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Example: Müller C, Büttner HJ, Petersen J, Roskomun H. A randomized comparison of clopidogrel and aspirin versus ticlopidine and aspirin after the placement of coronary-artery stents. Circulation 2000; 101: 590-3.

Instructions to Authors

Books

Section in a book: Sherry S. Detection of thrombi. In: Strauss HE, Pitt B, James AE, editors. Cardiovascular Medicine. 2nd ed. St Louis: Mosby; 1974. p.273-85.

Book with single author: Cohn PF. Silent myocardial ischemia and infarction. 3rd ed. New York: Marcel Dekker; 1993.

Editor(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

Article presented at a meeting: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. P. 1561-5.

Scientific or technical report: Smith P. Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX) Dept. of Health and Human Services (US). Office of Evaluation and Inspections: 1994 Oct. Report No: HHSIGOE 169200860.

Thesis: Kaplan SI. Post-hospital home health care: the elderly access and utilization (dissertation). St. Louis (MO): Washington Univ. 1995.

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Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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JOURNAL OF ACADEMIC RESEARCH IN MEDICINE

Contents

Prediction of Difficulty Level of Laparoscopic Cholecystectomy According to Preoperative Findings

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ABSTRACT

Objective: Laparoscopic cholecystectomy stands as the established surgical approach for gallbladder diseases. This study seeks to enhance the management of potential complications by anticipating the likelihood of operative complexity and the risk of requiring conversion to an open procedure.

Methods: In this retrospective study, a total of 811 cholecystectomy procedures were undertaken, segregating patients into case and control groups. The case group comprised 24 patients who initiated laparoscopically but ultimately underwent open conversion. Exclusion criteria encompassed direct open procedures, patients below 18 years of age, and cholecystectomies performed concurrently with other surgeries. A control group of 276 patients was chosen, matched for age, gender, and body mass index, to evaluate the influential aspects governing conversion likelihood.

Results: The study demonstrated a conversion rate of 3.09%. The preeminent determinant of conversion was the increased gallbladder wall thickness. Factors exerting influence on conversion included a history of endoscopic retrograde cholangiopancreatography, prior abdominal surgery, elevated C-reactive protein, lactate dehydrogenase, and direct bilirubin levels, instances of cholecystitis and cholangitis attacks, dense adhesions between the gallbladder and close organs, gallbladder hydrops, impacted stones, and a Callot dissection time exceeding 90 minutes. The Sugrue scoring system was also observed as a potentially valuable tool for predicting the likelihood of open conversion.

Conclusion: The capacity to foresee potential complications proactively empowers optimal preoperative preparations. This approach ensures patients are well-informed about the surgery, potential complications, and the prospect of conversion to an open procedure. Moreover, it enables the possibility of conducting the operation within a more experienced medical center when warranted.

Keywords: Laparoscopic cholecystectomy, open cholecystectomy, cholecystectomy complications

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J Acad Res Med 2023;13(2):50-7

Bayram et al.
Prediction of Difficulty Level of Cholecystectomy

51

INTRODUCTION

Laparoscopic cholecystectomy is currently the gold standard treatment for gallstones. Compared to open surgery, the laparoscopic method has several advantages such as early return of bowel functions, less postoperative pain, better cosmetic results, reduced hospital stay, and early return to daily life (1-3). However, in some patients, surgeons end up having to switch from the laparoscopic method to open surgery.

Some patient or gallbladder data may predict the risk of conversion to open surgery. Thus, this situation can be shared with the patient in advance, and the surgeon should be prepared for possible complications. In the literature, many factors such as male gender, age ≥60 years, history of abdominal surgery, hospitalization due to cholecystitis attacks, and increased gallbladder wall thickness on ultrasonography (USG) were expressed as warning signs that the operation would be difficult (4-7).

This study aims to predict whether the operation would be difficult, to predict the risk of conversion to open surgery, and to prevent or reduce possible complications by using the patient's laboratory test results, imaging methods, clinical findings and medical history.

METHODS

Our study was carried out, after it was approved, with the decision of Kafkas University Faculty of Medicine Ethics Committee dated 30.10.2019 and numbered 80576354-050-99/239. In our retrospective study, the 811 cholecystectomies performed at the General Surgery Clinic of Kafkas University Medical Faculty Research and Application Hospital between January 2015 and December 2018 were examined.

Two patient groups were formed using the patient and control method. Twenty-four patients in whom laparoscopic surgery was converted to open surgery were included as the patient group. According to the exclusion criteria we determined, patients in whom the surgery was started with open surgery, patients under the age of 18 and patients with cholecystectomy performed in conjunction with another operation were not included in the study. A control group was formed by selecting 276 patients in whom the surgery was started and finished laparoscopically according to criteria such as age, gender, and body mass index (BMI).

Age, gender, BMI, presence of additional disease, history of endoscopic retrograde cholangio-pancreatography (ERCP), previous abdominal surgery, previous cholecystitis and cholangitis attack, presence of pericholecystic fluid and gallbladder wall thickness in USG, increase in gallbladder wall thickness, preoperative serum liver enzymes, amylase, lipase, leukocyte count, C-reactive protein (CRP), direct bilirubin (DB) values during the operation were examined in order to determine the factors affecting exposure, gallbladder adhesion, presence of stones in the Hartman region, gallbladder hydrops, gallbladder scleroatrophy, and dissection time of Calot's triangle. In addition, the effectiveness of the Sugrue score was investigated (Table 1).

All these parameters were examined to determine the parameter that would make conversion to the open surgery more effective.

Statistical Analysis

Statistical analysis was performed using the SPSS version 22.0 software program for Windows. While descriptive statistics for numerical variables were expressed as mean, standard deviation, median, and minimum-maximum; percentage and frequency values were given for categorical variables. The conformity of the "Calot Dissection Time" variable to the normal distribution was tested with the Shapiro-Wilk test. In this context, the Mann-Whitney U test was used for two-group comparisons. Pearson chi-square and Fisher chi-square tests were used in the analysis of categorical data. Backward regression analysis was used to show the effect of independent variables on the conversion to open surgery and logistic analysis was used to show the variables that were found to be insignificant in the analysis. The results were evaluated within the 95% confidence interval, and the p<0.05 value was considered significant.

RESULTS

Eight hundred and eleven cholecystectomies were performed in our clinic between January 2015 and December 2018. Seven hundred and fifty-two of those were completed laparoscopically. In 24 of the other 59 patients, the operation was started laparoscopically and converted to open surgery while in 35 patients, it was directly started with open surgery for various reasons.

In order to reduce our type 2 error rate, we tried to get a high number of controls. Considering the inclusion and exclusion criteria between January 2015 and December 2018, all eligible patients were included in the study. Power analysis was not performed because all patients were included in the study.

There was no statistically significant difference between the age groups (≥60 years or <60 years), gender, BMI (BMI <30 vs. BMI >30) and comorbidity of the patients converted to open surgery (Table 2).

The risk of conversion to open surgery increased statistically in patients with a history of preoperative ERCP (p=0.001), those with previous abdominal surgery (p=0.001), those with a history of cholecystitis attack (p=0.002) and those who had a history of cholangitis attack (p=0.003) compared to those without ERCP, abdominal operation, cholecystitis and cholangitis attack histories (Table 3).

When liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP)] were examined within the scope of the study, no significant difference was found between those in whom laparoscopy was converted to open surgery and those in whom there was no conversion (Table 3).

In addition, there was no significant difference between the leukocyte counts between those in whom laparoscopy was converted to open surgery and those in whom there was no

52 Bayram et al.
Prediction of Difficulty Level of Cholecystectomy

J Acad Res Med 2023;13(2):50-7

conversion (p=0.449), but a statistically significant difference was observed in terms of CRP values (p=0.024) (Table 3).

The rate of conversion to open surgery was found to be higher in patients with high DB levels, and a statistically significant difference was found (p=0.001). A significant correlation was found between the conversion to open surgery and serum lactate dehydrogenase (LDH) levels (p=0.001) (Table 3). When ultrasonographic parameters were compared, no significant correlation was found between those who underwent laparoscopy and those who underwent open surgery in relation to the presence of pericholecystic fluid (p=0.401). A significant correlation was found between these two groups in terms of gallbladder wall thickness (p=0.001) and presence of hydrops (p=0.001) (Table 3). In terms of surgical findings, there was no difference between the two groups in terms of whether the gallbladder was scleroatrophic or not (p=0.053). However, it was determined that the adhesion to the organs around the gallbladder posed a risk of conversion to open surgery (p=0.001). It was determined that the presence of an impacted stone in the Hartman pouch also posed a risk of conversion to surgery (p=0.001). The dissection time of Calot's triangle was found out to be longer in patients who underwent open surgery compared to patients who underwent laparoscopy

(p=0.001) (Table 4). In addition, the calculated Sugrue score was found to be significantly higher in patients converted to open surgery (p=0.001) (Table 3).

Table 1. Difficulty grading of laparoscopic cholecystectomy (44)

Appearance of the gallbladder	Difficulty level, score
Less than 50% adhesions	1
Gallbladder not visible due to adhesions	3
Maximum	3
Distended/contracted gallbladder	1
Gallbladder cannot be grasped with atraumatic holding forceps	1
Enclaved >1 cm stone in Hartmann's pouch	1
Difficulty in entry	
BMI >30	1
Difficult to enter due to previous surgeries	1
Pus/biliary discharge from the gallbladder	1
Cystic artery/duct dissection >90 min	1

BMI: body mass index, min: minutes

Table 2. Characteristics of the patients

	Case, n (%)	p-value
Age <60 Age >60	12 (6.1%) 12 (11.5%)	p=0.100
Female Male	13 (6.1%) 11 (12.5%)	p=0.064
BMI <30 BMI >30	11 (7.7%) 13 (8.2%)	p=0.878
Comorbid disease (+) Comorbid disease (-)	11 (6.4%) 13 (10.2%)	p=0.221

BMI: body mass index

Table 3. Examined parameters related to the conversion to open surgery

	Case, n (%)	p-value
ERCP (-) ERCP (+)	19 (6.6%) 5 (41.7%)	p=0.001
Previous abdominal surgery (-) Previous abdominal surgery (+)	17 (6.1%) 7 (33.3%)	p=0.001
AST <32 U/L AST >32 U/L	20 (7.5%) 4 (11.4%)	p=0.502
ALT <33 U/L ALT >33 U/L	19 (7.7%) 5 (9.6%)	p=0.637
GGT <36 U/L GGT >36 U/L	16 (7.1%) 8 (10.7%)	p=0.326
ALP <104 U/L ALP >104 U/L	20 (8.1%) 4 (7.5%)	p=1.000
Leukocyte count <10.4 Leukocyte count >10.4	21 (7.7%) 3 (11.5%)	p=0.449
CRP <0.5 CRP <0.5	11 (5.5%) 13 (13%)	p=0.024
DB <0.3 DB >0.3	11 (5.5%) 8 (24.1%)	p=0.001
LDH <225 LDH >225	19 (6.7%) 5 (29.4%)	p=0.001
Amylase <100 Amylase >100	22 (7.7%) 2 (13.3%)	p=0.341
Lipase <60 Lipase >60	22 (7.7%) 2 (13.3%)	p=0.341
Cholecystitis attack (-) Cholecystitis attack (+)	5 (3.2%) 19 (13.1%)	p=0.002
Cholangitis attack (-) Cholangitis attack (+)	20 (6.9%) 4 (44.4%)	p=0.003
Pericholecystic fluid (-) Pericholecystic fluid (+)	21 (7.6%) 3 (13.6%)	p=0.401
Gallbladder wall thickness not increased Increased gallbladder wall thickness	5 (3.1%) 19 (13.9%)	p=0.001
Adhesion (-) Adhesion (+)	01 (0.6%) 23 (17%)	p=0.001
Hydropic gallbladder (-) Hydropic gallbladder (+)	7 (3.3%) 17 (19.5%)	p=0.001
Scleroatrophy (-) Scleroatrophy (+)	22 (7.5%) 2 (40%)	p=0.053
Stone in Hartmann's pouch (-) Stone in Hartmann's pouch (+)	10 (3.9%) 14 (32.6%)	p=0.001
Sugrue score: extreme difficulty Sugrue score: severe difficulty	8 (100%) 13 (33.3%)	p=0.001
Sugrue score: mild difficulty Sugrue score: moderate difficulty	2 (1.9%) 1 (0.7%)	

AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma glutamyl transferase, ALP: alkaline phosphatase, CRP: C-reactive protein, DB: direct bilirubin, LDH: lactate dehydrogenase

Bayram et al.
Prediction of Difficulty Level of Cholecystectomy

53

In the Backward regression analysis, 13 variables that significantly affected the conversion from laparoscopy to open surgery were included in the model. These variables are shown in Tables 2 and 3. The analysis was continued until the 9th step by adding the non-significant variables, and finally, 13 factors were included in the analysis (ERCP history, previous operation history, CRP, DB, LDH, cholecystitis attack, cholangitis attack, gallbladder wall thickness, gallbladder adhesion, hydrops, stone in Hartman, Sugrue score in those who underwent previous surgery, more than 10 years of previous abdominal operation, Calot's dissection). When patients who did not have previous abdominal surgery were taken as reference, conversion to open surgery was 10,663 times more in those who had previous surgery. When patients with a low DB level were taken as reference, conversion to open surgery was 9,402 times more in those with a high DB level. Considering those with normal gallbladder wall thickness as a reference, conversion to open surgery was 7,323 times more in those with increased gallbladder wall thickness (Table 5). Conversion to open surgery was 12,083 times more in those who had ERCP compared to those who did not.

When we compared patients with moderate/mild Sugrue scores with those with severe scores, it was found that the rate of conversion to open surgery was 379,219 times higher in those with severe Sugrue scores (Table 5). Model explanatory power was 71% at step 9 based on the Nagelkerke R2 value.

DISCUSSION

Open cholecystectomy was first performed by Carl Johann August Langenbuch in 1882 in a 43-year-old male patient who had had gallbladder disease for 16 years (8,9). Laparoscopic cholecystectomy has been used as the gold standard surgical treatment method in gallbladder pathologies since the day it was discovered by Philip Mouret. Although laparoscopic cholecystectomy has indisputable advantages, open cholecystectomy may be needed due to different problems arising during the operation. Some preoperative markers can help predict the difficulty of surgery and the risk of open cholecystectomy. Knowing these risk factors before surgery can help predict the risk of open cholecystectomy and can help the surgeon prepare for the operation and inform the patient about this situation and possible complications beforehand. The patient can be referred to more experienced centers if necessary. Conversion to open cholecystectomy should not be seen as a failure or a complication; on the contrary, it should be considered as the most important way to prevent possible complications.

In the literature, the rate of open cholecystectomy varies between 2-15% (4), but in the study of Gabriel et al. (5), in 61 patients (26.1%) out of 234 patients, the laparoscopic operation was converted to open cholecystectomy. In our study, the rate of conversion to open surgery was determined as 3.09% and it was compatible with the literature. Due to the fact that older patients are exposed to more cholecystitis attacks, it has been reported in many studies that the rate of conversion to open surgery is higher in older patients (6). In our study, we found that advanced age was not a predictive factor in the conversion to open surgery.

Most studies have reported that male gender is a risk factor for difficult gallbladder surgery. This outcome can be explained by finding an unexplained fibrotic Calot triangle during surgery (10) or by the higher number of "untreated attacks" due to fewer doctor visits in male patients according to a hypothesis. In a study conducted by Anuk and Kahramanca (11), it was found that gender was not a risk factor. In our study, we concluded that gender was not a significant factor for difficult cholecystectomies.

Table 4. The relationship between the dissection time of Callot's triangle and the rate of conversion to open surgery

Case	Number	Median (min-max)	Mean rank*	p-value
Conversion to open surgery	24	87.5 (35-210)	239.71	0.001
Laparoscopy	276	50 (20-100)	142.74	
	300	50 (20-210)		

min-max: minimum-maximum, *mean rank = average ordinal number

Table 5. Logistic regression analysis of the factors affecting conversion to open surgery

Independent variables	Dependent variables						
		B	SE	Wald	p-value	Odds ratio	%95 CI (lower bound-upper bound)
Previous surgery	(+)	2.209	1.026	4.633	0.031	9.107	1.218-68.078
	(-)	-	-	-	-	-	-
Direct Bilirubin level	>0.3	1.898	0.927	4.188	0.041	6.672	1.083-41.086
	<0.3	-	-	-	-	-	-
Gallbladder wall thickness	increased	2.588	1.238	4.370	0.037	13.308	1.175-150.690
	normal	-	-	-	-	-	-

CI: confidence interval, SE: standard error

54

Bayram et al.
Prediction of Difficulty Level of Cholecystectomy

J Acad Res Med 2023;13(2):50-7

Obesity has been used as an indicator of difficult cholecystectomy operations in the literature for reasons such as causing more inflammation and fibrosis, and thus making dissection difficult, problems with trocar entry due to the thickness of the subcutaneous adipose tissue, and the inability to fully determine the anatomy due to the density of intraperitoneal adipose tissue. In their study, Ammori et al. (12) showed that obese individuals had longer laparoscopic cholecystectomy durations compared to non-obese individuals due to the fatty Calot triangle, fatty right colonic flexure, fatty liver and increased body volume, but there was no difference in the rate of conversion to open surgery. Angrisani et al. (13) could not detect a difference between the rates of open surgery in morbidly obese and non-obese patients (obese 11.4%, non-obese 15.5%); In our study, it was determined that obesity was not a significant risk for difficult cholecystectomy.

Husain et al. (14) suggested in their prospective study with 108 patients that the presence of a history of comorbid disease in general was a predictive factor for conversion to open surgery, and Lipman et al. (15) suggested in their multivariate analysis of 1377 patients that the presence of a history of diabetes mellitus was a predictive factor for conversion to open surgery. In our study, it was determined that the history of additional disease was not a predictive factor for conversion to open surgery. However, in our study, we could state that the additional disease was not a predictive factor, inconsistent with the literature, since we kept the spectrum of additional diseases wide including diabetes mellitus, hypertension, heart failure, coronary artery disease, chronic obstructive respiratory disease and asthma.

In our study, it was observed that the rate of conversion to open surgery in patients who underwent ERCP was 12 times higher than those who did not. In the literature, it was stated that the shorter the time between ERCP and laparoscopic cholecystectomy, the lower the rate of conversion to open surgery (16). If cholecystectomy was performed within the first week after ERCP, it was reported as 13.2%, if it was performed less than forty-two days, 11.4%, and 20.6% if it was performed after forty-three days (16,17). Similarly, de Vries et al. (18) compared the rate of conversion to open surgery in cholecystectomies performed within two weeks after the ERCP procedure with the rate of conversion to open surgery in cholecystectomies performed within 2-6 weeks in their study, and the rate of conversion to open surgery was found to be statistically lower in the first group of patients. In their study, Boerma et al. (19) determined that laparoscopic cholecystectomy could be difficult and the rate of conversion to open surgery could increase 3 times 6 weeks after ERCP.

Many studies in the literature have shown that previous abdominal surgery is effective in the conversion to open surgery (6,20). This rate increases especially in those with a history of upper abdominal surgery (radical gastrectomy, perforated ulcer, vagotomy types, splenectomy, etc.). Adhesions between the liver bed and gallbladder and adjacent organs are common after such surgeries. The entrance to the abdomen will be dangerous due to possible intestinal adhesions, so as a precaution, the trocar should be inserted into the abdomen with the open method in these patients, and unnecessary dissection should be avoided by the surgeon. In our study, we found that patients with a history of previous abdominal surgery had 10 times more conversion to open surgery. Philip Rothman et al. (21) commented in their meta-analysis of 32 prospective studies that especially previous upper abdominal surgeries did not increase the rate of conversion to open surgery. According to the studies of Lee et al. (22), the rate of conversion to open surgery was 20 times higher especially in those who underwent upper abdominal surgery. Similarly, Hu et al. (23) reported in their review study that previous upper abdominal surgery was a serious risk factor, but previous lower abdominal surgery did not affect the rate of conversion to open surgery.

In their meta-analysis, Philip Rothman et al. (24) discussed the importance of leukocytosis in conversion to open surgery in 15 of 32 prospective studies. In 5 of these studies, it was stated that leukocytosis was an important risk factor for conversion to open surgery. In others, it was concluded that the increase in white cell count was not a significant risk factor for conversion to open surgery. Our findings suggested that increased white cell count was not associated with conversion to open surgery.

It has been observed that high liver enzymes (AST, ALT, GGT, ALP) do not constitute an important risk factor for conversion to open surgery in most of the studies in the literature (6,25-27). In our study, it was determined that high liver enzymes did not pose a significant risk for conversion to open surgery.

When Shapiro et al. (28) retrospectively analyzed 46 patients who were operated for acute cholecystitis, they found that elevated amylase lipase level was not a predictive parameter, but they stated that high LDH level was an important predictive factor in the conversion to open surgery. In our study, while the level of amylase lipase was not predictive of conversion to open surgery, the level of LDH was predictive of conversion to open surgery.

Wevers et al. (29) reported that CRP level above 165 mg/dL was predictive of conversion to open surgery. Jessica Mok et al. (30) found the mean CRP level of laparoscopic cholecystectomies which were converted to open surgery to be 286.2 mg/dL, the mean CRP level of difficult cholecystectomies to be 67.4 mg/dL, and the mean CRP level of completed laparoscopic cholecystectomies to be 7.05 mg/dL, and as a result it was considered that preoperative CRP level above 220 mg/dL was a predictive factor in the conversion to open surgery. Increased CRP level is a parameter that reveals the severity of inflammation. It is an important indicator of cholecystitis and is one of the most important causes of conversion to open surgery in inflammatory conditions. Gupta et al. found the mean CRP level to be 22.2±18.2 mg/dL in patients undergoing simple laparoscopic cholecystectomy, 56.5±32 mg/dL in patients undergoing difficult cholecystectomy, and 83.6±22.4 mg/dL in patients with conversion to open cholecystectomy. They concluded that high preoperative CRP level was an important predictive factor for difficult cholecystectomy and conversion to

Bayram et al.
Prediction of Difficulty Level of Cholecystectomy

55

open surgery (31). In our study, we found that the CRP level was predictive for the conversion to open surgery.

In our study, it was observed that the rate of conversion to open surgery as 9 times higher in those with a high DB level. In the literature, similar to our study, it has been revealed in various publications that there is a relationship between high serum bilirubin level and conversion to open surgery (10,15). Beliaev and Booth (32) reported that the elevation of DB and ALP levels was directly related to the conversion to open surgery, and the risk of conversion to open surgery in such patients increased 3 times.

Having a history of cholecystitis and cholangitis attacks may be a preoperative determinant of difficult gallbladder surgeries by making it difficult to reveal Calot's triangle with intense inflammation, widespread adhesions, and increased vascularity. Sudhir and Pruthvi (33) compared preoperative and intraoperative grading systems for difficult laparoscopic procedures in their prospective randomized study. In that study, they observed that patients with a history of hospitalization due to cholecystitis had a higher rate of conversion to open surgery (33). According to Nidoni et al. (34), it was reported that those who had more than two cholecystitis attacks experienced approximately 6 times more conversion to open surgery than those who did not. In our study, similar to the literature, a significant relationship was found between the history of cholecystitis and cholangitis, and the rate of conversion to open surgery.

Like Bunkar et al. (35), Lipman et al. (15) also identified pericholecystic fluid on USG as a predictive factor in conversion to open surgery, as stated in many studies in the literature. Randhawa and Pujahari (7) stated that pericholecystic fluid was a non-significant finding in the estimation of difficult gallbladder surgeries, similar to our study.

Bunkar et al. (35) developed a clinical, sonographic and history-based scoring system in a prospective study conducted with 100 patients over the course of 2 years. In this study, they determined that the gallbladder wall thickness of 4 mm and above in ultrasonography was a sign of difficult cholecystectomy operations (35). In our study, we observed that the gallbladder wall thickness of 4 mm and above was 7 times more associated with conversion to open surgery. The data in the literature support our study, and a significant relationship between gallbladder wall thickness and conversion to open surgery has been demonstrated (6,7).

In their study conducted in 450 patients, Awan et al. (36) stated that the most important reason for conversion to open surgery was intense adhesion. They argued that due to the healing of acute cholecystitis with scar and fibrosis, dense fibrotic adhesions between the omentum and gallbladder and dense fibrotic adhesions between the ductus cysticus and the main bile ducts made it difficult to reveal the anatomy of the Calot's triangle, leading to the inability to perform a "safe cholecystectomy" and leading to conversion to open surgery. In a study in which 6147 patients were examined by Singh and Ohri (37), it was stated that

intense adhesion in the Calot triangle was the most important reason for conversion to open surgery. In our case series, in 0.6% of the patients without dense adhesions, the operation was converted to open surgery, while in 17% of the patients with dense adhesions, it was converted to open surgery. A statistically significant correlation was found between the presence of adhesion and the rate of conversion to open surgery.

Chand et al. (38) and Sudhir and Pruthvi (33) reported in their study that there was a significant relationship between gallbladder hydrops and conversion to open surgery. Cho et al. (39), showed in their study that the volume of the gallbladder >50 cm³, difficulty in traction and manipulation with graspers due to thickened (edematous) and fragile gallbladder wall during an acute cholecystitis attack, and are difficult, and that pouring of the liquid and stone contents of the gallbladder into the environment, prolonging operation time could cause conversion to open surgery. In our study, a significant relationship was found between gallbladder hydrops and the rate of conversion to open surgery, and the rate of conversion to open surgery was found to be 19.5% in patients with hydrops, which was consistent with the average literature data, but it could not be evaluated as an independent variable in the regression analysis.

Z'graggen et al. (40) reported that the presence of scleroatrophic cholecystitis and the presence of stones in the main biliary tract during an acute cholecystitis attack greatly increased the rate of conversion to open surgery. It is known that the scleroatrophic appearance of the gallbladder macroscopically in laparoscopy is due to many previous episodes of acute cholecystitis. In our study, we observed that, contrary to many studies in the literature, scleroatrophic gallbladder was not effective in the conversion to open surgery.

Although the presence of an impacted stone in Hartmann's pouch was found to be unrelated to conversion to open surgery by Randhawa and Pujahari (7), in many studies in the literature, similar to our study, conversion to open surgery was found to be more common in patients who developed Mirizzi syndrome associated with an impacted stone in Hartmann's pouch (41-43).

