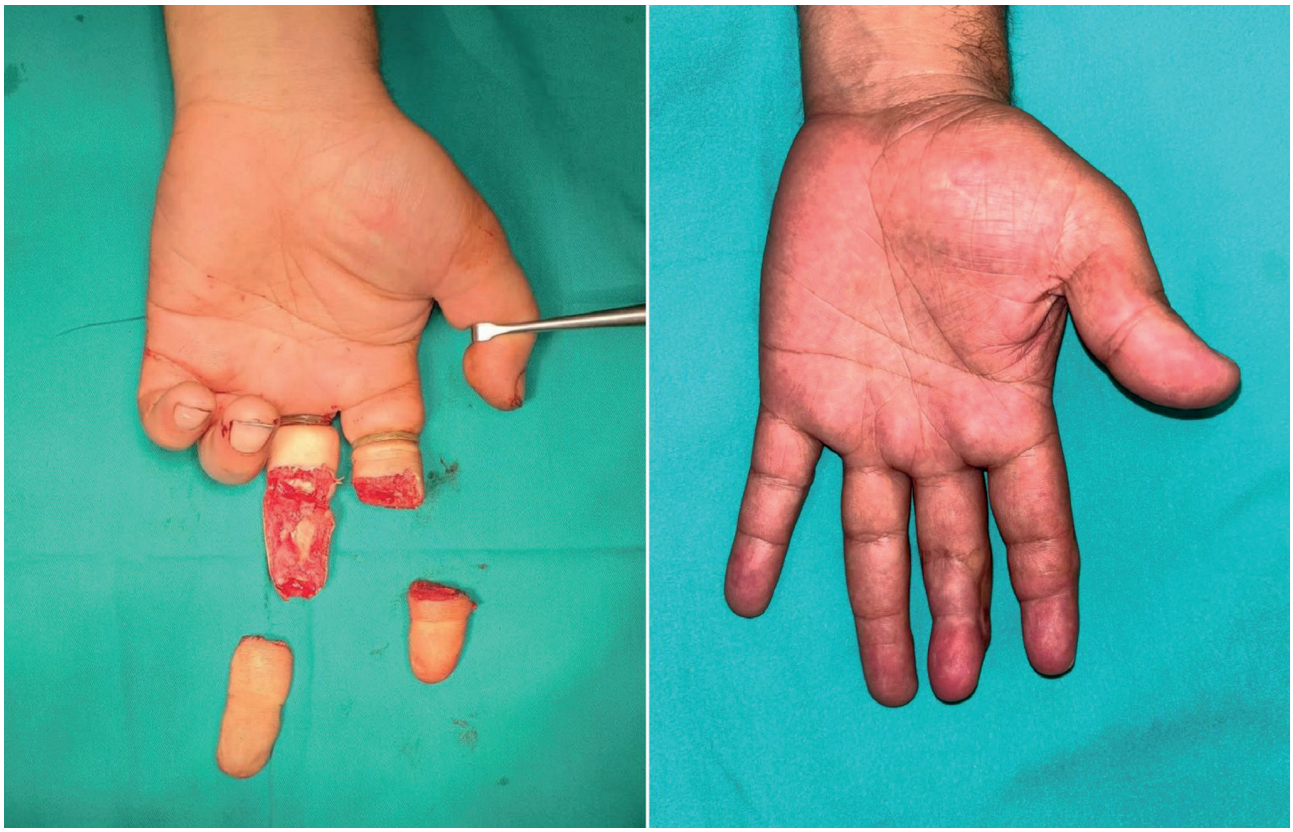

Figure 1. One year post operative result after replantation of two-digit amputation

