HAMIDIYE MEDICAL JOURNAL
The Official Journal of University of Health Sciences Türkiye, Hamidiye Faculty of Medicine
E-ISSN: 2718-0956
December - 2022
Volume - 3
Issue - 3

ORIGINAL ARTICLES

galenos Publishing House
www.hamidiyemedj.com

hamidiye med j

HAMIDIYE MEDICAL JOURNAL

2022
Volume 3

Editorial Board

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 İstanbul, Türkiye
Phone: +90 (212) 621 99 25 **Fax:** +90 (212) 621 99 27 **E-mail:** info@galenos.com.tr
Web: www.galenos.com.tr **Publisher Certificate Number:** 14521
Online Publication Date: December 2022 **E-ISSN:** 2718-0956
International periodical journal published three times in a year.

hamidiye med j

2022/Volume 3

HAMIDIYE MEDICAL JOURNAL

Aims and Scope

Hamidiye Medical Journal (Hamidiye Med J) is a peer-reviewed open-access international journal that publishes conducted in all fields of medicine, interesting case reports, and clinical images, invited reviews, editorials, letters, comments and letters to the Editor including reports on publication and research ethics. The journal is the official scientific publication of the University of Health Sciences Türkiye, Hamidiye Faculty of Medicine, İstanbul, Türkiye. It is published three times a year in April, August, and December. The language of the journal is English.

The journal is based on independent and unbiased double-blinded peer-reviewed principles. Only unpublished papers that are not under review for publication elsewhere can be submitted. Hamidiye Medical Journal does not accept multiple submissions and duplicate submission even though the previous one was published in a different language. The authors are responsible for the scientific content of the material to be published. Hamidiye Medical Journal reserves the right to request any research materials on which the paper is based.

Hamidiye Medical Journal encourages and enables academicians, researchers, specialists, and primary care physicians to publish their valuable research in all branches of medicine. The primary aim of the journal is to publish original articles with high scientific and ethical quality and serve as a good example of medical publications in the Türkiye as well as in the world.

Hamidiye Medical Journal is indexed in **EBSCO Host, J-Gate** and **Ulakbim TR Index.**

English Title: Hamidiye Medical Journal

Official abbreviation: Hamidiye Med J

E-ISSN: 2718-0956

OPEN ACCESS POLICY

This journal provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

Author (s) and copyright owner (s) grant access to all users for the articles published in Hamidiye Medical Journal as free of charge. Articles may be used provided that they are cited.

Open Access Policy is based on rules of Budapest Open Access Initiative (BOAI). By "open access" to [peer-reviewed research literature], we mean its free availability on the public internet, permitting any users to read, download, copy, distribute, print, search, or link to the full texts of these articles, crawl them for indexing, pass them as data to software, or use them for any other lawful purpose, without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. The only constraint on reproduction and distribution, and the only role for copyright in this domain, should be to give authors control over the integrity of their work and the right to be properly acknowledged and cited.

Creative Commons

A Creative Commons license is a public copyright license that provides free distribution of copyrighted works or studies. Authors use the CC license to transfer the right to use, share or modify their work to third parties. This journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC-ND 4.0) (https://creativecommons.org/licenses/by-nc-nd/4.0/) which permits third parties to share and adapt the content for non-commerical purposes by giving the apropriate credit to the original work.

Open access is an approach that supports interdisciplinary development and encourages collaboration between different disciplines. Therefore, Hamidiye Medical Journal contributes to the scientific publishing literature by providing more access to its articles and a more transparent review process.

Advertisement Policy

Potential advertisers should contact the Editorial Office. Advertisement images are published only upon the Editor-in-Chief's approval.

Material Disclaimer

Statements or opinions stated in articles published in the journal do not reflect the views of the editors, editorial board and/or publisher; The editors, editorial board and publisher do not accept any responsibility or liability for such materials. All opinions published in the journal belong to the authors.

Contact
Editorial Office
Address for Correspondence

Selimiye Mah. Tıbbiye Cad. 34668 Üsküdar, İstanbul/Türkiye

Phone: +90 216 418 96 16

E-mail: tipfakultesi@sbu.edu.tr

Publisher/Issuing Body

Galenos Yayınevi Tic. Ltd. Şti.

Molla Gürani Mah. Kaçamak Sok. No: 21, 34093, Fındıkzade, İstanbul, Turkiye

Phone: +90 212 621 99 25

Fax: +90 212 621 99 27

Web page: http://www.galenos.com.tr

E-mail: info@galenos.com.tr

OPEN ACCESS

hamidiye med j

HAMIDIYE MEDICAL JOURNAL

2022
Volume 3

Contents

ORIGINAL ARTICLES

CASE REPORT

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.15238

Hamidiye Med J 2022;3(3):162-170

Investigation of the Change in the Correct Classification Ratios by Using the Richard Link Function in Logistic Regression: A Research on the Determination of Risk Factors in COPD

Lojistik Regresyonda Richard Link Fonksiyonu Kullanımı ile Doğru Sınıflama Oranlarındaki Değişimin İncelenmesi: KOAH'da Risk Faktörlerinin Belirlenmesi Üzerine Bir Araştırma

Kürşad Nuri Baydili[1], Mustafa Çörtük[2], Ahmet Dirican[1]

[1]*İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Biostatistics, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye Hamidiye Faculty of Medicine; İstanbul Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Chest Diseases and Tuberculosis, İstanbul, Türkiye*

ABSTRACT

Background: Regression analyses are used to explain the relationship between a dependent variable and independent variables using mathematical models. Logistic regression, which is used in cases where the dependent variable is categorical, is often used in analyzing health-related data.

Materials and Methods: It is well known that the inflection point of the logistic regression curve sometimes corresponds to smaller or larger values on the horizontal axis, resulting in incorrect classifications. The present study aimed to increase correct classification rates by using the Richards link function to determine the most suitable inflection point for data.

Results: In order to evaluate the performance of the Richards link function, four different simulation scenarios and applications were carried out with a total of 1.005 individuals, of whom 505 were non-chronic obstructive pulmonary disease (COPD) and 500 were COPD individuals. The data were divided into learning and test data. A logistic regression model was obtained from the learning data, and an increase in the correct classification rates was observed with the use of the Richards link function in this model. The model was applied to the test data with the m-value determined for the learning data set and achieved a higher correct classification rate than the current method.

Conclusion: The present study indicated that certain percentage increases can be achieved in correct classification rates by using the Richards link function. However, it would be beneficial to conduct studies in which applications are made with data sets containing fewer and more independent variables, different sample sizes, and combinations of independent variable types.

Keywords: Logistic regression, Richard link function, correct classification

ÖZ

Amaç: Bir bağımlı değişken ile bağımsız değişkenler arasındaki ilişkinin matematiksel modeller kullanılarak açıklanması için regresyon analizleri kullanılır. Bağımlı değişkenin kategorik olduğu durumlarda kullanılan lojistik regresyon özellikle sağlık alanında sıklıkla kullanılır.

Gereç ve Yöntemler: Lojistik regresyon eğrisinin bükülme noktasının yatay eksende bazen olması gerekenden daha küçük ya da daha büyük değerlere karşılık geldiği, bunun sonucunda da hatalı sınıflandırmalar yaptığı bilinmektedir. Araştırmada; verilere en uygun büküm noktasının tespiti için Richard link fonksiyonu kullanılarak doğru sınıflama oranlarında artışlar sağlanması hedeflenmiştir.

Bulgular: Richard link fonksiyonunun performansını değerlendirmek amacıyla; dört farklı simülasyon senaryosu ve 505'i kronik obstrüktif akciğer hastalığı (KOAH) olmayan 500'ü ise KOAH olan toplamda 1,005 bireyden oluşan gerçek verilerle uygulamalar gerçekleştirilmiştir. Gerçek verilerle gerçekleştirilen uygulamalarda ise tüm verilerin kullanılmasıyla elde edilen modelde Richard link fonksiyonu kullanımı ile birlikte doğru sınıflama oranlarında artışlar olduğu saptanmıştır. Verilerin öğrenme ve test verileri

Address for Correspondence: Kürşad Nuri Baydili, İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Biostatistics, İstanbul, Türkiye
Phone: +90 541 739 23 23 E-mail: kursadnuri.baydili@sbu.edu.tr **ORCID ID:** orcid.org/0000-0002-2785-0406
Received: 06.09.2022 **Accepted:** 26.09.2022

ÖZ

olarak ikiye ayrılmış, öğrenme verilerinden lojistik regresyon modeli elde edilmiş, bu modelde de Richard link fonksiyonu kullanımı ile birlikte doğru sınıflama oranlarında artış gözlemlenmiştir. Öğrenme veri seti için belirlenen m değeri için model test verilerine uygulanmış ve mevcut yöntemden daha yüksek doğru sınıflama oranına ulaştığı görülmüştür.

Sonuç: Richard link fonksiyonu kullanılarak doğru sınıflama oranlarında belirli oranlarda artışlar sağlanabileceği görülmüştür. Ancak, daha az ve daha çok sayıda bağımsız değişkenler içeren, farklı örneklem büyüklüklerinde ve bağımsız değişken türlerinde kombinasyonların denendiği veri setleriyle uygulamaların yapıldığı çalışmaların yapılmasında fayda görülmektedir.

Anahtar Kelimeler: Logistik regresyon, Richard link fonksiyonu, doğru sınıflama

Introduction

Establishing a cause-effect relationship between the dependent variable and the independent variable(s) is one of the aims of scientific research (1). Univariate methods used when investigating causality make the comparison by assuming that all other factors other than the variable are homogeneous or constant. However, sometimes it is not possible to achieve homogeneity or stability in the real world. A variable can often change with one or more variables. The problem can be solved by including these co-variations through multivariate statistical analysis (2), which aims to predict an outcome with multiple independent variables (3).

Multivariate statistical analysis includes many techniques depending on the purpose of the study, the type of dependent and independent variable(s), and the fulfillment of certain conditions (2). Regression analysis is used to estimate relationships between a dependent variable and a set of independent variables using mathematical models (4). Researchers in the field of health generally aim to classify their observations and make inferences about future observations based on existing observations (5). The first known classification methods were cluster analysis, which was originated by Driver and Kroeber (6) in social and life sciences, and two-group discriminant analysis proposed by Fisher (7). The primary techniques used to classify observations are cluster analysis, discriminant analysis, and logistic regression analysis (8). In cluster analysis, where the number of groups is unknown, data are assigned to groups according to certain criteria (9). In discriminant analysis and logistic regression, although the number of groups is known, data are assigned to groups by using this information (8). In logistic regression, assumptions such as normality and homogeneity of variances required in discriminant analysis are not sought (4).

Berkson (10) was the first to publish an application of the logistic model in the field of biology. Logistic regression aims to reveal the model that has the highest fit with the least number of variables (9). Logistic regression can be used to estimate and summarize data, as well as for classification by examining the relationship between the dependent variable and the independent variable(s). Logistic regression is used when the dependent variable is in the form of qualitative data (4). There are three different types of logistic regression: Binary logistic regression is used when the dependent variable has two categories, multinomial logistic regression is used when the dependent variable has more than two categories, and ordinal logistic regression is used when the dependent variable is measured at the ordinal level (2,4,11).