Lal et al. (41) found in a prospective study consisting entirely of elective operations that total surgery time over 90 minutes, Calot dissection time over 20 minutes, and bile or stones in the bile coming out of the gallbladder significantly increased the risk of conversion to open surgery. In our study, we found that there was a significant relationship between the conversion to open surgery and the Calot dissection time being 90 minutes or more.

Sugrue et al. (44) developed an operative scoring model with surgical findings in their study. While most of the previous studies were conducted with preoperative findings, the scoring made by Sugrue et al. (44) was formed with operative findings. In our study, our patients were evaluated with the scoring made by Sugrue et al. (44).

56 Bayram et al.
Prediction of Difficulty Level of Cholecystectomy

J Acad Res Med 2023;13(2):50-7

Study Limitations

The limitation of our study was that our study was a single-center retrospective study and the conversion to open surgery group included a small number of patients in our study.

CONCLUSION

In our patients, a history of ERCP, previous abdominal surgery, elevated CRP, LDH and DB levels, history of previous cholangitis or cholecystitis attack, increased gallbladder wall thickness, adhesion of the gallbladder to adjacent organs, presence of hydropic gallbladder and impaction of the stone on the Hartmann's pouch caused the operation to be difficult and was found to increase the risk of conversion to open surgery.

ERCP, previous abdominal operation history, high DB level and thick gallbladder wall were considered as independent variables.

The scoring system developed by Sugrue was proven to be reliable in our patients. This scoring system can be further improved with new studies and a consensus can be achieved in all clinics.

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Synthesis of Graphene Oxide and *in vitro* Evaluation of Its Cytotoxic Effect

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ABSTRACT

Objective: Graphen oxide, a carbon-based material, is considered as a potential material for many biomedical applications such as cell labeling, cell imaging, biosensors, drug release studies, due to its physical and chemical properties. Graphene oxide can be synthesized by more than one method. In this study, it was aimed to evaluate the cytotoxic effects of the graphenoxide samples we synthesized.

Methods: Synthesis of graphene oxide was done by Hummers method. The cytotoxic effects of graphene oxide were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay in human embryonic kidney cells (HEK293). After 24 hours, 48 hours and 72 hours of graphene oxide incubation at different doses, the IC50 values were calculated which 50% percentage of cell viability.

Results: After 24 hours, 48 hours and 72 hours, IC50 values were measured as 206.18 μg/mL, 108.98 μg/mL, and 55.54 μg/mL, respectively. It was determined that the IC50 value decreased as the incubation time increased.

Conclusion: It was determined that the cytotoxic effect of the synthesized graphene oxide varies depending on the exposure time.

Keywords: Graphene, graphen oxide, HEK293, toxicity

INTRODUCTION

Carbon is one of the most abundant elements in the earth's crust. Carbon-based materials have many different application areas due to their high chemical resistance ability, efficient mechanical properties, low weight characteristics and high dispersibility in the body. Materials with different properties are formed by the bonding of carbon atoms to each other in different ways. Multilayer graphene, graphene oxide (GO), carbon nanotubes, nano diamonds and reduced GOs are carbon derivatives used in biomedical systems (1).

Graphene was first described as crystal graphitic films by Nobel laureates Andre Geim and Konstantin Novoselov. Graphene, which has a two-dimensional structure, is 1 atom thick and has the appearance of a honeycomb formed by the bonding of sp^2 hybrid carbon atoms. Graphenes, specific surface area (2630 m^2G^{-1}), high charge mobility (200,000 $cm^2 V^{-1} \cdot S^{-1}$), high Young's modulus (~1.0 TPa), high thermal conductivity (~5000 $Wm^{-1} \cdot K^{-1}$) and high optical transmittance (~97.7%) have unique thermal, electrical and mechanical properties (2-4). Because of these properties, the use of graphene-based materials in various biomedical applications, including tissue engineering, biosensing, gene delivery and cancer therapy, is increasing day by day (1,5).

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GO is a chemically modified form of graphene and graphene is hydrophobic while GO is hydrophilic. This feature of GO has made it suitable for use as a drug delivery agent, cellular imaging probes and biosensors in biological systems (1,6,7). GO contains both aromatic (sp^2) and aliphatic (sp^3) domains. Its surface is even wider to allow electrostatic interaction, and it allows drug binding. Therefore, it is also compatible with drug release studies (5,8). The functionality of GO as a toxic or biocompatible material depends on its composition, size, surface, shape, functional groups, charge, coating and solute medium. In the literature, more than one method is used for the synthesis of GO (9,10).

In this study, it was aimed to synthesize GO and evaluate the cytotoxic effect of synthesized GO in embryonic kidney cells.

METHODS

Graphen Oxide Synthesis

GO synthesis was carried out by the modified Hummers method (11). All chemicals used in the synthesis of GO were used in analytical purity: Sulfuric acid (H_2SO_4) (MERCK, 7664-93-9, Germany), phosphoric acid (H_3PO_4) (MERCK, 7664-38-2, Germany), Graphite, Potassium Permanganate ($KMnO_4$) (ISOLAB, 7722-64-7, Germany), hydrogen peroxide (H_2O_2) (30% wt) (MERCK, 1085972500), hydrochloric acid (HCl) (38% wt) (ISOLAB, 7647-01-0, Germany), anhydrous ethanol (ISOLAB, 64-17-5, Germany), sodium hydroxide (NaOH) (MERCK, 106498.1000, Germany).

For the synthesis of GO, 360 mL of H_2SO_4 and 40 mL of H_3PO_4 were placed in a beaker and mixed at constant temperature at 200 rpm. Then 3 g of graphite and 18 g of $KMnO_4$ were slowly added to the solution. This reaction was stirred at 40-45 °C for 16 hours. The suspension, which was stirred for 16 hours for the synthesis of GO, was transferred to a beaker containing 400 g of ice and mixing was continued. While the suspension was mixed with ice, 3 mL of H_2O_2 (30% by weight) was added dropwise. The resulting mixture was centrifuged at 3000 rpm for 45 minutes. After centrifugation, the supernatant (acid) was discharged into the waste bin. GO was obtained by washing the pellets and then drying them in an oven.

Characterization of Graphen Oxide

In order to determine the functional groups in the obtained GO structure, Fourier Transform Infrared (FT-IR) analysis was performed in the range of 4000-400 cm^{-1} using FT-IR spectroscopy (Nicholet FT-IR Spectrometer). The images of the samples were taken with the Transmission Electron Microscope (Jeol JEM 2100 plus).

Cell Culture

Commercially available human embryonic kidney cells HEK293T (CRL-1573) (The American Type Culture Collection-ATCC; Manassas, VA, USA) were cultured in a 37 °C, 5% CO_2 incubator in the medium prepared with 89% Dulbecco's modified eagle medium, 9% heat-inactivated fetal bovine serum (FBS) and 1% antibiotic solution (100 U/mL penicillin and 100 U/mL streptomycin). When the cells reached 70-80% density, they were first washed with Dulbecco's phosphate buffer saline and then removed from the flask with 0.25%. Trypsin-EDTA. The trypsin-EDTA-cell suspension was centrifuged at 120xg for 5 min. After centrifugation, the supernatant was discarded and fresh medium was added to the cell pellet. Cells were incubated by seeding in 96-well plates (1x10^4 cells/well). All chemicals were commercially available (Euroclone S.p.a.; Via Figino-Italy).

Application of Graphene Oxide and MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) Test

The MTT test was used to measure cellular metabolic activity in the evaluation of cell viability, proliferation and cytotoxicity. This colorimetric assay uses metabolically active cells to convert the yellow tetrazolium salt MTT into purple formazan crystals. NAD(P)H-dependent oxidoreductase enzymes in living cells convert MTT to formazan. Using dimethyl sulfoxide (DMSO) to dissolve insoluble formazan crystals, the absorbance of the resulting colored solution is measured in a spectrophotometer. Darker wells contain more metabolically active living cells, and the color is lighter in wells with reduced cell viability (12,13).

In our study, in order to determine the effective dose (IC$_{50}$) of GO, cells were seeded in 96-well plates at 1x10^4 cells/well. Twenty four hours after cell cultivation, control group was treated with fresh medium, experimental groups were treated with GO at different concentrations (1000-500-250-125-62.5-31.25-15.62-7.81-3.90 µg/mL). It was incubated for 24, 48 and 72 hours. At the end of the experiment, the medium was removed from all wells. 100 µL of fresh medium and 10 µL (5 mg/mL) of MTT solution were added to the wells and the cells were incubated for 3 hours. At the end of the incubation period, 100 µL of DMSO was added to each well and absorbance values were measured at 570 nm in an ELISA microplate reader (Multiskan GO-Thermo). By using optical density values, dose (IC$_{50}$) values for GO, which reduced cell viability by 50%, were determined with the GraphPad Prism 9 program.

Measurements were made in 3 repetitions and the results were expressed as mean values, statistical evaluation was not made.

RESULTS

Fourier Transform Infrared Spectroscopy Analysis

FTIR Spectra are given in Figure 1. Between 3000-3500 cm^{-1}, a broad peak is observed, corresponding to the stretching and bending vibration of the OH groups of water molecules adsorbed on GO. This peak indicates that GO has a strong hydrophilic structure. The characteristic peaks at 1735 cm^{-1} indicates the carboxyl C=O group, and the peak at 1630 cm^{-1} indicates the aromatic C=C group. The absorption peaks at 2920 cm^{-1} and 2850 cm^{-1} correspond to the stretching vibrations of CH$_2$. The absorption peaks at 1200 cm^{-1} indicate the stretching vibration of the C-O bond of the carboxylic acid. It shows the presence of absorption bonds in the range of 2925 cm^{-1}, 2119 cm^{-1}, 1768 cm^{-1}, 1631 cm^{-1}, 1380 cm^{-1}, 916 cm^{-1} and 534 cm^{-1}.

Morphological Characterization

Looking at Figure 2, it is seen that GO has a partially uniform transparent morphology due to its characteristic monolayer feature. Due to the large specific surface area formed by the oxygenated functional groups during the oxidation process, numerous bends and irregular wrinkles are observed on the surface (14).

MTT Results

HEK293 cells were treated with different concentrations of GO (1000-500-250-125-62.5-31.25-15.62-7.81-3.90 μg/mL). It was observed that GO decreased cell viability depending on time and dose (Figure 3). IC_{50} values were calculated as μg/mL and given in Table 1.
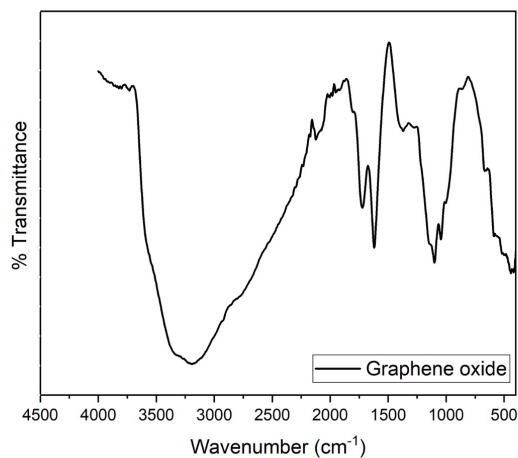
Figure 1. FT-IR spectroscopy of graphene oxide
FT-IR: Fourier Transform Infrared
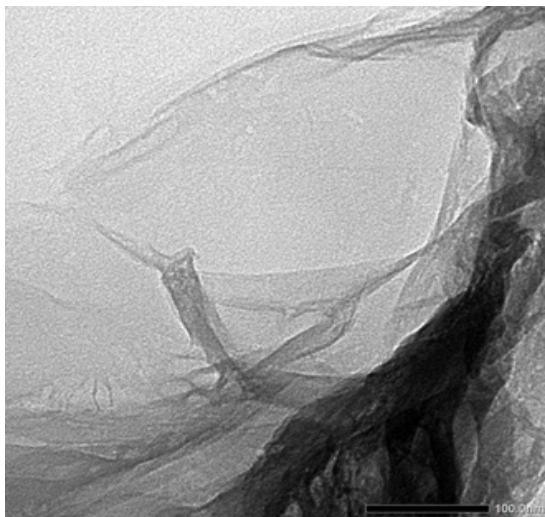
Figure 2. TEM photograph of graphene oxide
TEM: transmission electron microscope

DISCUSSION

Since carbon is one of the most common elements in nature, it is known that the biocompatibility of carbon materials is higher than other inorganic materials. In particular, graphite has been used in our daily lives for hundreds of years without critical toxicity issues. Due to the unique physical and chemical properties of graphene, which is a single layer of graphite, its use in biological studies is increasing day by day (1,2,3,7).

In this study, it was aimed to determine the cytotoxic effect of the synthesized GO on the viability of human embryonic kidney cells. For this purpose, the effects of 1000-500-250-125-62.5-31.25-15.62-7.81-3.90 μg/mL concentrations of GO on HEK293 cells were evaluated by MTT analysis, which was evaluated depending on mitochondrial activity. According to the results obtained, cell viability was found to be over 75% at the incubation period of 24 and 48 hours and at the concentrations of 125-62.5-31.25-15.62 μg/mL. When GO incubation was 72 hours, cell viability decreased below 65% at values above 62.5 μg/mL concentration. IC_{50} values showing 50% viability concentrations for 24, 48 and 72 hour incubations were calculated as 206.18 μg/mL, 108.98 μg/mL and 55.54 μg/mL, respectively. It was determined that when the incubation period with GO increased, mitochondrial damage increased, thus negatively affecting cell viability.

Today, there are many *in vitro* and *in vivo* studies investigating the biological effects of different graphene derivatives (4,6,9). Zhang et al. (15) found in their study on PC12 cells that the cytotoxic effect of graphene derivatives with different structures was directly related to the graphene structure. In another study investigating the effects of graphene, Wu et al. (16) showed that nano zinc oxide did not show toxic effects on cell viability, oxidative stress, mitochondrial depolarization and membrane damage in A549 cells when bonded with graphene.

Table 1. IC_{50} values for 24, 48 and 72 hours incubations in HEK293 cells treated with graphene oxide

	24 hours	48 hours	72 hours
IC_{50} value	206.18 μg/mL	108.98 μg/mL	55.54 μg/mL
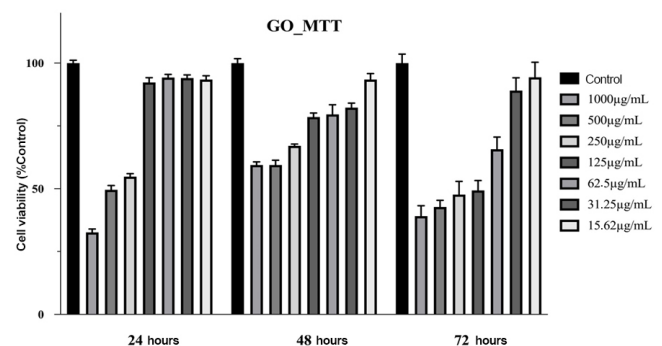
Figure 3. Time and dose dependent effect of graphene oxide on HEK293 cells

GO, one of the graphene derivatives, is interesting for its use in biological systems due to its hydrophilic structure. Due to the wide application area of GO, a comprehensive understanding of the cytotoxicity of GO is required for the safe and sustainable development of GO-based technologies (6). In this study, we aimed to observe the effect of GO, which we synthesized by using the modified Hummers method, on HEK293 cells.

Wang et al. (17) evaluated the biocompatibility of GO, which they synthesized by using the modified Hummers method, in human fibroblast cells and mice. While the application of GO at a dose of less than 20 µg/mL did not show toxicity in human fibroblast cells, it showed significant cytotoxic effects such as decreased cell adhesion, inducing apoptosis, penetrating into lysosomes, mitochondria and cell nuclei at doses above 50 µg/mL. On the mice, it was determined that low and medium doses of GO did not show significant toxicity, but at high doses (0.4 mg) it exhibited chronic toxicity with accumulation in the liver, spleen and kidney, and caused lung granuloma. In a study on human erythrocytes, it was reported that GO showed dose-dependent hemolytic activity, and the particle size of GO samples also played a critical role in hemolic activity (18). In a study investigating the effects of nanoGO on cardiomyoblast cell line, incubation with 20, 40, 60, 80 and 100 µg/mL nano-GO for 24 hours caused mitochondrial hyperpolarization, free radical production, and DNA damage at concentrations of 40, 60, 80, 100 µg/mL (19). Lammel et al. (20) showed that the cytotoxic effect of GO was dose-dependent through plasma membrane damage, and that GO damaged the structural integrity of the plasma membrane by establishing a strong physical interaction with the phospholipid bilayer. They also reported that GO could penetrate the plasma membrane, resulting in altered cell morphology and an increased number of apoptotic cells (20).

Ünal et al. (4) investigated the effect of different graphene derivatives in their study and showed that the viability of A549 human lung epithelial carcinoma cells was 17% even at the highest dose of 1000 µg/mL of GO. GO showed the greatest effect on MCF-7 breast cancer cells at the lowest dose tested, 1.96 µg/mL, and it was found to cause 11% death. However, they reported that the same graphene derivatives did not show a significant cytotoxic effect in healthy 293-T human embryonic kidney epithelial cells (4). However, Hu et al. (21) stated that the effect of GO was greatly attenuated by incubation with 10% fetal bovine serum, due to the extremely high protein adsorption ability of GO. In another *in vitro* study, it was reported that low-dose GO had no effect on the morphology, viability and membrane integrity of the A549 cell line, but it could cause a dose-dependent oxidative stress and cause a decrease in cell viability at high doses (22). Similar to the studies, in our study, it was observed that cell viability of HEK293 cells that applied different concentrations of GO decreased depending on time and dose.

Study Limitations

Molecular studies are needed to determine the cytotoxic effect of GO and to elucidate its interaction with the cell. In this sense, our study can be considered as a preliminary study to investigate the effect of GO.

CONCLUSION

As a result, more studies are needed to increase the usability of graphene derivatives synthesized by different methods in biomedical applications and to evaluate long-term toxicity/biocompatibility data.

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Cyberchondria's Possible Relationship with Problematic Internet Use and eHealth Literacy

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ABSTRACT

Objective: This study aims to reveal the possible relationship between cyberchondria, a new phenomenon occuring with the frequent use of the Internet for health-related information, and problematic internet use (PIU) and electronic health (eHealth) literacy. Our results are important for raising physicians' awareness, preventing false referrals and reducing health expenses.

Methods: Two hundred seventy one subjects who applied to the Esenler Education Family Health Centre-University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital Family Medicine Clinic on the dates between 01.06 and 30.06.2022 and who met the inclusion criteria were included in the study. Cyberchondria Severity scale-Short Form, Problematic Internet Usage questionnaire-Short Form (PIUQ-SF-6), eHealth Literacy scale, and sociodemographic form were applied. The results were evaluated at a significance level of $p<0.05$.

Results: A statistically significant relationship was found between the Cyberchondria Severity scale and its subcales, the PIUQ-SF-6 and its subscales, and the eHealth literacy scale ($p<0.05$). The severity of cyberchondria increased as PIU and eHealth literacy scores increased. A statistically significant relationship was found between PIUQ-SF-6 and eHealth literacy ($p<0.05$). Those with high PIU scores also had high eHealth literacy scores. PIU, cyberchondria severity, and eHealth literacy scores were higher in the 18-30 age group, and cyberchondria severity and eHealth literacy scores were higher among those who had a college degree.

Conclusion: The increase in cyberchondria as PIU increases shows that conscious use of the Internet will have positive effects on cyberchondria. Filtering and disseminating correct information in eHealth resources and increasing eHealth literacy will ultimately have a positive impact.

Keywords: Cyberchondria, problematic internet use, eHealth literacy, internet, health anxiety, health literacy

INTRODUCTION

Nowadays, as technology is developing rapidly, the use of internet is increasing day by day. According to the data from the beginning of 2023, the total number of internet users reached 5.16 billion, which corresponds to 64.6% of the world population (1). Due to this frequent use of the internet; it has become an important source for obtaining information from all fields. Health-related searches also have an important place in information seeking. In

2015, according to the data from Google, one of the most popular search engines in the world, one in twenty Google searches was health-related (2).

The advantages of obtaining health-related information via the internet include fast, inexpensive, and easy access. It also offers many options in a variety of formats, such as informative written texts, e-mail, and chat rooms (3). There are also some disadvantages of acquiring health information over the internet. An important one of these disadvantages is cyberchondria, which

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can be defined as the health anxiety triggered by seeking health information over the internet (4).

Cyberchondria refers to the phenomenon wherein individuals attempt to self-diagnose or self-treat by conducting online searches for information, documents, and treatment modalities pertaining to the illnesses they perceive themselves to have (5). This concept, which has a history of approximately 20 years, finds its place in the literature because of the increase in internet use, internet addiction and the widespread problematic internet use.

Problematic internet use can be defined as the use of the internet that leads to psychological, social, educational, and/or occupational difficulties in a person's life (6). Easy access to the internet via smartphones and having an internet connection almost anywhere in the world has led to more studies on problematic internet use.

Another important concept that comes to mind in the context of accessing health information via the internet and cyberchondria is electronic health (eHealth) literacy. eHealth literacy is defined as the ability to search, find, understand, and evaluate health information from electronic sources and the ability to use this information to solve a health problem (7).

Because cyberchondria is a new concept in the literature and restrictions such as the fact that the diagnostic criteria are still in the developmental stage and the use of scales to identify cyberchondriac patients is required, new studies are needed.

It is essential to reduce the problematic use of the internet in the age of technology and to increase the level of eHealth literacy in society. Studies have shown that societies can be protected from disinformation with conscious internet use (5).

The aim of this study was to evaluate cyberchondria's possible relationship with problematic internet use and eHealth literacy, to find solutions to the problem and to contribute to the literature.

METHODS

Our research is a single-centre, observational, descriptive, cross-sectional study. Two hundred seventy one people who applied to the Esenler Education Family Health Centre affiliated to University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital Family Medicine Clinic on the dates between 01.06-30.06.2022 and who met the inclusion criteria were included in the study. Three scales were used; Cyberchondria Severity scale-Short Form, Problematic Internet Usage questionnaire-Short Form (PIUQ-SF-6) and eHealth Literacy scale. Also, a sociodemographic form prepared by us was applied to the participants (8-10). Ethical approval was obtained from the Clinical Research Ethics Committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital, and the study was planned in accordance with the Declaration of Helsinki (decision no: 124, date: 22.04.2022). Informed consent was obtained from all participants in the study.

Turkish citizens over 18 years of age who voluntarily participated were included in the study. Three hundred and eighty three

patients who met the criteria during the specified period were reported, 271 of whom voluntarily participated in the study and completed the questionnaires.

Individuals who participated in our study were asked to answer 8 sociodemographic questions, PIUQ-SF-6 consisting of 6 questions, Cyberchondria Severity scale-Short Form consisting of 12 questions, and eHealth Literacy scale consisting of 10 questions making the questionnaire 36 questions in total.

PIUQ-SF-6; It is a Likert-type scale consisting of 6 questions. The scale has 3 subscales: obsession (obsessive thinking about the internet and psychological disturbance when not using the internet), neglect (neglecting even basic needs and daily activities due to internet use), and control disorder (inability to control internet use). The score obtained from the scale varies from 6 to 30 and as the score increases, the problematic internet use increases.

Cyberchondria Severity scale-Short Form consists of 12 questions. It is evaluated that a very low score is between 12 and 24 points; a low score is between 24 and 36 points; a medium score is between 36 and 48 points; and a high score cyberchondria severity is between 48 and 60 points. There are 4 different subscales. These subscales are; excessiveness, reassurance-seeking, compulsion, and distress.

The eHealth Literacy scale has 8 Likert-type questions and 2 open-ended questions. The minimum score that can be obtained from the scale is 8, and the maximum score is 40. It is assumed that the higher the score, the higher the eHealth literacy.

Statistical Analysis

In this study, statistical analyses were performed using the programme IBM SPSS 21. In the analysis of the data, in addition to descriptive statistical methods (mean, standard deviation, median, quartiles, frequency and percentage distributions), the distribution of variables was examined with the Shapiro-Wilk normality test. The Mann-Whitney U test was used for the two-group comparisons of the non-normally distributed variables, and the Kruskal-Wallis test was used for the multiple-group comparisons. The linear relationship between the scale scores was examined using the Spearman rho correlation coefficient. The reliability of the answers given to the scale was evaluated with Cronbach's alpha coefficient. The results were evaluated at a significance level of $p < 0.05$.

RESULTS

Descriptive statistics obtained using sociodemographic questions are shown in Table 1.

The Cronbach alpha values for each of the three scales used and the average scores of the scales and subscales are shown in Table 2. Accordingly, an average of 11.07 points was obtained from the PIUQ-SF-6, where 6-30 points can be obtained. Also, an average of 25.89 points from the eHealth Literacy scale, which has a scoring from 8 to 40 points. Problematic internet use and eHealth literacy levels tend to increase as the highest score that can be obtained from these scales is approached. Furthermore, an average of

Başhan Aslantaş and Altuntaş.
Cyberchondria, Problematic Internet Use and eHealth

65

25.52 points was obtained from the Cyberchondria Severity scale, which can be scored between 12 and 60, indicating a low level of cyberchondria severity among participants.

Table 3 presents statistics on the scales and their subscales according to age groups and education levels. Our findings reveal a significant difference in the PIUQ-SF-6 scores among the age groups (p<0.05). Specifically, this difference is between the 18-30 age group and all other groups and between 30-45 and 45-60 age groups. Upon further examination of the subscales, a significant difference was found between the 18-30 and 45-60 age groups in obsession scores (p<0.05). Additionally, a significant difference was found between 18-30 age group and all other groups in neglect scores (p<0.05). The control disorder scores showed a significant difference between the 18-30 and 45-60, 30-45 and 45-60, 18-30 and 60+ age groups (p<0.05).

In the results of the Cyberchondria Severity scale for age groups; a significant difference was found in cyberchondria severity scores (p<0.05). Accordingly, this difference was observed between 18-30 and 45-60, 18-30 and 60+, 30-45 and 60+ age groups. When the subscales were examined, a significant difference was also found between 18-30 and 45-60, 18-30 and 60+, 30-45 and 60+ age groups in excessiveness scores (p<0.05). Moreover, distress and reassurance-seeking scores showed a statistically significant difference (p<0.05). This difference was observed between the age groups of 18-30 and 60+.