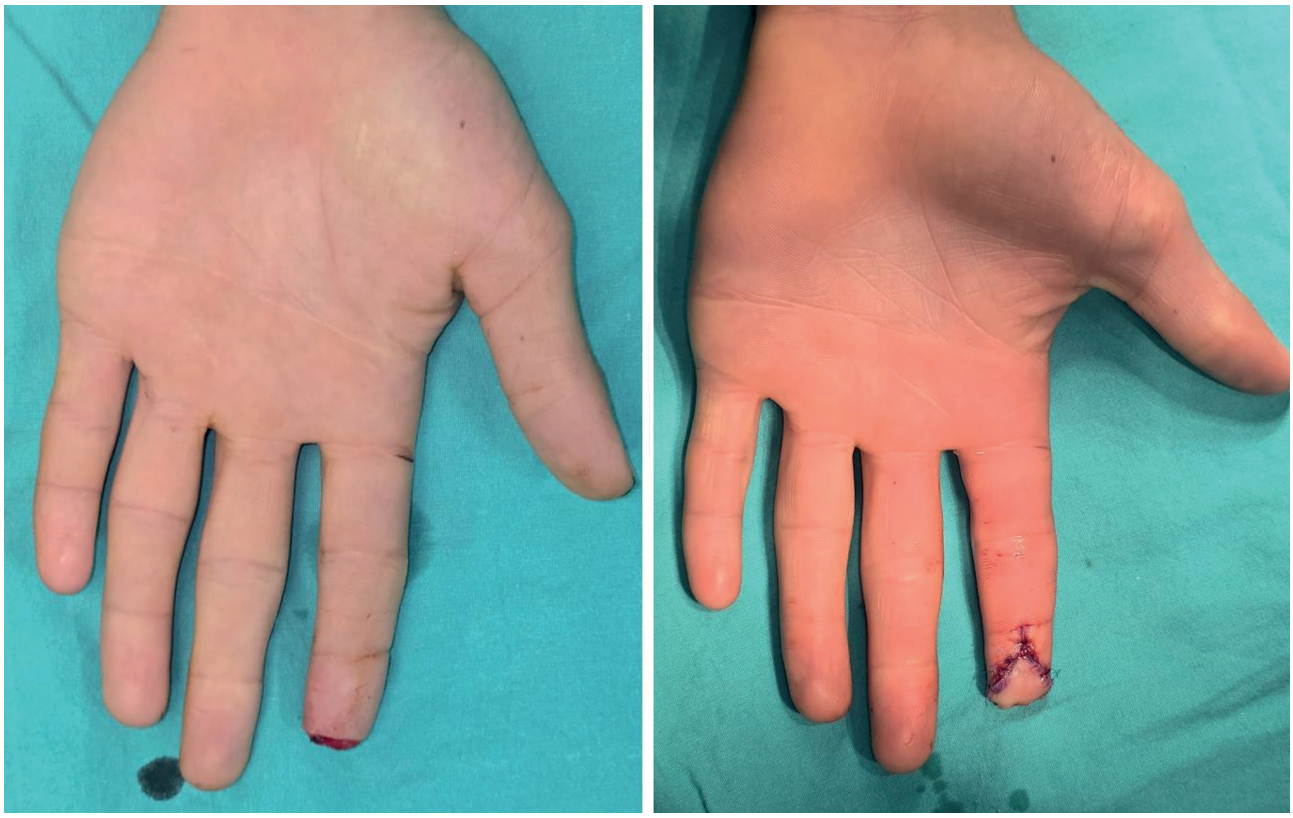

Figure 2. Acute stump repair after amputation injury

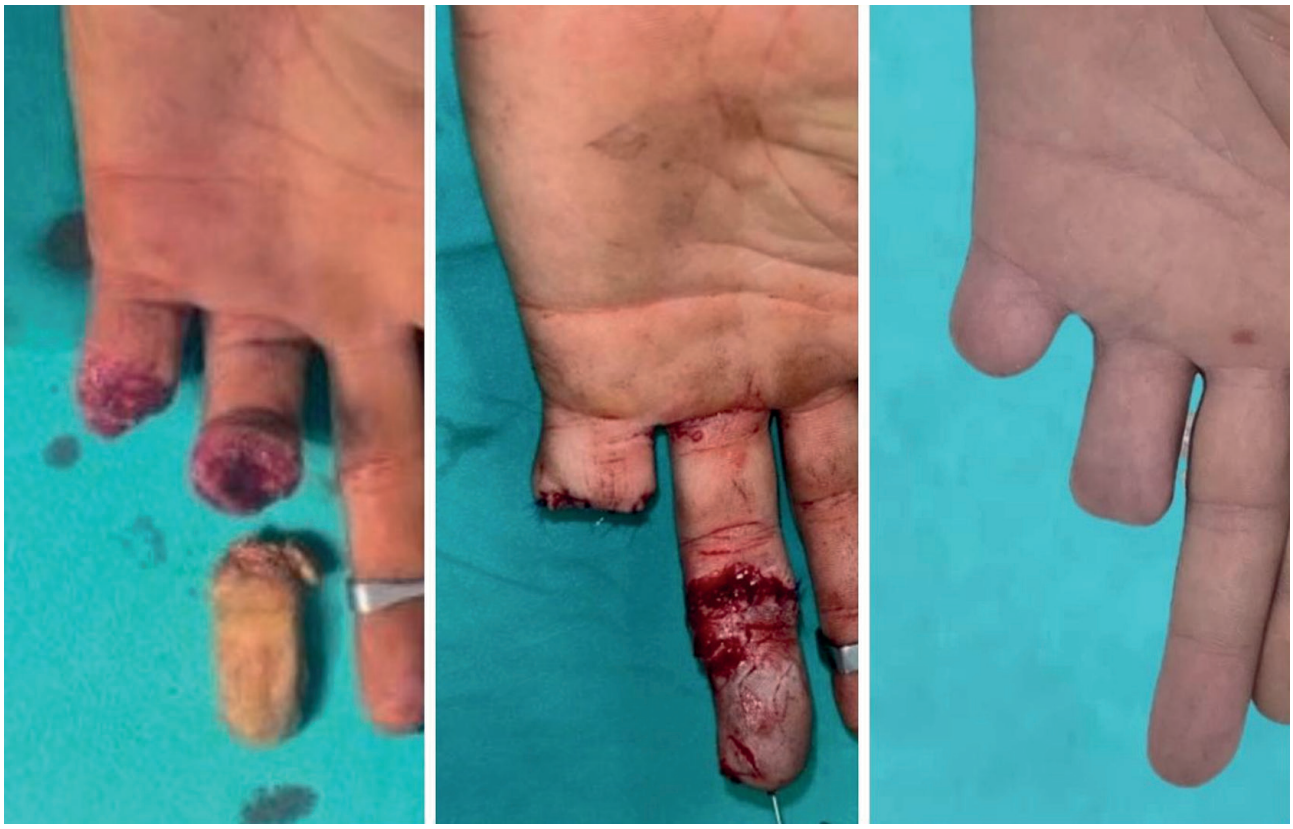

Figure 3. Two-digit amputation, 4th digit had revision amputation after circulatory failure following replantation, 5th digit had acute stump repair

The Semmes-Weinstein monofilament test [9] for sensory assessment was conducted during clinical visits and cold intolerance [10] was evaluated subjectively.

Statistical analysis included the Kolmogorov-Smirnov and Shapiro-Wilk tests to assess normal distribution differences in amputation level and digit involvement. The chi-square test was used for categorical variables when parametrical assumptions were not met. The Mann-Whitney U and Kruskal-Wallis tests were applied to compare quantitative data. SPSS 24.0 (IBM, New York, USA) was used for all statistical analyses, with significance set as $p < 0.05$.

RESULTS

This retrospective study was included 150 patients with 167 digital amputations. Among them, 82% were male and 18% were female. Ages ranged from 19 to 92, with a mean of 45.5 years. Right-hand dominance was observed in 90% of cases whereas 10% of patients were left-handed. Right-hand trauma occurred in 52% of patients, while 48% had left-hand injuries (Table 1).

Single-digit amputations were seen in 88% of cases, while 12% had multiple finger amputations, all involving two digits. The most common amputation mechanism was sharp injury (87 patients), followed by crush trauma (35 patients) and blunt