Logistic regression is commonly used in such fields as economics, education and health (12). Binary logistic regression has become an increasingly employed statistical tool in medical research, and is generally concerned with whether there is a risk, such as disease (13), and is coded as 1 and 0. An odds ratio is used in risk estimation in retrospective studies. The significance of the odds ratio is determined by examining the confidence intervals. If the confidence interval for the odds ratio does not include the number 1, then the calculated odds ratio is considered statistically significant. If the calculated odds ratio is found to be significant, an odds ratio greater than 1 indicates that the factor is a risk factor, while an odds ratio of less than 1 indicates that it is a protective factor (4). In binary logistic regression, a logistic regression model is created [$\pi(x)$] by calculating the probability of Y being 1 $P(Y=1|X=x)$ when the value of the independent variable (X) is known.

The logistic regression graph is an S-shaped sigmoid curve (14,15). The logistic curve was first used by Verhulst (16) to describe the growth in a population. The inflection point of this curve may sometimes correspond to smaller or larger x-values than it should be. Gürcan et al. (17) stated that in such cases, by using various link functions, the x-values corresponding to the inflection point of the curve may be more optimal, and thus, there may be an increase in the rates of correct classification. They found the inflection point by analyzing its second derivative of the curve proposed by Richards (18), and aimed to increase the correct classification rate of the model by applying the inflection points separately for the misclassified observations.

Material and Methods

Ethics committee approval with the number 18/1 was obtained from the Hamidiye Scientific Research Ethics Committee at the meeting numbered 2022/18 for the research. The present study aimed to increase the correct classification rate by using an alternative link function to the existing method used in binary logistic regression. In logistic regression, instead of the $P = \frac{e^a}{1+e^a}$ forwmula, the model was changed with 0.01 increments in the m (1,3) interval, the probability values were calculated using the Richards link function with the $P = (1 + (m-1).(e^{-a}))^{\frac{1}{1-m}}$ formula, and the estimated classification values were obtained according to these probability values. Data were collected through face-to-face interviews using a questionnaire for a total of 1005 individuals, 505 without chronic obstructive pulmonary (COPD) and 500 with COPD, to evaluate the performance of the Richards link function. The demographic characteristics of the study participants are presented in Table 1. Applications were carried out in two different ways. In the first method, all the data were included in the logistic regression model and the probability and class values were obtained with the proposed method. Then, probability calculations were made using the Richards link function with the same coefficients. Subsequently, the m-value, which maximizes the correct classification percentage, was determined. In the second method, 74.6% (n=750) of the data were included in the logistic regression model, and the correct classification numbers and ratios for all m-values were presented with the coefficients. Next, probability and classification values were obtained for the remaining 25.4% (n=255) of the data using the m-value, which maximizes the correct classification percentage.

Table 1. Demographic information of participants

	Learning data set	Test data set	Total
	n (%)	n (%)	n (%)
Gender			
Male	461 (61.5)	176 (69)	637 (63.4)
Female	289 (38.5)	79 (31)	368 (36.6)
Packs of cigarettes smoked per year>10 per year			
No	406 (54.1)	149 (58.4)	555 (55.2)
Yes	344 (45.9)	106 (41.6)	450 (44.8)
COPD in relatives			
No	495 (66)	184 (72.2)	679 (67.6)
Yes	255 (34)	71 (27.8)	326 (32.4)
Lung disease other than COPD in relatives			
No	575 (76.7)	190 (74.5)	765 (76.1)
Yes	175 (23.3)	65 (25.5)	240 (23.9)
Place of residence			
Other	227 (30.3)	104 (40.8)	331 (32.9)
Metropolis	523 (69.7)	151 (59.2)	674 (67.1)
Duration of daily exercise>1 hour			
≤60 min	607 (80.9)	244 (95.7)	851 (84.7)
>60 min	143 (19.1)	11 (4.3)	154 (15.3)
COPD			
No	375 (50)	130 (51)	505 (50.2)
Yes	375 (50)	125 (49)	500 (49.8)
	Med (min-max)	**Med (min-max)**	**Med (min-max)**
Age	52.5 (14-91)	52 (16-94)	52 (14-94)
BMI	26.40 (15.59-45.71)	25.54 (15.62-57.11)	26.13 (15.59-57.11)
Duration of exposure to wood, dung or coal smoke	0 (0-50)	0 (0-70)	0 (0-70)

COPD: Chronic obstructive pulmonary disease, BMI: Body mass index

Results

In the data set in which all observations were included, the variables were individually included in the logistic regression model to select the variables suitable for the logistic regression model. It was concluded that the variables of gender (p<0.001), age (p<0.001), body mass index (p<0.001), duration of exposure to wood, dung or coal smoke (p<0.001), smoking status over 10 packs/year (p<0.001), having a relative with COPD (p<0.001), having a recent lung disease other than COPD (p<0.001), place of residence (p<0.001), and daily exercise for more than 1 hour (p<0.001) should be included in the model (Table 2).

Logistic regression in which all variables were included in the model showed that the variables of gender (p=0.946) and body mass index (p=0.307) had no effect on COPD status. It was found that a 1-unit increase in age was a 1.148-fold greater risk (p<0.001), and a 1-unit increase in exposure time to wood, dung or coal smoke was a 1.027-fold greater risk (p=0.011). It was determined that smoking more than 10 packs/year was a 7.832-fold greater risk (p<0.001), having COPD in first-degree relatives was a 2.792-fold greater risk (p<0.001), having individuals with lung disease other than COPD in first-degree relatives was a 4.068-fold greater risk (p<0.001), living in a metropolis was a 7.664-fold greater risk (p<0.001), and exercising for more than 1 hour a day was a 0.04-fold greater risk (25-fold protective factor) (p<0.001) (Table 3).

The correct classification rate obtained with the available variables was 93% (n=935) (Table 4). By using the Richards link function in probability calculations, it was found that the correct classification rate for the 10 value of m in the range (1.42,1.51) was higher than the correct classification rates for the other m-values. The results of the observations with changes in the estimated classification values for m=1.42 are presented in Table 5.

By using the Richards link function, for 6 observations with a change in classification values for m=1.42, the m-values that gave the highest correct classification rate for values varying in the range (1,6) were determined. It was determined that the current method gave the correct result

Table 2. Univariate logistic regression results

	B	S.E.	Wald	p	OR (95% CI)
Gender (ref: Male)	-1.117	0.137	66.06	<0.001*	0.327 (0.25-0.428)
Age	0.167	0.011	241.765	<0.001*	1.182 (1.157-1.207)
BMI	0.105	0.016	42.175	<0.001*	1.111 (1.076-1.147)
Duration of exposure to wood, dung or coal smoke	0.088	0.007	153.198	<0.001*	1.092 (1.077-1.107)
Packs of cigarettes smoked per year>10 per year (ref: No)	2.716	0.159	292.175	<0.001*	15.12 (11.074-20.645)
COPD in relatives (ref: None)	1.289	0.145	79.451	<0.001*	3.629 (2.734-4.818)
Lung disease other than COPD in relatives (ref: None)	0.94	0.155	36.594	<0.001*	2.561 (1.888-3.473)
Place of residence (ref: Metropolis)	2.034	0.161	159.907	<0.001*	7.643 (5.576-10.475)
Duration of daily exercise>1 hour (ref: No)	-3.364	0.393	73.276	<0.001*	0.035 (0.016-0.075)

*p<0.05, OR: Odds ratio, CI: Confidence interval, COPD: Chronic obstructive pulmonary disease, S.E.: Standard error

Table 3. Multivariate logistic regression table

	B	S.E.	Wald	p	OR (95% CI)
Gender (ref: Male)	-0.02	0.299	0.005	0.946	0.98 (0.545-1.76)
Age	0.138	0.013	110.701	<0.001*	1.148 (1.119-1.178)
BMI	-0.034	0.033	1.046	0.307	0.967 (0.907-1.031)
Duration of exposure to wood, dung or coal smoke	0.027	0.011	6.402	0.011*	1.027 (1.006-1.048)
Packs of cigarettes smoked per year>10 per year (ref: No)	2.058	0.303	46.135	<0.001*	7.832 (4.325-14.184)
COPD in relatives (ref: None)	1.027	0.281	13.349	<0.001*	2.792 (1.61-4.844)
Lung disease other than COPD in relatives (ref: None)	1.403	0.322	18.989	<0.001*	4.068 (2.164-7.646)
Place of residence (ref: Metropolis)	2.037	0.319	40.702	<0.001*	7.664 (4.1-14.329)
Duration of daily exercise>1 hour (ref: No)	-3.207	0.551	33.834	<0.001*	0.04 (0.014-0.119)
Constant	-7.65	1.094	48.916	<0.001*	0

*p<0.05, S.E.: Standard error, COPD: Chronic obstructive pulmonary disease, BMI: Body mass index, OR: Odds ratio, CI: Confidence interval

in two of these 6 observations, while the proposed method gave the correct result in four of them. With the proposed method, it was observed that an increase of approximately 0.2% (n=2) occurred in the correct classification rates (Table 5).

In the selection of the variables suitable for the logistic regression model, the variables were examined one by one by including them in the logistic regression model. It was concluded that the following variables should be included in the model: Gender (p<0.001), age (p<0.001), body mass index (p<0.001), duration of exposure to wood, dung or coal smoke (p<0.001), smoking status over 10 packs/year (p<0.001), having a relative with COPD (p<0.001), having a relative with lung disease other than COPD (p<0.001), place of residence (p<0.001), and exercising for more than 1 hour daily (p<0.001) (Table 6).

The logistic regression model showed that gender (p=0.727) and body mass index (p=0.643) had no effect on having COPD. It was determined that a 1-unit increase in age was a 1.195-fold greater risk (p<0.001) and 1-unit increase in exposure time to wood, dung or coal smoke was 1.069-fold greater risk for having COPD (p<0.001). It was determined that smoking more than 10 packs/year was a 16.446-fold greater risk (p<0.001), having COPD in first-degree relatives was a 3.348-fold greater risk (p=0.002), having individuals with lung disease other than COPD in first-degree relatives was a 9.797-fold greater risk, living in a metropolitan area was a 17.288-fold greater risk (p<0.001), and exercising for more than 1 hour a day was a 71.43-fold protective factor (p<0.001) (Table 7).

Table 4. Classification table of logistic regression equation

	Estimated		Correct classification rate
Observed	**Absence of COPD**	**Presence of COPD**	
Absence of COPD	466	39	92.3
Presence of COPD	31	469	93.8
			93.0

COPD: Chronic obstructive pulmonary disease

Table 5. Observations with changes in estimated classification values for m=1.42

p	Class	$p_{m=1.42}$	$Class_{m=1.42}$	Observed
0.521	1	0.460	0	1
0.540	1	0.483	0	0
0.540	1	0.483	0	0
0.507	1	0.442	0	1
0.545	1	0.489	0	0
0.538	1	0.480	0	0

Table 6. Univariate logistic regression results in learning data set

	B	S.E.	Wald	p	OR (95%CI)
Gender (ref: Male)	-1.404	0.161	75.89	<0.001*	0.246 (0.179-0.337)
Age	0.195	0.015	165.484	<0.001*	1.215 (1.179-1.251)
BMI	0.121	0.019	38.738	<0.001*	1.128 (1.086-1.172)
Duration of exposure to wood, dung or coal smoke	0.124	0.01	155.665	<0.001*	1.132 (1.11-1.154)
Packs of cigarettes smoked per year >10 per year (ref: No)	2.866	0.187	233.796	<0.001*	17.563 (12.164-25.36)
COPD in relatives (ref: None)	1.531	0.17	81.25	<0.001*	4.622 (3.313-6.447)
Lung disease other than COPD in relatives (ref: None)	1.041	0.184	32.023	<0.001*	2.832 (1.975-4.061)
Place of residence (ref: Metropolis)	2.007	0.192	109.004	<0.001*	7.438 (5.103-10.84)
Duration of daily exercise >1 hour (ref: No)	-3.567	0.425	70.333	<0.001*	0.028 (0.012-0.065)

*p<0.05, COPD: Chronic obstructive pulmonary disease, BMI: Body mass index, S.E.: Standard error, OR: Odds ratio, CI: Confidence interval

The correct classification rate of the equation with the available variables was 94.5% (n=709) (Table 8). By using the Richards link function in probability calculations, it was determined that the correct classification rate for 23 different values of m in the (1.33; 1.44) and (1.56; 1.66) ranges was higher than the correct classification rates for the other m-values. The results of the observations with changes in the estimated classification values for m=1.66 are presented in Table 9.