A significant difference was found between 18-30 and 60+ age groups in the results of the eHealth Literacy scale according to age groups (p<0.05).

When the findings were examined, a statistically significant distinction emerged among different educational levels in relation to scores on the PIUQ-SF-6 (p<0.05). Spesifically; a significant difference was observed between individuals with primary education and those with a bachelor's degree, as well as between individuals with secondary education/high school and those with a bachelor's degree. When the subscales were examined, there was a significant difference in obsession scores between individuals with basic literacy skills and those with a bachelor's degree, as well as between individuals with secondary education/ high school and those with a bachelor's degree (p<0.05). Also, a significant difference was identified between individuals with primary education and those with a bachelor's degree in neglect and control disorder scores (p<0.05).

In the results of the Cyberchondria Severity scale; significant differences were found between different education levels. A significant difference was found between individuals with basic literacy skills and those with a bachelor's degree, individuals with primary education and those with a bachelor's degree in cyberchondria severity scores, and subscales of excessiveness and reassurance-seeking (p<0.05). In addition, a significant difference was observed in distress scores between individuals with primary education and those with a bachelor's degree groups (p<0.05).

Table 1. Descriptive statistics

		n	%			n	%
Age	18-30	77	28.4	Marital status	Single	71	26.2
	30-45	95	35.1		Married	185	68.3
	45-60	53	19.6		Divorced/widow	15	5.5
	60+	46	17	Income level	Below minimum wage	28	10.3
Gender	Female	151	55.7		Minimum wage	59	21.8
	Male	120	44.3		Up to twice the minimum wage	89	32.8
Educational status	Literate	3	1.1		Up to 2-3 times the minimum wage	45	16.6
	Primary education	24	8.9		3 times the minimum wage or more	50	18.5
	Secondary Education/ Highschool	60	22.1				
	Bachelor's degree	151	55.7				
	Master's degree and above	33	12.2				

Table 2. Cronbach's alpha values and mean scores of scales

Scale	Mean ± SD	Cronbach's alpha	Scale	Mean ± SD	Cronbach's alpha
PIUQ-SF-6	11.07±4.19	0.814	Cyberchondria severity	25.52±9.68	0.897
- Neglect	4.25±1.8	0.628	- Distress	6.69±3.28	0.811
- Control disorder	3.57±1.59	0.537	- Reassurance-seeking	6.06±2.92	0.75
- Obsession	3.25±1.52	0.627	- Compulsion	4.4±2.08	0.727
eHealth literacy	25.89±8.31	0.953	- Excessiveness	8.37±3.47	0.85

SD: standard deviation, eHealth: electronic health, PIUQ-SF-6: Problematic Internet Usage questionnaire-Short Form

66 Başhan Aslantaş and Altuntaş.
Cyberchondria, Problematic Internet Use and eHealth

J Acad Res Med 2023;13(2):63-9

Table 3. Statistics of scales according to age groups and education levels

Scale	Age					Educational status					
	18-30	30-45	45-60	60+	p-value	Literate	Primary education	Secondary education/ Highschool	Bachelor's degree	Master's degree and above	p-value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
PIUQ-SF-6	12.83±4.23	10.99±3.84	9.36±3.44	10.26±4.6	<0.001	6.33±0.58	8.88±2.98	10.1±4.06	11.91±4.22	11±4.12	<0.001
- Neglect	4.97±1.74	4.25±1.71	3.58±1.57	3.78±1.91	<0.001	2.33±0.58	3.29±1.43	3.87±1.55	4.63±1.87	4.06±1.71	<0.001
- Control disorder	4.13±1.63	3.62±1.56	2.85±1.1	3.37±1.74	<0.001	2±0	2.71±1.3	3.23±1.59	3.83±1.58	3.76±1.54	<0.001
- Obsession	3.73±1.76	3.12±1.38	2.92±1.4	3.11±1.35	0.014	2±0	2.88±1.19	3±1.69	3.45±1.53	3.18±1.33	0.016
Cyberchondria severity	27.56±8.7	26.76±9.75	23.53±9.53	21.87±10.09	<0.001	13±1.73	20.33±9.59	24.18±8.5	27.36±9.33	24.48±11.28	<0.001
- Distress	7.29±3.52	6.99±3.18	6.09±3.22	5.76±2.87	0.018	3.67±1.15	5.25±2.86	6.4±3.23	7.17±3.25	6.36±3.47	0.004
- Reassurance-seeking	6.49±2.74	6.33±3.08	5.62±2.84	5.3±2.86	0.026	3±0	5.13±2.85	5.8±2.81	6.45±2.88	5.73±3.21	0.011
- Compulsion	4.53±2.14	4.53±2.13	4.13±1.74	4.24±2.23	0.47	3±0	4.04±1.78	3.95±1.72	4.58±2.13	4.79±2.55	0.113
- Excessiveness	9.25±3.09	8.92±3.59	7.68±3.29	6.57±3.3	<0.001	3.33±0.58	5.92±3.01	8.03±3	9.16±3.37	7,61±3.82	<0.001
eHealth literacy	28.13±7.47	26.54±7.53	24.98±8.57	21.87±9.46	0.006	14.33±10.97	20±9.37	24.55±8.56	27.46±7.29	26.48±8.54	0.001

SD: standard deviation, eHealth: electronic health, PIUQ-SF-6: Problematic Internet Usage questionnaire-Short Form, p: Mann-Whitney U test

A significant difference was found on eHealth Literacy scores according to education levels ($p < 0.05$). This difference was between the primary education and bachelor's degree groups.

There was no significant difference between the genders in terms of scales and subscales ($p > 0.05$).

Table 4 presents the statistics between the scales and subscales used in our study. We observed a positive and moderate linear relationship between cyberchondria severity and PIUQ-SF-6. Furthermore, a positive, weak/moderate linear relationship was found between all subscales of the cyberchondria severity scale and all subscales of PIUQ-SF-6. Additionally, a positive and weak linear relationship was identified between cyberchondria severity and eHealth literacy. Moreover, a positive, very weak/weak linear relationship was found between all subscales of the cyberchondria severity scale, except for compulsion and eHealth literacy.

Table 5 shows the statistical analysis between PIUQ-SF-6 and eHealth literacy. A positive, very weak linear relationship was found between PIUQ-SF-6 and eHealth literacy. Spesifically, we found that eHealth literacy was weakly correlated with neglect, one of the PIUQ-SF-6 subscales, and very weakly linearly correlated with control disorder.

DISCUSSION

In our results, Cyberchondria Severity scale and PIUQ-SF-6 and all their subscales showed a statistically significant relationship. Accordingly, as problematic internet use increases, the severity of cyberchondria also increases. In a study on cyberchondria and problematic internet use in 2017, a significant relationship was found between them, similar to our study (11). In a research conducted by Starcevic et al. (12), who developed the Cyberchondria Severity scale, a positive statistically significant relationship was found between problematic internet use and cyberchondria. This article attributes the strong relationship between these two concepts highlighting the challenge of reducing engagement in online activities. Our findings, consistent with existing literature, demonstrate a significant relationship between cyberchondria and problematic internet use. As mentioned earlier, repetitive online searches form the basis of cyberchondria which aligns with the basis of problematic internet use. Individuals with high severity of cyberchondria tend to spend more time on the internet, thus further substantiating the link between these phenomena.

A positive statistically significant relationship was found between eHealth literacy and the Cyberchondria Severity scale and its subscales excessiveness, distress, andreassurance-seeking. A study conducted on healthcare workers showed that the level of eHealth literacy increased as the level of excessiveness increased (13). Contrary to our study, a decrease in cyberchondria-related responses was observed in individuals with high eHealth literacy in another

Table 4. The relationship of cyberchondria severity with PIUQ-SF-6 and eHealth literacy

		Excessiveness	Distress	Reassurance-seeking	Compulsion	Cyberchondria severity
Obsession	r	0.272	0.314	0.308	0.333	0.36
	p	<0.001	<0.001	<0.001	<0.001	<0.001
Neglect	r	0.374	0.341	0.296	0.301	0.401
	p	<0.001	<0.001	<0.001	<0.001	<0.001
Control disorder	r	0.284	0.306	0.229	0.258	0.327
	p	<0.001	<0.001	<0.001	<0.001	<0.001
PIUQ-SF-6	r	0.377	0.386	0.325	0.348	0.433
	p	<0.001	<0.001	<0.001	<0.001	<0.001
eHealth literacy	r	0.28	0.182	0.187	0.117	0.248
	p	<0.001	0.003	0.002	0.054	<0.001

eHealth: electronic health, PIUQ-SF-6: Problematic Internet Usage questionnaire-Short Form, p: Spearman rho correlation

Table 5. The relationship between PIUQ-SF-6 and eHealth literacy

		eHealth literacy
Obsession	r	0.090
	p	0.138
Neglect	r	0.250
	p	<0.001
Control disorder	r	0.128
	p	0.035
PIUQ-SF-6	r	0.198
	p	0.001

eHealth: electronic health, PIUQ-SF-6: Problematic Internet Usage questionnaire-Short Form, p: Spearman rho correlation

study conducted on this subject (14). Later in this article, it is noted that people with high levels of eHealth literacy can use online health resources to make accurate decisions and are less likely to develop anxiety during their internet searches even if they have health concerns. In another study, a statistically significant positive correlation was found between cyberchondria and eHealth literacy levels, similar to our study (15). According to the author's comment, since studies in the literature indicate that people with high eHealth literacy levels spend more time on the internet, it is possible that this situation may increase the level of cyberchondria, albeit at a low level.

A positive statistically significant relationship was found between PIUQ-SF-6 and its subscales: neglect, control disorder, and eHealth literacy. In a study examining the relationship between participants' time spent on the internet and their eHealth literacy levels, eHealth literacy was found to be higher in individuals who used internet daily (16). As the results of our study overlap with the literature, it appears that the increase in time spent on the Internet has a positive effect on eHealth literacy. However, this time spent on the internet leads to problematic internet use; is an undesirable outcome.

Our results shows that as the age of the group gets younger, the scores of PIUQ-SF-6 and the cyberchondria severity scale increase, in other words; the problematic internet use and the severity of cyberchondria increase. According to our study, the group with the most frequent problematic internet use is the 18-30 age group. In a study using different scales to assess problematic internet use in Japan in 2018, the age of problematic internet users was found to be significantly lower than that of regular internet users, which is similar to the results of our study (17). This result could be due to the fact that they came into contact with the internet earlier than other groups and have a higher level of technological knowledge. Another research found that cyberchondria decreases as age increases, which is consistent with the results of our study (18). A 2021 publication; using the same scale as in our study found that the severity of cyberchondria was higher in the 20-30 age group compared to other groups (19). It can be assumed that this situation is due to the fact that young people have easier access to the internet and technological devices.

The results of our study show that as the level of education increases, so does problematic internet use. In a study conducted in Slovenia in 2016, it was found that problematic internet use increased as the education level increased, which is consistent with our study (20). In another research conducted in Bangladesh with 13,525 participants during the coronavirus-2019 pandemic in 2020, it was also found that problematic internet use is more common among young people and people with higher levels of education (21).

The bachelor's degree education level has the highest cyberchondria severity. When the literature is examined, it was stated in a study conducted with 1196 participants in Turkey in 2021 that the level of cyberchondria severity decreased as the level of education increased (5). Again, a study in Turkey found that as educational level and age increased, the level of cyberchondria decreased and the level of health literacy increased (22). This difference between studies can be attributed to the difference in the number of participants and the single-center nature of our study.

It was found that the highest level of eHealth literacy was found among participants with a bachelor's degree.

When the literature is examined, there are studies that did not find a significant relationship between education level and eHealth literacy, whereas there are also publications that found a significant relationship similar to our study (15,23). Since eHealth literacy is defined as the ability to understand, interpret, and analyze readings based on the fundamentals, it can be considered as an area where those with a high level of education may be more successful than those with a low level of education.

Study Limitations

The most important limitation of our study is that it is single-centered. Our results may not be valid for the entire population. Therefore, further studies with larger populations are needed to confirm our results.

CONCLUSION

The use and spread of the internet are increasing day by day. This situation has led to the introduction of new concepts into our lives. The term 'problematic internet use' is more common than in the past due to frequent and uncontrolled internet use, 'eHealth literacy' is a more popular concept nowadays because of the increase in health-related internet searches and anxiety triggered by health-related searches over the internet, resulting in the term 'cyberchondria' within the last 20 years. According to our results, the risk of cyberchondria and problematic internet use is higher among individuals in the younger age group and in those with a high level of education. It is possible that this result is because the participants in this group use more technological devices or the internet than the other groups. Based on the same point; more intensive internet use may have indirectly increased the level of eHealth literacy in these groups.

The average level of cyberchondria of all participants as 'low level' with a score of 25.52±9.68 is a positive result, but it shows that cyberchondria is a subject that needs to be carefully examined, especially by physicians. As physicians, it will be beneficial to colloborate with the patient, to provide safe and accurate information by reminding them that they can access false and incomplete information on the internet and to direct them to the right information sources if necessary. Thus, negative situations such as treatment discontinuation can be prevented, and unnecessary healthcare expenditures can be reduced by preventing patients from repeatedly turning to healthcare facilities. The fact that cyberchondria is positively correlated with problematic internet use reminds us that we need to focus on correct and appropriate internet use again. Pathological internet use can exacerbate conditions such as cyberchondria, which encompass anxiety and obsession.

The mean eHealth literacy score of all participants was determined as 25.89±8.31 out of a maximum of 40 points. The importance of eHealth literacy is increasing as the internet becomes more widespread and powerful. With a high level of eHealth literacy, it is possible for patients to take responsibility for their own diseases and participate effectively and competently in treatment processes. The increase in eHealth literacy among individuals with a high level of education shows that we should attach importance to policies that raise people's education levels. High eHealth literacy will reduce healthcare expenditures in different aspects and reduce the burden on the healthcare system. For this reason, attempts should be made to ensure proper filtering in online health information resources and arrangements should be made to ensure that the public has access to accurate health information.

It is valuable for physicians to know the pathologies and similar situations that we have just learned about in our globalized and technology-dependent world, which is the subject of this study. Keeping cyberchondria, problematic internet use, and eHealth literacy in mind in daily practice will benefit both our healthcare system and our patients.

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Would Women Who Have Been Informed About Automated Breast Ultrasound Prefer This Method to Hand-held Ultrasound?

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ABSTRACT

Objective: To search, whether women would prefer a new method after they have been informed about automated breast ultrasound (US) examination, their perspective on technological developments and whether they make decisions based on their psychological and physical conditions.

Methods: Between 17.02.2023-17.03.2023, 118 women have visited our clinic for breast examination and 91 of them filled the questionnaire-based survey and have been included into the study. Questions of the survey consisted of three parts. Questions related to hand-held breast US were: "Do you feel relaxed while chatting with the physician during the examination?", "Do want to get information from the physician during or after the US examination?", "Do the physician's facial expressions have an effect on you?". Questions related to automated US were: "Would you change your mind if there was pain while the procedure was applied and your breasts were compressed?", "Would you think that this machine will give you more detailed information because it is a newer technology?", "Does any machine need to be widely used to be reliable or does it need to be available in a low number of imaging clinics?". Questions related to both methods were: "Does it make any difference to you whether the physician performs the procedure or not?", "Do you have any tolerance level for the duration of the US examination?", "Do you decide according to your own preference or the preference of the physician who referred you to have an US examination?".

Results: Mean age was 47.8 (22-74). Twelve out of 91 (13.1%) women had a history of breast cancer in their family. Seventy nine (86.8%) had a previous breast examination. Previous interventional procedures such as cyst aspiration or biopsy were applied for 5 (5.4%) women. When evaluating the questions about the physician as yes/no; the ratio of 'yes' answer was very high: Do want to get information from the physician during or after the US examination? (97.8% vs. 2.1%), Do you feel relaxed while chatting with the physician during the examination? (82.4% vs. 17.4%), Do the physician's facial expressions have an effect on you? (83.5% vs. 16.4%), does it make any difference to you whether the physician performs the procedure or not (83.5% vs. 16.5%). When both examinations were evaluated jointly; the ratio of the women who do not care about the duration of the procedure was higher than the ratio of who care (85.7% vs. 14.2%), and who care about the decision of the physician who refers her was higher than her own choice (80.1% vs. 19.7%).

Conclusion: Breast imaging is quite important and stressful for women. A significant number of the women care about obtaining information about the examination and being in direct contact with the physician as well as getting the results of the examination. However; although automated breast examination is a new technology and is not used very commonly in our country; it has been evaluated as testable for women.

Keywords: Automated US, hand-held US, breast imaging, ultrasonography

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Burçin Tutar.
Hand-held or Automated Breast US?

71

J Acad Res Med 2023;13(2):70-5

INTRODUCTION

While technological developments are advancing rapidly in our age, new breast imaging techniques are being added day by day. The fact that breast cancer (BC) is the most common cancer in women and that early diagnosis reduces BC-related deaths and changes in the course of treatment play an important role in the rapid increase in developments.

In addition to mammography, ultrasonography (US) is used in BC screening. US can detect early stage, small invasive cancers that cannot be detected on mammography in women with dense breast parenchyma (1,2). However, the hand-held breast US (HHBUS) examination, which is used as a standard today, has some disadvantages. These disadvantages shown in some studies include dependence on the physician performing the procedure, its being time consuming for radiologists, high false-positive rates, and not being cost-effective (2-5). Due to these disadvantages, the use of automated three-dimensional breast US has been increasing in breast imaging clinics recently. Automated breast US (ABUS) allows the image to be stored digitally and is not user dependent and provides technical standardization (6-8).

The aim of this prospective study was to evaluate whether women would prefer a new method for breast US examination after being informed about ABUS examination, their distance from technological developments, and whether they decide according to their mental and physical conditions.

METHODS

Between 17.02.2023 and 17.03.2023, 118 women who were admitted to our clinic for breast US examination were evaluated. Foreign nationals were not included in the study due to problems that might arise from incomplete communication and women with a history of BC and/or implants were not included in the study due to the lack of data on ABUS application.

An anonymous questionnaire was prepared by an experienced breast radiologist who had worked with several different ABUS devices. The forms were made available only in Turkish, printed on paper. All of the women who were admitted to our clinic were asked to fill out a questionnaire consisting of personal information (age, "do you have BC or does one of your relative have BC?", "do you have any complaint about your breast?", "has an interventional procedure been applied to the breast before?") before the breast US examination and the data were recorded routinely. Information on personal characteristics used in the study was obtained from these forms. The questionnaire form prepared for this study was filled by the physician who would perform the HHBUS examination and who was also the investigator, after giving a brief verbal information about the ABUS. The physician also explained what the survey questions were about and informed that it would take approximately 7-8 minutes to answer. Women participated in the survey voluntarily and no coercion was applied to those who could not complete the survey.

It was stated that the questions of the questionnaire were aimed at the differences between the two methods, even if the ABUS device was used instead of the HHBUS device available in the clinic. Then, about the ABUS, it was told that the radiology technician would perform the imaging similar to mammography (but the evaluation would be done by the physician on the screen and the whole breast image might be saved), that the process might take longer than HHBUS, and that there might be pain because the breast would be compressed during the procedure, but the general procedure was found to be tolerable. It was told that these devices were not widely used and that they were available in a limited number of centers in Turkey.

The questions consisted of three groups: those related to HHBUS, those related to ABUS, and those that were related to both. The questions related to HHBUS were as follows: "Does the conversation with the doctor relax you?", "Do you want to get information from the doctor during or after the US examination?", and "Does the facial expression of the doctor affect you during the procedure?". The questions related to ABUS were as follows: "Does compression of the breast and its being a painful procedure change your decision about the imaging method used?", "Do you think that ABUS gives more detailed information because the device is a new technology?" and "Which is reassuring? Widely usage of a device or its use in a limited number of centers?" The questions regarding both procedures were as follows: "Does it make any difference to you if the doctor performs the procedure or not?", "Do you have a tolerance level for the duration of the US examination?" (if possible, <10-15 minutes or I don't care about the time) and "do you act according to the preference of the doctor who referred you or according to your own preference when having an imaging examination?".

Descriptive statistics, numbers and percentage distributions were used for statistical analysis. İstanbul Medipol University Non-Interventional Clinical Research Ethics Committee approved the study (decision no: 178, date: 16.02.2023). Consent for the study was obtained from the patients.

Statistical Analysis

When the answers regarding HHBUS were evaluated as yes/no; the rate of patients who answered "yes" was found to be very high: "Do you want to get information from the doctor during or after the procedure?" (97.8% vs. 2.1%), "Does the conversation with the doctor relax you?" (82.4% vs. 17.4%), "Does the facial expression of the doctor affect you?" (83.5% vs. 16.4%) and "Does it make a difference for you if the doctor or technician does the extraction?" (83.5% vs. 16.5%).

72   Burçin Tutar.
Hand-held or Automated Breast US?

J Acad Res Med 2023;13(2):70-5

When both processes were evaluated jointly, the rate of those who said "I don't care about the time" in terms of tolerance level was found to be higher than those who said "I care" (85.7% vs. 14.2%). The rate of those who said "I care about the doctor's preference" when choosing for the procedure was found to be higher than those who said "I act according to my own preferences" (80.1% vs. 19.7%).

RESULTS

Of the women not included in the study, 5 had breast cancer, 12 were foreign nationals, and 8 had silicone. Two women over 75 years old, one was not included in the study because she was non-cooperative and the other thought she could not fill out the questionnaire. A total of 91 out of 118 women were included in the study.

When the sociodemographic characteristics of the women were evaluated, the mean age was 47.8 (22-74). Twelve (13.1%) women had a family history of breast cancer. The number of those who had breast imaging before was 79 (86.8%). Five (5.4%) women had previously undergone an interventional procedure as biopsy or cyst aspiration.

While only 3 (3.2%) out of 91 women were primary school graduates, 8 (8.7%) were high school graduates, 80 (87.9%) women were university graduates. While only 2 (2.1%) women lived in the small city, 89 (97.8%) women lived in İstanbul.

Seventy six out of 91 women, 76 women (83.5%) stated that they would prefer the doctor to perform the imaging, and 89 (97.8%) stated that they would like to get information about the procedure.

While talking with the physician was comforting for 75 (82.4%) women, 76 (83.5%) women stated that the facial expression of the physician affected them.

Twenty-three women stated that they would not want a painful procedure, whether they previously had a mammogram or not. Of the women, 53 (58.2%) stated that ABUS would give better information because it was a new technology, while 61 (67%) stated that it was more reassuring to have the device in fewer centers.

While making a choice for the procedure, 73 (80.1%) women stated that they cared about the preference of the referring physician. The majority of them (61/91) stated that they only considered the preference of the physician. In terms of duration, there were 13 (14.2%) women who said that they would like the procedure to take a short time, while 78 (85.7%) women stated that they would not care about the duration (Tables 1-3).

DISCUSSION

Breast imaging is very important and also stressful for women (9,10). Anxiety levels are usually high during the procedure, but sometimes even days before the procedure. During HHBUS examination, women are curious about what the examining physician will say, and they may sometimes look into the eyes of the physician or carefully examine the physician's facial expressions. Although there were not enough studies in the literature on this subject, it was shown in a study that while anxiety levels were high before breast US was performed, the level of anxiety decreased significantly when information about the procedure was given after the procedure (11).

Table 1. Answers for the questions about hand-held breast ultrasonography

	n=91 (%)
Do you want to get information from the doctor during or after the procedure?	
Yes	89 (97.8%)
I would like to receive information during the procedure	57
I would like to receive information when the process is completed	30
I would like both	2
No (I get information from the doctor who referred me)	2 (2.1%)
Does having a conversation with a doctor during the procedure relax you?	
Yes	75 (82.4%)
Absolutely	69
I do not want to interrupt the examination, otherwise it does	6
It does not matter	13 (14.2%)
No	3 (3.2%)
Does the doctor's facial expression affect you?	
Yes	76 (83.5%)
Always	63
Sometimes	13
No	15 (16.4%)

Burçin Tutar.
Hand-held or Automated Breast US?

73

Table 2. Answers for the questions about automated breast ultrasonography

	n=91 (%)
Do you think it gives better information because it is a new technology?	
Yes	53 (58.2%)
No	10 (10.9%)
I can't decide/I don't know	28 (30.7%)
Does compression of the breast and pain affect your decision?	
Yes, it affects	23 (25.2%)
No, it does not affect	68 (74.7%)
Does the widespread use of a device or the fact that it is used in a limited number of centers give confidence?	
Its widespread use	7 (7.6%)
Its use in a limited number of centers	61 (67.0%)
I can't decide/I don't know	23 (25.2%)

Table 3. Answers for the questions about both methods

	n=91 (%)
Do you have a tolerance level related to the duration of the US examination?	
I prefer it to last a short time (less than 10-15 minutes)	13 (14.2%)
I don't care about the time	78 (85.7%)
Does it matter if a doctor or a technician is performing the imaging?	
Yes, I want the doctor to perform the imaging	76 (83.5%)
Absolutely, the doctor should perform the imaging	72
It is better if the doctor performs the imaging	4
No, it does not matter	14 (16.4%)
Technician can perform the imaging	1 (1.0%)
Is the preference of the referring physician or your own preference important when conducting a US examination?	
Doctor's preference	61 (67.0%)
My own preference	18 (19.7%)
Both of my and the doctor's preference	12 (13.1%)
US: ultrasonography	

It is reassuring for women to be in direct contact with the physician and to receive information about their breasts during or after the procedure in countries where the physician performs the US procedure, as in our country (6,12). In this study, 83.5% (76/91) of the women stated that they would prefer the doctor to perform the imaging, and 97.8% (89/91) of them would like to get information about the procedure. Conversation with the physician was found to be relaxing for 82.4% (75/91) of the women. One of the important results of this study was that the facial expression of the physician affected 83.5% (76/91) of the women. When the answers about HHBUS were evaluated, the rate of those who gave 'Yes' answer was very high.