trauma (28 patients). The most frequent amputation level was the distal interphalangeal joint (77 cases), followed by the proximal interphalangeal joint (45 cases), proximal phalanx (37 cases), middle phalanx (6 cases), and metacarpophalangeal joint (2 cases).

EQ-5D-5L scores were highest in the acute stump repair group (74.4), followed by the revision amputation group (72.69) and replantation group (72.4), though differences were not statistically significant ($p>0.05$) (Table 2).

Table 1. Patient demographics and characteristics of injury

	n (%)
Gender	
Male	123 (82)
Female	27 (18)
Age (years)	
Mean	45,52
Range	19-92
Injured Hand	
Right	78 (52)
Left	72 (48)
Dominant Hand	
Right	135 (90)
Left	15 (10)
Mechanism of injury	
Sharp injury	87 (58)
Crush trauma	35 (23,3)
Blunt Trauma	28 (18,7)
Type of amputation	
Multiple digits involved	17 (12)
Single digit injured	133 (88)
Amputation level	
Distal Interphalangeal Joint	77 patients
Metacarpophalangeal joint	2 patients
Midphalanx	6 patients
Proximal Interphalangeal Joint	45 patients
Proximal phalanx	37 patients

The Quick DASH score was reported as 10.5 in the replantation group, 11.2 in the revision amputation and 12.6 in acute stump repair groups but the differences were not significant ($p>0.05$) (Table 2).

Sensory function as assessed by Semmes Weinstein monofilament test was 3.82 in the replantation group, 3.75 in the revision amputation group and 3.56 in acute stump repair group with no significant differences ($p>0.05$) (Table 2).