By using the Richards link function, 6 observations with changes in classification values for m=1.66 were determined from 23 different m-values, which gave the highest correct classification rate for values varying in the (1,3) range. The current method gave the correct result in one of these six observations, while the proposed method gave the correct results in five of these six observations. It was observed that there was an increase of approximately 0.67% (n=5) in the correct classification rate with the proposed method (Table 9).

The application of the models from the training data to the test data gave the following results: Correct classification was made for 221 observations with the current method and 222 observations with the proposed method. The probability value calculated with the current method was found to be above 0.5, while the probability values calculated by the proposed method for the observation estimated to be COPD were found to be below 0.5. The correct classification was made in the direction of not having COPD. As a result, the model, which increased the rate of correct classification by 0.67% in the training data, also provided an increase in correct classification of approximately 0.4% in the test data (Table 10).

Table 7. Multivariate logistic regression table in learning data set

	B	S.E.	Wald	p	OR (95% CI)
Gender (ref: Male)	0.145	0.415	0.122	0.727	1.156 (0.513-2.607)
Age	0.178	0.023	61.601	**<0.001***	1.195 (1.143-1.249)
BMI	-0.022	0.047	0.215	0.643	0.978 (0.892-1.073)
Duration of exposure to wood, dung or coal smoke	0.067	0.019	12.841	**<0.001***	1.069 (1.031-1.109)
Packs of cigarettes smoked per year >10 per year (ref: No)	2.8	0.437	41.115	**<0.001***	16.446 (6.988-38.705)
COPD in relatives (ref: None)	1.208	0.394	9.39	**0.002***	3.348 (1.546-7.251)
Lung disease other than COPD in relatives (ref: None)	2.282	0.51	19.992	**<0.001***	9.797 (3.603-26.639)
Place of residence (ref: Metropolis)	2.85	0.479	35.416	**<0.001***	17.288 (6.762-44.195)
Duration of daily exercise >1 hour (ref: No)	-4.281	0.712	36.153	**<0.001***	0.014 (0.003-0.056)
Constant	-10.701	1.737	37.932	**<0.001***	0

*p<0.05, OR: Odds ratio, CI: Confidence interval, COPD: Chronic obstructive pulmonary disease, BMI: Body mass index, S.E.: Standard error

Table 8. Classification table of logistic regression equation in learning data set

	Estimated		Correct classification rate
Observed	**Absence of COPD**	**Presence of COPD**	
Absence of COPD	353	22	94.1
Presence of COPD	19	356	94.9
			94.5

COPD: Chronic obstructive pulmonary disease

Table 9. Observations with changes in the estimated classification values for m=1.66

p	Class	$p_{m=1.42}$	$Class_{m=1.66}$	Observed
0.506	1	0.471	0	1
0.532	1	0.499	0	0
0.516	1	0.481	0	0
0.502	1	0.466	0	0
0.519	1	0.485	0	0
0.504	1	0.468	0	0

Table 10. Probability and classification values obtained as a result of applying the model to the test data

p	Yp	C	p	Yp	C	p	Yp	C	p	Yp	C	p	Yp	C
0.996	0.996	1	0.994	0.994	1	0.984	0.984	1	0.290	0.233	0	<0.001	<0.001	0
1.000	1.000	1	1.000	1.000	1	0.937	0.936	1	0.688	0.673	0	<0.001	<0.001	0
0.997	0.997	0	1.000	1.000	1	0.889	0.887	1	0.003	<0.001	0	<0.001	<0.001	0
1.000	1.000	1	0.949	0.948	1	0.997	0.997	1	0.845	0.841	0	<0.001	<0.001	0
1.000	1.000	1	0.979	0.979	1	0.992	0.991	1	0.010	0.002	0	<0.001	<0.001	0
0.999	0.999	1	0.994	0.994	1	0.258	0.199	0	<0.001	<0.001	0	<0.001	<0.001	0
1.000	1.000	1	0.995	0.995	1	1.000	1.000	1	<0.001	<0.001	0	<0.001	<0.001	0
1.000	1.000	1	0.690	0.675	1	0.965	0.965	1	0.047	0.018	0	0.001	<0.001	0
0.997	0.997	1	1.000	1.000	1	0.707	0.693	0	<0.001	<0.001	0	<0.001	<0.001	0
0.994	0.994	1	0.978	0.978	1	0.965	0.965	1	0.001	<0.001	0	<0.001	<0.001	0
0.985	0.985	1	1.000	1.000	1	0.636	0.616	1	0.003	<0.001	0	0.002	<0.001	0
0.902	0.900	1	1.000	1.000	1	0.984	0.984	0	<0.001	<0.001	0	0.001	<0.001	0
0.997	0.997	1	0.636	0.616	1	0.794	0.787	1	<0.001	<0.001	0	0.161	0.104	0
0.032	0.010	1	0.695	0.681	1	0.999	0.999	1	0.169	0.112	0	<0.001	<0.001	0
0.393	0.345	1	0.997	0.997	1	0.999	0.999	1	0.004	<0.001	0	0.341	0.288	0
0.970	0.970	1	0.944	0.944	1	0.999	0.999	1	0.001	<0.001	0	<0.001	<0.001	0
1.000	1.000	1	0.971	0.971	1	0.982	0.981	1	0.052	0.020	0	0.002	<0.001	0
0.999	0.999	1	0.988	0.988	1	0.999	0.999	1	<0.001	<0.001	0	<0.001	<0.001	0
0.973	0.973	0	0.992	0.992	1	0.998	0.998	1	<0.001	<0.001	0	<0.001	<0.001	0
0.990	0.990	0	0.949	0.949	1	0.261	0.202	1	<0.001	<0.001	0	0.001	<0.001	0
0.853	0.849	0	1.000	1.000	1	0.001	<0.001	1	<0.001	<0.001	0	<0.001	<0.001	0
0.456	0.414	1	0.998	0.998	1	0.984	0.984	1	0.073	0.033	0	0.002	<0.001	0
0.974	0.974	1	0.935	0.934	1	0.998	0.998	1	<0.001	<0.001	0	<0.001	<0.001	0
0.963	0.963	1	0.695	0.681	1	0.999	0.999	1	<0.001	<0.001	0	0.003	<0.001	0
0.996	0.996	0	1.000	1.000	1	0.998	0.998	1	0.002	<0.001	0	0.600	0.575	0
0.024	0.006	0	0.999	0.999	1	0.998	0.998	1	<0.001	<0.001	0	<0.001	<0.001	0
0.757	0.748	1	1.000	1.000	1	0.890	0.888	1	<0.001	<0.001	0	0.007	0.001	0
0.003	0.000	1	0.002	<0.001	0	0.766	0.757	1	0.001	<0.001	0	0.002	<0.001	0
0.828	0.823	1	0.999	0.999	1	0.999	0.999	1	<0.001	<0.001	0	0.021	0.005	0
0.970	0.970	1	0.984	0.984	1	0.997	0.997	1	<0.001	<0.001	0	0.029	0.009	0
0.011	0.002	1	0.994	0.994	1	0.997	0.997	1	0.001	<0.001	0	0.045	0.017	0
0.994	0.994	1	0.270	0.212	0	0.997	0.997	1	0.001	<0.001	0	0.009	0.002	0
0.950	0.949	1	0.849	0.845	1	0.917	0.916	1	<0.001	<0.001	0	<0.001	<0.001	0
0.999	0.999	1	0.993	0.993	1	0.999	0.999	1	<0.001	<0.001	0	<0.001	<0.001	0
0.005	0.001	0	0.997	0.997	1	0.980	0.980	1	<0.001	<0.001	0	0.883	0.881	0
0.846	0.842	1	0.006	0.001	1	0.996	0.996	1	0.054	0.021	0	0.955	0.955	0
0.996	0.996	1	0.993	0.993	1	<0.001	<0.001	0	<0.001	<0.001	0	0.922	0.921	0
0.999	0.999	1	0.999	0.999	1	<0.001	<0.001	0	<0.001	<0.001	0	0.836	0.832	0
0.889	0.887	1	0.969	0.969	1	0.062	0.027	0	<0.001	<0.001	0	0.934	0.933	0
0.948	0.948	1	0.080	0.038	1	0.005	0.001	0	<0.001	<0.001	0	0.638	0.618	0
0.936	0.935	1	0.999	0.999	1	<0.001	<0.001	0	0.001	<0.001	0	0.094	0.048	0
0.985	0.985	1	0.596	0.571	1	0.035	0.011	0	<0.001	<0.001	0	0.314	0.259	0

Table 10. Continue

p	Yp	C	p	Yp	C	p	Yp	C	p	Yp	C	p	Yp	C
0.982	0.982	**1**	1.000	1.000	**1**	0.232	0.172	**0**	<0.001	<0.001	**0**	0.010	0.002	**0**
0.828	0.823	**1**	0.970	0.970	**1**	0.017	0.004	**0**	<0.001	<0.001	**0**	0.003	<0.001	**0**
0.992	0.992	**1**	0.005	0.001	**0**	0.989	0.989	**0**	<0.001	<0.001	**0**	0.008	0.001	**0**
0.996	0.996	**1**	0.896	0.894	**1**	0.672	0.655	**0**	<0.001	<0.001	**0**	0.895	0.893	**0**
0.318	0.263	**1**	0.969	0.969	**1**	0.001	<0.001	**0**	0.025	0.007	**0**	0.104	0.056	**0**
0.498	0.462	**1**	0.983	0.983	**1**	<0.001	<0.001	**0**	0.011	0.002	**0**	0.096	0.050	**0**
0.989	0.989	**1**	1.000	1.000	**1**	<0.001	<0.001	**0**	0.001	<0.001	**0**	0.942	0.942	**0**
0.961	0.961	**1**	0.999	0.999	**1**	<0.001	<0.001	**0**	<0.001	<0.001	**0**	0.526	0.493	**0**
0.001	0.000	**1**	0.892	0.890	**1**	0.145	0.090	**0**	<0.001	<0.001	**0**	0.941	0.941	**0**

C: Status of having chronic obstructive pulmonary disease
p: Probability value obtained with current application
Yp: Probability value obtained with proposed application

Discussion

Human beings have been trying for centuries to instill some human skills in inanimate beings (19). In the 20th century, there has been an increasing interest in this subject among scientists. At the beginning of the second half of the 20th century, the Turkish scientist Arf (20) raised the question "Can Machines Think and How Can They Think?". One of the tasks undertaken by artificial intelligence is to give machines the ability to make inferences and decisions based on past experiences (21). As in many fields, in the field of health, researchers aim to make inferences about future observations with the data of existing observations by classifying these observations (5). Methods such as discriminant analysis, cluster analysis, and logistic regression are some of the methods used for classification (8). Logistic regression is mostly used when the classes of observations are known (22). Many studies have been carried out to improve the predictions made with logistic regression and increase the rate of correct classification (12,23,24). The logistic regression plot is an S-shaped sigmoid curve (25). The inflection point of this curve may sometimes take smaller or larger values than it should be in logistic regression. Gürcan et al. (17) stated that if the inflection point of the logistic curve corresponds to smaller or larger x-values than it should be, a more optimal inflection point can be determined by using various link functions. They found the inflection point by analyzing its second derivative of the curve proposed by Richards (18), and aimed to increase the correct classification rate of the model by applying the inflection points separately for the misclassified observations. This study aimed to make the inflection point of the logistic regression curve more ideal by using the link function $\pi(x) = (1 + (m-1).(e^{-a}))^{\frac{1}{1-m}}$ equation proposed by Richards (18). Probability values were calculated separately for all observations and assigned to classes according to these values. Then, the m-values that maximized the correct classification rate of the model were determined. Application of the model with all data indicated that the percentage of correct classification, which was 93% with the current method, increased by approximately 0.2% for m=1.42 using the Richards link function. Then, the data were split into training (n=750) and test (n=255) data sets. In the training data set, the correct classification rate, which was 94.5% with the current method, was found to be 95.07% for 23 different m-values in the (1.33; 1.44) and (1.56; 1.66) intervals using the Richards link function. For these values, the same model was applied to the test data for m=1.66. Correctly classifying 1 observation that was misclassified by the current method provided an increase of approximately 0.4% in the correct classification rate for the test data.