The use of ABUS for both screening and diagnostic purposes is increasing (13-15). A method must be accepted by the female population, especially when it will be used for screening purposes (12). There are few studies evaluating the perspective of the procedure and the tolerance of the patients after having the ABUS procedure (6,12,16,17). The most important disadvantage of ABUS is the feeling of discomfort due to pressure and pain. However, it was shown to be less painful in one study compared to mammography (18). Considering all these studies, the procedure was generally found to be tolerable. In this study, it was told to the patients that the procedure could be painful and could take a long time, but it was emphasized that it was tolerable in these respects. In terms of breast compression and a painful procedure,

74   Burçin Tutar.
Hand-held or Automated Breast US?

J Acad Res Med 2023;13(2):70-5

23 women (25.2%) stated that they would not want a painful procedure, whether they previously had a mammogram or not. Although the ability to save the whole breast image is an important advantage of ABUS, it may be viewed with suspicion in women because it is a new technology. However, it was seen in this study that more than half of the women (53/91) with a rate of 58.2% stated that ABUS would give better information because it was a new technology and that they would prefer a new technology instead of the traditional method and would like to try it. It was emphasized that while the widespread use of a device was not important for most women in terms of trust, it was more important to trust the center where the device was located and the physician who referred them. In such a situation, having the device in fewer centers was more reassuring for 67% (61/91) of women.

Patients are admitted to imaging clinics, usually by referral from different clinics. And if they trust the referring physician, they also trust the center where they will undergo the imaging. It was also seen in this study that while making a choice for the procedure, 80.1% (73/91) of the women stated that they cared about the preference of the physician who referred them. The majority of them (67%) stated that they only considered the preference of the physician. In terms of the duration of the procedure, the rate of those who said "I don't care about the duration" was very high (85.7% vs. 14.2%).

Study Limitations

Some of the limitations of this study could be listed as follows: The number of participants was relatively small and the questionnaire was administered in the light of the verbal information given, taking into account the advantages and disadvantages of the ABUS device, which was not routinely used in daily practice. However, the aim of the study was to assess how well the device would be accepted before placing it in the clinic. On the other hand, the reason why a new technology was viewed so positively might be that while patients were informed that the ABUS device was a new technology, they were not informed about the results in breast cancer detection.

CONCLUSION

It may be helpful to survey patients before placing a new device in imaging clinics. Especially in terms of breast imaging, a significant portion of women care about getting information about the examination and being in direct contact with the physician as much as they care about the result of the examination.

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J Acad Res Med 2023;13(2):70-5

Burçin Tutar.
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75

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The Relationship of Multiple Sclerosis with Brain Lobes and Related Structures: A Volumetric Analysis Study

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ABSTRACT

Objective: Magnetic resonance (MR) imaging techniques are at the center of studies on multiple sclerosis (MS), an autoimmune disorder characterized by demyelination and neurodegeneration in the central nervous system. We aimed to show the differences in the white matter (WM) and cerebrospinal fluid (CSF) volumes in addition to the frontal, temporal, and occipital lobes (OLs) in MS disease and their relations with each other through MR images.

Methods: Images of 50 MS and 50 control group patients randomly selected for use in our study were obtained from Biruni University Faculty of Medicine. Using volBrain software, the volumes of brain structures were calculated on T1-weighted MR images in accordance with the determined parameters. Data analysis was performed using the IBM SPSS 25 package programs.

Results: Volumetric calculations with statistically significant results between groups: abnormal appearance white matter (AaWM) (p<0.001), total volume of WM and gray matter (WMGM) (p=0.001), CSF (p=0.002) and frontal lobe (FL) (p=0.024) volumes. Parameters with high correlation according to correlation analysis; WMGM-CSF (r=0.999), FL supplementary motor cortex (SMC) (r=0.742), FL-temporal lobe (TL) (r=0.827), FL-temporal pole (TP) (r=0.652), SMC-TL (r=0.663), TL-TP (r=0.875), OL-occipital pole (r=0.649) and FL-frontal pole (r=0.686) volumes.

Conclusion: The presence of a decrease in FL and WM against the volumetric increase in AaWM and CSF is the most important of the predicted and significant results in this study. The fact that the significance levels in other parameters are comparatively low indicates that further studies should focus on more specific structures and methods, and all the results obtained can give clinicians a clue for the diagnosis step of MS disease.

Keywords: Multiple sclerosis, MRI, CSF, white matter, brain, T1-weighted

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Karip et al.
Brain Lobe Volume in Multiple Sclerosis

77

INTRODUCTION

Multiple sclerosis (MS) is a common and popular disease in society. By definition, the disease begins when a chronic autoimmune deficiency affects the central nervous system. Unlike most important pathologies, its symptoms (inflammation, T cell-mediated demyelination, and axonal loss) appear very early (1,2). Many factors can be effective in the appearance or progression of this disease, the most important of which are environmental, genetic, and epigenetic factors. Although there are more than 2.5 million MS patients worldwide, it is stated that the prevalence of MS in Turkey is between 41.1 and 101.4 per 100,000 people (3). Despite many studies, the large number of factors with which it can interact indicates that there is still a need for new studies on MS (4).

In the clinical reflection of MS, cognitive abnormalities, impairments in attention, and information processing speed can be counted among the major functional disorders. It is known that mental disorders can occur with anatomical damage or lesions in the frontal lobe (FL) of the brain. This situation can be seen as a factor that emphasize the pathology or morphology of the FL of the brain (5). In addition, pathologies in areas where some lesions or changes may compress the optic nerve, such as the retrobulbar region in visually impaired patients, can be seen in the magnetic resonance imaging (MRI) findings of MS patients (6).

Symptoms such as poor memory use and processing of new information in the memory and brain indicate that MS affects the hippocampal region in particular and the temporal lobe (TL) in general (7). One of the most distinctive differential diagnoses of neuromyelitis optica spectrum diseases, which is a type of MS that shows radiologically similar symptoms to MS, is the positive relationship between peripapillary retinal nerve fibers and the gray matter (GM) of the occipital lobe (OL) (8). This may indicate that MS disease may cause the OL (visual cortex) to be affected or that pathology in the relevant region may give a symptom of MS.

Clinical findings and MRI are the two main parameters used by neurologists to diagnose MS. Cerebrospinal fluid (CSF) and evoked potentials are sometimes used to confirm the diagnosis. There is no specific treatment for MS yet, and this varies from patient to patient. Most treatments are aimed at slowing the progression of the disease or stopping it at its current stage. It can also prevent new attacks and the emergence of new symptoms (9).

MS disease should be evaluated from multiple perspectives. The symptoms are seen in the brain, which is the most complex and sensitive area of the human body. When considered holistically, the brain is examined in 4 main sections; the cerebral cortex, limbic system, basal nuclei, and olfactory system (10). The cerebral cortex is divided into four lobes: FL, parietal lobe, TL, and OL (11,12).

Our study aimed to examine the statistical significance of volumetric measurement calculations of the FL, TL, OL, and related structures of MS patients and healthy individuals, which we think may be closely related to the clinical symptoms of MS and their relationships with each other.

METHODS

Ethics committee approval (decision no: 2023/77-38, date: 06.01.2023) was obtained from Biruni University Non-Interventional Clinical Research Ethics Committee for our study. MRI images were obtained from Biruni University Faculty of Medicine, Department of Radiology. A total of 50 MS and 50 control group patients (25-60 years old) were randomly selected. MR image of a patient in the MS group was not included in the study due to artifacts. The images of 99 patients (35 males, 64 females) including 49 MS (16 males and 33 females) and 50 controls (19 males and 31 females) were subjected to three-dimensional volume measurements in the "volBrain" program (www.volbrain.upv.es).

Parameters included in our study among the outputs obtained from the program (Figure 1) included abnormal appearance of white matter (AaWM), total volume of WM and GM (WMGM), CSF, FL, frontal pole (FP), supplementary motor cortex (SMC), TL, temporal pole (TP), OL and occipital pole (OP). The data obtained for relevant parameters are in the form of percentages. This was a retrospective radiological study. Therefore, the patient consent form was not needed.

Statistical Analysis

Analysis of the data was carried out using IBM SPSS 25 package programs. Qualitative variables frequency and percentage values are presented. The conformity of the data to the normal distribution was tested with the Shapiro-Wilk test. The Mann-Whitney U test was used for comparisons between two-category qualitative and quantitative variables. Chi-square test was used for comparisons between two qualitative variables and Spearman correlation was used for comparisons between two quantitative variables. Type I error rate was taken as 0.05 in the study.

Tissue	Volume (cm^3/%)
White Matter (WM)	595.25 (36.209)
Normal Appearing White Matter	595.14 (36.202)
Abnormal Appearing White Matter	0.11 (0.007)
Grey Matter (GM)	818.71 (49.802)
Subcortical Grey Matter	46.10 (2.804)
Cortical Grey Matter	654.38 (39.806)
Cerebellar Grey Matter	118.22 (7.192)
Cerebro Spinal Fluid (CSF)	208.41 (12.678)
Brain (WM+GM)	1413.96 (86.011)
Intracranial Cavity (IC)	1643.93 (100.000)
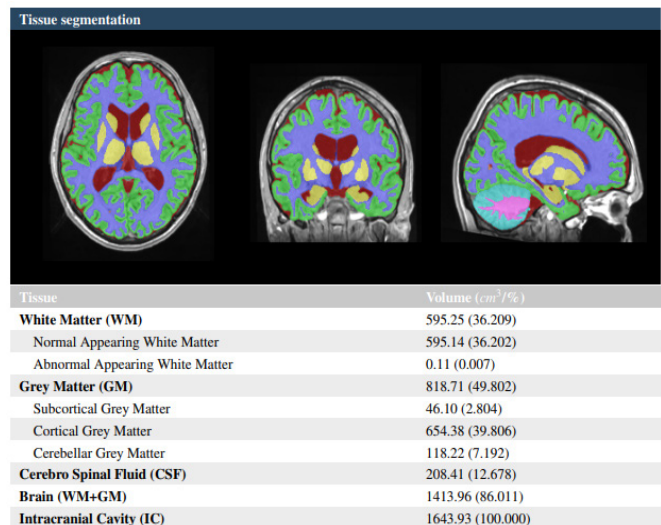
Figure 1. VolBrain PDF report example

78

Karip et al.
Brain Lobe Volume in Multiple Sclerosis

J Acad Res Med 2023;13(2):76-81

RESULTS

The volumetric measurements of the brain lobes, which is one of the basic parameters of the study, showed that the FL had the highest volume compared to the other lobes studied, while the OL was found to have the smallest volume (Table 1).

Because of the comparisons between MS and control groups; AaWM (p<0.001) and CSF (p=0.002) values were higher in the MS group; WMGM (p=0.001) and FP (p=0.024) values were higher in the control group. It was concluded that there was no significant difference between the groups in terms of FL (p=0.170), SMC (p=0.099), TL (p=0.113), TP (p=0.094), OL (p=0.659) and OP (p=0.671) values (Table 2).

Because of examining the existence of a relationship between the percentile values, the relationships between the relevant parameters were determined (Table 3). Parameters with positive and high correlation; FL with SMC (p<0.001; r=0.742), FL with TL (p<0.001; r=0.827), FL with TP p<0.001; r=0.652), SMC with TL (p<0.001; r=0.663), TL with TP (p<0.001; r=0.875), and OL with OP (p<0.001; r=0.649). Negative and highly correlated parameters; WMGM with CSF (p<0.001; r=0.999) and FL with FP (p<0.001; r=0.686).

Parameters with positive and moderate correlation; OL with FL (p<0.001; r=0.497), SMC with FP (p<0.001; r=0.485), TL with FP (p<0.001; r=0.542), TP with SMC (p<0.001; r=0.591), TL with OL (p<0.001; r=0.562), TP with OL (p<0.001; r=0.499), WMGM with FL (p<0.001; r=-0.458), WMGM with FP (p<0.001; r=0.489), FL with CSF (p<0.001; r=-0.453), and AaWM with CSF (p<0.001; r=0.408). Negative and moderately correlated parameters; AaWM with WMGM (p<0.001; r=-0.414), AaWM with FL (p<0.001; r=-0.524), AaWM with FP (p<0.001; r=-0.437), AaWM with TL (p<0.001;

r=-0.574), TP with AaWM (p<0.001; r=-0.571), OL with AaWM (p<0.001; r=-0.414), and FP with CSF (p<0.001; r=-0.484).

Parameters with positive and weak correlation; WMGM with SMC (p<0.001; r=0.365), WMGM with TL (p<0.001; r=0.399), WMGM with TP (p<0.001; r=0.362), FP with TP (p<0.001; r=0.388) and OL

Table 1. Descriptive statistics

	n (%)
Group	
MS	49 (49.5)
Control	50 (50.5)
Gender	
Male	35 (35.4)
Female	64 (64.6)
Parameters	**Median (min-max)**
AaWM	0.09 (0-10.82)
WMGM	84.93 (63.45-93.98)
CSF	13.62 (4.38-35.49)
FL	12.11 (5.65-15.05)
FP	0.44 (0.18-0.76)
SMC	0.69 (0.08-0.92)
TL	7.91 (2.4-9.44)
TP	1.29 (0.38-1.85)
OL	5.04 (1.91-6.21)
OP	0.25 (0.1-0.44)

MS: multiple sclerosis, AaWM: abnormal appearing white matter, WMGM: total volume of WM and gray matter, CSF: cerebrospinal fluid, FL: frontal lobe, FP: frontal pole, SMC: supplementary motor cortex, TL: temporal lobe, TP: temporal pole, OL: occipital lobe, OP: occipital pole, min-max: minimum-maximum

Table 2. Comparisons between patient and control groups

Parameters	MS Median (min-max)	Control Median (min-max)	Z	p-value
AaWM	0.15 (0.01-10.82)	0.035 (0-10.81)	-3.725	<0.001*
WMGM	82.2 (63.45-93.98)	86.9 (69.11-93.68)	-3.212	0.001*
CSF	16.42 (4.59-35.49)	11.685 (4.38-29.54)	-3.153	0.002*
FL	11.71 (5.65-14.21)	12.175 (7.04-15.05)	-1.372	0.170
FP	0.43 (0.18-0.6)	0.465 (0.24-0.76)	-2.253	0.024*
SMC	0.67 (0.21-0.82)	0.7 (0.08-0.92)	-1.649	0.099
TL	7.69 (2.4-9.44)	8.14 (2.82-9.2)	-1.585	0.113
TP	1.28 (0.38-1.63)	1.32 (0.53-1.85)	-1.677	0.094
OL	5 (2.07-5.86)	5.105 (1.91-6.21)	-0.441	0.659
OP	0.26 (0.11-0.44)	0.245 (0.1-0.4)	-0.424	0.671
Gender	**MS n (%)**	**Control n (%)**	**Chi-square**	**p-value**
Male	16 (45.7)	19 (54.3)	0.310	0.578
Female	33 (51.6)	31 (48.4)		

*p<0.05, MS: multiple sclerosis, AaWM: abnormal appearing white matter, WMGM: total volume of WM and gray matter, CSF: cerebrospinal fluid, FL: frontal lobe, FP: frontal pole, SMC: supplementary motor cortex, TL: temporal lobe, TP: temporal pole, OL: occipital lobe, OP: occipital pole, min-max: minimum-maximum

Karip et al.
Brain Lobe Volume in Multiple Sclerosis

79

J Acad Res Med 2023;13(2):76-81

with SMC (p<0.001; r=0.390). Parameters with negative and weak correlations were AaWM with SMC (p<0.001; r=-0.359), AaWM with OP (p=0.017; r=-0.240), CSF with SMC (p<0.001; r=-0.359), CSF with TL (p<0.001; r=-0.391) and TP with CSF (p<0.001; r=-0.355).

OL with WMGM (p=0.113), OP with WMGM (p=0.121), OL with CSF (p=0.122), OP with CSF (p=0.129), OP with FL (p=0.132), FP with OL (p=0.095), FP with OP (p=0.423), SMC with OP (p=0.203), TL with OP (p=0.077), and TP with OP (p=0.181) were not significantly correlated.

DISCUSSION

The presence of focal lesions or plaques is one of the most important distinguishing features of MS (13). For many years, sequences such as double inversion recovery on T2-weighted images have been used to detect disease-related lesions during MS diagnosis, especially in the analysis of WM content (13,14). This method is very important in terms of revealing the abnormal situation in WM. A different condition seen in almost 1 out of every 4 patients in MS pathology is diffusely abnormal WM, which can sometimes be called 'dirty WM' in the clinic (15). T1-weighted images are thought to be associated with more disability progression than T2-weighted images (16). In contrast to the standard, T1-weighted images were used to measure volume independently of the detection of focal lesions or plaques, the goal of our study. One of the parameters we determined on these images is AaWM. Significantly (p<0.001) higher volume of AaWM

was detected in MS (0.15) patients compared to the control group (0.035) patients. This finding agrees with the WM abnormality that is mostly seen at the diagnostic stage in MS disease.

Undoubtedly, one of the most important steps in the diagnosis of MS is the analysis of CSF biomarkers. Studies have shown that cerebral blood volume in MS decreases in the later stages of MS prognosis, however, it predicts that there may be an increase in CSF volume (17,18). In our study, CSF volumes were compared between the patient and control groups, and it was observed that the CSF volumes of MS (16.42) patients were significantly higher (p=0.002) than the control group (11.68). In cases where all diagnostic steps cannot be performed, a simple brain MRI image, can be a clue for the early diagnosis of MS.

The negative correlation of the total volume of WM and GM in the brain structure with the CSF volume (p<0.001; r=0.999) can be shown as a finding that proves that the CSF volume may increase in parallel with the knowledge of the decrease in the WM volume in MS patients. In addition, the moderate negative correlation between AaWM and WMGM (p<0.001; r=-0.414); Since AaWM can be interpreted as causing a decrease in healthy WM volume, a positive moderate relationship between AaWM and CSF (p<0.001; r=0.408) is also parallel to the correlation between WMGM and CSF.

Especially cognitive disorders can occur with anatomical damage or lesions in the FL of the brain. This situation can be seen as a factor that emphasize the pathology or morphology of the FL of the brain. An important example is the damage that may

Table 3. Correlation analysis between percentile values of parameters

		a	b	c	d	e	f	g	h	i
b	r	-0.414**								
	p	<0.001								
c	r	0.408**	-0.999**							
	p	<0.001	<0.001							
d	r	-0.524**	0.458**	-0.453**						
	p	<0.001	<0.001	<0.001						
e	r	-0.437**	0.489**	-0.484**	0.686**					
	p	<0.001	<0.001	<0.001	<0.001					
f	r	-0.359**	0.365**	-0.359**	0.742**	0.485**				
	p	<0.001	<0.001	<0.001	<0.001	<0.001				
g	r	-0.574**	0.399**	-0.391**	0.827**	0.542**	0.663**			
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001			
h	r	-0.571**	0.362**	-0.355**	0.652**	0.388**	0.591**	0.875**		
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
i	r	-0.414**	0.160	-0.156	0.497**	0.169	0.390**	0.562**	0.449**	
	p	<0.001	0.113	0.122	<0.001	0.095	<0.001	<0.001	<0.001	
j	r	-0.240*	0.157	-0.154	0.152	0.082	0.129	0.178	0.136	0.649**
	p	0.017	0.121	0.129	0.132	0.423	0.203	0.077	0.181	<0.001

*p<0.05, **p<0.01, a: abnormal appearing white matter, b: total volume of WM and gray matter, c: cerebrospinal fluid, d: frontal lobe, e: frontal pole, f: supplementary motor cortex, g: temporal lobe, h: temporal pole, i: occipital lobe, j: occipital pole

80

Karip et al.
Brain Lobe Volume in Multiple Sclerosis

J Acad Res Med 2023;13(2):76-81

occur in the connections between the FLs and the subcortical structures, which can cause dementia in the relevant region (5,6). Our volumetric measurements show a significant change in MS patients in FL compared with the control group, and a significant change in FP (p=0.024). According to the correlation analysis of SMC, which is also included in FL, in our study groups, although SMC and TL, unlike FL, did not show a significant difference in comparison of study groups, in our study, it was found that SMC was positively and highly correlated with FL (p<0.001; r=0.742) and TL (p<0.001; r=0.663). This may indicate that motor disability, which is frequently evaluated and observed in MS patients, may also be reflected in the volumetric relationships in brain structures (19,20).

Although it is thought that SMC may not have a relationship with OL on a volumetric basis due to the distance between them, it has been reported that changes may occur in the occipital cortex in addition to motor cortex involvement in MS and similar diseases (amyotrophic lateral sclerosis) (21). This may prove the positive and weak relationship between SMC and OL (p<0.001; r=0.390), which emerged because of the correlation analysis in our study.

Because of the statistical analyzes, the parameters that are not significant within themselves, between the groups, and according to the correlation analyzes and their relations with each other are related to the processing of T1-weighted images, which are less frequently used in MR imaging, which is only one of the steps of the diagnosis of MS disease in our study. Based on this, it is not possible to say that parameters that are not significant are not associated with MS disease.

Study Limitations

Even if some MR images had T1-weighted sequences, we could not use some because their section thicknesses were not taken as 1 mm. On the other hand, the images were taken in DICOM format. However, volBrain software can function in NIFTI format which was not applicable to certain images in our study group.

CONCLUSION

In addition to individuals with MS disease, studies with randomly selected images compared with control groups show the volumetric decrease in the FL, where cognitive disorders occur, and the SMC, which is included in its content, and their positive correlations with each other. As a result of volumetric analysis, it is seen that a decrease in WM and an increase in CSF can occur. It is foreseen that these situations can be an important guide for clinicians.

Our study provides a clue about MS disease, which needs more academic studies from diagnosis to treatment, especially in the early diagnosis stage, that practical programs such as 'volBrain', which are relatively easy to use and provide limited but free access, can be easily used in hospitals with access to MRI.

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Karip et al.
Brain Lobe Volume in Multiple Sclerosis

81

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Original Investigation

Can Acellular Dermal Matrix and Kombucha Cellulose Membrane Be Used in Calvarial Reconstruction?

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ABSTRACT

Objective: Our objective was to study the applicability of acellular dermal matrix (ADM) and Kombucha membrane (KM) in repairing calvarial dura mater defects.

Methods: In a study conducted on Wistar rats, six groups were formed. A dorsal calvarial subperiosteal pocket was created in all rats. Two groups were implanted with an ADM and two groups with a KM. The other two groups were designated as control groups. Half of the six groups were sacrificed on day 30, while the other half were sacrificed on day 60. Tissue grafts were removed from the subperiosteal pocket and subjected to histological analysis. Neovascularization and calcification intensity were examined by preparing samples with hematoxylin-eosin and periodic acid Schiff stains and observing them under a light microscope.

Results: In the control group, intense calcification was observed, but neovascularization was not observed. In the KM group, intense calcification was observed in almost all animals in both the 30-day and 60-day samples, and while neovascularization was absent in the 30-day animals, signs of neovascularization were present in half of the 60-day animals. In the ADM group, while no signs of neovascularization were observed in the 30-day and 60-day tissues, calcification was seen in more than half of the 30-day animals and disappeared in the 60-day animals.

Conclusion: This study demonstrates the potential use of KM in calvarial reconstruction.

Keywords: Acellular dermal matrix, kombucha, calvarial reconstruction

INTRODUCTION

The reconstructive surgery of the calvarial tissue is as old as the history of brain surgery. After many successful and unsuccessful experiences over the years, this problem is now being attempted to be solved with various methods (1-4). The search for solutions that cause less damage to the tissue and provide better adaptation always leads to ongoing research in this field (2,5). Today, the goal of reconstructing bone defects is to close the defect with a material that does not create a foreign body reaction, is not resorbed in the late period, provides the required volume as close as possible, without creating functional loss in another part of the body, and can be obtained at the lowest possible cost, as quickly as possible.

One of these materials is acellular dermal matrix (ADM) grafts. The initial clinical studies on the use of ADM started with the

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restoration of full-thickness skin defects due to burns (6,7). After the successful results in this area, the areas of graft usage expanded rapidly, especially in periodontology (8,9), plastic and reconstructive surgery (10,11), neurosurgery (12,13), hand surgery (14), and otolaryngology (15) for various purposes. ADM is a dermal graft devoid of the epidermis and all other cellular elements to prevent tissue rejection and graft failure (16). They are prepared using the de-cellularization process of human cadaver donor skin (allogeneic) or mammalian skin donor sources (xenogeneic) natural dermal tissue (17). This reduces the need for autologous grafts and eliminates problems with donor site wounds (17).

Another reconstructive material is kombucha, a drink made by fermenting tea and sugar with a symbiotic culture of bacteria and yeast (SCOBY) (18) typically for 7-10 days (19). SCOBY is a biofilm of microorganisms that resembles a mushroom head (19). The popularity of kombucha has increased due to its anti-inflammatory potential, antioxidant activity, lowering of cholesterol levels and blood pressure, reduction in cancer spread, and improvement in gastrointestinal, liver, immune system, and function (20-23).

In this study, we investigated the effects of ADM and kombucha on mouse calvarium and thus their usability in human calvarium.

METHODS

Permission was obtained from the İstanbul University Animal Experiments Local Ethics Committee (decision no: 2013/105, date: 30.09.2013) for the study of Wistar rats. Six groups were created. A sagittal incision of 2 cm in the midline was made on the dorsal calvarial skin of all rats. After entering through this incision, the periosteum was first reached, and then a periosteal incision was made at the projection of the same incision under the microscope, after the periosteum was visualized. Using a periosteal elevator, the bone under the periosteum was separated to create a subperiosteal pocket (Figure 1). In two groups, a 0.5x1 cm piece of ADM graft was cut and placed into the subperiosteal pocket. A new three-dimensional pig-derived ADM composed of natural type I and III collagens (Mucoderm, botiss gmbh, Berlin, Germany) was used (Figure 2) (24). In the other two groups, a 0.5x1 cm piece of natural cellulose membrane obtained by Kombucha fermentation and sterilized with UV was placed into the subperiosteal pocket. "Kombucha" is a beverage made by fermenting tea and sugar with a SCOBY for 7-10 days, and a cellulose biofilm membrane is formed on the top of the beverage by extended fermentation (18). These membranes were dried and sterilized with UV light and used as grafts. The remaining two groups were designated as control groups, and only subperiosteal pockets were created without graft tissue implantation. Half of the six groups (Table 1) were sacrificed on the 30th day and the other half were sacrificed on the 60th day. Tissue grafts taken from the subperiosteal pocket were removed and sent for histological analysis. They were examined under a light microscope after being prepared with hematoxylin-eosin and periodic acid Schiff stains. Histological examination was performed using cross sections taken in the medial-lateral plane along the long axis of the graft to determine histological changes.