Cold intolerance was reported in 39% of patients in replantation group, 30.3 % in the revision

Table 2. Results of patient reported outcomes

	EQ-5D-5L Score	Quick DASH Score	Semmes-Weinstein Monofilament Test	Cold Intolerance (%)
Replantation	72.4	10.5	3.82	39
Acute stump repair	74.4	12.6	5.56	20
Revision amputation	72.69	11.2	3.75	30.3
p value	**> 0.05**	**> 0.05**	**> 0.05**	**< 0.05**

amputation group and 20% in the acute stump repair group. Cold intolerance was significantly higher in replantation group compared to other two groups ($p<0.05$) (Table 2).

DISCUSSION

Digital amputations constitute a significant portion of emergency department visits due to hand trauma and predominantly affect young and working-age male patients [11]. These injuries may severely impair occupational performance and daily activities [12]. This results in both revenue loss andhave long-term financial implications [13]. Additionally, due to the highly visible nature of the fingers, amputations can lead to social withdrawal and diminished quality of life [14]. The psychological impact varies based on the patient's mental health, social support, and financial stability, but digital amputations have been associated with depression, anxiety, reduced self-esteem, and, particularly in cases of multiple amputations, a negative outlook on the future [15].

Digital amputations are treated either by acute stump repair or replantation [15]. Acute stump repair is a relatively fast procedure requiring less postoperative rehabilitation; however, digit shortening may compromise hand aesthetics, grip strength, and dexterity. Advances in microsurgery have enabled replantation to restore hand aesthetics and most of its functional abilities. However, digital replantation is a technically demanding procedure requiring prolonged rehabilitation and workforce loss. Furthermore, maintaining high success rates in this complex surgery necessitates experienced surgical teams performing high number of procedures and with 24/7 availability [3,15]. Postoperatively, vascular complications may arise, potentially leading to total necrosis of the replanted digit. The survival of a replanted digit is affected by factors such as patient's comorbidities, smoking habits, and the mechanism of trauma. If necrosis occurs despite all efforts, revision amputation becomes necessary [16].

There is significant heterogeneity in the literature regarding outcome measures and classification systems for traumatic digital amputations. Consequently, studies comparing replantation and revision amputation have reported varying results. For instance, Tessler et al. [17] emphasized the superior outcomes of replantation, whereas another study [18] found no significant difference between these treatment modalities. The paradigm for assessing outcomes in hand surgery has shifted towards patient-reported measures, including general health-related quality of life questionnaires and those specifically targeting upper extremity function [19]. Within this framework, our study aimed to evaluate patient-centered outcomes using questionnaires especially targeted evaluating the general quality of life and upper extremity functions for digital amputations managed with replantation, acute stump repair, and revision amputation over a five-year period in our clinic. The assessment also focused on sensory recovery and cold intolerance.

The EQ-5D-5L questionnaire evaluates health in five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression [20]. In our study, patients who underwent acute stump repair had higher mean EQ-5D-5L scores compared to those in the replantation and revision amputation groups, but the differences were not statistically significant. Similarly to our results, Pyörny et al. used the EQ-5D-5L scale in their study and reported comparable outcomes between replantation, revision amputation, and acute stump repair, concluding that successful replantation was not associated with worse patient-reported outcomes. It was also reported that if the amputated tissue was severely damaged or replantation surgery was unsuccessful, the treatment resulted in revision amputation, which was not associated with worse outcomes than successful replantation [21].

The Quick DASH questionnaire was designed to assess overall health, upper extremity injuries and associated symptoms, and focuses mostly on range of motion, grip strength, and skin sensitivity. This tool aids in evaluating the impact of an intervention on the entire upper extremity, providing crucial insights into daily activity limitations with specific questions [8,19]. In our study, Quick DASH scores were slightly lower in the replantation group compared to the other two groups, though the difference was not statistically significant. This could be clinically important in the decision-making process. Prior studies have similarly demonstrated

better Quick DASH scores in replantation patients compared to amputation groups, likely due to the preservation of finger length and improved range of motion [6,15,22].

The Semmes-Weinstein monofilament test is a well-established sensory assessment tool used to detect abnormal sensory function in specific areas and can be performed with a mobile device. Since its first innovation, Semmes-Weinstein monofilament test is proven to gain trust in detecting abnormal functioning in peripheral nerves. This method has been validated for evaluating nerve dysfunction and remains widely utilized [23]. In our study, sensory outcomes in the replantation group were slightly decreased compared to the revision amputation and acute stump repair groups, though no statistically significant differences were observed. Sensory-functional return is one of the main goals of surgical treatment of finger amputations, and the mechanism of trauma and the condition of the amputated finger should be taken into consideration. Literature findings also indicate poorer sensory recovery in replantation cases compared to amputation groups. For instance, Bott et al. reported that 37.8% of patients in the amputation group retained normal tactile sensation, whereas it was decreased to 21% in the replantation group [24]. Another study analyzing 111 patients found superior sensory recovery in amputation cases compared to replantation [25].