Conclusion

The present study was carried out to make predictions with a higher percentage of correct classification in logistic regression. It can be concluded that certain percentage increases in the correct classification rates can be achieved by using the Richards link function. However, it would be beneficial to carry out studies in which simulation scenarios are made with data sets containing fewer and more independent variables, different sample sizes, and combinations of independent variable types.

Information: This study is derived from the thesis study of Kürşad Nuri Baydili, PhD student of İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Biostatistics.

Ethics

Ethics Committee Approval: Ethics committee approval with the number 18/1 was obtained from the Hamidiye Scientific Research Ethics Committee at the meeting numbered 2022/18 for the research.

Informed Consent: Retrospective study.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.D., Concept: K.N.B., M.Ç., A.D., Design: K.N.B., M.Ç., A.D., Data Collection or Processing: K.N.B., M.Ç., A.D., Analysis or Interpretation: K.N.B., A.D., Literature Search: K.N.B., A.D., Writing: K.N.B., M.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Karagöz Y. SPSS AMOS META Uygulamalı Nitel-Nicel Karma Bilimsel Araştırma Yöntemleri ve Yayın Etiği, 2. Basım, Ankara: Nobel Yayıncılık; 2019. [Crossref]
2. Özdamar K. Paket Programlar ile İstatistiksel Veri Analizi 2, Kaan Kitabevi, Eskişehir; 2010. [Crossref]
3. Katz MH. Multivariable analysis: a practical guide for clinicians and public health researchers. Cambridge university press, 2011. [Crossref]
4. Alpar R. Uygulamalı çok değişkenli istatistiksel yöntemler. Detay Yayıncılık, 2017. [Crossref]
5. Karakoyun M, Hacıbeyoğlu M. Biyomedikal Veri Kümeleri İle Makine Öğrenmesi Sınıflandırma Algoritmalarinin İstatistiksel Olarak Karşılaştırılması. Mühendislik Bilimleri Dergisi. 2014;16:30-42. [Crossref]
6. Driver HE, Kroeber AL. Quantitative expression of cultural relationships (vol. 31, no. 4). Berkeley: University of California Press; 1932. [Crossref]
7. Fisher RA. The use of multiple measurements in taxonomic problems. Annals of eugenics, 1936;7:179-188. [Crossref]
8. Tatlıdil H. Uygulamalı Çok Değişkenli İstatistiksel Analiz. Cem Web Ofset, 1996. [Crossref]
9. Bircan H. Lojistik regresyon analizi: Tıp verileri üzerine bir uygulama. Kocaeli Üniversitesi Sosyal Bilimler Enstitüsü Dergisi. 2004;2:185-208. [Crossref]
10. Berkson J. Application of the logistic function to bio-assay. Journal of the American Statistical Association. 1994;39:357-365. [Crossref]
11. Şenel S, Alatlı B. Lojistik regresyon analizinin kullanıldığı makaleler üzerine bir inceleme. Journal of Measurement and Evaluation in Education and Psychology. 2014;5:35-52. [Crossref]
12. Sancar N, Inan D. A new alternative estimation method for Liu-type logistic estimator via particle swarm optimization: an application to data of collapse of Turkish commercial banks during the Asian financial crisis. J Appl Stat. 2021;48:2499-2514. [Crossref]
13. Tabachnick BG, Fidell LS, Ullman JB. Using multivariate statistics. Boston, MA: Pearson; 2007;481-498. [Crossref]
14. Seber GAF, Wild CJ. Nonlinear regression, 1989. [Crossref]
15. Başarır G. Çok Değişkenli Verilerde ayrımsama sorunu ve lojistik regresyon Analizi. Hacettepe Üniversitesi Sosyal Bilimler Enstitüsü, Yayınlanmamış Doktora Tezi, 1990. [Crossref]
16. Verhulst PF. Notice on the law that the population follows in its growth. Corresp Math Phys. 1938;10:113-126. [Crossref]
17. Gürcan M, Kaya MO, Halisdemir N. Lojistik İncelemede Ayrımsama Performansının Değerlendirilmesi. Avrupa Bilim ve Teknoloji Dergisi. 2019;1008-1013. [Crossref]
18. Richards FJ. A flexible growth function for empirical use. Journal of experimental Botany. 1959;10:290-301. [Crossref]
19. Öztürk K, Şahin ME. Yapay sinir ağları ve yapay zekâ'ya genel bir bakış. Takvim-i Vekay. 2018;6:25-36. [Crossref]
20. Arf C. Makineler Düşünebilir mi ve Nasıl Düşünebilir? Atatürk Üniversitesi 1958-1959 Öğretim Yılı Halk Konferansları. 1959;91-103. [Crossref]
21. Demirhan A, Kılıç YA, İnan G. Tıpta yapay zeka uygulamaları. Yoğun Bakım Dergisi. 2010;9:31-41. [Crossref]
22. Kleinbaum DG, Kupper LL, Muller KE, Nizam A. Applied Regression Analysis and Other Multivariable Methods, 4th ed. Thomson Learning. Inc., Belmont: California; 2007. [Crossref]
23. Kibria BG, Shukur G. On Liu estimators for the logit regression model. Economic Modelling. 2012;29:1483-1488. [Crossref]
24. Kang K, Gao F, Feng J. A new multi-layer classification method based on logistic regression. In 2018 13th International Conference on Computer Science & Education (ICCSE) (pp. 1-4). IEEE. 2018. [Crossref]
25. Eberhardt LL, Breiwick JM. (2012). Models for population growth curves. International Scholarly Research Notices, 2012. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.72473

Hamidiye Med J 2022;3(3):171-176

Our Experiences in Percutaneous Cannulation and Monitoring in Minimal Invasive Cardiac Surgery

Minimal İnvaziv Kalp Cerrahisinde Perkütan Kanülasyon ve Monitorizasyon Deneyimlerimiz

Mustafa Şimşek, Türkan Kudsioğlu

İstanbul Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital, Clinic of Anesthesiology and Reanimation, İstanbul, Türkiye

ABSTRACT

Background: The aim of the study was to evaluate the patients who underwent minimally invasive cardiac surgery and percutaneous internal jugular vein catheterization in our center, and to discuss the catheterization results and complications in the literature.

Materials and Methods: Between January 2015 and September 2019, 70 female (59.3%) and 48 (40.7%) male patients, who underwent minimally invasive cardiac surgery and percutaneous internal jugular vein cannulation in our center, had a mean age of 37.2±14.5 (19-74 years), data of 118 cases were evaluated retrospectively. It was noted that 17 F jugular venous catheter was placed in patients with body surface area (BSA) <1.87, and 19 F venous catheter was placed in patients with (BSA) >1.87. Cannula positions and echocardiographic findings of the patients during cannulation were evaluated with transesophageal echocardiography (TEE).

Results: All surgical interventions were performed minimally invasively by thoracotomy. Since the adequate surgical field of vision could not be achieved in 3 (2.5%) of the patients, the operation was reverted to sternotomy. No mortality due to cannulation was observed in any of the patients. Local hematoma (1.6%) developed due to carotid artery puncture in 2 patients, transient atrial fibrillation (1.6%) in 2 patients, and pneumothorax (0.8%) in one patient.

Conclusion: Minimally invasive cardiac surgical interventions have become popular nowadays and their importance has increased due to reasons such as faster recovery of patients, less complications, and smaller surgical incision area. Internal jugular cannulation is required in minimally invasive cardiac surgery procedures. In order to avoid possible complications in the percutaneous cannulation process and therefore to reduce mortality, it is very important that cannulation procedures be performed by an experienced team and evaluated with TEE during this time.

Keywords: Minimally invasive surgical procedures, cannulation, jugular veins

ÖZ

Amaç: Çalışmanın amacı, merkezimizde minimal invaziv kalp cerrahisi uygulanan ve perkütan internal juguler ven kateterizasyonu yapılan hastaların değerlendirilmesi, kateterizasyon sonuçları ve meydana gelen komplikasyonların literatürler eşliğinde tartışılmasıdır.

Gereç ve Yöntemler: Ocak 2015-Eylül 2019 tarihleri arasında merkezimizde minimal invaziv kalp cerrahisi geçiren ve perkütan internal juguler ven kanülasyonu yapılan, 70'i kadın (%59,3), 48'i erkek (%40,7) hastadan oluşan ve yaş ortalaması 37,2±14,5 (19-74 yaş) olan 118 olgunun verileri retrospektif olarak incelendi. Vücut yüzey alanı (VYA) <1,87 olan hastalara 17 F juguler venöz kateter, (VYA) >1,87 olan hastalara 19 F venöz kateter yerleştirildiği kaydedildi. Hastaların kanülasyon sırasında kanül pozisyonları ve ekokardiyografik bulguları transözefageal ekokardiyografi (TÖE) ile değerlendirildi.

Bulgular: Uygulanan cerrahi girişimlerin tümü torakotomi ile minimal invaziv olarak gerçekleştirilmiştir. Hastalardan 3'ünde (%2,5) yeterli cerrahi görüş alanı sağlanamadığından operasyon sternotomiye dönmüştür. Hiçbir hastada kanülasyona bağlı mortalite gözlenmemiştir. Hastaların 2'sinde karotis arter ponksiyonu nedeniyle lokal hematom (%1,6), iki hastada geçici atriyal fibrilasyon (%1,6), birinde de pnömotoraks (%0,8) gelişmiştir.

Sonuç: Minimal invaziv kalp cerrahisi girişimleri, hastaların daha çabuk derlenmesi, daha az komplikasyona neden olması, cerrahi kesi alanının küçülmesi gibi nedenlerden dolayı, günümüzde popüler hale gelmiş ve önemi artmıştır. Minimal invaziv kalp cerrahisi

Address for Correspondence: Mustafa Şimşek, İstanbul Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital, Clinic of Anesthesiology and Reanimation, İstanbul, Türkiye

Phone: +90 505 778 99 80 E-mail: mustafasimsek73@gmail.com **ORCID ID:** orcid.org/0000-0001-9903-5307

Received: 10.05.2022 **Accepted:** 05.10.2022

ÖZ

girişimlerinde internal jugular kanülasyon gerekmektedir. Perkütan kanülasyon sürecinde olası komplikasyonlardan kaçınmak ve mortaliteyi azaltmak için kanülasyon işlemlerinin deneyimli bir ekip tarafından yapılması ve bu sırada TÖE ile değerlendirilmesi oldukça önemlidir.