They were visualized under low- and high-power microscopes to evaluate their macroscopic and microscopic appearances. The presence of microcalcifications or neovascularization was examined. For calcification, values such as '-' for none, '+' for low density, and '++' for high density were assigned based on the density of osteoid formation and microcalcification. For
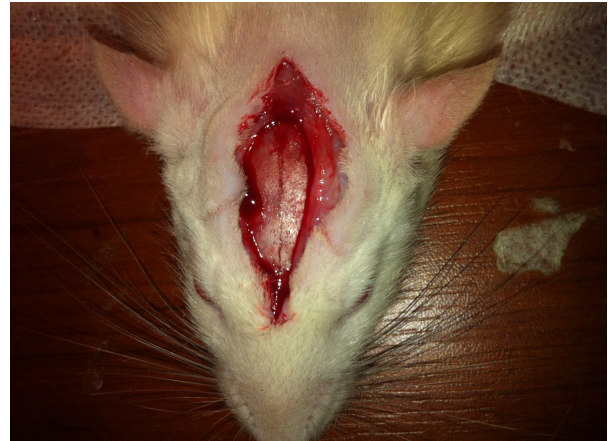
Figure 1. Creation of subperiosteal pocket

Figure 2. Implantation and detection of acellular dermal matrix

Table 1. Characteristics of the experimental groups

Group	Characteristics
Group-1	Control group, sacrificed on day 30
Group-2	Control group, sacrificed on day 60
Group-3	Implanted acellular dermal matrix, sacrificed on day 30
Group-4	Implanted acellular dermal matrix, sacrificed on day 60
Group-5	Kombucha cellulose membrane implanted, sacrificed on day 30
Group-6	Kombucha cellulose membrane implanted, sacrificed on day 60

neovascularization, values such as '-' for none, '+' for low density, and '++' for high density were assigned based on the density of lymphocyte infiltration and chronic inflammation.

Statistical Analysis

Statistical analysis was not performed in the study. Microscopic data was evaluated.

RESULTS

After completing the sacrifice process on days 30 and 60, calvarial tissue samples were taken from 6 groups and sent for histological examination. No infection or malignancy was found in the 48 subjects. When the results of the control group at 30 and 60 days were examined, intense calcification was observed but no neovascularization was seen. In the Kombucha membrane (KM) group, intense calcification was observed in almost all the tissues

of both 30-day and 60-day animals. When KM was examined for neovascularization, no neovascularization findings were found in the 30-day subjects, whereas signs of neovascularization were present in half of the 60-day subjects. In the ADM group, no signs of neovascularization were observed in the 30-day and 60-day tissues. While calcification was observed in more than half of the 30-day subjects, it was observed that they disappeared in the 60-day subjects (Table 2) (Figures 3, 4, 5, 6).

DISCUSSION

In our study, we applied ADM and KM grafts to the calvarium of experimental animals to investigate their suitability for human calvaria. The two primary factors we investigated for suitability were neovascularization and calcification. Neovascularization was evaluated by assessing the presence of adequate scaffold function that allowed for re-epithelialization, neoangiogenesis, cellular

Table 2. Histological sections prepared from bone tissues left to heal without any treatment on their periosteums (control groups) and bone tissues left to heal by placing acellular dermal matrix and kombucha cellulose natural membrane under the periosteum were examined under a light microscope after 30-60 days. The sections were evaluated for lymphocyte infiltration and calcification

	30-day treatment		60-day treatment	
Control	Neovascularization	Calcification	Neovascularization	Calcification
1 Rat	-	++	-	++
2 Rat	-	++	-	++
3 Rat	-	++	-	++
4 Rat	-	++	-	++
5 Rat	-	++	-	++
5 Rat	-	++	-	++
7 Rat	-	++	-	++
8 Rat	-	++	-	++
Kombucha				
1 Rat	-	++	++	++
2 Rat	-	++	-	-
3 Rat	-	+	-	++
4 Rat	-	++	+	++
5 Rat	-	++	-	++
6 Rat	-	++	++	++
7 Rat	-	++	-	++
8 Rat	-	++	-	++
Acellular dermal matrix				
1 Rat	-	-	-	-
2 Rat	-	+	-	-
3 Rat	-	-	-	-
4 Rat	-	+	-	-
5 Rat	-	++	-	-
6 Rat	-	-	-	-
7 Rat	-	++	-	-
8 Rat	-	++	-	+

Figure 3a. In the control group, histological sections were prepared from the tissue left to heal and renewal stages were observed in the light microscopic images taken 30 days later. Hematoxylin-eosin staining was used
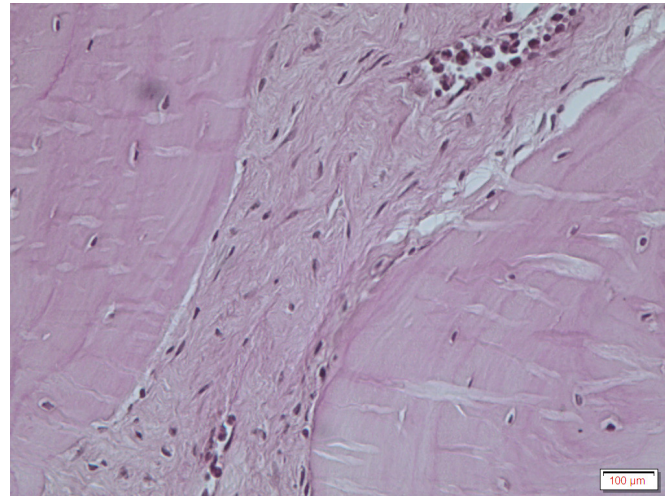
Figure 4. In the control group, histological sections were prepared from the healing tissue left to heal for 60 days and renewal stages were observed in the light microscopic images obtained. Hematoxylin-eosin stain was used
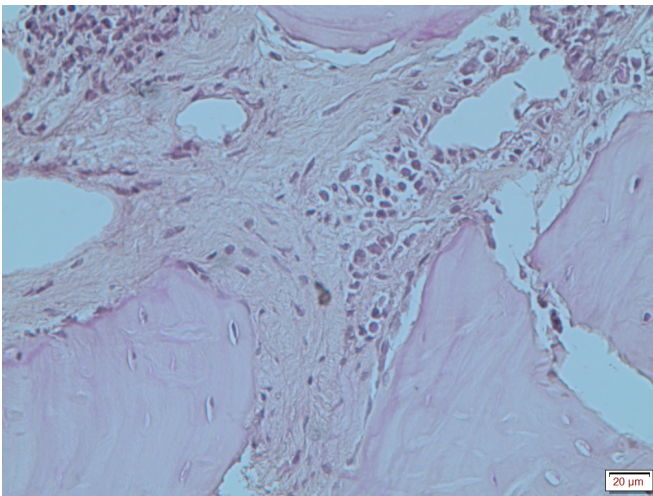
Figure 3b. In the control group, histological sections were prepared from the healing tissue left to recover and microscopic images were taken 30 days later, showing the stages of regeneration, using hematoxylin-eosin staining
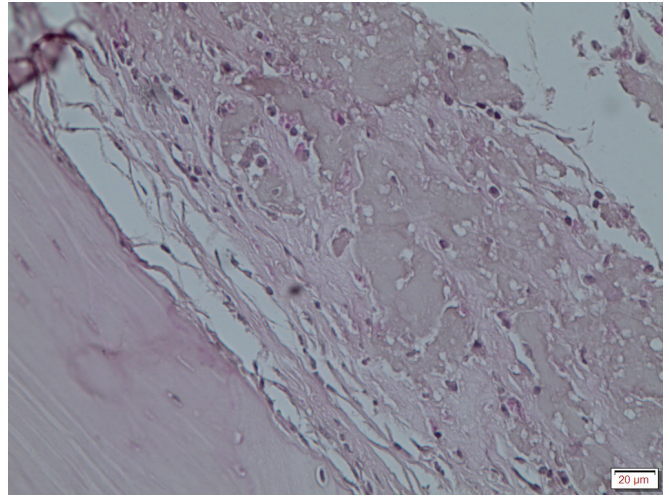
Figure 5. In histological sections prepared 30 days after implantation of acellular dermal matrix adjacent to the periosteum under the skin, light microscopic images showed cell-matrix interactions in the bone tissue. Periodic acid-Schiff staining was used

migration, and revascularization. Calcification was important for the restoration of the defect in the calvarial region for continued stability.

In our study, we found that KM had a strong calcification property, and signs of neovascularization began to appear in the late stage (60 days). However, we did not observe any neovascularization findings in ADM, and while there were signs of calcification at 30 days, these signs had disappeared at 60 days. These findings suggest that KM may be suitable for the calvarial defect reconstruction.

When looking at the literature, it is seen that many studies using ADM and KM have moved from animal experimental studies to studies on different tissues in humans with varying results. Studies on neovascularization and calcification with ADM and KM can only be seen in patients who undergo revision surgery. Our study allowed us to evaluate neovascularization and calcification results in a suitable number of experimental subjects. Revision surgery results are found only in a few case report series, and these results contradict each other (25,26). Additionally, studies have shown that ADM is useful in different areas, and there is less contraction

in wounds where it is used (27). Studies have also shown that ADM gradually replaces intact collagen fibers with host collagen, thereby supporting the healing process and minimizing the scar tissue formation (17).

KM has been used successfully in general surgical procedures to prevent adhesions, and it has also been shown to be an antimicrobial natural barrier (28-30). Although KM has various applications in modern medicine, no studies have been found in the literature on calcification and bone tissue reconstruction (31). In our results, we observed significant calcification and neovascularization began to develop after approximately 60 days.

Study Limitations

Although studies on experimental animals are one of the cornerstones of scientific research, it is difficult to make a clear decision without using the materials used in the study on humans. Apart from this, the number of test animals could be increased, or different materials could be trialed.

CONCLUSION

This study demonstrates the potential use of KM in calvarial reconstruction. Based on our promising and encouraging results, we are optimistic about the contribution of KM to calvarinal reconstruction. However, we believe that larger-scale experiments are needed to conduct clinical trials.

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Overdiagnosis of Endometrium Cancer: A Retrospective Study

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ABSTRACT

Objective: The deep myometrial invasion (MI) is a risk factor for lymph node metastases in endometrial cancer (EC). There is no consensus regarding which diagnostic method should be preferred for evaluating deep MI. Preoperative magnetic resonance imaging (MRI) and intraoperative frozen section (FS) examinations are the most definitive two diagnostic methods for evaluating deep MI. This study was designed to compare the diagnostic accuracy of preoperative MRI and intraoperative FS examinations in predicting deep MI and review their impact on clinical management and cost on health care.

Methods: MRI and FS findings of 65 patients with surgically staged EC between 2016 and 2019 were evaluated for deep MI. A definitive diagnosis of paraffin sections was used as the gold standard diagnosis.

Results: For detection of deep MI, accuracy, sensitivity, and specificity of MRI were 53.06%, 61.9%, and 65.9% respectively and significantly low consistency was observed between the final pathology results (p=0.034). Significant strong consistency was observed between the FS and the final pathology results for the detection of deep MI. Accuracy, sensitivity, and specificity were 77.18%, 85.7%, and 95.5%, respectively (p=0.000). Laparotomy rate (p=0.026), operation time (p=0.047), total days of hospitalization (p=0.004), rate of intensive care administration (p=0.027), and the total health-care cost were significantly higher in the MRI inconsistent group (p=0.015).

Conclusion: For the diagnostic approach and staging algorithm of EC, each clinic should take into account the accuracy of their diagnostic tests and individualize on a patient and clinical basis.

Keywords: Cost and cost analysis, endometrial neoplasms, frozen sections, magnetic resonance imaging, overdiagnosis, overtreatment

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Yeniocak et al.
Overdiagnosis of Endometrium Cancer by MRI

89

INTRODUCTION

The World Health Organization reported 380,000 new cases of endometrial cancer (EC) diagnosed in 2018 worldwide. Overall, EC is the sixth most common cancer in women after breast, colon, lung, cervical, and thyroid cancer. On the other hand, among the gynecological malignancies in women, EC is the most common cancer in developed countries and the second most common cancer in developing countries after cervix carcinoma (1). Pathologically, the most common type of EC is the endometrioid type with a better prognosis (2). Clinically the cancer is divided into two types. Type I tumors are low-grade [Federation of Gynecology and Obstetrics (FIGO) grade 1 and 2] endometrioid type ECs and have a better prognosis. Type II tumors include FIGO grade 3 endometroid and non-endometrioid EC and are associated with a poor prognosis. Poor prognostic factors showing high risk of lymph node metastasis are type II tumors, deep myometrial invasion (MI) ≥50%, lymphovascular space invasion (LVSI), and cervical stromal invasion (3). Tumor size greater than 2 cm is associated with an increased risk of lymph node metastasis in endometrioid-type EC regardless of the stage (4). According to the 2009 FIGO guidelines; EC staging is performed surgically and every patient should be operated unless there is a contraindication for surgery. Despite technological developments today, no imaging method can replace surgical staging with histological tissue diagnosis confirmation (3,5). Today, during surgical staging, complete lymphadenectomy, which was performed routinely, is now accepted as a treatment option for high-risk patients. In the MRC ASTEC study, it was shown that lymphadenectomy has no therapeutic benefit in early-stage EC (3,6,7). Preoperative magnetic resonance imaging (MRI) and intraoperative frozen section (FS) examinations are the most definitive diagnostic methods, often used together, in the evaluation of deep MI and cervical stromal invasion (8). The primary aim of this study was to compare the diagnostic accuracy of preoperative MRI and intraoperative FS examinations in predicting deep MI. Our secondary aim is to investigate the accuracy of existing diagnostic tests in the detection of low-risk patients and their impact on clinical management and cost on health care in our clinic.

METHODS

Study Design

The study was planned retrospectively and cross-sectionally. The study population consisted of 68 patients who were surgically staged for EC between 2015 and 2019 in our clinic. Three patients whose MRI reports could not be obtained were excluded from the study. Ethics approval with thesis subjects number E.9224 was obtained from Taksim Training and Research Hospital Clinical Research Ethics Committee on 27.02.2019 (decision no: 3). The diagnosis of EC was made by pathological examinations obtained from Pipelle biopsy, dilation curettage, or hysteroscopic biopsy materials. Final pathology results were determined by paraffin block results. Patients were staged according to the FIGO 2009 criteria. The demographic data of the patients and additional

risk factors for EC, pathology reports, MRI reports, and final pathology reports were accessed from the clinical database of our hospital. The duration of the operation, the need for blood product replacement perioperative or postoperative period, the need for intensive care follow-up after the operation, the length of stay in the hospital, and the pre-operative and postoperative hemoglobin levels were obtained from the surgery and anesthesia reports. Total costs for patients were calculated by Turkish Lira (TL) and converted into US dollars ($) according to the exchange rate of the Central Bank of the Republic of Turkey on the discharge date of the patients and used in cost analysis.

MRI Examination

All patients underwent preoperative routine contrast-enhanced pelvic MRI examinations before the operation in our hospital with a General Electric (GE signa HD Milwaukee, USA) 1.5 Tesla device. The imaging protocol was axial and sagittal T2-weighted fast-spin echo. MRI sequences were performed with slice thicknesses of 5 mm, intersectional gaps of 1.0 mm, and fields of 20-50 cm. Dynamic contrast-enhanced MRIs were obtained with axial fat-saturated T1-weighted GRE imaging before and 30, 60, and 120 seconds after intravenous administration of contrast agent using 0.1 mmol/kg meglumine gadoterate (Magnescope®; Guerbet Japan). MRI examination was reported by radiologists specialized in gynecologic oncology at our hospital. The MRI data of each patient was examined by at least two radiologists, and the final MRI diagnoses were determined through their discussion of observations.

FS Examination

The hysterectomy and bilateral salpingo-oopherectomy material was perioperatively carefully examined and the endometrium was sliced at 3-5 mm intervals, and full-thickness endometrial tissue, which is the deepest visible macroscopic invasion area, was examined microscopically. After the entire specimen was fixed on the coverslip with a cryostat, it was placed in a pre-chilled Hystobath (Nesland Instruments Newington, NH). After 4-6 μm-thick tissue slices were cut with a acrotome (Leica CM 1900, Leica Microsystems, Numsloch, Germany), they were quickly stained with hematoxylin-eosin staining, and the stage of deep MI of the tumor was microscopically evaluated by the expert pathologists of our hospital.

Surgical Staging for EC

The choice of abdominal surgery or endoscopic surgery was made by the patient, surgeon, and anesthesiologist, with the patient's informed consent, in a multidisciplinary manner, taking into account the patient's American Society of Anesthesiology (ASA) score and additional comorbidities. All procedures in our clinic were performed by gynecological oncology surgeons. As the operating procedure, after exploration by laparoscopy or laparotomy, hysterectomy and bilateral salpingo-opherectomy material were sent to the pathology department of our hospital for FS examination. In case of EC with suspicious lymph node

90 | Yeniocak et al.
Overdiagnosis of Endometrium Cancer by MRI

J Acad Res Med 2023;13(2):88-94

involvement in preoperative MRI examinations, or with deep MI of the tumor above 50% in the pre-operative MRI or intraoperative FS examination, or in type II tumors, complete lymph node dissection was performed rather than suspicious lymph node sampling.

Statistical Analysis

Mean, standard deviation, median, lowest, highest, frequency, and percentage values were used in the descriptive statistics. The distribution of variables was measured using the Kolmogorov-Smirnov test. Independent sample t-test and Mann-Whitney U test were used in the analysis of quantitative independent data.

Pearson's chi-square test was used in the analysis of qualitative independent data, and Cohen's kappa test was used for the consistency analysis. SPSS 22.0 (IBM Corporation, Armonk, NY, USA) program was used in the analysis. A p-value of <0.05 was considered significant.

RESULTS

Demographic Data

Demographic and pathological data of all patients (n=65) are given (Table 1a, b). The age range of the patients was between 42

Table 1a. Demographic and pathological data of the patients

	Min-max	Median	Mean ± SD
Age	42.0-80.0	61.0	61.1±8.1
Gravida	0.0-15.0	5.0	4.7±3.0
Parity	0.0-11.0	3.0	3.6±2.3
Operation time (minutes)	120-480	240	241±68
Hospital stay (days)	3.0-27.0	11.0	10.9±5.1
Cost in TL	1204-6194	2790	2953±1182
Cost in $	207-2431	836	871±384
Admission Hb	9.3-15.0	12.0	12.1±1.3
Discharge Hb	7.2-14.0	11.0	10.9±1.3
Tumor size	0.2-12.0	4.0	4.7±2.8

TL: Turkish Lira, $: US Dollar, Hb: hemoglobin, SD: standard deviation

Table 1b. Demographic and pathological data of the patients

		Number	%
ASA score	I	16	24.6%
	II	37	56.9%
	III	12	18.5%
Surgery type	Laparoscopy	29	44.6%
	Laparotomy	36	55.4%
ES Transfusion	(-)	53	81.5%
	(+)	12	18.5%
Post-op ICU	(-)	45	69.2%
	(+)	20	30.8%
Final pathology FIGO surgical stage	Ia	40	61.5%
	Ib	9	13.8%
	II	9	13.8%
	IIIb	1	1.5%
	IIIc1	4	6.2%
	IIIc2	1	1.5%
	IV	1	1.5%
Tumor location	Fundus/corpus	53	81.5%
	Lower segment	12	18.5%
PB histopathological type	Grade I-II	58	89.2%
	Grade III	7	10.8%

ASA: American Society of Anesthesiology, FIGO: Federation of Gynecology and Obstetrics, ES: erythrocyte suspension, ICU: intensive care unit, PB: pipelle biopsy

Yeniocak et al.
Overdiagnosis of Endometrium Cancer by MRI

91

and 80 years, and the mean age was 61.1±8.1 years. The number of patients with an ASA score of III was 12 (18.5%). The number of patients with previous abdominal surgery was 25 (38%). While laparoscopic intervention was preferred in 29 patients (44.6%), surgery was performed by laparotomy in 36 patients (55.4%). The mean operation time was 241±68 minutes [minimum-maximum (min-max): 120-480 minutes], while the mean hospital stay was 10.9±5.1 days (min-max: 3.0-27 days). While the total cost in TL was 2953±1182 TL on average (min-max: 1204-6194 TL), it was $871±$384 in $ (min-max: $207-$2431). While the mean hemoglobin values before the operation were 12.1±1.3 mg/dL (min-max: 9.3-15.0 mg/dL), the mean hemoglobin values after the operation were 10.9±1.3 mg/dL (min-max: 7.2-14.0 mg/dL). Erythrocyte suspension transfusion was administered to 12 (18.5%) patients preoperatively or postoperatively. Twenty patients (30.8%) were admitted to the intensive care unit (ICU) of our hospital after the operation. In the final postoperative pathological staging, 40 patients were FIGO stage Ia (61.5%), 9 patients were FIGO stage Ib (13.8%), 9 patients were FIGO stage II (13.8%), 1 patient was FIGO stage IIIb (1.5%), 4 patients were FIGO stage IIIc1 (6.1%), 1 patient was FIGO stage IIIc2 (1.6%), and 1 patient was FIGO stage IV (1.6%). The mean tumor size was 4.7±2.8 cm (min-max: 0.2-12.0 cm). The tumor was located in the fundus/corpus in 53 (81.5%) patients, while it was located in the lower segment in 12 (18.5%) patients.

Deep MI Detection Results of MRI and FS Examinations

In our study, when the pre-operative MRI examinations and the final pathology results were compared in terms of deep MI of the tumor, a significantly low consistency was observed, accuracy, sensitivity, and specificity were 53.06%, 61.9%, and 65.9% respectively (p=0.034). When the intraoperative FS examinations and the final pathology results were compared in terms of the degree of deep MI of the tumor, a significant strong consistency was observed, accuracy, sensitivity, and specificity were 77.18%, 85.7%, and 95.5%, respectively (p=0.000) (Table 2).

In the literature, the presence of adenomyosis or uterine myoma is associated with low consistency of preoperative MRI examination with the final pathology results in detecting deep MI (9,10). In our study, when the pathologies that could increase the discordance between the pre-operative MRI examinations and final pathology results for detecting deep MI were compared; the presence of uterine fibroid or adenomyosis rate, tumor size, and location of the tumor did not differ significantly between the two groups; the preoperative MRI examinations and final pathology results were consistent or inconsistent in terms of detecting deep MI (p>0.05) (Table 3a, b).

Impact on Clinical Management and Cost on Health Care for Patients

When the two groups of patients whose preoperative MRI examinations and final pathology results were consistent and inconsistent were compared; the two groups did not differ significantly in age or ASA score (p>0.05). In those with preoperative MRI examinations and the final pathology results are inconsistent; laparotomy rate (p=0.026), operation time (p=0.047), total days of hospitalization (p=0.004), need for ICU administration after the operation (p=0.027) and total cost for patients in TL (p=0.015)

Table 2. Comparison of MRI and frozen examinations with final pathology results

		Myometrial invasion based on final pathology		Accuracy	Sensitivity	Specificity	Kappa	p-value
		(-)	(+)					
MRI invasion	(-)	29	8	53.06%	61.9%	65.9%	0.256	0.034
	(+)	15	13					
Frozen invasion	(-)	42	3	77.18%	85.7%	95.5%	0.822	0.000
	(+)	2	18					
At least one of the MRI or frozen tests	(-)	27	3	86.36%	62.79%	70.77%	0.429	0.000
	(+)	16	19					

Cohen's kappa analysis; MRI: magnetic resonance imaging

Table 3a. Comparison of demographic data of the groups whose MRI and final pathology results were compatible and inconsistent and their effects on patient management

	Pathology-MR consistent		Pathology-MR inconsistent		p-value
	Mean ± SD	Median	Mean ± SD	Median	
Age	61.1±8.4	62.0	61.0±7.8	60.0	0.964[t]
Operation time (minutes)	233±72	210	256±60	240	0.047[m]
Total length of stay (days)	9.5±4.5	8.0	13.3±5.4	11.0	0.004[m]
Cost (TL)	2699±1108	2640	3418±1194	3170	0.015[m]
Cost ($)	774±335	778	1048±410	952	0.005[m]

[t]t-test, [m]Mann-Whitney U test. TL: Turkish Lira, $: US Dollar, SD: standard deviation, MRI: magnetic resonance imaging

92

Yeniocak et al.
Overdiagnosis of Endometrium Cancer by MRI

J Acad Res Med 2023;13(2):88-94

and $ (p=0.005) were significantly higher compared to the group whose preoperative MRI examinations and final pathology results were consistent (Table 4a, b).

DISCUSSION

Evaluating Each Diagnostic Test Separately

Investigating 65 patients who were surgically staged for EC within 4 years in our clinic, when each diagnostic test was considered separately; we performed advanced surgical staging in 15 patients (23%) with a low risk of EC due to the low specificity of the pre-operative MRI examinations we routinely requested. This number was two patients (3%) for the FS analysis. On the other hand, if we had planned the surgical staging without preoperative MRI examinations but only with intraoperative FS examinations, we would have underestimated the surgical staging of three patients (4.6%). The first case, total lymph node dissection was already planned preoperatively for the patient's operation since the endometrial biopsy result was reported as high-grade serous cancer. Our second patient, a 64-year-old FIGO stage I endometrioid type adenocarcinoma, was 4x2.5 cm

in size and located in the fundus on ultrasound examination. In both pre-operative MRI, intraoperative FS, and postoperative paraffin section examinations, deep MI was <50%; however, in the pre-operative MRI examination, possible pelvic lymph node involvement was reported. Therefore, total lymph node dissection was performed. In the final pathology results of 25 pelvic and paraaortic lymph node examinations, one pelvic lymph node was reported as positive in the final pathology results, and the patient was surgically reported as FIGO stage IIIC1. In our last patient, who was 57 years old, with endometrioid adenocarcinoma FIGO stage I, there was a polypoid mass of 4x4.5 cm in the posterior fundus on ultrasound examination. Although the deep MI of the tumor was <50% in the pre-operative MRI and intraoperative FS examinations, the deep MI of the tumor was ≥50% in the final paraffin examinations. Because the tumoral involvement was not detected in the suspicious pelvic lymph node sampling of the patient, the patient was surgically reported as FIGO stage Ib.