Cold intolerance is defined as an "icy cold sensation lasting for hours and potentially progressing to pain," triggered by exposure to low temperatures [26]. Long-term cold intolerance is among the most frequently reported issues following digital replantation [27]. In a study, cold intolerance was found to be twice as prevalent in the replantation group compared to the stump repair group. Peripheral nerve injury, vascular dysfunction and other factors such as central and humoral mechanisms could be the cause of the higher cold intolerance in the replantation group [25]. Similarly, our study revealed a statistically higher significance of cold intolerance in the replantation group compared to the revision amputation and acute stump repair groups. However, some studies suggest that cold intolerance is primarily attributed to the nature of the trauma itself rather than the reconstruction method used. Thus, it has been argued that cold intolerance should not be considered an absolute contraindication for replantation or a decisive factor in treatment selection [10]. The limitations of our study include its retrospective design and it was conducted at a single center.

CONCLUSION

Digital amputations are most frequently observed in male patients, particularly in the third digit at the distal interphalangeal joint. Postoperative evaluations using EQ-5D-5L, the Semmes-Weinstein monofilament test, and cold intolerance assessments indicate decreased outcomes in the replantation group compared to the other two groups, though Quick DASH scores were better. Prospective studies focusing on individualized patient assessments based on injury characteristics and severity are necessary to suggest specific treatment modalities, improve treatment strategies and functional outcomes.

Author contribution

Study conception and design: EB, HT, YNB, MÇ; data collection: EB, HT, YNB; analysis and interpretation of results: EB, HT, YNB, UK; draft manuscript preparation: EB, YNB, MÇ, UK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ethics Committee of Ankara Training and Research Hospital (Protocol no. E-24-33/22.02.2024).

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Conflict of interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Anemia in the patients with acute myeloid leukemia revisited: Prognostic importance of anemia on treatment-naïve patients

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ABSTRACT

Objective: To determine the independent prognostic influence of pre-treatment anemia severity in patients with acute myeloid leukemia.

Patients and Methods: This was a retrospective evaluation of AML patients between January 2002 and May 2018 at a university hospital hematology clinic. The patients were divided into four groups: intensive treatment achieving complete remission (CR), intensive treatment without CR, non-intensive treatment, and supportive treatment. Baseline clinicodemographic features, laboratory data including serum hemoglobin levels, were collected. Baseline and post-treatment hemoglobin levels were compared according to treatment and across groups. A logistic regression analysis was also made to evaluate the influence of anemia on achieving a complete remission.

Results: The mean hemoglobin level at the time of diagnosis was 8.5 g/dL (6.4 – 14.4). Although hemoglobin value was lower in the secondary AML subgroup, there was no significant difference between the groups at the time of diagnosis ($p = 0.082$). Hemoglobin values after induction chemotherapy were significantly different between treatment groups ($p < 0.001$). When the variables predicting complete remission are examined by logistic regression, per 1 gr/dL increase in hemoglobin level at the time of diagnosis increased the probability of remission significantly ($p = 0.047$, OR = 1.13, 95% CI 1.07 - 1.24).

Conclusion: A patient's baseline pre-treatment serum hemoglobin level can predict the achievement of complete remission in AML patients. Anemia improves with induction chemotherapy, even without complete remission.

Keywords: acute myeloid leukemia, anemia, prognosis, induction chemotherapy

INTRODUCTION

Acute myeloid leukemia (AML), which results from maturation block, abnormal proliferation, and differentiation of hematopoietic stem cells and myeloid progenitor cells, accounts for 80% of acute leukemias in adults [1]. Despite tremendous advances in the understanding of the molecular basis of the disease and a corresponding increase in the therapeutic armamentarium, mortality rates are still far more than acceptable. A national registry from England reported median survival as 0,6 years. 1-year and 5-year overall survival rates were 39.8% and 18.8%, respectively [2].

Not all AML patients fare similar. Variation of the prognostic markers in individual patients determines the fate of the disease. Modern prognostic classification systems such as ELN 2022 mostly rely on the presence or absence of molecular genetic abnormalities [3]. On the other

hand, several easily attainable clinical markers including blood counts, might inform additional prognostic data [4].