Anahtar Kelimeler: Minimal invaziv cerrahi girişimler, kanülasyon, juguler venler

Introduction

Conventional cardiac surgery is performed through median sternotomy, which provides a larger surgical area, allowing easy access to cardiac structures for cannulation and cardiopulmonary bypass (CPB). Currently, the ability to perform operations with a less invasive approach through smaller surgical incisions have become more important; rendered possible by the development of technology, technical innovations in surgery and anesthesia and the use of intraoperative transesophageal echocardiography (TEE) Minimally invasive surgery (MICS) significantly reduces surgical stress response, length of stay in the hospital and intensive care unit, scar tissue formation and transfusion requirement. Thus, a significant reduction in the hospital costs occurs (1).

Normally, CPB is established after sternotomy via central arterial and venous cannulation made by the surgeon. However, with the definition of minimally invasive cardiac surgery (MICS), new cannulation techniques have come forward. With use of these techniques, which are performed through percutaneous peripheral cannulation, CPB can be established with a smaller sternal incision or a thoracotomy incision without the need for median sternotomy. Subclavian, internal jugular, femoral and iliac veins or arteries can be used for cannulation (2).

For example, minimally invasive surgeries that do not require right atriotomy, such as coronary artery bypass graft surgery, can be performed with a single venous cannula placed in the right atrial cavity via the femoral vein. However, the placement of the venous cannula into the atrial cavity is not suitable in open heart surgery procedures that require right atriotomy or left atriotomy, such as atrial septal defect (ASD) repair, mitral valve replacement (MVR). In these cases, bicaval cannulation can be provided through the femoral and internal jugular veins (IJVs) (2). Our study aims to analyze the percutaneous IJV cannulations applied in MICSs performed in our institution, in the light of current literature.

Material and Methods

After the approval of the Ethics Committee İstanbul Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital, (E-28001928-604.01.01), 118 patients who underwent MICS with percutaneous IJV cannulation in our center between January 2015 and September 2019 were included in the study.

The mean age was 37.2±14.5 years (minimum-maximum: 19-74 years), 70 were female (59.3%) and 48 were (40.7%) male. Patients with MICS contraindication criteria; ascending aortic aneurysm (AA >4 cm), presence of moving plaque in the aorta, severe mitral annular calcification, patients with radiotherapy history, as well as patients who are morbidly obese [body mass index (BMI) >35] and patients requiring emergency surgery were excluded from the study.

Written informed consent was obtained from each patient. The study protocol was approved by the hospital scientific committee. The study was conducted in accordance with the principles of the Declaration of Helsinki. Patient data were scanned retrospectively from perfusion and anesthesia records and patient files in our hospital data system.

Anesthesia Management and Catheterization

All patients routinely underwent IV catheterization with 16 G wide peripheral IV catheter (The Introcan Safety® IV Catheter B. Braun Medical Inc. USA), then 20 G radial artery catheterization (Arrow® Seldinger Arterial Catheter, USA). Monitorization was provided with 5-electrode electrocardiography, peripheral oxygen saturation (SpO_2) and near infrared spectroscopy (Covidien, USA). External defibrillator pads were placed for defibrillation.

After preoxygenation, anesthesia was induced with 2-3 mg/kg propofol, 5 mcgr/kg fentanyl, 1 mg/kg rocuronium. Female patients shorter than 165 cm were intubated with a 35 F, female patients taller than 165 cm were intubated with a 37 F left double lumen tube (Handan FCH Medical Device Technology, China), while male patients taller than 170 cm were intubated at 41 F, male patients shorter than 170 cm were intubated with a 39 F left double lumen tube. An 8.5 F three-way central catheter (Multi-Lumen Central Venous Catheter, Arrow International Inc.PA, USA) was also placed through the left IJV and central venous pressure (CVP) monitoring was performed.

Propofol, fentanyl and sevoflurane were administered for anesthesia maintenance. 1 g vancomycin was routinely given to the patients who will undergo mechanical valve

replacement, for the prophylaxis of infective endocarditis, as recommended by the infectious diseases committee of our center. 1 g IV tranexamic acid was given to all patients and followed by continuous infusion at 7.5 mg/kg/hour.

TEE (Vivid 7 GE Vingmed Ultrasound AS Horten, Norway) probe was placed in all patients for intraoperative TEE.

Before cannulation, 1 mg/kg heparin was administered to the patients. For percutaneous central venous cannulation, the patient's head was deviated 45° to the left, the IJV was punctured with the palpation technique, the guide wire was advanced, and the location of the guide wire was confirmed by TEE, and cannulation was performed with the seldinger technique.

The tip of the cannula was advanced and fixed at the cava-atrial junction with TEE guidance (Figure 1). 17 F jugular venous catheter (Bio-Medicus venous canulae Medtronic, Minneapolis, USA) was used for patients with body surface area (BSA) <1.87, and 19 F venous catheter was used for patients with BSA >1.87 (Figure 2).

Femoral artery and femoral vein cannulation was performed by the surgical team under the guidance of TEE. Before initiating CPB, 3 mg/kg heparin was administered to the patients, to ensure an ACT measurement >450 sec. All operations were performed at 32 °C moderate hypothermia. Myocardial protection was provided by cold blood cardioplegia, given every 20 minutes.

The operations were performed with thoracotomy. At the end of the operation, hemodynamic parameters and blood gas results (pH, PaO_2, $PaCO_2$, hemoglobin, hematocrit, SaO_2, lactate) were evaluated and CPB was terminated under TEE monitoring. Heparin was neutralized 1:1 with protamine and the IJV cannula was withdrawn, the cannulation incision was sutured with circular stitches and jugular compression was applied for approximately 15 minutes. Femoral decannulation was made afterwards, hemostasis was achieved and the incisions were closed. The patients were transferred to the intensive care unit after the operation.

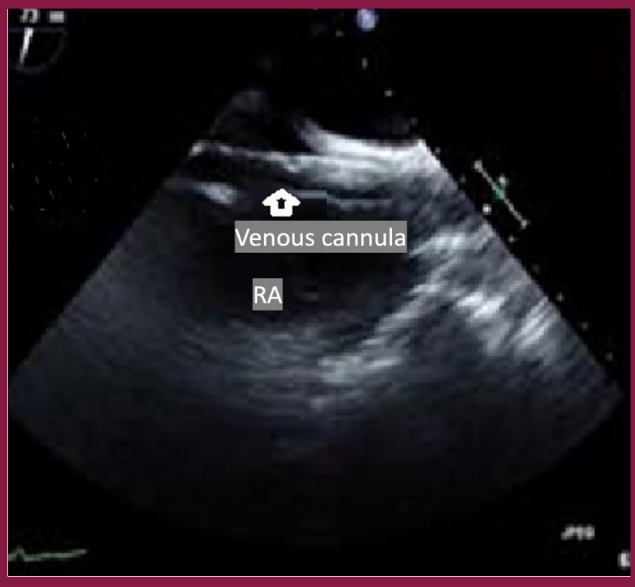

Figure 1. Venous cannula in the bicaval right atrium image at the middle esophageal level in transesophageal echocardiography

Statistical Analysis

Categorical variables are presented as numbers and frequencies. Continuous variables showing normality are expressed as mean ± standard deviation. SPSS software (version 25.0, IBM Corporation, Armonk, NY, USA) was used for statistical analysis.

Results

A total of 118 (n=118) patients, including 70 women (59.3%) and 48 (40.7%) men, were included in the study. Their mean age was 37.2±14.5 (minimum-maximum: 19-74 years), mean body weight was 69.5±12.4 kg, and mean BMI was 25.5±4.3. The mean BSA was 1.77±0.17, and the mean preoperative left ventricular ejection fraction was 57.4±5.8 (Table 1).

Performed surgical operations were; 32 ASD closure, 2 ASD closure + tricuspid ring annuloplasty (TRA), 1 ASD closure + TRA + partial venous return anomaly (PAPVD) correction, 64 MVR, 6 MVR + TRA, 1 MVR+coronary fistula closure, 8 minimally invasive direct coronary artery bypass (MIDCAB), 4 atrial myxoma removal (Table 2). The operation be performed by thoracotomy. The mean duration of CPB

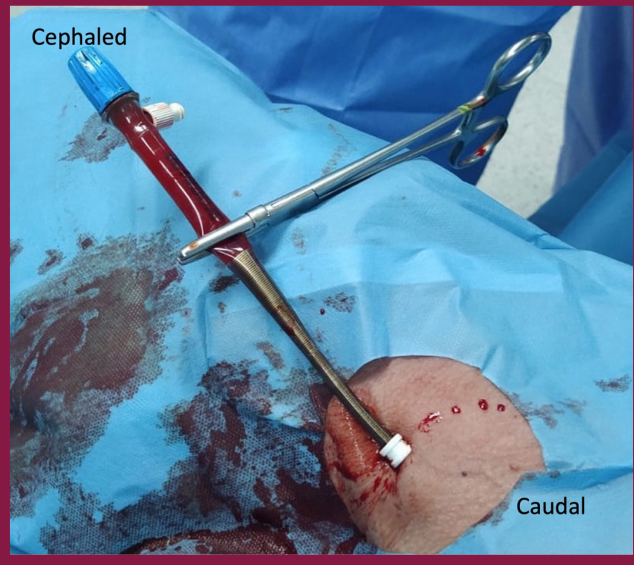

Figure 2. Internal jugular cannulation

was 128.7±76 minutes, the cross-clamp time was 84.4±49 minutes, the pump flow was 2.3±0.17 Lt/m^2/min, and the postoperative stay in the intensive care unit was 1.9±3.2 days (Table 1). The study flow chart is given in Figure 3.

The operation was reverted to sternotomy and central cannulation in 3 patients (2.5%) due to insufficient surgical field of vision, two patients (1.6%) had local hematoma due to carotid artery puncture, and 2 patients (1.6%) had temporary atrial fibrillation and pneumothorax were observed in 1 patient (0.8%). Complications such as vascular injury, hemothorax, mediastinal hematoma that required surgery were not encountered in the patients. Mortality was not observed in any patient due to cannulation (Table 3).

Table 1. Patient characteristics

Characteristics	n (%)/Mean ± SD
Sex (F/M)	70 (59.3%)/48 (40.7%)
Age (year)	37.2±14.5
Weight (kg)	69.5±12.4
BMI (kg/m^2)	25.5±4.3
BSA (m^2)	1.77±0.17
EF (%)	57.4±5.8
Operation data	
CPB duration (min)	128.7±76
Cross clamp duration (min)	84.4±49
Mean pump flow (L/m^2/min)	2.3±0.17
Mean CVP (mmHg) 17 F cannula/19 F cannula	2.9±0.3/3.3±0.4
Maximum CVP (mmHg) 17 F cannula/19 F cannula	6.8±1.2/7.8±1.6
Intensive care unit stay (day)	1.9±3.2

BMI: Body mass index, BSA: Body surface area, EF: Left ventricle ejection fraction, CPB: Cardiopulmonary bypass, CVP: Central venous pressure, SD: Standard deviation

Table 2. Types of operation

Operation	n (%)
ASD	32 (27.11%)
ASD+TRA	2 (1.6%)
ASD+TRA+PAPVD	1 (0.8%)
MVR	64 (54%)
MVR+TRA	6 (5.08%)
MVR+coronary fistula repair	1 (0.8%)
ATRIAL MYXOMA	4 (3.3%)
MIDCAB	8 (6.7%)

ASD: Atrial septal defect repair, TRA: Tricuspid ring annuloplasty, MVR: Mitral valve replacement, PAPVD: Partial anomalous pulmonary venous return, MIDCAB: Minimally invasive direct coronary artery bypass grafting

Discussion

Traditional cardiac surgery techniques are in the direction of evolving into smaller surgical incisions and less invasive approaches supported by the advancements of technology, surgery and anesthesiology, and the use of intraoperative TEE.