The Mayo Criteria

Grade I and II endometrioid type EC, size less than 2 cm in the pre-operative radiological examinations and deep MI <50% in

Table 3b. Comparison of demographic data of the groups whose MRI and final pathology results were compatible and inconsistent and their effects on patient management

		Pathology-MR consistent		Pathology-MR inconsistent		p-value
		Number	(%)	Number	(%)	
ASA	I	12	28.6%	4	17.4%	0.547$^{X^2}$
	II	22	52.4%	15	65.2%	
	III	8	19.0%	4	17.4%	
Surgical	Laparoscopy	23	54.8%	6	26.1%	0.026$^{X^2}$
	Laparotomy	19	45.2%	17	73.9%	
Need for ICU after surgery	(-)	33	78.6%	12	52.2%	0.027$^{X^2}$
	(+)	9	21.4%	11	47.8%	

$^{X^2}$Pearson's chi-square test. ASA: American Society of Anesthesiology, MRI: magnetic resonance imaging, ICU: intensive care unit

Table 4a. Comparison of pathologies that may increase the inconsistency of MRI examination with final pathology results

		Pathology-MR consistent		Pathology-MR inconsistent		p-value
		Number	%	Number	%	
Presence of fibroid/adenomyosis in the pathology report	(-)	26	61.9%	17	73.9%	0.328$^{X^2}$
	(+)	16	38.1%	6	26.1%	
Tumor location in pathology report	Located in the fundus/corpus	33	78.6%	20	87.0%	0.405^m
	Located in the lower segment	9	21.4%	3	13.0%	

^{m}Mann-Whitney U test, $^{X^2}$Pearson's chi-square test. MRI: magnetic resonance imaging

Table 4b. Comparison of pathologies that may increase the inconsistency of MRI examination with final pathology results

	Pathology-MR consistent		Pathology-MR inconsistent		p-value
	Mean ± SD	Median	Mean ± SD	Median	
Tumor size in pathology report	4.4±2.8	4.0	5.1±2.8	4.5	0.454^m

^{m}Mann-Whitney U test, MRI: magnetic resonance imaging, SD: standard deviation

J Acad Res Med 2023;13(2):88-94

Yeniocak et al.
Overdiagnosis of Endometrium Cancer by MRI

93

the intraoperative FS examinations, was defined as "low risk" by Mariani et al. (11). In this patient group, the risk of retroperitoneal lymph node metastasis was found to be 5% or lower. Therefore, routine lymph node dissection can be ignored in this patient group. Later, this idea was supported by a community-based study conducted by Vargas et al. (4). In this patient population, the risk of retroperitoneal lymph node metastasis was reported to be approximately 1%. When we evaluated our three patients who had false-negative intraoperative FS results, we considered the existing criteria because one of our patients had non-endometrioid-type EC on endometrial biopsy and the tumor size was greater than 2 cm in the pre-operative ultrasonographic examinations of our other two patients. We would have included these three patients in the high-risk group for lymph node metastasis. As a result, we could avoid any undertreatment for surgical staging using Mayo criteria.

Patient Management and Cost Analysis

In our clinic, evaluating patients by a multidisciplinary team, surgical intervention is planned endoscopically if there are no contraindications. In the LAP2 randomized controlled study of the Gynecologic Oncology Group (12), 1696 patients between clinical stages I-IIA were randomized to the laparoscopy group and 920 patients to the laparotomy group. Similar rates of intraoperative complications were found in the laparoscopy group. In the laparoscopy group, a longer operative time (mean, 204 vs. 130 minutes) and a lower postoperative complication rate (14% vs. 21%) were found. In the same study, the >2-day length of hospital stay (52% vs. 94%) and the need for lymph node dissection (8% vs. 4%) were lower in the laparoscopy group; however, no significant difference was found in terms of the total number of lymph nodes removed between the two groups. In our study, although the rate of laparotomy was significantly higher in the group whose preoperative MRI examinations and final pathology results were inconsistent compared to the consistent group, the operation time was 30 minutes longer on average (210 vs. 240 minutes) and the total number of days of hospitalization was 3 days longer on average (8 days vs. 11 days). Likewise, the costs for patients in the group with inconsistent MRI examination results were significantly higher on average by 530 TL ($74) compared to the group with consistent preoperative MRI examination results in terms of deep MI of the tumor (p=0.015).

The Possible Cause of Low Consistency of MRI According to the Current Literature

The role of FS examination as a diagnostic test in showing the degree of deep MI, shown as a limitation of selective lymph node dissection in the literature, is controversial due to variable sensitivity and specificity results (13,14). The meta-analysis by Andreano et al. (15) reported a sensitivity of 86% and specificity of 86% for dynamic MRI for the diagnosis of deep MI. In our retrospective analysis, the possible cause of our low consistency of preoperative MRI findings and the final pathology results in detecting the deep MI of the tumor is, the pre-operative MRI

sequences were performed with slice thicknesses of 5 mm. On the other hand, in intraoperative FS examinations, the endometrium was sliced at 3-5 mm intervals. In the current literature review, reports with high sensitivity and specificity of preoperative MRI for detecting deep MI of the tumor, imaging technique was performed with 3-5 mm intervals.

The current guidelines of the European Society of Gynecological Oncology, the European Society for Radiotherapy and Oncology, and the European Society of Pathology recommend, for predicting deep MI of EC, preoperative MRI examinations are used but not intraoperative FS examination (16,17). However, these guidelines do not show sufficient evidence because there is no satisfactory data directly comparing both methods on the same subjects. We think that the Mayo criteria can be safely used in centers such as our clinic where intraoperative FS examination shows strong consistency in determining deep MI of the tumor. Without combining two diagnostic tests for detecting deep MI but keeping preoperative MRI examination for high-risk tumors for adjacent/distant organ involvement, high-risk patients for LVSI or cervical involvement.

Study Limitations

The main limitation of this study is the relatively small study population and design retrospectively. Any further prospective large series will support our findings.

CONCLUSION

Creation and implementation of ideal follow-up and treatment plans in EC emerges as a responsibility, not only a consideration of deontological perspective but also a need in health-care economics in the practice of medicine. Considering current scientific data, we think that our study is important in the EC diagnostic approach and patient management algorithm, as it emphasizes the need for treatment centers to individualize their current diagnostic tests on a patient and clinical basis, considering the accuracy of existing tests.

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94 Yeniocak et al.
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Optimizing Signal to Cut-off Values (S/Co) of Anti-HCV in Prediction of Viremia: A Retrospective Analysis with HCV-RNA

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ABSTRACT

Objective: The prevalence of chronic hepatitis C worldwide is defined in three categories: low (<2.5%), medium (2.5-10%) and high (>10%) while the prevalence of it is seen between 0.5-1.9% in Turkey. This study aims to identify the most appropriate signal to cut-off (S/Co) value to observe viremia and decrease the number of unnecessary hepatitis C virus-ribonucleic acid (HCV-RNA) analyzes due to false positive anti-HCV results.

Methods: A retrospective analysis was performed regarding the relationship of HCV-RNA and anti-HCV results between January 2019-September 2021. Adult patients (18+ age) with reactive anti-HCV and HCV-RNA results were included in the study.

Results: A total of 626 patients were included (median age 49.7; standard deviation ±18.2), of which 352 (56.2%) were female and 274 (43.8%) were male. Despite their seropositivity, 595 (95%) patients were HCV-RNA negative and only 31 (%5) patients were positive. Non-parametric Mann-Whitney U test was used to compare anti-HCV results regarding HCV-RNA positive and negative patients. Anti-HCV levels in the HCV-RNA positive group of patients (median =13.7) were significantly higher compared with the HCV-RNA negative group (median =1.1) (p=0.001). HCV-RNA was accepted as the gold standard in receiver operator characteristic (ROC) analysis to define the most accurate cutoff value which was found to be 8.9 S/Co. Sensitivity and specificity were 93% and 91%, respectively [area under the curve (AUC): 0.94] (95% confidence interval: 0.907-0.974). AUC was significantly over 0.5 (p=0.001).

Conclusion: It is essential for laboratories to modify reference values according to their patient populations and prevalence. 8.9 S/Co was defined as the most appropriate value for anti-HCV in our laboratory and below this, additional interventions and retesting should be performed prior to the report.

Keywords: Hepatitis C, serological diagnosis, PCR, seropositivity

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INTRODUCTION

Hepatitis C virus (HCV), first identified in 1989, remains a public health issue, annually affecting almost 2 million individuals worldwide. It is one of the major causes of chronic liver disease, including hepatocellular carcinoma (1,2). Only in the USA, there are 3.2 million individuals estimated to be living with HCV infection, whereas half a million citizens are infected in Turkey (approximately 1%), suggesting the second leading reason for liver transplantation (2-4). Its prevalence is at 45%, 8-17%, and 6% in intravenous (IV) drug addicts, imprisoned individuals, and hemodialysis patients, respectively (4).

HCV is a member of the Flaviviridae family in the hepacivirus genus with a single-strand ribonucleic acid (RNA) and envelope. The most frequent type worldwide (including Turkey) is genotype 1 subtype 1b (2,4). The whole pathogenesis has not been totally understood; however, it is known that the natural progression of the disease takes a long time. Almost all untreated acute cases turn into chronic viral hepatitis, of which 5-15% convert to eventually cirrhosis and 1-4% cause hepatocellular carcinoma (5). Symptomatic cases rarely become chronic and most patients are diagnosed incidentally because individuals in the subclinic phase (transforming progress from acute to chronic) are usually not aware of their infected status (4-6).

HCV infection is diagnosed by testing for specific antibodies, which actually do not state whether the infection is acute, chronic, or resolved. In addition, an "antibody-undetectable" window period was defined in the first weeks of infection (5). Screening is another terminology, since The Centers for Disease Control and Prevention (CDC) recommends it at least once in a lifetime for all adults during each pregnancy (7). In HIV-infected individuals, persons with persistently abnormal alanine aminotransferase (ALT) levels, hemodialysis patients, children born from HCV-infected mothers, IV drug addicts, and blood donors, routine periodic testing is strongly recommended (7). As stated above, additional testing is required to distinguish between a resolved and current infection to consider antiviral treatment. Hence, nucleic acid testing [HCV-RNA polymerase chain reaction (PCR)] is a good marker to observe current viremia, and the CDC published an algorithm to diagnose the infection (8).

In the stated algorithm, CDC particularly pointed to possible false positivity of antibody tests (8). Enzyme immunoassay (EIA) and chemiluminescent immunoassay (CLIA) are routinely used with highly beneficial potential to predict viremia, since high signal to cut-off (S/Co) ratios were reported to be associated with HCV-RNA positivity, however, it is noted that especially high rates of false positivity can be observed in low prevalence (<2.5%) areas (9). In such cases, the usage of S/Co ratios in reflex supplemental testing algorithms is recommended by CDC (1). This study aims to identify the most appropriate and accurate S/Co value to observe viremia and decrease the number of unnecessary HCV-RNA analysis due to false positive anti-HCV results. With this S/Co value, we plan to provide self-algorithms for our laboratory.

METHODS

Materials: A retrospective-analysis was performed regarding the relationship of HCV-RNA and anti-HCV results between January 2019-September 2021 in Balıkesir Atatürk City Hospital. Adult patients (18+ age) whose anti-HCV was reactive and who were simultaneously investigated for HCV-RNA were included in the study. Patients that did not fit these criteria were excluded.

Methods: Anti-HCV analysis was performed with the CLIA method using an Architect i2000 analyzer (Abbott Diagnostics, Abbott Park, IL, USA). Isolation of HCV-RNA was done with a Magnesia 16 device and kit (Anatolia Geneworks, Istanbul, Turkey), and PCR was applied with Bosphore HCV Quantification Kit v2.0 by a Montania 4896 Real Time PCR device (Anatolia Geneworks, İstanbul, Turkey).

Statistical Analysis

Median S/Co values were calculated for both HCV-RNA positive and negative groups. Due to non-normal distribution of anti-HCV results, a nonparametric Mann-Whitney U test was used to compare anti-HCV results regarding HCV-RNA positive and negative patients. HCV-RNA was accepted as the gold standard in receiver operator characteristic (ROC) curve analysis to define the most accurate cut-off value.

Ethical approval: Approved by the Ethical Board of İstanbul Medipol University Non-Invasive Clinical Research (decision no: 1306, date: 23.12.2021).

RESULTS

A total of 626 patients were included (median age 49.7; standard deviation ±18.2), of which 352 (56.2%) were female and 274 (43.8%) were male. Almost all patients were under a follow-up schedule for infectious disease and gastroenterology services.

Five hundred ninety five (95%) of patients were HCV-RNA negative, while 31 (5%) patients were positive. Anti-HCV levels were significantly higher in the positive HCV-RNA group (median: 13.7) compared with the negative HCV-RNA-group (median: 1.1) (p=0.001) (Figure 1).

In the ROC analysis, the most accurate cut-off value was found to be 8.9 S/Co (Table 1). Sensitivity was 93%, specificity was 91%, and area under curve (AUC) was found to be 0.94 (confidence interval: 95%; 0.907-0.974), which was significantly higher from 0.5 (p=0.001) (Figure 2).

DISCUSSION

At present, isolation and culture of HCV from clinical specimens are extremely difficult, and HCV-RNA is accepted as a gold standard in order to show viremia; especially the quantitative one, which is important for treatment monitoring. However, the test requires experienced staff, is time-consuming, and costs high, and for these reasons, serological analysis has come forward as a simpler and cheaper screening procedure (10,11). On the other hand, anti-HCV IgMs could be detected in patients with acute
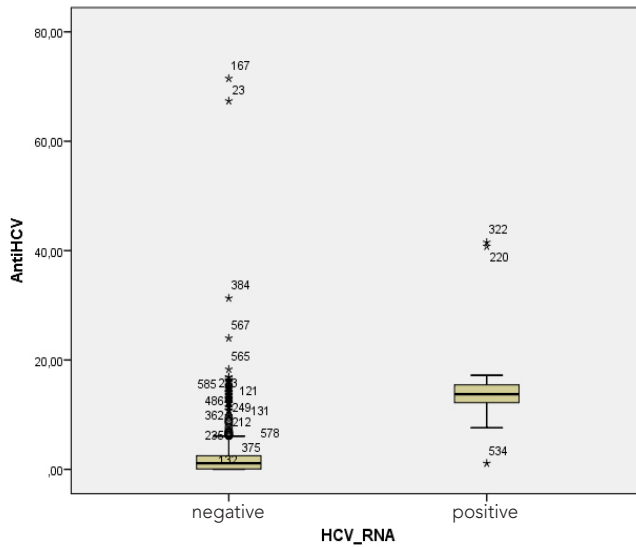
Figure 1. Median analysis of anti-HCV levels
HCV-RNA: hepatitis C virus-ribonucleic acid
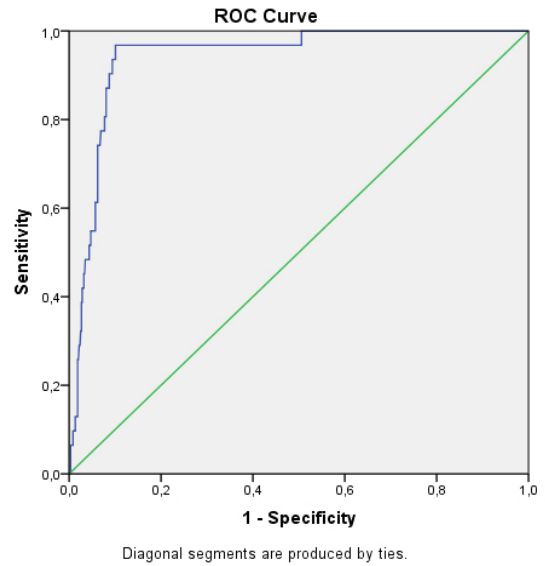
Figure 2. ROC analysis
ROC: receiver operator characteristic

Table 1. ROC analysis results

Anti-HCV S/Co	Sensitivity, %	Specificity, %
1.01	100	46
2.02	96	70
3.05	96	79
4.06	96	83
5.08	96	85
6.02	96	87
7.03	96	89
8.9	**93**	**91**
9.07	90	91
10.1	90	92

ROC: receiver operator characteristic, HCV: hepatitis C virus, S/Co: signal to cut-off

hepatitis C and chronic patients. Thus, anti-HCV IgM cannot be used as a reliable marker for active infection, which prevents its routine usage for screening purposes. Currently, screening and diagnostic assays via EIA or CLIA for anti-HCV total antibody are widely used in clinical practice (11). In general, the CLIA shows better specificity than the EIA, but both methods have high false-positive rates, especially in low-prevalence populations (1). In addition, as previously stated, anti-HCV total antibody seropositivity may indicate previous exposure to the virus, active infection, or false positivity. A window period of false negativity was also reported. These conditions strongly limit "diagnostic" value of the total antibody (5,10). The World Gastroenterology Organization Global Guideline on Diagnosis, Management and Prevention of Hepatitis C states that acute hepatitis C diagnosis depends on "marked elevation of alanine aminotransferase (ALT; more than 10x)", "with or without jaundice", "detectable serum HCV-RNA" and "followed by anti-HCV seroconversion weeks later" (12).

Serology has an important mission as a screening procedure, which the CDC strongly recommends at least once in an adult's lifetime, especially in particular patient groups (7). Screening tests are actually slightly different from diagnostic tests because a high negative predictive value (negative likelihood ratio) is mainly focused on "slightly tolerated" false positives. The main expectation from a screening test is not to miss even a single case, so suspected positive results are exposed to confirmatory tests such as HCV-RNA, which are mainly used as diagnostic tests with high specificity (2,11). The accuracy of a test is not based only on sensitivity and specificity, since overall accuracy generally shows a prevalence-dependent feature, which is a problematic descriptor of test validity. Other terms to describe the validity of tests were defined, such as positive likelihood ratio, negative likelihood ratio, and AUC, which do not vary with disease prevalence (13).

CDC notified the importance of S/Co value, particularly in low prevalence countries (like Turkey), to optimize the interpretation of assay results and recommended laboratories to define their own ratio (2). In such cases, no further testing is required for diagnosis. Studies have reported that higher S/Co value correlates with higher positive predictive value (1). Regarding this, the S/Co values for Abbott Architect (Abbott Diagnostics, Abbott Park, IL, USA), Ortho Vitros (Ortho Clinical Diagnostics, Raritan, NJ, USA), and the Siemens Advia Centaur (Siemens Healthineers AG, Erlangen, Germany) anti-HCV assays were stated by CDC as 5.0, 8.0, and 11.0 respectively (3). Kim et al. (3) suggested 19.0 for Elecsys assay (Roche Diagnostics International AG, Basel, Switzerland) (ELISA vs recombinant immunoblot assay-RIBA and HCV-RNA). Similarly, there are studies from Turkey indicating

98

Gülseren et al.
Anti-HCV S/Co Value Optimization

J Acad Res Med 2023;13(2):95-9

S/Co values, such as Altuğlu et al. (14) (3.27 with CLIA vs line immunoassay), Şanlıdağ et al. (15) (5.0 with CLIA vs HCV-RNA), Karakoc et al. (16) (8.1 with Ortho EIA and 3.4 with microEIA vs RIBA and PCR), and Aydın et al. (2) (7.13 with CLIA vs HCV-RNA). Confirmatory tests other than PCR may be evaluated as "overdated". On the other hand, results compared with HCV-RNA, including this study (8.9 with CLIA vs HCV-RNA), show variability. Differences between these values would not reflect any differences in terms of analytical performances, but they can be based on assay differences (methods and molecules utilized for signal generation and detection) and/or sample size. In a wide study by Lai et al. (17), below 3.0 S/Co values were indicated as negative with CLIA vs RIBA, along with 3.0-19.9 values as the gray zone, which requires additional confirmatory tests.

Defining valid S/Co value is important to increase accuracy. However, as shown, there is a hidden danger in creating an individual laboratory-based S/Co value, since this might corrupt standard procedures at the national base and might also cause confusion in interpretations. This is because of not only variations between laboratories even when the same devices are used but also because of high-fixed S/Co values that can cause the missing of true positives, which is totally undesirable for a screening test. On the other hand, low S/Co values can make the assay less specific, which makes the test insufficient to establish the clinical diagnosis of the disease (18), since the CDC recommended additional testing for laboratories with low S/Co values (1). A wide study from Turkey directly supports this suggestion that high S/Co values are more appropriate (9). On the other hand, there are also studies indicating that S/Co definition does not sufficiently optimize the tests and thus additional tests are required (1,19). Furthermore, HCV antibody assays vary according to their antigens, test platforms, and performance characteristics, so it is hard to suggest a standard S/Co value (19).

Study Limitations

The major limitation of this study was the lack of all HCV-RNA results of all patients during the study period. Our center became active in 2017 and in our investigation time zone, we could not reach the HCV-RNA data of all seropositive patients. Secondly, our study was based on a single CLIA device, and hence we could not conduct multiple device antibody testing.

CONCLUSION

Even though there are controversial points about assay interpretations via S/Co values, the CDC still recommends laboratories to do so, especially when focusing on a population-based perspective. In Turkey, anti-HCV false positivity is a common condition that creates unnecessary workload, costs, and a requirement for molecular analysis. In this study, a high S/Co value of 8.9 showed a direct correlation with true positivity without any further testing to detect viremia. For values below this value, additional sampling and testing are recommended before HCV-RNA PCR.

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99

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Screw-only Fixation of Calcaneal Fractures Using the Sinus Tarsi Approach

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ABSTRACT

Objective: Calcaneus is the most commonly fractured tarsal bone. This type of fracture may result from high-energy trauma or axial loading. The ideal treatment method for calcaneal fractures remains controversial in terms of surgical approach and the material that will be used for fracture fixation. This study aims to evaluate the outcomes of screw-only fixation in intra-articular calcaneal fractures using the sinus tarsi approach.

Methods: Patients who were treated between 2014 and 2021 for calcaneal fractures were retrospectively investigated. Functional outcomes were evaluated using the American Orthopaedic Foot & Ankle Society (AOFAS) hindfoot-ankle score, and Gissane and Böhler angles were measured and compared.

Results: The study included 32 patients (23 male, 9 female) who underwent screw fixation through the sinus tarsi approach for intra-articular calcaneal fractures. The mean age of the patients was 45.2 years. The fractures were classified according to the Sanders Classification, with 15 patients having type 2 fractures, 12 with type 3 fractures, and five with type 4 fractures. AOFAS hindfoot-ankle score revealed nine patients achieving excellent results, 14 patients achieving good results, and nine patients achieving fair results. AOFAS scores were lower in patients with Sanders type 4 fractures when compared to type 3 and type 2 fractures (p=0.005 and p<0.001 respectively).

Conclusion: This study provides evidence supporting the favorable results of the sinus tarsi approach using screw-only fixation for treating intra-articular calcaneal fractures.

Keywords: Sinus tarsi, screw-only fixation, mini incision, calcaneus, Sanders type 4

INTRODUCTION

The most commonly fractured tarsal bone is the calcaneus, and this fracture may result from high-energy trauma or axial loading (1). Displaced intra-articular fractures account for 60% to 75% of these fractures. The Sanders Classification, which takes the displacement and number of fragments in the posterior facet into consideration, is commonly used for classifying calcaneal fractures (2). When the posterior facet is fractured, a subtalar intra-articular fracture occurs, and achieving anatomical restoration of the joint surface is crucial in this situation. Open reduction is recommended for restoring normal anatomy and fracture reduction (3). Traumatic arthritis of the talocalcaneal joint, which results from the inability to achieve anatomical restoration of the joint surface, leads to a painful joint that impairs walking (4). However, the ideal treatment method for calcaneal fractures remains controversial,

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Özden and Aybar.
Screw Fixation Calcaneal Fractures Sinus Tarsi

101

and studies advocating conservative treatment also exist due to the high complication rates associated with surgical treatments (5). However, these complications are often associated with the extended lateral approach (6).

While the extended lateral approach can restore the calcaneus geometry and anatomical surface, it can also present problems with the surgical site (6,7). Recently, the sinus tarsi approach, which allows anatomical reduction and fixation of the subtalar joint while encountering fewer wound complications, has become more favorable in the surgical treatment of calcaneal fractures (8-10). This approach enables minimally invasive plate applications, screw osteosynthesis and osteosynthesis using only K-wires (11-13). While standard calcaneal plates are used in the extended lateral approach, modified plates can be used in the sinus tarsi approach, and there is limited research on osteosynthesis without using plates (12-14).

This study evaluated the radiological and functional outcomes of patients with Sanders type 2, 3 and 4 calcaneal fractures, where osteosynthesis was achieved by screws only through the sinus tarsi approach.

METHODS

Patients who were treated between 2014 and 2021 for calcaneal fractures were retrospectively investigated after obtaining approval from University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital Clinical Research Ethics Committee (decision no: 345, date: 06.10.2021). The calcaneus fractures that underwent intra-articular fragment fixation using only screws through the sinus tarsi approach were included in the study. Patients with accompanying tarsal bone fractures or pre-existing pathologies that could cause preoperative mobility limitations were excluded from the study because they could affect the outcomes. In addition, isolated tongue-type fractures and patients who underwent fracture fixation using the extended lateral incision were excluded. Fractures that were treated by plate osteosynthesis were also excluded from the study.

Surgical Technique

After administering prophylactic cefazolin antibiotics, patients with isolated calcaneal fractures were placed in the lateral decubitus position. In contrast, patients with bilateral calcaneal fractures were placed in the supine position, and a pneumatic tourniquet was applied to the thigh. A 4 cm straight incision was made beneath the lateral malleolus leading to the fourth metatarsal base (Figure 1). While preserving the lateral sural cutaneous nerve, the peroneal tendons were retracted posteroinferior and the lateral cortex of the calcaneus was exposed (Figure 2). When the talocalcaneal joint became visible, the posterior facet, usually found collapsed anteroinferior, was reduced with the help of an elevator and temporarily fixated with K-wires. The Achilles tendon should be loosened to facilitate the reduction and the

calcaneal tuberosity should be able to advance distally. Therefore, knee flexion is essential. After reduction of the posterior facet, it was stabilized to the constant fragment using one or two 4 mm short-threaded cannulated screws (Figure 3). Varus malalignment of the heel was checked with fluoroscopy (Figure 4), and then two 6.5 mm cannulated screws were placed in the posterior-to-anterior direction for fixation (Figure 5). No graft was applied to the remaining defective area after reduction of the posterior facet. Following anatomical closure, a dressing and a short leg cast were applied, and a drain was inserted. The drain and cast were removed after 24 hours and early mobilization was initiated.