Anemia is a near-universal finding in AML patients at the time of the diagnosis [5,6]. The pathophysiologic mechanism is generally assumed as the damaging of erythroid progenitors by myeloblast cells in the bone marrow [7]. Normal erythropoiesis is significantly reduced or absent on bone marrow examination in treatment-naive patients [8].

Few studies examined the independent effect of anemia on the prognosis of AML patients. Tsimberidou et al. evaluated the prognostic significance of beta-2 microglobulin in addition to several clinical and laboratory parameters in AML patients [9]. They found that having a serum hemoglobin (Hgb) level lower than 8 g/dL was associated with a worse prognosis in AML patients who were <60 years old. Data regarding the influence of anemia on different AML subtypes are scarce.

Thus, we intended to evaluate the prognostic importance on anemia in treatment-naive AML patients with a emphasis on AML subtypes.

PATIENTS AND METHODS

Patients and setting

All adult (>17 years old) patients diagnosed with acute myeloid leukemia (AML) treated at the Department of Hematology of Hacettepe University hospitals from January 2002 to May 2018 were retrospectively screened for eligibility for the study. Missing data regarding induction treatment type and remission status, failure to complete the scheduled treatment regimen due to any problem, and history of erythrocyte transfusion within 1 month before diagnosis were determined as exclusion criteria. Hacettepe University Ethics Committee approved the study protocol with the document number 18/428-12.

Data collection

The data had been retrospectively recorded from electronical and/or hard-copy medical records of the patients. We collected patient characteristics, such as age, sex, subtypes of AML, bone marrow findings before and after the treatment, blast percentages in the peripheral blood, type of AML treatment, number of blood transfusions, and blood counts, including serum Hgb levels before and after induction chemotherapy from electronic hospital database system and patient charts. Hgb levels were recorded within one month of completion of treatment and at the end of short-term myelosuppressive episodes following chemotherapy. The maximum Hgb level achieved by patients within one month after completion of treatment, within an increasing trend and not attributable to a recent erythrocyte suspension transfusion, is recorded as the zenith Hgb value. This is used to refer to the post-induction Hgb status.

Chemotherapy regimens

AML was categorized into three groups: acute promyelocytic leukemia (APL), AML other than APL (non-APL AML), and secondary AML.

Complete remission (CR) was defined as per ELN International Working Group criteria [10].

Various intensive chemotherapy regimens with the aim of achieving CR were used, such as idarubicin + cytarabine, mitoxantrone + cytarabine, daunorubicin + cytarabine, etoposide + mitoxantrone + high-dose cytarabine, high-dose cytarabine + mitoxantrone, idarubicin + all-transretinoic acid, and regimens containing high-dose cytarabine. Non-intensive regimens without the aim of CR included hydroxycarbamide, azacitidine, and subcutaneously administered cytosine-arabinoside.

For complete remission evaluation, blast percentages and morphologic features of the bone marrow, cytogenetic analysis, if available, flow cytometry results, and complete blood count findings after the scheduled treatment regimen were used. According to CR evaluation, patients were divided into four groups as those who achieved CR with intensive chemotherapy, those who did not achieve CR with intensive chemotherapy, those who received a non-intensive regimen without a CR intent, and those who received only supportive therapy.

Statistical analysis

The conformity of the variables to normal distribution was evaluated using histograms,

probability graphs, and the Kolmogorov-Smirnov and the Shapiro-Wilk tests. Descriptive statistics were presented as "mean" and "standard deviation (SD)" for normally distributed variables; "median", "minimum-maximum," and "interquartile range (IQR)" for variables not conforming to the normal distribution, and "frequency tables" for nominal and ordinal variables. In case of more than 2 subgroups in categorical variables, the comparison of continuous variables was made by the One way ANOVA test if the normality assumption was met, and with the Kruskal-Wallis test if it was not. The relationship between continuous variables was investigated by the Pearson correlation analysis if the normality conditions were met and by the Spearman correlation analysis, if they were not. We also performed multivariable logistic regression after univariate analyses to determine independent predictors of complete remission. The IBM SPSS Statistics v25 was used for statistical analyses. Statistical significance level was accepted as "$p < 0.05$".

RESULTS

Patients, treatment regimens, and remission

Overall, 276 AML patients (163 males, 59.1%) were retrospectively examined and included in this study. Median age was 53 years (range 40 – 87 years) in the whole group. Table 1 displays the clinical features and demographic characteristics of the study participants.