Cosgrove and colleagues described the first minimally invasive valve interventions in 1996 and grouped several different approaches as "MICS" (3). Today, MIDCAB, robotic-assisted cardiac surgery, atrial fibrillation ablation surgery and minimally invasive approaches to the mitral valve, left and right atrium and aortic valve, are considered within the scope of MICS.

A long, two-stage superior vena cava (SVC)/inferior vena cava (IVC) cannula, inserted via the femoral vein, is used for venous cannulation of the surgical procedures in which the heart cavities are not completely opened. However, in

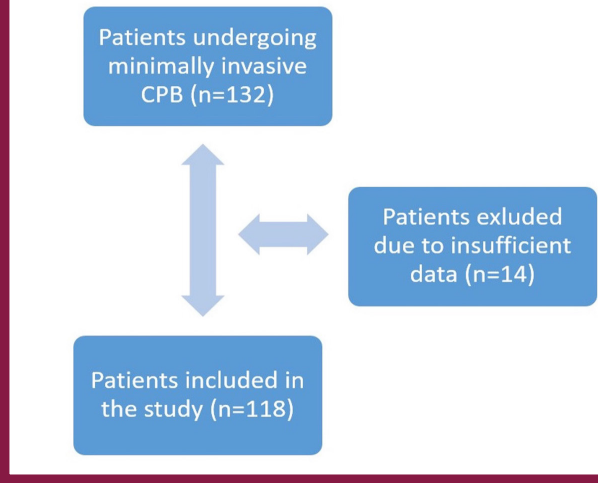

Figure 3. Study of flowchart
CPB: Cardiopulmonary bypass

Table 3. Intraoperative complications

Intraoperative complications	n (%)
Local hematoma	2 (1.6%)
Transient atrial fibrillation	2 (1.6%)
Vascular injury	0 (0%)
Hemothorax	0 (0%)
Pneumothorax	1 (0.8%)
Mediastinal hematoma	0 (0%)
Edema in the upper extremity	0 (0%)
Failed minimally invasive surgery	3 (2.5%)
Mortality	0 (0%)

operations that require opening of the right side of the heart or the left atrium, a possible air leak into the CPB circuit may cause a dangerous air lock in the system. SVC/IVC cannula is also required in transseptal or transatrial MVR, as well as in surgical interventions involving the right atrium, such as tricuspid valve surgery, atrial myxoma removal, ASD or patent foramen ovale repair. The right IJV, which anatomically has a linear path close to the right atrium, is preferred for SVC cannula insertion (4,5,6,7).

In our case series, jugular venous catheterisation preferences were made according to the BSA of the patients. A 19 F jugular venous catheter was inserted in patients with BSA >1.87, and a 17 F jugular venous catheter was inserted in patients with BSA <1.87.

IJV cannulation can be performed with the palpation technique or under the ultrasonography guidance (6,7). In our center, cannulation is performed using the palpation technique. Studies have reported that the palpation method provides 60-95% success, depending on the cannulation site and the physical characteristics of the patient (6,7). It has been reported that although more than 5 million central venous catheters (IJV, SVC, and FV) are inserted annually in the United States alone, mechanical complications occur at a rate of 5% to 19%, and these complications are mostly due to lack of experience and the physical characteristics of the patients (6,7,8,9,10). In our study, local hematoma that did not require surgical intervention due to the carotid artery puncture (1.6%) occurred in 2 patients, pneumothorax that regressed spontaneously in one patient (0.8%), and transient atrial fibrillation (1.6%) in two patients due to a long guide wire, were observed. In the presence of recent data, our complication rate is considerably lower than the complication rates reported in the literature.

However, during IJV cannulation, other than puncture-related complications, more serious and mortal conditions can be seen, especially depending on the cannula size and diameter. In the case report of Hirose et al. (8), a 23 F Avalon cannula was inserted into the IJV in a patient who was planned to have VV-ECMO due to ARDS, but sudden hemodynamic deterioration and cardiac tamponade were encountered.

The emergent operation revealed the cannulation related injury in the apex of the right ventricle (8). The most fair way to avoid such complications is to place the cannula under TEE guidance. Jankovic et al. (9) also reported that V-V bypass was planned due to hepatic cirrhosis, 21 F femoral and 21 F internal jugular catheters were inserted, the IJV cannula punctured the vena cava and penetrated the right pleura approximately 4 cm deep into the right thorax. It has been reported that 1.8 liters of hemorrhagic effusion and 0.7 liters of hemorrhagic effusion were drained from the left thorax (9).

Therefore, the use of large diameter and long cannula without TEE guidance may lead to mortal result (10,11). Hemothorax and vascular injury did not occur in our study. In addition, it is important to provide an effective venous drainage for CPB. For this reason, the diameters of the cannula used must be large enough and drainage holes must be present to prevent vacuuming. Inadequate SVC drainage results in impaired venous return in the head and neck, conjunctival edema, and elevated CVP (12,13,14,15). Such complications did not develop in the patients included in the study. Our mean CVP rates were 2.9±0.3 mmHg for 17 F catheters and 3.3±0.4 mmHg for 19 F catheters, while our maximum CVP values were 6.8±1.2 mmHg for 17F catheters, and 7.8±1.6 mmHg for 19 F catheters.

Study Limitations

The limitations of this study was the limited number of patients.

Conclusion

Nowadays, MICS offers serious advantages to the patients and to the healthcare system. During these interventions, the new cannulation techniques supported by the technology and applications such as TEE are substantial in the prevention of many possible complications. Based on our experience and many studies on this subject, we believe that; an appropriate cannula selection according to the type of the intervention and the physical characteristics of the patient, and use of the appropriate techniques are very important for achieving a successful operation.

Ethics

Ethics Committee Approval: After the approval of the Ethics Committee İstanbul Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital, (E-28001928-604.01.01), 118 patients who underwent MICS with percutaneous IJV cannulation in our center between January 2015 and September 2019 were included in the study.

Informed Consent: Written informed consent was obtained from each patient.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.Ş., Concept: M.Ş., Design: M.Ş., Data Collection or Processing: M.Ş., T.K., Analysis or Interpretation: M.Ş., T.K., Literature Search: M.Ş., Writing: M.Ş., T.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Dieberg G, Smart NA, King N. Minimally invasive cardiac surgery: A systematic review and meta-analysis. Int J Cardiol. 2016;223:554-560. [Crossref]
2. Ateş M, Sensöz Y. [Toward the perfect heart-lung machine]. Anadolu Kardiyol Derg. 2002;2:253-258. [Crossref]
3. Cosgrove DM, Sabik DF, Navia JL. Minimally invasive valve operations. Ann Thorac Surg. 1998;65:1535-1539. [Crossref]
4. Turker G, Kaya FN, Gurbet A, Aksu H, Erdogan C, Atlas A. Internal jugular vein cannulation: an ultrasound-guided technique versus a landmark-guided technique. Clinics (Sao Paulo). 2009;64:989-992. [Crossref]
5. Troianos CA, Hartman GS, Glas KE, Skubas NJ, Eberhardt RT, Walker JD, et al. Guidelines for performing ultrasound guided vascular cannulation: recommendations of the American Society of Echocardiography and the Society of Cardiovascular Anesthesiologists. J Am Soc Echocardiogr. 2011;24:1291-1318. [Crossref]
6. Iribarne A, Easterwood R, Russo MJ, Chan EY, Smith CR, Argenziano M. Comparative effectiveness of minimally invasive versus traditional sternotomy mitral valve surgery in elderly patients. J Thorac Cardiovasc Surg. 2012;143(Suppl 4):S86-90. [Crossref]
7. Maltepe F, Metin KS, Hepaguşlar H, Hancı V, Uğurlu B, Oto Ö. Percutaneous internal jugular vein cannulation for cardiac surgery. Türk Göğüs Kalp Damar Cerrahisi Dergisi. 2013; 21:959-965. [Crossref]
8. Hirose H, Yamane K, Marhefka G, Cavarocchi N. Right ventricular rupture and tamponade caused by malposition of the Avalon cannula for venovenous extracorporeal membrane oxygenation. J Cardiothorac Surg. 2012;7:36. [Crossref]
9. Jankovic Z, Boon A, Prasad R. Fatal haemothorax following large-bore percutaneous cannulation before liver transplantation. Br J Anaesth. 2005;95:472-476. [Crossref]
10. McGee DC, Gould MK. Preventing complications of central venous catheterization. N Engl J Med. 2003;348:1123-1133. [Crossref]
11. Prempeh ABA, Jacques S, Justiaan LS. Transesophageal echocardiography in minimally invasive cardiac surgery. Curr Opin Anaesthesiol. 2020;33:83-91. [Crossref]
12. Ni Y, Leskosek B, Shi L, Chen Y, Qian L, Li R, et al. Optimization of venous return tubing diameter for cardiopulmonary bypass. Eur J Cardiothorac Surg. 2001;20:614-620. [Crossref]
13. Mack MJ, Leon MB, Thourani VH, Makkar R, Kodali SK, Russo M, et al. Transcatheter aortic-valve replacement with a balloon-expandable valve in low-risk patients. N Engl J Med. 2019;380:1695-1705. [Crossref]
14. Wilbring M, Charitos E, Silaschi M, Treede H. Minimally invasive mitral valve surgery. Indian J Thorac Cardiovasc Surg. 2018;34:113-116. [Crossref]
15. Kayir S, Ozyalcin S, Dogan G, Diken AI, Turkmen U. Internal Jugular Vein Catheterization: The Landmark Technique versus Ultrasonography Guidance in Cardiac Surgery. Cureus. 2019;11:e4026. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.83702

Hamidiye Med J 2022;3(3):177-183

Qualitative and Quantitative Evaluation of Videoendoscopic Simulation in Surgical Residents Training

Cerrahi Asistanlarının Videoendoskopi Simülasyon Eğitiminin Kalitatif ve Kantitatif Olarak Değerlendirilmesi

Sezen Milli Avtan[1], Esra Akdeniz[2], Wafi Attaallah[3]

[1]*University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Department of Medical Education, İstanbul, Türkiye*

[2]*Marmara University Faculty of Medicine, Department of Medical Education, İstanbul, Türkiye*

[3]*Marmara University Faculty of Medicine, Department of General Surgery, İstanbul, Türkiye*

ABSTRACT

Background: In our study, we aimed to evaluate the contribution of video-endoscopy simulation for the development of gastroscopy and colonoscopy skills of residents in surgical resident training, using qualitative and quantitative methods.

Materials and Methods: Sixteen novice surgeons were trained with an endoscopic virtual reality simulator. Ten colonoscopy and 10 gastroscopy cases were overviewed in this training. Three hundred-twenty gastroscopy and colonoscopy simulation modules were evaluated. Continious variables are presented as median and interquartile range. Wilcoxon signed-rank test was used to detect changes during the training. For the qualitative data of the study, in-depth interviews were conducted with the residents who completed the modules. At the end of the interviews, the answers were documented directly in the same day. The themes and sub-themes related to the content were determined by two medical evaluators.