Figure 1. 4 cm straight sinus tarsi incision on a patient in lateral decubitus position

Figure 2. Flexor tendons are protected

Figure 3. Posterior facet is reduced and fixed with one or two canulated screws

102

Özden and Aybar.
Screw Fixation Calcaneal Fractures Sinus Tarsi

J Acad Res Med 2023;13(2):100-5

Partial weight bearing was started in the first month, and full weight bearing was allowed in the second month.

Outcomes of Interest

The American Orthopaedic Foot & Ankle Society (AOFAS) hindfoot-ankle score was used to evaluate functional outcomes (15). Boehler and Gissane angles were measured and compared on preoperative and postoperative radiographs. Wound complications and the presence of reflex sympathetic dystrophy were assessed.

Statistical Analysis

Three groups were created according to the Sanders classification, and the normal distribution of the data was assessed by Shapiro-

Figure 4. Fluoroscopy position for the Harris view

Figure 5. Two 6.5 mm cannulated screws were placed in the posterior-to-anterior direction

Wilk test for each group by using the SPSS 25.0 (SPSS Inc, IBM, Chicago, IL) software. Mean, standard deviation (SD), median, and interquartile range (IQR) values were used as descriptive analysis when presenting the data. Non-normally distributed (non-parametric) variables were evaluated between groups using the Kruskal-Wallis test, and the Wilcoxon test was used to compare the pre-operative and postoperative calcaneus angles if any of the parameters were nonnormally distributed. However, normally distributed (parametric) variables were compared using the paired sample t-test. AOFAS scores between groups were analyzed using the ANOVA test, and post-hoc (Tukey) test results were given. Results where the p-value was below 0.05 were considered statistically significant.

RESULTS

A total of 32 patients, including nine (28.13%) females and 23 males (71.88%) who underwent fixation of intra-articular calcaneal fractures using only screws through the sinus tarsi approach, were included in this study. The mean age was 45.2 years (range: 19-68, SD: 11.5). Among the patients, there were 15 Sanders type 2 fractures (46.88%), 12 type 3 fractures (37.50%), and 5 type 4 fractures (15.63%). The mean ± SD values for normally distributed data and median (IQR) values for non-normally distributed data and the comparison of preoperative and postoperative angles for every fracture type are presented in Table 1. Statistically significant differences were observed between the preoperative and postoperative Gissane and Böhler angles in all types of fractures (p<0.05).

According to the AOFAS scores, nine patients had excellent results (28.13%), 14 had good results (43.75%), and nine had fair results (28.13%). However, the AOFAS scores were lower in patients with Sanders type 4 fractures when compared with type 3 and type 2 (p=0.005, p<0.001 respectively) fractures. Type 2 fractures had better results than type 3 fractures, and this difference was found to be statistically significant (p=0.025) (Table 2).

DISCUSSION

This study evaluated the outcomes of calcaneal intra-articular fractures that were treated by screws only fixation, using the sinus tarsi approach. The sinus tarsi approach allows for anatomical reduction and fixation of the subtalar joint, and cannulated screws can be used alone for osteosynthesis of calcaneal fractures in selected cases.

After Griffin et al. (16) reported no significant difference in symptoms and functional outcomes between surgical and conservative treatments in 2014, an increased tendency toward conservative treatment emerged. Similarly, Shah et al. (17) reached the same conclusion in 2021. However, both studies used the extensile lateral approach described by Letournel (18). It has been observed that wound complications with this approach
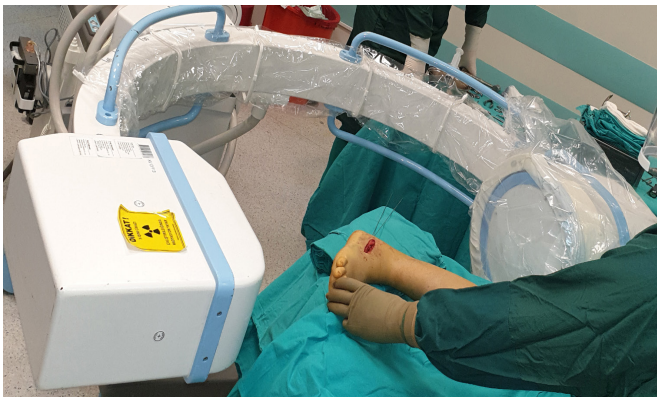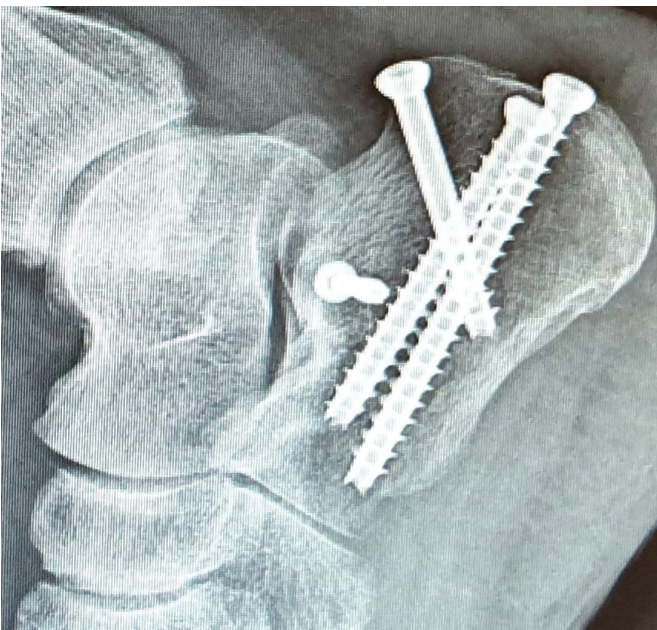
Özden and Aybar.
Screw Fixation Calcaneal Fractures Sinus Tarsi

103

J Acad Res Med 2023;13(2):100-5

Table 1. Comparison of pre-operative and postoperative angles

	Sanders type 2	Sanders type 3	Sanders type 4	p[1]
	Mean ± SD Median (IQR)	Mean ± SD Median (IQR)	Mean ± SD Median (IQR)	
Follow-up period as months	24 (8)	28.67±7.89	24.2±6.72	0.390
Böhler angle pre-operative	7.2±3.17	7.08±2.61	3 (6)	0.424
Böhler angle postoperative	31.07±2.94	27.5 (6)	29.2±5.12	0.174
p[2]	**0.017**			
P[3]		**0.002**	**0.043**	
Gisanne pre-operative	110 (14)	105.17±3.76	108.6±7.23	0.684
Gisanne postoperative	116.13±6.08	118.67±6.53	124.6±7.8	
p[2]		**<0.001**	**0.004**	
p[3]	**0.003**			

p[1]: Kruskal-Wallis test, p[2]: paired sample t-test, p[3]: Wilcoxon test (paired sample t-test was used if both groups are normally distributed & Wilcoxon test was used if any one of the groups was not normally distributed). IQR: interquartile range, SD: standard deviation

Table 2. ANOVA test post-hoc analyse for AOFAS scores between groups

	AOFAS	p-value
Sanders type 2	89.8±7.06	0.025
Sanders type 3	83.33±5.31	
Sanders type 2	89.8±7.06	<0.001
Sanders type 4	72.2±3.27	
Sanders type 3	83.33±5.31	0.005
Sanders type 4	72.2±3.27	

AOFAS: American Orthopaedic Foot & Ankle Society

occur at around 25% and are exceptionally high in individuals with risk factors such as smoking and diabetes (19).

The incision described by Palmer (20), which is more limited compared to the extensile approach, was modified by Gupta et al. (21), and it became well-known as the sinus tarsi approach. This approach has recently gained popularity in the surgical treatment of calcaneal fractures (8,22). In addition, it is associated with fewer wound complications compared with the extended lateral approach (6,23). Subsequently, several studies compared the sinus tarsi approach to the lateral extensile approach and demonstrated that the rates of wound complications with the sinus tarsi approach have lowered (24-26).

Although the sinus tarsi approach is widely used today, there is an ongoing search for suitable fixation materials for this incision because the plate used in the extensile approach is incompatible. In this regard, Eltabbaa et al. (12) used K-wires, Huang et al. (22) used a modified calcaneal plate, Xia et al. (4) used a combined locking plate, and Kikuchi et al. (8) used a one-third tubular plate, locking/non-locking plate, and in some cases, only cannulated

screws. The sinus tarsi approach without plating has been studied less, with limited research on the outcomes of such fixation methods (8,12,13). In our study, we achieved osteosynthesis using only screws and initiated early mobilization.

Guo et al. (27) compared a less invasive plate with the screw fixation method. They found no significant difference in functional outcomes but emphasized that screw fixation alone is more cost-effective. In another study where only screws were used, Abdelazeem et al. (28) concluded that screw-only fixation is sufficient for Sanders type 2 and 3 fractures. Our results also demonstrated significant improvements in the Gissane and Böhler angles after surgery, indicating successful anatomical restoration of the subtalar joint surface. These findings suggest that the sinus tarsi approach with screw fixation can effectively achieve anatomical reduction and restoration of the subtalar joint surface.

Most of the patients in this study achieved excellent or good results according to the AOFAS scores, indicating satisfactory functional outcomes following surgery. These results demonstrate the positive impact of the sinus tarsi approach with screw fixation on functional recovery.

Abdelazeem et al. (28) stated that there was no significant difference between the AOFAS score and Sanders type, but they did not include any type 4 fracture. Alajmi et al. (29) concluded that Sanders type 4 fractures are more prone to complications than other types, even when the sinus tarsi approach is used. We observed a correlation between the Sanders Classification and AOFAS scores, with type 4 fractures associated with poorer AOFAS scores (p<0.001). This finding highlights the importance of considering fracture severity and displacement when assessing functional outcomes in calcaneal fractures.

Regarding complications, we encountered no wound-related issues in our patient cohort. In addition, there were two reflex

104

Özden and Aybar.
Screw Fixation Calcaneal Fractures Sinus Tarsi

sympathetic dystrophies, that were resolved after partial weight bearing. These data suggest that the sinus tarsi approach with screw-only fixation has a low risk of wound complications.

Study Limitations

It is essential to acknowledge the limitations of our study. The relatively small sample size may have affected the generalizability of our findings as this was a retrospective study. Additionally, the lack of a control group limited our ability to directly compare the outcomes of the sinus tarsi approach with other surgical techniques or conservative management.

CONCLUSION

Our study provides evidence supporting the sinus tarsi approach using screw-only fixation for treating intra-articular calcaneal fractures. Our study suggests that the sinus tarsi approach is effective and safe for treating calcaneus fractures without using plates. This technique allows the anatomical reduction of the subtalar joint and demonstrates favorable outcomes.

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Screw Fixation Calcaneal Fractures Sinus Tarsi

105

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Ministry of Health Data Reveals Age, Gender, and Clinic-specific Differences in CEA Test Outcomes for Colorectal Cancer Detection

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ABSTRACT

Objective: Carcinoembryonic antigen (CEA) is a tumor marker associated with various malignancies, including colorectal cancer. This study analyzes the use and outcomes of CEA testing in Türkiye, with a focus on colorectal cancer detection. The objective of this study is to examine the values of CEA in Türkiye, how it is used in cancer diagnosis and treatment, and how it can be optimized.

Methods: Data from a five-year period (2017-2021) were analyzed, including a total of 27,394,778 tests from 4,016,178 individuals. The CEA test results were obtained through immunoassay method and extracted from the National Health Database System of the Turkish Ministry of Health.

Results: The study found that the number of CEA tests and the number of tests per 100,000 population increased progressively between 2017 and 2019, followed by a decrease in 2020 and 2021. The rate of tests exceeding the reference range was highest in the over-65 age group and in men. The study also found that the number of CEA tests requested was highest in the Marmara region and Central Anatolia region and lowest in the Southeastern Anatolia region.

Conclusion: This study provides valuable insights into the application and outcomes of CEA tests in the detection of colorectal cancer across various demographic groups in Türkiye. Despite its limitations, this study reveals gender, age, and clinic-specific disparities in test application and outcomes, and underscores the potential value of CEA as a biomarker in cancer detection. Future research should aim for a more comprehensive data collection that encompasses lifestyle and genetic factors, longitudinal tracking of individuals in order to capture disease progression, and to explore additional biomarkers for colorectal cancer.

Keywords: Carcinoembryonic antigen, colorectal cancer, health data

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INTRODUCTION

Carcinoembryonic antigen (CEA) is a type of tumor marker associated with many types of cancer and some nonmalignant conditions. First identified in 1965 by Dr. Phil Gold and Dr. Sam Freedman, CEA is typically a glycoprotein found on the surface of cancerous cells. It shows an increase in many malignancies, including colorectal, stomach, pancreatic, lung, and breast cancers (1).

It is a glycoprotein with a molecular weight of 200 kDa and is normally derived from embryonic endodermal epithelium in the fetus, controlled by fetal oncogenes. It usually disappears from serum after birth; however, small quantities of CEA may remain in colon tissue. CEA and related genes (29 of which 18 are normally expressed) constitute the CEA family in human beings and are clustered on chromosome 19q13.2 (2). Because it is associated with various types of malignant and nonmalignant medical conditions, elevated serum CEA levels are not a definitive marker of a particular site of cancer origin (3).

CEA is a tumor marker used in colorectal, stomach, esophageal adenocarcinoma, non-small-cell lung cancer, and breast cancer. CEA is the tumor marker with the highest value in the follow-up of patients with colorectal cancer and its use is recommended in international guidelines (4).

Various meta-analyses highlight that CEA plays a significant role in the early detection of relapse in patients diagnosed with colorectal cancer, subsequently having positive effects on survival. CEA is also a preferred marker in the evaluation of treatment response and the early detection of progression in patients with metastatic disease. A continuous increase above the basal CEA value suggests disease progression, even in the absence of confirmatory imaging methods (5).

Given Türkiye population, access to healthcare, and efforts in cancer diagnosis and treatment, the development of a database related to the use of CEA is significant. The establishment of this database can enhance the quality of health services in Türkiye. Moreover, this database could play a critical role in providing more information on how CEA can be better utilized in cancer treatment.

This study aims to examine the values of CEA in Türkiye, how it is used in cancer diagnosis and treatment, and how it can be optimized. This study will also explore how the broader use of CEA could affect patients in Türkiye.

This introduction will discuss efforts to understand the use of CEA in Türkiye based on a comprehensive literature review, extensive data collection, and analysis. It will also corroborate how this study could contribute to cancer treatment by identifying possible directions and issues for future research.

METHODS

Data from a five-year period (2017-2021) were analyzed, including a total of 27,394,778 tests from 4,016,178 individuals. The test counts, test rates per population, and rates of exceeding the reference range were assessed based on gender, age groups, geographic regions, and healthcare institution types.

The CEA test results were obtained through immunoassay method and extracted from the data transferred to the National Health Database System of the Turkish Ministry of Health. The healthcare database service in Türkiye is referred to as e-Nabız. The transmission of health data set packages is facilitated through XML web services. This database encompasses the health records of patients who have sought medical services from all public, private and university healthcare institutions in Türkiye. The data include all the demographic characteristics, laboratory data, medication usage, and comorbidities of these patients.

Database and E-pulse

E-pulse is a platform developed by the Ministry of Health in Türkiye. It allows individuals to store and manage their health information digitally. For this study, patient information and health records were collected from the e-Pulse system. During the data collection process, personal information was protected, and the principle of privacy was fully respected.

This platform allows users to use 30 different services for prevention, treatment, health promotion, and health-related areas. In addition, some statistics belonging to the relevant categories are also included in the e-pulse system (6).

Health Coding Reference Server (HCRS) and International Classification of Disease (ICD) Codes

HCRS is a data recording and reporting system used by the Ministry of Health in Türkiye. This system aids in the more effective management of health services. In this study, data pulled from the HCRS, and ICD codes were used to analyze disease diagnoses, treatment plans, and the overall state of health services. ICD codes are a standard disease and health problem classification system created by the World Health Organization and used worldwide. These codes are an important tool for identifying, monitoring, and treating diseases.

The study population: The study population consisted of individuals who underwent CEA testing during the study period. Both men and women were included in the analysis.

Statistical Analysis

Descriptive statistics were used to analyze the data. The test counts, test rates per population, rates of exceeding the reference range, and cancer diagnosis rates were calculated and compared across different variables, including gender, age groups, geographic regions, and healthcare institution types.

Ethical considerations: The study adhered to ethical guidelines and protected the privacy and confidentiality of the individuals included in the data. Institutional review board approval was obtained, and all data were anonymized to ensure the privacy of the individuals involved in this study.

RESULTS

Between 2017 and 2021, CEA testing was requested from 4,016,178 individuals with the total number of tests amounting to 27,394,778. This results in an average of 6.82 tests per individual and 33,150 tests per 100,000 population. Among the tumor markers used in our CEA study, it holds the highest test number per 100,000 population. When comparing the number of CEA tests year by year, the number of tests and the number of tests per 100,000 population increased progressively between 2017 and 2019, followed by a noticeable decrease in 2020 and 2021 (Table 1). For women, the pattern is similar. The number and rate of requested tests increased from 2017 to 2019 and then significantly decreased in 2020 and 2021. It ranks sixth among all tumor markers tested in women across all years. In men, the test request pattern mirrors that of the general population, with the number and rate of tests increasing progressively from 2017 to 2019 and decreasing noticeably in 2020 and 2021. Comparing the number of tests for women to men year by year, the ratio was 2.01 in 2017, 2.01 in 2018, 2.03 in 2019, 1.96 in 2020 and 1.96 in 2021. When examining the rates of exceeding the reference range by gender, it was found to be positive at a rate of 12.82% overall, 18.01% in men and 10.14% in women. In men, the rate of exceeding the reference range for tests is second (Table 2).

When comparing test request numbers across different age groups, CEA was most frequently requested in the 18-64 age group, followed by the over-65 age group, and was least requested in the 0-17 age range. The ratio of test requests between the 18-64 age group and the over-65 group was 2.46 in 2017, 2.43 in 2018, 2.35 in 2019, 2.37 in 2020 and 2.25 in 2021. The consumption ratio of tests per 100,000 individuals between the 18-64 age group and the over-65 group was 1/2.99 in 2017, 1/2.96 in 2018, 1/2.96 in 2019, 1/2.79 in 2020 and 1/2.94 in 2021. Among the over-65 group, CEA was the second most requested tumor marker per 100,000 individuals.

Between 2017 and 2019, as the years progressed, both the number of test requests and the consumption per 100,000 individuals in the over-65 group increased, but there was a significant decrease in 2020 and 2021. When examining the rates of exceeding the reference range by age group, it was found to be the highest at 18.56% in those over 65, second at 10.44% in the 18-64 age group, and third at 1.20% in the 0-17 age group. When compared by the age group, a positivity ratio of 15.4/8.7/1 was found. When examining the rates of exceeding the reference range by admission status, the highest rate is 17.25% for inpatients, 16.22% for day-case patients, and 12.30% for outpatient patients; when rated in order, a ratio of 1.40/1.31/1 is calculated (Table 3).

In the Ministry Health's e-Nabız and Sina data, among the 4,016,178 individuals examined for CEA within 27,39,778 tests, significant differences were found in terms of age and gender when evaluated using the chi-square test for association and

Table 1. Number of tests and the ratio of the population by years

CEA	2017		2018		2019		2020		2021	
	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population
	5,124,970	6,342	5,915,615	7,214	6,531,332	7,854	4,757,144	5,689	5,065,717	6,058

CEA: carcinoembryonic antigen

Table 2a. Number of test requests in women by years

CEA	2017		2018		2019		2020		2021	
	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population
	3,423,742	8,501	3,955,933	9,681	4,379,233	10,569	3,153,069	7,562	3,357,328	8,051

CEA: carcinoembryonic antigen

Table 2b. Number of test requests in men by years

CEA	2017		2018		2019		2020		2021	
	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population
	1,701,228	4,197	1,959,682	4,763	2,152,095	5,158	1,604,075	3,827	1,708,388	4,076

CEA: carcinoembryonic antigen

additional assessment among those who were not tested for CEA (p<0.0001) (Table 4).

When comparing the rate of receiving a cancer diagnosis at any time in patients who were tested for the CEA tumor marker, the cancer detection rate increased as the years progressed from 2017 to 2020. In 2017, 36% of individuals, in 2020, 47% of individuals, and in 2021, 42% of individuals received a cancer diagnosis. When comparing cancer detection rates in people who were tested for tumor markers, CEA ranks second in terms of diagnostic percentage in all years. When analyzing the timing of test requests for individuals tested for tumor markers at the time of diagnosis, it was found that requests were at most predominantly made before diagnosis, second at the same time as diagnosis, and least frequently after diagnosis. The rate of pre-diagnosis test requests increased as the years progressed up to 2020, with a rate of 16.64% in 2017 and 37.67% in 2020, dropping to 34.41% in 2021. When examining the rates of exceeding the reference range for tests based on whether a cancer diagnosis has been made, a total of 12.84% of individuals tested positive, 20.16% of the positive patients had a cancer diagnosis, and 5.48% did not have a cancer diagnosis (Table 5).

In 2017, 7.20% of the individuals who had a CEA test received a CEA-related cancer diagnosis, while 37.73% received a CEA-unrelated cancer diagnosis. These rates increased until 2020, when 9.34% of patients received a CEA-related cancer diagnosis, and 49.73% received a CEA-unrelated cancer diagnosis. In 2021, these rates were 8.50% and 45.19% respectively. When comparing

those who received a CEA-related cancer diagnosis with those who received a CEA-unrelated cancer diagnosis, the ratio was 1/5.24 in 2017, 1/5.27 in 2018, 1/5.30 in 2019, 1/5.32 in 2020, and 1/5.31 in 2021.

The CEA test was most frequently requested in the Marmara region across all years. It was second most frequently requested in the Central Anatolia region, with a ratio of 1.65 between the two regions in 2021. The region with the least test requests in all years was the Southeastern Anatolia region. When comparing the top region (Marmara) with the bottom region (Southeastern Anatolia) in 2021, the ratio was 11.64. As in the rest of Türkiye, the number of test requests increased from 2017 to 2019 in all regions except Southeastern Anatolia, and there was a significant decrease in test requests from 2020 to 2021 (the decrease in Southeastern Anatolia started in 2019). When analyzing the number of tests per 100,000 population across regions, the Central Anatolia region had the highest request rate across all years. The region with the lowest request rate across all years is Southeastern Anatolia. In 2021, when comparing the highest test rate region of Central Anatolia with the second-highest, Marmara, the ratio was 1.16. When compared with the lowest region, Southeastern Anatolia, the ratio was 4.81. When examining the rates of exceeding the reference range by geographical region, the highest rate belonged to the Aegean region, which ranked third in all years for test requests and tests per 100,000 population, at 15.4%. The Mediterranean region, which had the second lowest test per 100,000 population from 2019 to 2021, was second with 13.6%, and the Black Sea

Table 3. Test consumption per 100,000 persons by years and age groups

CEA	2017			2018			2019			2020			2021		
	0-17	18-64	65+	0-17	18-64	65+	0-17	18-64	65+	0-17	18-64	65+	0-17	18-64	65+
	215	7,080	21,214	239	8,007	23,730	247	8,623	25,534	148	6,284	17,583	149	6,589	19,434

CEA: carcinoembryonic antigen

Table 4a. Relationship between CEA status and gender group

	Men	Women	Total	p-value
CEA non-requested	77.4%	22.6%	100.0%	0.0001
CEA requested	33.9%	66.1%	100.0%	
Total	64.0%	36.0%	100.0%	

CEA: carcinoembryonic antigen; chi-squared test

Table 4b. Relationship between CEA status and age group

	0-17	18-64	65+	Total	p-value
CEA non-requested	1.7%	66.1%	32.2%	100.0%	0.0001
CEA requested	0.8%	69.3%	29.9%	100.0%	
Total	1.4%	67.1%	31.5%	100.0%	

CEA: carcinoembryonic antigen; chi-squared test

Table 5. Distribution of cancer diagnosis related to CEA

Year	Related cancer diagnosis		Non-related cancer diagnosis		Total number of people tested
2017	75,049	7.20%	393,440	37.73%	1,042,719
2018	87,176	7.29%	459,697	38.46%	1,195,399
2019	95,019	7.35%	503,707	38.97%	1,292,611
2020	88,433	9.34%	471,002	49.73%	947,186
2021	89,720	8.50%	477,209	45.19%	1,056,044

CEA: carcinoembryonic antigen

region was third with 12.9%. The lowest rate belonged to the Eastern Anatolia region, which had the second lowest number of test requests, at 10.5% (Table 6).

When provinces were analyzed based on the number of test requests, İstanbul was the city with the highest number of test requests across all years, followed by Ankara. Bursa and İzmir were all in the third place. Looking at the number of tests per 100,000 population, Sinop had the highest number in 2017, Erzurum in 2018, Kırşehir in 2019, and Isparta in 2020 and 2021. Ankara, which has the highest number of test requests, is not in the top 7 cities.

When clinics were compared based on the number of test requests, the clinic requesting the most tests was Internal Medicine in 2017-2018 and Medical Oncology in 2019-2021. The second most frequent were Medical Oncology and Internal Medicine, and the third was Obstetrics and Gynecology. General surgery ranked 4th, while gastroenterology ranked 5th. Family medicine ranked 7th in 2017-2019 and 2021, and emergency medicine ranked 8th in the same years.

When examining the rate of tests exceeding the reference range in clinics requesting CEA tests, the highest rate is 22.07% in the Medical Oncology clinic; this is followed by 12.92% in the Hematology clinic; and the third is 12.60% in the Emergency Medicine clinic. The rate in the Internal Medicine clinic, which was the highest in terms of test request numbers in 2017-2018, is 8.49%, the rate in the Medical Oncology clinic, which was the highest in 2019-2021, is 22.07% (second), and the rate in the Obstetrics and Gynecology clinic, which was third in all years, is 2.81% (the lowest rate in the top ten clinics). Family medicine had the second lowest rate among the 10 clinics (Table 7).