The most common AML subtype was non-APL AML (194 patients, 70.3%). Number of patients who had APL and secondary AML were 37 (23.4%) and 45 (16.3%), respectively. Regardless of AML type, 229 (83%) patients were treated with an intensive induction chemotherapy regimen with the goal of CR, 28 (10.1%) with the non-intensive regimen, and 19 (6.9%) with supportive care, taking into account their age, comorbidities, and performance status. Patients in the secondary leukemia subgroup received non-intensive chemotherapy regimens more commonly than APL and non-APL AML patients (35.6%, 0%, and 6.2%, respectively. $p<0.0001$). According to the bone marrow and peripheral blood findings performed for response evaluation after the completion of the induction chemotherapy, complete remission (CR) was not achieved in 149 patients (54%), and complete remission criteria were met in 127 patients (46%). As expected, the majority of patients who achieved CR were in the patient group receiving intensive chemotherapy (Table 2). Twenty-eight patients who received chemotherapy did not seek CR, and 19 patients merely received supportive care.

Hemoglobin levels

The patients were divided into three treatment groups, as mentioned above, to better understand the interaction between chemotherapy and serum Hgb levels. Hgb levels at the time of diagnosis did not significantly differ across the four groups ($p=0.888$), although it was lower in the secondary leukemia group.

Table 1. Basic characteristics, treatment type, and remission status of the study participants

		Number (%)	Type of treatment		
			Non-intensive	Intensive	Supportive
Sex	Male	163 (59.1%)	--		
	Female	113 (40.9%)	--		
Type of AML	APL	37 (13.4%)	0	33 (89.2%)	4 (10.8%)
	Non-APL	194 (70.3%)	12 (6.2%)	175 (90.2%)	7 (3.6%)
	Secondary	45 (16.3%)	16 (35.6%)	21 (46.7%)	8 (17.8%)
Type of treatment	Intensive	229 (83.0%)	--		
	Non-intensive	28 (10.1%)	--		
	Supportive	19 (6.9%)	--		
Status of complete remission	Yes	127 (46.0%)	--		
	No	149 (54.0%)	--		

AML: Acute Myeloid Leukemia, APL: Acute Promyelocytic Leukemia

Table 2. Pre- and post-treatment serum Hgb levels in groups according to AML subtype, treatment type, and CR attainment status

Patient group	Subgroup	Pre-Treatment serum hemoglobin (g/dL)	Post-Treatment serum hemoglobin* (g/dL)	p-value
AML type	APL	8.7 ± 2.0	10.4 ± 1.6	p=0.063**
	NonAPL	8.5 ± 1.7	10.3 ± 1.5	
	Secondary	8.0 ± 1.0	8.4 ± 1.6	
Treatment type	Intensive	8.5 ± 1.8	10.3 ± 1.5	**p=0.006*** **
	Non-intensive	8.2 ± 1.4	9.2 ± 1.9	
	Supportive	8.2 ± 0.7	8.3 ± 1.1	
Remission status	Complete remission	8.8 ± 1.9	10.8 ± 1.4	**p=0.048[α]**
	No Complete remission	8.2 ± 1.4	9.5 ± 1.6	p=0.062[β]

*Zenith Hgb level achieved after chemotherapy, **comparison of pre-treatment serum hemoglobin level in three leukemia subtypes, ***Comparison of post-treatment serum hemoglobin levels between non-intensive chemotherapy and supportive therapy groups. [β] Comparison of pre-treatment serum hemoglobin levels. βComparison of post-treatment serum hemoglobin levels between patients with and without CR. APL: acute promyelocytic leukemia.

However, following treatments, these three groups' Hgb levels varied statistically significantly from one another (Table 2, p<0.001). The CR group that received intensive chemotherapy had the greatest Hgb level (10.3 g/dL) following the treatments. Patients who achieved CR and those who were not following intensive treatment showed a statistically significant difference in post-chemotherapy Hgb levels (p=0.001). The intensively treated group that did not achieve CR had the second-highest post-treatment Hgb levels. Between the non-CR intensive treatment group and the non-intensive treatment group, there was no statistically significant difference as per post-treatment serum Hgb levels (p=0.696). Hgb levels were greater in the non-intensive treatment group than in the supportive therapy group, and this difference was statistically significant (p=0.006).

Effect of pre-treatment Hgb value on complete remission

The variables affecting complete remission were analyzed in multivariable logistic regression. Higher Hgb value at the time of diagnosis increased the likelihood of achieving remission [p = 0.047, OR = 1.13 (95% CI 1.07 - 1.24)].