Results: A significant improvement was observed both in colonoscopy and gastroscopy modules. While the time to reach the cecum in the colonoscopy module decreased from 20 minutes to 3 minutes on average, and the time to reach the duodenum in gastroscopy from 4 minutes to 3.6 minutes. The percentage of mucosal surface examined increased both in gastroscopy and colonoscopy. The time spent to obtain quality images did not change in gastroscopy, but there was an obvious increase in colonoscopy. The percentage of effective usage of the screen increased both in gastroscopy and colonoscopy. Qualitative data proved that all participants were satisfied by the training and benefited from it.

Conclusion: The results of our study indicate the importance and advantage of the utilization of simulators in trainings that require interventional skills, before patient encounter. It has been shown that video-endoscopy simulation supports the dexterity of residents in gastroscopy and colonoscopy applications.

Keywords: Medical simulation, video-endoscopy, medical education

ÖZ

Amaç: Çalışmamızda cerrahi asistan eğitiminde, video-endoskopi simülasyonunun asistanların gastroskopi ve kolonoskopi becerilerinin gelişimine katkısını kalitatif ve kantitatif yöntemlerle değerlendirilmeyi amaçladık.

Gereç ve Yöntemler: Çalışmada daha önce video-endoskopi deneyimi olmayan 16 genel cerrahi asistanınına bir sanal gerçeklik simülatöründe, eğitmen gözetiminde 10 kolonoskopi ve 10 gastroskopi olgusundan oluşan simülasyon eğitimi verildi. Toplam 320 modül değerlendirildi. Eğitmenler tarafından seçilen orta zorlukta bir modül ilk modül olarak çalıştırıldı ve tüm eğitim tamamlanınca tekrar edildi. Veriler medyan, birinci çeyreklik ve üçüncü çeyreklik olarak verildi. Eğitim süresince değişikliklerin saptanması için Wilcoxon rank-sign test kullanıldı. Çalışmanın niteliksel verileri için, asistanlarla yüz yüze derinlemesine görüşmeler yapıldı. Görüşme sonunda aynı gün yazıya döküldü. İki değerlendirmeci tarafından içeriğe ilişkin temalar ve alt temalar belirlendi.

Bulgular: Hem kolonoskopi hem de gastroskopi modüllerinde anlamlı iyileşme gözlendi. Kolonoskopi modülünde çekuma ulaşma süresinin 20 dk'den ortalamada 3 dk'ye gastroskopide duedonuma ulaşma süresinin 4 dk'den 3,6 dk'ye indiği görüldü. Gastroskopi ve kolonoskopide değerlendirilen mukozal alan yüzdesi anlamlı olarak arttı. Kaliteli görüntü elde etmek için harcanan süre

Address for Correspondence: Sezen Milli Avtan, University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Department of Medical Education, İstanbul, Türkiye
Phone: +90 532 725 73 70 E-mail: sezen.avtan@sbu.edu.tr **ORCID ID:** orcid.org/0000-0003-2528-8760

Received: 24.03.2022 **Accepted:** 11.10.2022

ÖZ

gastroskopide değişmezken, kolonoskopi uygulamaları sırasında anlamlı oranda arttığı izlendi. Ekranın etkin kullanım yüzdesi hem gastroskopide, hemde kolonoskopide arttı. Niteliksel veriler değerlendirildiğinde yapılan yüz yüze görüşmeler sonucunda katılımcıların tümü simülasyon eğitimini faydalı bulduklarını ifade ettiler.

Sonuç: Girişimsel beceri gerektiren eğitimlerde, hasta deneyiminden önce simülatör kullanımının faydalı olduğunu, video-endoskopi simülasyonunun asistanların gastroskopi ve kolonoskopi uygulamalarında el becerilerinin gelişmesine katkı sağladığını destekler niteliktedir.

Anahtar Kelimeler: Medikal simülasyon, video-endoskopi, tıp eğitimi

Introduction

Healthcare providers must be able to competently perform a wide range of clinical skills. These skills include taking a patient's history, performing a physical examination, and performing procedures. While some procedural skills are unique to certain fields, proficiency in the execution of skills is necessary to provide safe patient care (1). The effectiveness of skills training is controversial, and data show that novice healthcare professionals are overconfident in their ability to teach practical skills (2,3). This may cause undesirable consequences during patient intervention. The use of simulators and task trainers provides the opportunity to safely train and practice procedural skills before applying them to the patient. The simulation was derived from the Latin word simulare. Simulating means making something look like the real thing. Nowadays, simulation is widely used, especially in the medical field. Patient simulations, which provide significant benefits especially in the field of medical teamwork, and surgical simulations that are effective in developing technical skills,are some of the important medical simulations. Thanks to simulation, a technical skill develops much better and facilitates the transfer to the real clinical environment (2,4,5). High-quality simulations supported by visual elements have been identified in systematic review as the most successful technique in arranging adult learner needs and procedural skills training (6). As it is known, the most valuable motivators for adult learning are the practical use of the information to be learned and the security principles provided for the application environment. Six principles have been developed for the effective learning and application of procedural knowledge.

1. Learn: Knowledge acquisition
2. See: Observation of the procedural skill
3. Practice: To make a practice using simulation
4. Prove: To assesss the competency
5. Do: The technique is carried out on a patient under direct supervision until the student is trusted to carry it out on his or her own.
6. Maintain: To make continuing clinical practical skills, with simulation-based training as a supplement (4).

As stated in this example, simulation is a training model that is applied not only to teach a skill but also to reinforce it. Before becoming competent at a skill, learners go through a succession of phases. In the acquisition of skills, there are four levels: 1) Unconsciously incompetent, 2) Consciously incompetent, 3) Consciously competent, 4) Unconsciously competent (1). The 4 phases of skill acquisition are also shown in (Figure 1) (7).

Just like using a car, we need to reach an unconsciously competent level in applications that require medical intervention. We think that one of the most important educational elements that facilitates access to this level is medical simulation. We will try to demonstrate this in our own working practice. In line with the results we have obtained, we will discuss the inclusion of simulation in the curriculum.

Material and Methods

In this study, a "virtual reality (VR)" simulator with a special video-endoscopy software, the same fiber system as the original endoscope models, was used as the simulator (Figure 2). The simulator was a simulator given as a donation to the simulation center. Three hundred-twenty gastroscopy and colonoscopy modules were evaluated. Sixteen surgical

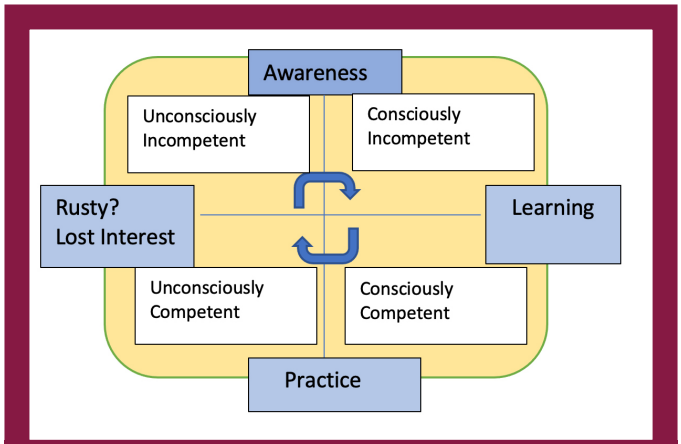

Figure 1. Development of competency (Peyton, 1998)

residents, who had not previously performed video-endoscopy on the patient, carried out 20 video-endoscopy cases, 10 of which were colonoscopy and 10 gastroscopies. Modules were structured in increasing difficulty. A medium difficulty module selected by the instructors was run as the first module and repeated when all modules were completed. Third module for gastroscopy and 5th module (Figure 3, 4) for colonoscopy were chosen as the reference. It was started with this module first and after 9 consecutive modules were studied, the first module was run again. Improvement trends were also examined in other modules other than the reference module.

For the qualitative data of the study, in-depth interviews were conducted with the residents who completed the modules. The answers given to the semi-structured questions prepared by the trainers with the support of the literature were recorded. The interviews lasted about 35-40 minutes. Before the interviews, verbal and written consents were obtained from the sixteen participants. It was informed that the interviews will be recorded. At the end of the in-depth interview, recordings were transcribed in the same day. The themes and sub-themes related to the content were determined by two medical evaluators. The expressions indicating the theme and sub-themes were written in the words of the participant. Ethical Permissions were obtained from Marmara University Health Sciences Institute (22.03.2021-45).

Figure 2. Virtual reality video-endoscopy simulator, lower endoscopy set up

Statistical Analysis

The results of the 160 gastroscopy and 160 colonoscopy applications consisting 10 different modules with gradual difficulty, which were demonstrated by each of the 16 surgical assistants were evaluated. Continuous variables were summarized according to their distribution with mean and standard deviation or median and interquartile range (IQR). R was used as a statistical program, $p<0.05$ was considered significant. Wilcoxon rank-sign test was used to detect changes during the training.

Results

Ten tasks with different difficulty levels were run for gastroscopy training. Case 3, which has a medium difficulty level, was given as the first task, and followed by Cases 1,2,4,5,6,7,8,9,10 and Case 3 was given again as a last task. Success and improvement in technical skills between the repeated Case 3 tasks were evaluated in terms of percent

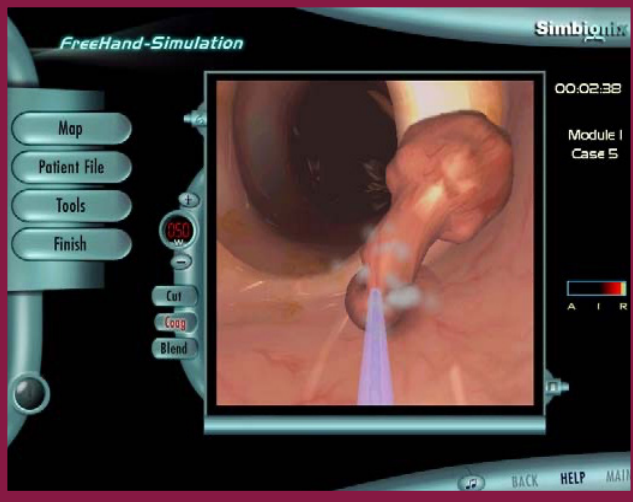

Figure 3. Polypectomy exercise module

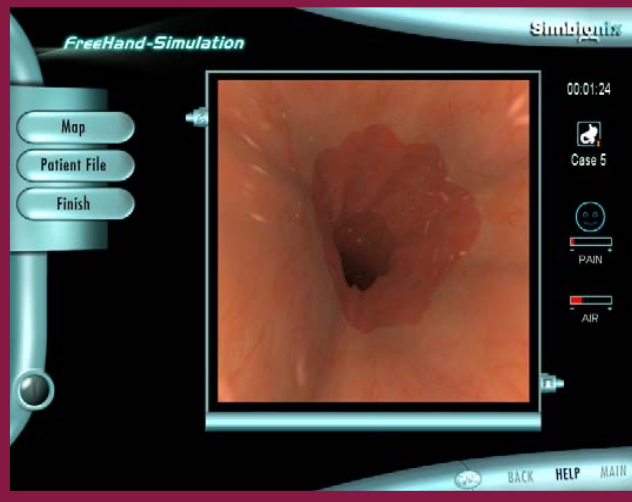

Figure 4. The view of gastroesophageal-junction

of mucosal surface examined, percent of time spent with clear view, time to reach the duodenum, the efficiency of screening.