When the rates of exceeding the reference range were compared by years, the highest rate was in 2020 at 14.40%, and the lowest rate was in 2017 at 11.81%. Among tumor markers, CEA has the third highest rate of exceeding the reference range.

When the rates of exceeding the reference range were compared by institution tier, the highest rate was at tertiary institutions at 13.72%, secondary institutions were second at 11.31%, and primary institutions were third at 6.19%. When examining the rates of exceeding the reference range by institution type, the total rate was 12.83%, with university hospitals having the highest rate at 15.10% while private health institutions ranked second at 14.76%, and public hospitals third at 11.04%.

DISCUSSION

Our study provides a comprehensive analysis of various aspects related to CEA test requests, distribution, and outcomes across different demographics, regions, and clinical contexts within Türkiye. It has identified several disparities and patterns, offering crucial insights into the use of this particular biomarker in healthcare landscape. From the geospatial distribution of test requests, demographic-specific test outcomes to institutional variables and financial implications, the results contribute to the growing body of evidence surrounding CEA testing. These findings invite discussion on a variety of fronts including the practice of diagnostic testing, interpretation of results, allocation of healthcare resources, and the role of disease-specific contexts in driving these trends. Let us delve deeper into these areas to understand the implications of these results and their potential impact on the healthcare system.

The geographical disparity in the demand for CEA tests across different regions of Türkiye, specifically the high demand in Central Anatolia and the low demand in Southeastern Anatolia, illuminates the uneven distribution of healthcare resources across the country, raising important questions about the accessibility and availability of diagnostic testing. Furthermore, changes in the demand for CEA tests among different clinics, particularly the shift from Internal Medicine to Medical Oncology over the years, hint at evolving disease patterns and a growing focus on oncology.

Table 6. CEA geographical distribution by years and test per 100,000 population

2017		2018		2019		2020		2021	
Marmara region	7,496	Central Anatolia region	8,728	Central Anatolia region	9,989	Central Anatolia region	7,216	Central Anatolia region	8,151
Eastern Anatolia region	7,341	Marmara region	8,407	Marmara region	9,192	Marmara region	6,680	Marmara region	6,993
Central Anatolia region	7,156	Aegean region	7,656	Aegean region	8,334	Aegean region	6,444	Aegean region	6,826
Aegean region	7,105	Eastern Anatolia region	7,389	Eastern Anatolia region	7,479	Black Sea region	5,450	Black Sea region	5,901
Black Sea region	6,323	Black Sea region	7,172	Black Sea region	7,314	Eastern Anatolia region	4,878	Eastern Anatolia region	5,193
Mediterranean region	4,519	Mediterranean region	5,789	Mediterranean region	6,551	Mediterranean region	4,669	Mediterranean region	4,768
Southeast Anatolia region	2,406	Southeast Anatolia region	2,671	Southeast Anatolia region	2,578	Southeast Anatolia region	1,728	Southeast Anatolia region	1,694

CEA: carcinoembryonic antigen

Table 7. CEA top 10 clinics by years and number of test requests

2017		2018		2019		2020		2021	
Internal medicine	1,165,988	Internal medicine	1,292,526	Medical oncology	1,469,374	Medical oncology	1,426,489	Medical oncology	1,339,452
Medical oncology	979,236	Medical oncology	1,235,601	Internal medicine	1,364,917	Internal medicine	846,849	Internal medicine	999,363
Gynecology and obstetrics	812,500	Gynecology and obstetrics	914,926	Gynecology and obstetrics	994,944	Gynecology and obstetrics	661,358	Gynecology and obstetrics	697,154
General surgery	678,596	General surgery	740,201	General surgery	739,870	General surgery	493,935	General surgery	513,263
Gastroenterology	317,402	Gastroenterology	367,268	Gastroenterology	359,896	Gastroenterology	270,256	Gastroenterology	308,765
Radiation oncology	216,422	Radiation oncology	247,889	Radiation oncology	250,914	Radiation oncology	153,949	Radiation oncology	149,572
Family medicine	110,356	Family medicine	159,380	Family medicine	186,530	Emergency medicine	100,433	Family medicine	118,395
Emergency medicine	78,214	Emergency medicine	88,076	Emergency medicine	129,557	Family medicine	93,819	Emergency medicine	93,486
Chest diseases	63,096	Neurology	67,930	Neurology	82,088	Gynecological oncology	62,207	Gynecological oncology	78,929
Urology	60,225	Urology	67,134	Gynecological oncology	79,668	Neurology	56,111	Neurology	65,644

CEA: carcinoembryonic antigen

The higher rates of CEA test results exceeding the reference range among males and individuals above 65 indicate the potential impact of demographic factors on disease prevalence. Observations regarding the high rates of abnormal CEA levels among inpatients and outpatient daycare patients suggest a possible correlation between disease severity and elevated CEA levels. Lastly, the steady trend observed in Türkiye Health Practice Communiqué and unit costs over the years calls for a discussion on the efficiency and financial implications of current testing practices. This comprehensive analysis offers valuable insights for shaping future healthcare policies and practices.

Colorectal cancer is the third most common type of cancer, with about 1.5 million cases diagnosed worldwide each year, according to GLOBOCAN 2018 data. Approximately 750,000 patients have been lost due to this disease. The incidence rate of colon cancer per 100,000 people is 35.7 for men and 29 for women (7).

While colorectal carcinoma is more commonly seen in men, our study found that the number of tests requested for women has been higher in all years when the female-to-male test number ratio is considered: 2.01 in 2017, 2.01 in 2018, 2.03 in 2019, 1.96 in 2020, and 1.96 in 2021. When we looked at the rates of tests exceeding the reference range by gender, we generally found a positive rate of 12.82%, with 18.01% in men and 10.14% in women. In men, the rate of tests exceeding the reference range ranks second.

Age is the most important risk factor for sporadic colorectal cancer as it is rarely seen under the age of 40, and the incidence increases from 40 to 50 years onward. 90% of cases are seen in patients over the age of 50, and this rate is 10% in men and 15% in women over the age of 80. The risk of colorectal cancer increases with age, and in the period of 2015-2019, individuals were divided into age groups with 5-year intervals. The incidence of colorectal cancer in each age group increases by 80-100% for every 5-years up to the age of 50, and by 20-30% for every 5-years in those aged 55-59 and above (8). In our study, when the number of test requests per year was compared by age group, CEA was most frequently requested in the 18-64 age group, second most frequently in the over 65 age group, and least frequently in the 0-17 age range.

CEA is the tumor marker with the highest value in the follow-up of patients with colorectal cancer, and its use is recommended in international guidelines. The general opinion in the guidelines is that CEA monitoring should be performed after surgery. When the test request times at the time of diagnosis for individuals who had tumor markers requested were analyzed in our study, it was found that in all years, the tests were mostly requested before the diagnosis, second most frequently at the same time as the diagnosis, and least frequently after the diagnosis (9).

In Zhang et al. (10), the specificity rate of CEA in colorectal diagnosis was calculated as 91%. In another study by Ding et al. (11) in 2022, the sensitivity was calculated as 70.59%.

In our study, 7.20% of the individuals who underwent the CEA test in 2017 received a CEA-related cancer diagnosis and 37.73% received a CEA-unrelated cancer diagnosis. There has been an

increase in these rates up to 2020, with 9.34% of patients receiving a CEA-related cancer diagnosis and 49.73% receiving a CEA-unrelated cancer diagnosis. In 2021, these rates are seen at 8.50% and 45.19%, respectively. When the rates of tests exceeding the reference range are compared by year, the highest rate is in 2020 at 14.40%, and the lowest rate is in 2017 at 11.81%. Among tumor markers, it ranks as the third highest marker in terms of the rate of exceeding the reference range. When the rates of tests exceeding the reference range are compared by institutional level, the highest rate is in tertiary institutions, followed by secondary and primary institutions. When examining the rates of tests exceeding the reference range by institution type, the total rate was 12.83%, with the highest rate in university hospitals, followed by private health institutions, and finally public hospitals.

While this study provides valuable insights into the prevalence and detection of colorectal cancer, it also exposes certain limitations that should be addressed in future research. These include the risk of selection bias due to reliance on available medical records and performed tests, a lack of consideration for lifestyle and genetic factors influencing colorectal cancer incidence, and the cross-sectional nature of the study that limits understanding of disease progression. Also, the heavy reliance on CEA as a biomarker may mask the full picture due to its known limitations in specificity and sensitivity. Consequently, future research should aim for a more comprehensive data collection that encompasses lifestyle and genetic factors, longitudinal tracking of individuals to capture disease progression, exploration of additional biomarkers for colorectal cancer, ensuring sample representativeness to mitigate selection bias, and considering disparities in healthcare access that could impact test requests and diagnosis rates.

Study Limitations

This study has several limitations. First, the data were obtained retrospectively, which means that pre-study design control could not be implemented. Additionally, there may be missing or erroneous data in the dataset. The scope of the study focused solely on the use of the CEA test and did not consider the impact of other potential factors or variables.

CONCLUSION

This study contributes valuable insights into the application and outcomes of CEA tests in the detection of colorectal cancer across various demographic groups. It reveals gender, age and clinic-specific disparities in test application and outcomes, highlights the prevalence of CEA tests conducted before cancer diagnosis, and

underscores the potential value of CEA as a biomarker in cancer detection despite its limitations. Although there are challenges due to potential selection bias, lack of comprehensive information on influencing factors, and constraints of the cross-sectional study design, the findings shed light on critical areas for future research and policymaking. These include a need for more nuanced approaches to testing strategies, further exploration of potential biomarkers, and consideration of broader contextual factors in colorectal cancer detection and treatment. Ultimately, these insights have significant implications for improving colorectal cancer detection strategies, enhancing patient outcomes, and advancing the field of cancer research.

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Relationship Between the Protrusion of the Optic Nerve and Internal Carotid Artery and Sphenoid Sinus Volume

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ABSTRACT

Objective: This study was conducted to investigate the relationship between the protrusion of the optic nerve (ON) and internal carotid artery (ICA) and sphenoid sinus volume.

Methods: A total of 142 patients (284 sphenoid sinuses) were included in the study. The study population was classified into the four groups according to ON and ICA protrusion into the sphenoid sinuses as follows: no protrusion of any structure (group 1); protrusion of ICA (group 2); protrusion of ON (group 3); and protrusion of both ICA and ON (group 4). A three-dimensional volumetric assessment of the bilateral sphenoid sinuses was performed with ITK-SNAP 3.8.0 software separately for both sides.

Results: Group 1 was the most common group and included 138 sinuses (48.6%), while group 3 was the rarest group and included 25 sinuses (8.8%). Statistically significant differences in the volume of sphenoid sinus were found between group 1 and group 2 (p=0.002) and group 1 and group 4 (p<0.001).

Conclusion: Larger sphenoid sinuses have a higher probability of ICA protrusion compared to smaller sphenoid sinuses. Although ON protrusion is considered to be rarer than ICA protrusion, it can be observed regardless of the volume of the sphenoid sinuses.

Keywords: Sphenoid sinus, optic nerve, internal carotid artery, paranasal sinus, sphenoid sinus volume, protrusion

INTRODUCTION

The sphenoid sinuses (SS) are intricate anatomical formations that display substantial variations in their dimensions and structural characteristics (1). These variations can be observed within the two sides of a single individual (2). The anatomical variations of the SS become especially significant for patients who are subjected to procedures such as functional endoscopic sinus surgery or trans-sphenoidal pituitary surgery due to their close proximity to neighboring neurovascular formations, including the optic nerve (ON) and internal carotid artery (ICA) (3).

The paranasal computed tomography (CT) scan serves as an indispensable imaging technique, providing an intricate evaluation of the structural composition of the paranasal sinuses. Widespread pneumatization within the SS has the potential to cause the ON and/or ICA to protrude into the sinus space (4). In light of the potential catastrophic consequences of iatrogenic injury to these vital structures, a pre-operative radiological evaluation of the SS and the surrounding ON and ICA is essential (5).

The aim of this study is to investigate the connection between SS volume and the extension of the ON and ICA into the sinus.

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METHODS

All the paranasal CT scans and clinical records of patients admitted to the Kırklareli Training and Research Hospital Otolaryngology Department between January 2020 and January 2021 were retrospectively analyzed. Patients with nasal polyposis, signs of sinusitis, craniofacial deformities, a history of maxillofacial trauma, prior sinonasal surgery, or systemic diseases that could affect the anatomical configuration of the paranasal sinuses were excluded from the study. After these exclusions, 142 patients (comprising 284 SS) were enrolled in the study. This retrospective study was approved by the Kırklareli University Local Ethics Committee (approval date: 15.03.2021, decision no: 11).

All the paranasal CT scans were performed utilizing identical CT scanners (Somatom go.Now, Siemens, Forchheim, Germany) adhering to the following specific acquisition parameters: kV: 130; mAs: 80; reconstruction filters: H40 smooth for soft tissues and H70 sharp for bone; and slice thickness: 0.75 mm. The segmentation and three-dimensional (3D) volume analysis of the SS on both sides were independently carried out for each side utilizing ITK-SNAP 3.8.0 open-source software (Figure 1). ITK-SNAP enables semi-automatic segmentation of the paranasal sinuses based on gray levels, produces 3D models, and computes the volumes of these models (6). This software has been validated and is widely used for the volumetric assessment of paranasal sinuses (7,8). For the creation of the 3D model and volumetric assessment, an automatically increasing "seed" was placed for measurement in each sinus. These seeds then permeated the entire airspace within the boundaries of the bone walls. All volumetric measurements were repeated twice with a 14-day interval, and the average of the measurements was utilized.

For each patient, the extensions of the ON and ICA into the SS were assessed using both coronal and axial CT scans of the left and right SS. Based on the protrusion of the ON and ICA, the subjects of the study were categorized into the following four distinct groups: no protrusion of either structure (group 1), protrusion of only the ICA (group 2), protrusion of only the ON (group 3), and protrusion of both the ICA and ON (group 4). (Figure 2). The proportion of cases allocated to each group was determined in conjunction with the median volume of the SS.

Statistical Analysis

The analysis was conducted using the Statistical Package for the Social Sciences 15.0 for Windows (SPSS Inc., Chicago, IL), and the results are depicted as medians and interquartile ranges, where relevant. Categorical variables are described by their numbers and percentages. The Pearson chi-square test was used to compare the independent groups. The normal distribution of the data was evaluated using the Kolmogorov-Smirnov test. When the numerical variables across the groups did not meet the normal distribution criteria, the Kruskal-Wallis test was used for comparisons among more than two independent groups. Paired comparisons of groups were performed utilizing Dunn's post-hoc test. A significance level of $p < 0.05$ was deemed meaningful.

RESULTS

The study retrospectively analyzed paranasal sinus CT scans from 142 patients, encompassing a total of 284 SS. Within this population, there were 72 females (144 sinuses) (50.7%) and 70 males (140 sinuses) (49.3%), and there was no significant difference among the four groups in terms of age (p=0.627) and gender (p=0.958) (Table 1).

Group 1 was the most common, with 138 sinuses (48.6%), and median SS volume was 3.35 cm³. Group 2 was the second most common, with 65 sinuses (22.9%), and median SS volume was 4.95 cm³. Group 3 was the least common, with 25 sinuses (8.8%), and median SS volume was 5.77 cm³. Group 4 included 56 sinuses (19.7%), and median SS volume was 6.77 cm³ (Table 1, Figure 3). The analysis revealed a statistically significant difference in SS volume among the groups (p<0.001).
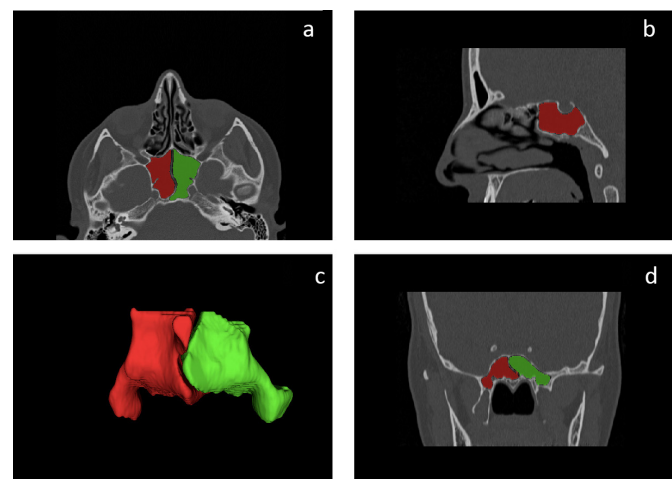
Figure 1. Three-dimensional (3D) volumetric analysis of sphenoid sinuses with ITK-SNAP Software. **a.** Cross-sectional view in the transversal plane of the sphenoid sinuses. **b.** Longitudinal section in the sagittal plane of the sphenoid sinuses. **c.** Visualization of a 3D model showing the right (red) and left (green) sphenoid sinuses. **d.** Frontal section in the coronal plane of the sphenoid sinuses
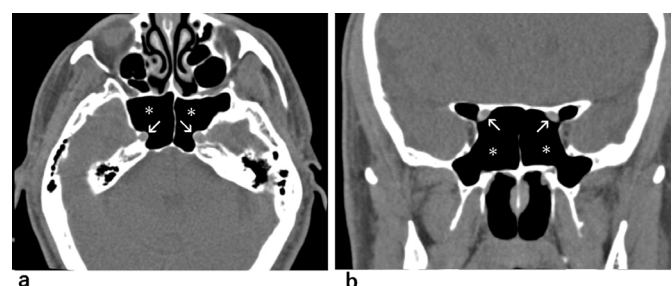
Figure 2. Axial and coronal computed tomography (CT) images of a group 4 subject. **a.** Axial CT image highlighting the protrusion of right and left internal carotid artery (arrow) into the sphenoid sinus cavity (*). **b.** Coronal CT image illustrating the protrusion of right and left optic nerve (arrow) into the sphenoid sinus cavity (*).

Zülküf Burak Erdur.
Optic Nerve and Carotid Artery Protrusion

115

Table 1. Prevalence, age and gender distribution and sphenoid sinus volumes of each group

	Number (%)	Age (years)	Gender	Sphenoid sinus volume (cm³)
		Median (IQR)	Female/male n (%)	Median (IQR)
Group 1	138 (48.6)	30 (16)	71 (51.4)/67 (48.6)	3.35 (3.03)
Group 2	65 (22.9)	28 (16.5)	34 (52.3/31 (47.7)	4.95 (4.29)
Group 3	25 (8.8)	30 (19)	12 (48.0)/13 (52.0	5.77 (4.82)
Group 4	56 (19.7)	31.5 (15.5)	27 (48.2)/29 (51.8)	6.77 (4.81)
		*p=0.627	**p=0.958	*p<0.001

*Kruskal-Wallis test, **Pearson chi-square test, IQR: interquartile range

Table 2. Paired comparisons of groups

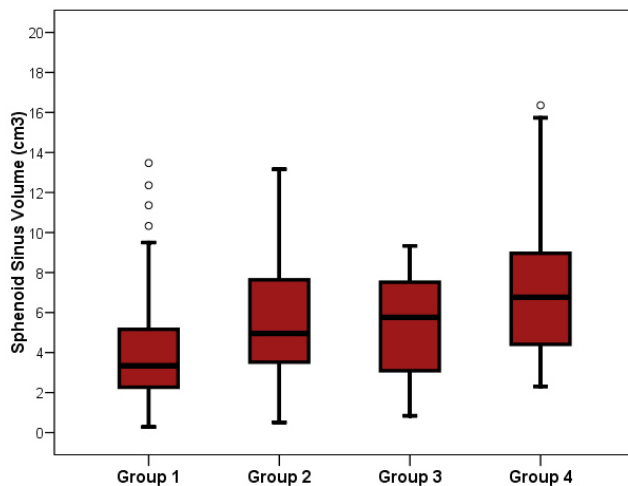|	p-value*
Group 1 vs. group 2	0.002
Group 1 vs. group 3	0.063
Group 1 vs. group 4	<0.001
Group 2 vs. group 3	1.00
Group 2 vs. group 4	0.057
Group 3 vs. group 4	0.349

*Dunn's post-hoc test

Figure 3. Sphenoid sinus volumes of each group

Table 2 displays the outcomes and comparisons of the groups. Significant statistical differences in SS volume were observed between group 1 and group 2 (p=0.002) and group 1 and group 4 (p<0.001). In contrast, there were no significant volume disparities found between the subjects without protrusions (group 1) and those with only ON protrusion (group 3) (p=0.063), between the subjects with only ICA protrusion (group 2) and those with only ON protrusion (group 3) (p=1.00), between the subjects with only ICA protrusion (group 2) and those with protrusion of the ICA and the ON (group 4) (p=0.057), and between the subjects with isolated ON protrusion (group 3) and those with protrusion of both the ICA and the ON (group 4) (p=0.349).

DISCUSSION

The SS, a complex and fluctuating structure, is centrally positioned within the base of the skull. It has a close association with numerous vital neurovascular components, including the ON and the ICA (9). The optic canal, hosting the ON, resides in the superolateral portion of the sinus and frequently composes its roof. The absence of bone covering the ON, a condition known as dehiscence, can lead to the ON's direct contact with the sinus in 4% to 30% of instances (10,11). Conversely, the ICA follows a path that is inferolateral to the ON and is a substantial contributor to the SS's lateral wall (11). Variations in the ICA's course and the pneumatization of the SS may cause dehiscence of the bone over the ICA. This can expose the ICA to the sinus cavity, thereby heightening the potential for injury during surgical interventions (11).

While functional endoscopic sinus surgery and trans-sphenoidal pituitary surgery are generally considered safe, rare but significant complications can occur, including injuries to the ICA and the ON. The close proximity to the surgical field of these vital structures renders them susceptible to damage during surgical maneuvers. Although the prevalence of ICA injury in endoscopic sinus surgery is relatively low, the potential consequences are severe. Studies have reported the incidence of ICA injury to be below 0.1% of cases (12). Despite its rarity, ICA injury can lead to significant morbidity and mortality due to severe hemorrhage and potential vascular complications such as stroke, carotid-cavernous fistula formation, cranial nerve palsies, and even death (13). Another rare but serious complication of endoscopic sinus surgery is ON injury. The reported incidence varies widely, ranging from 0.03% to 0.1% (14). ON injury can lead to visual impairment or even permanent blindness, significantly affecting a patient's quality of life (14). It is important to note that the prevalence and incidence of these complications can vary based on several factors, including the surgeon's experience, patient anatomy, and the complexity of the surgical procedure. To minimize the risk of these complications, surgeons must thoroughly understand the anatomical relationships and employ meticulous surgical techniques.

The existing literature reveals considerable variation in the reported prevalence of ICA and ON protrusions. Estimates for ICA protrusion range from 3.9% to 46.2%, while ON protrusions have been reported to vary between 2.8% and 41.5% (15-17). A potential explanation for this significant discrepancy may lie in the

116 Zülküf Burak Erdur.
Optic Nerve and Carotid Artery Protrusion

J Acad Res Med 2023;13(2):113-7

lack of a universally accepted definition of "protrusion." Some authors have characterized protrusions as situations in which the invagination into the sphenoid air cavities extends beyond half the diameter of a specific neural or vascular structure (11,17). However, others in the field do not adhere to a specific definition for this term. In the context of our study, we opted to encompass all instances in which either the ICA or the ON resulted in an indentation of any magnitude within the SS. We adopted this broad approach to ensure comprehensive coverage of all potential cases. In the current study, we detected ICA protrusions in 42.6% of sinuses and ON protrusions in 28.5% of cases.

A recent investigation spearheaded by Fadda et al. (18) revealed the incidence of ICA and ON protrusion to be more common in instances of significant SS pneumatization. They observed a higher frequency of ICA and ON protrusion within the sellar and postsellar classifications (18). Similarly, research conducted by Dal Secchi et al. (19) identified a direct association between the extensive lateral pneumatization of the SS and a heightened probability for ICA protrusion. Additionally, Fatihoglu et al. (4) delineated that the pneumatization of the anterior clinoid process is correlated with an increased prevalence of protrusions involving both the ICA and ON.

Though numerous investigations have delved into the relationship between the pneumatization of the SS and that of the ICA and ON, only a select few have specifically focused on the connection between the SS's volume and these vital neurovascular elements. A study conducted by Gibelli et al. (17), employing a classification methodology analogous to our own, determined that the SS volume was higher in categories displaying ICA protrusions, whether occurring alone or in combination with the ON, relative to other groupings. Interestingly, they did not detect a statistically significant variation in SS volume between groups with no protrusions of any kind and those with only ON protrusion (17).

Our findings are consistent with the previously mentioned study, revealing a correlation between the SS volume and the protrusion of certain anatomical structures. Specifically, cases exhibiting a concomitant protrusion of both the ICA and ON were linked with the largest volume of the SS. Conversely, we did not discern a statistically significant disparity in the volume of the SS between the groups with no protrusions of any anatomical structures and those with an isolated protrusion of the ON. Additionally, we identified a significant increase in SS volume in cases with isolated ICA protrusions compared to the group with no protrusions. From a surgical standpoint, these results suggest that larger sinuses have a higher probability of ICA protrusion than do smaller SS. However, ON protrusion, which is less common than ICA protrusion, can be observed in both small and larger SS dimensions.

Study Limitations

The present study is characterized by certain limitations, most notably the relatively small sample size of 142 patients. This restriction necessitates caution in extrapolating the findings to a broader population. Further investigations employing a

more extensive and diversified cohort are required to uncover additional variables that may influence the protrusion of the ICA and the ON into the SS. Replication of these results in larger and more heterogeneous samples would contribute to a more comprehensive understanding and validation of the complex interplay between SS volume and the proximity of these vital neurovascular structures.

CONCLUSION

Larger SS have a higher probability of ICA protrusion compared to smaller SS. Although ON protrusion is considered to be rarer than ICA protrusion, it can be observed regardless of the volume of the SS. This implies that even in cases with smaller SS, the possibility of ON protrusion should not overlooked or underestimated.

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