DISCUSSION

The results of the current study showed that (i) chemotherapy improves anemia significantly in one month in AML patients compared to pre-treatment levels. (ii) Predictably, post-treatment serum zenith Hgb levels within one month were significantly higher in patients who achieved complete remission compared to those who did not. (iii) Multivariable logistic regression analysis showed that pre-treatment serum Hgb level was an independent predictor of achieving complete remission. Per 1 gr/dL increase in serum Hgb level at the time of diagnosis increased the probability of remission significantly.

To our knowledge, this is the first study in the literature demonstrating the independent prognostic significance of baseline anemia in different subtypes of AML patients.

Anemia is a constant feature of newly diagnosed AML, regardless of subtype. There are several causes of anemia in AML, including but not limited to reduced red blood cell lifespan and reduced bone marrow production. The latter is the most prominent mechanism of anemia in these patients. As a result of bone marrow invasion by blastic cells, erythrocyte morphology shows slight abnormalities in addition to the presence of nucleated erythrocytes. Consistent with the peripheral blood picture, bone marrow examination reveals several abnormalities in erythroblastic progenitor cells. These include normoblasts of extreme size, with nuclear fragmentation or location.

Anemia has long been excluded from the prognostic classification schemes for AML because it is a near-universal finding at baseline and is considered a mere consequence of bone marrow invasion by blast cells. Moreover, the flood of genetic molecular discoveries left little room for blood counts and some clinical features in the prognosis estimation of AML patients.

The impact of untoward effects of anemia was shown in solid tumors [11]. The relative risk of death was increased between 19% and 75% in several cancers, including lung, head and neck, and prostate cancer. The same holds true for several hematologic malignancies including lymphoma, chronic myeloid leukemia and childhood acute lymphoblastic leukemia [11-13].

Anemia has not been rigorously studied as a prognostic marker in AML. This is especially true for subtypes of AML patients. Vucinic et al. evaluated prognostic value of red cell distribution width in newly diagnosed AML patients [14]. The authors found that the higher the RDW value, the worse the prognosis. Interestingly, the predictive ability of RDW was robust enough to remain in the multivariate analysis that included ELN2017 as a covariate. In our opinion, RDW here serves as a surrogate marker for the abnormalities in the erythroblastic precursors in the bone marrow. Yanada and colleagues [15] evaluated the prognostic value of blood counts (platelet, neutrophil counts and Hgb value) at the time of complete remission in AML patints. The authors revealed that platelet and neutrophil counts but not Hgb levels were independent predictors of relaps-free survival.

Some other studies did not find serum Hgb level as an independent predictor of outcomes in pre-treatment AML. For instance, Colovic et al. [16] reported patient age, comorbidities, performance status, leukocytosis, hepatomegaly, lactate dehydrogenase, and cytogenetics as independent predictors of prognosis. Serum Hgb was not among them.

The discordance among studies regarding anemia status or serum Hgb level as an independent prognostic variable may be due to the inclusion of different established prognostic markers in the logistic regression models and to differences in sample sizes of the studies.

Our results showed that those with secondary AML had the lowest mean Hgb level at baseline. In addition, these patients still had the lowest, but not significantly different from other groups, mean serum Hgb level after chemotherapy. Secondary AML includes patients whose pre-existing hematologic disease has progressed to AML. This heterogeneous group of patients traditionally has a worse prognosis compared to other AML subtypes [17]. Predictably, the resistance of this type of AML to treatment is responsible for the inadequate clearance of blasts from the bone marrow and the resulting anemia. Regardless of the underlying AML subtype, patients who received intensive chemotherapy had significantly higher serum Hgb levels at the end of chemotherapy in our study.

The present study has several limitations worth mentioning. The retrospective design of the study has inherent limitations thereof. Blood transfusions and some concomitant disease states that may affect serum Hgb levels might have been missed out. When considering subgroups, the sample size of the study was relatively small. We did not include molecular genetic abnormalities because we included patients who were treated decades ago, when modern genetic analyses were not widely available.

CONCLUSION

In conclusion, our results revealed worse anemia in secondary AML as well as better anemia improvement with intensive chemotherapy. More importantly, pre-treatment serum Hgb level was an independent predictor of prognosis in AML patients.

Author contribution

Study conception and design: ICH, OEC, and HG; data collection: OEC, UYM and EAK; analysis and interpretation of results: YB, HD and ICH; draft manuscript preparation: OEC and HG. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Hacettepe University Ethics Committee (Protocol no. 18/428-12).

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Conflict of interest

The authors declare that there is no conflict of interest.

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