Wilcoxon signed rank-test results showed that for gastroscopy, the percentage of mucosal area evaluated increased significantly in the repeated case [(Md=89) (IQR=8) and Md=94 (IQR=4.5) respectively, p=0.002]. Percent of time spent with clear view had not changed [Md=97 (IQR=2.2) and (Md=96.5) (IQR=3.7) respectively p=0.365]. The time to reach the duodenum had shortened [(Md=241.5 (IQR=213.5) and Md=221.5 (IQR=60.7) respectively p=0.024]. The efectiveness of screen use also had increased [Md=23 (IQR=35) and (Md=58.5) (IQR=16.7) recpectively p=0.008]. The trainer's observational notes also indicate that technical skills improve in repetitive tasks (Table 1).

For colonoscopy, the tasks were started with Case 5, which was determined to be of medium difficulty by the trainer, and Case 5,1,2,3,4,6,7,8,9,10, and Case 5 (R) were repeated, respectively. The trend towards improvement in achievement and technical skills between Case 5 and Case 5 (R) was evaluated. Success and improvement in technical skills between the repeated Case 5 tasks were evaluated in terms of percent of mucosal surface examined, percent of time spent with clear view, time to reach cecum, efficiency of screening.

For colonoscopy, the percentage of mucosal area evaluated increased significantly in the repeat case (Case 5 R) [Md=83.5 (IQR=35.8) and (Md=88.5) (IQR=14.5) respectively p=0.031]. The time to reach the cecum had significantly shortened [Md=1425 (IQR=1406.5) and (Md=180) (IQR=205) respectively p=0.001]. The percentage of time spent producing clean images [Md=76.5 (IQR=13.5) and (Md= 87.5) (IQR=10.5) respectively p=0.004] and the effectiveness of screen use have also increased [(Md=20) (IQR=33) and (Md=84) (IQR=16.7) respectively p=0.001]. The trainer's observational notes also indicate that technical skills improve in repetitive tasks (Table 2).

The themes and sub-themes obtained from the qualitative data are shown in (Table 3).

Table 1. Gastroscopy case 3 results

	First time	Second time	p-value
Percent of mucosal surface examined (%) Median (Q1-Q3) Min-max	89.0 (83.0; 91.0) (67.0; 95.0)	94.0 (91.5; 96.0) (89.0; 98.0)	0.002
Percent of time spent with clear view (%) Median (Q1-Q3) Min-max	97.0 (96.0; 98.2) (85.0; 99.0)	96.5 (93.5; 97.2) (87.0; 98.0)	0.365
Time to reach duodenum (sec) Median (Q1-Q3) Min-max	241.5 (163.0; 376.5) (11.0; 602.0)	221.5 (183.5; 244.2) (97.0; 397.0)	0.024
Effciency of screening Median (Q1-Q3) (sec) Min-max	23.0 (20.0; 55.0) (16.0; 78.0)	58.5 (50.5; 67.2) (32.0; 78.0)	0.008

Q1: First quantile, Q3: Third quantile, p-value: Wilcoxon signed-rank test p-value, sec: Second, %: Percentage

Table 2. Colonoscopy case 5 results

	First time	Second time	p-value
Percent of mucosal surface examined (%) Median (Q1-Q3) Min-max	83.5 (53.2; 89.0) (13.0; 94.0)	88.5 (78.7; 93.2) (73.0; 97.0)	0.031
Percent of time spent with clear view (%) Median (Q1-Q3) Min-max	76.5 (71.2; 84.7) (64.0; 89.0)	87.5 (84.2; 94.7) (64.0; 96.0)	0.004
Time to reach cecum (sec) Median (Q1-Q3) Min-max	1425.0 (568.5; 1975.2) (249.0; 3016.0)	180.0 (125.0; 330.0) (122.0; 456.0)	0.001
Effciency of screening (%) Median (Q1-Q3) Min-max	20.0 (19.2.0; 52.2) (18.0; 87.0)	84.0 (50.5; 67.2) (63.0; 97.0)	0.001

Q1: First quantile, Q3: Third quantile, p-value: Wilcoxon signed-rank test p-value, sec: Second, %: Percentage

Table 3. Qualitative data which were obtained from in-depth interview	
Themes	1- Factors that facilitate simulation and increase success 2- Negative aspects of simulation
Sub-themes	1a- The presence of an instructor giving feedback 1b- No repeat limit, providing a self-learning environment 1c- High fidelity, virtual reality simulator 1d- Increasing the feeling of trust in the practitioner physician 2a- The difficulty of going to another place outside the hospital environment 2b- Technical problems may reduce the training time and quality

The statements of the assistants who participated in the in-depth interviews are as follows:

Participant 1, P(1): "The most important feature of simulation for me is to be able to repeat as many times as I want in a stress-free environment"."In addition, having someone to consult with me when I need it increases the feeling of trust".

P(3): "I was very comfortable working on the patient as the fiber optic part of the simulator is the same as the real video endoscopy device".

P(7): "I think the simulator application before the patient experience is extremely necessary, but studying in a separate location was a waste of time for me. I wish this simulator was in our hospital".

P(9): "The simulator broke down very often while I was working. I had to rebuild the modules. I wasted a lot of time".

Qualitative data also show that; By making their first experience on the simulator, the residents experienced increased confidence in patient intervention. The high reality feature of the simulator was stated by most of the participants as a factor facilitating adaptation in patient practice.

While a simulation center that is isolated and outside of the working environment is considered a waste of time, very few participants stated that receiving training in a separate place is a break from their busy work schedule. Generally, because of the qualitative study, the idea that simulation increases successful patient outcomes, creates familiarity in terms of devices and interventions, reinforces the feeling of trust in physicians and patients. In addition, the answers given to the evaluation questions asked in the interviews with two trainers support our findings.

Discussion

As it is known, video-endoscopy is a very important and difficult training due to the difficulty in selecting the appropriate patient for resident training and the inadequacy of the cases. Unfortunately, it is not easy to achieve adequate technical skills for video-endoscopy in the early stages of surgical residency (8).

Competence is defined as "the minimum level of ability, knowledge, and/or expertise that is required to safely and efficiently perform a task or a procedure gained via training and experience"(8). In recent years, competence and skill development in endoscopy has been considered as an important problem (9,10,11). Different models and task trainers have been used in endoscopy training for many years. The aim of simulator-based training is to shorten the learning time in endoscopy for beginners and to eliminate the possible harm that can be given to the patient. For physicians who do not perform endoscopic interventions very often, working with a simulator before patient application, provides serious benefits (12). The performance of novice endoscopists using this simulator, improved significantly between pre- and post-training, according to this study. It is shown that surgical residents improve psychomotor and endoscopic skills with simulation-based education. Other researchs have investigated the impact of virtual endoscopic training. A study from Texas showed significant improvements in cecal intubation time, total time, and percentage of surface area scanned from pre- to post-training. In this study, the same VR simulator was used, but unlike ours, this study was conducted with senior surgical residents (13). In a study, took placed Nederlands, surgical assistants improved their performance in both the VR colonoscopy and the endobubble task. Unlike our study, there was no instructor guidance during the training (14). In another randomized controlled study, two groups were compared. One group consisted of novice residents who had less than 10 endoscopies, and the other group was senior residents. Then, these two groups are randomly seperated and simulation training is given. The total procedure time and the time to reach cecum were significantly reduced in the simulation-trained group (15). In our study, unlike this study, we evaluated the comparison of the results of the same people before and after the training as more meaningful. In a randomized study conducted in Denmark, an intermediate task was chosen, as in our study, and when all modules were completed, the same task was run again, and improvements were evaluated. Improvements in skills such as total time and clean surface area monitored by endoscopists were

determined (16). In our study, based on the literature, we evaluated parameters such as the cleanly observed surface area, the percentage of the scanned mucosal area, the time to reach the cecum, and the time to reach the duedonum. We determined the shortening of the time to reach the cecum and duodenum and the percentage of mucosal area observed and the percentage of the area observed as clean as skill development criteria. Although simulators are seen as expensive devices, general maintenance costs are not very high after the initial purchase costs. The first and most comprehensive study examining the cost-effectiveness values of VR simulators was conducted by Barsuk et al. (17). On central line-associated bloodstream infections (CLABSI). Ninety-two assistants received a simulation-based training with an instructor. Surprisingly, CLABSI rates were reduced by more than sixfold after simulation training (0.50 infections per 1000 catheter days) compared to the same uninterrupted unit (3.20 per 1000 catheter days). The annual cost of the simulation-based intervention was $112,000, reducing the net savings from lower CLABSI rates to about $700,000 (18).

Although there are studies evaluating simulation-based surgical assistant training in the literature, we have not encountered both a qualitative and quantitative evaluation (19,20,21). In this sense, we find it meaningful to support our study with qualitative data. From the results of the in-depth interviews, it is understood that the surgical residents benefited from the simulation training. Accurate feedback and unlimited repeatability provided by simulation were other important issues emphasized by the residents.

Conclusion

We found that simulation shortens the application time and increases the application skills of endoscopists in colonoscopy and gastroscopy applications that require interventional skills. In our study, we got the idea that simulation video-endoscopy training can fill a very important gap in surgical assistants training and it would be beneficial to add it to the curriculum. Quantitative data support that this training provides positive contributions in dexterity, time use and treatment management. Qualitative data also show that with this training, the trust of the residents increased, and they also gained more awareness about the sensitivity and seriousness of the intervention they performed. We believe that the malpractice problems, experienced especially in recent years, will decrease with the spread of simulation application centers.

Ethics

Ethics Committee Approval: Ethical Permissions were obtained from Marmara University Health Sciences Institute (22.03.2021-45).

Informed Consent: Written consents were obtained from the sixteen participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.M.A., W.A., Concept: S.M.A., W.A., Design: S.M.A., W.A., Data Collection or Processing: S.M.A., E.A., W.A., Analysis or Interpretation: S.M.A., E.A., W.A., Literature Search: S.M.A., W.A., Writing: S.M.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Burgess A, Van Diggele C, Roberts C, Mellis C. Tips for teaching procedural skills. BMC Med Educ. 2020;20(Suppl 2):458. [Crossref]
2. Kantak SS, Winstein CJ. Learning-performance distinction and memory processes for motor skills: a focused review and perspective. Behav Brain Res. 2012;228:219-231. [Crossref]
3. Wall D, McAleer S. Teaching the consultant teachers: identifying the core content. Med Educ. 2000;34:131-138. [Crossref]
4. Sawyer T, White M, Zaveri P, Chang T, Ades A, French H, et al. Learn, see, practice, prove, do, maintain: an evidence-based pedagogical framework for procedural skill training in medicine. Acad Med. 2015;90:1025-1033. [Crossref]
5. DeBourgh GA. Psychomotor skills acquisition of novice learners. A case for contextual learning. Nurse Educ. 2011;36:144-149. [Crossref]
6. Huang GC, McSparron JI, Balk EM, Richards JB, Smith CC, Whelan JS, et al. Procedural instruction in invasive bedside procedures: a systematic review and meta- analysis of effective teaching approaches. BMJ Qual Saf. 2016;25:281-294. [Crossref]
7. Peyton JWR. The learning cycle. In: Peyton JWR, editor. Teaching and learning in medical practice. Rickmansworth: Manticore Europe Ltd; 1999. p. 13-19. Crossref]
8. ASGE Standards of Practice Committee, Faulx AL, Lightdale JR, Acosta RD, Agrawal D, Bruining DH, et al. Guidelines for privileging, credentialing, and proctoring to perform GI endoscopy. Gastrointest Endosc. 2017;85:273-281. [Crossref]
9. Bini EJ, Firoozi B, Choung RJ, Ali EM, Osman M, Weinshel EH. Systematic evaluation of complications related to endoscopy in a training setting: a prospective 30-day outcomes study. Gastrointest Endosc. 2003;57:8-16. [Crossref]
10. McCashland T, Brand R, Lyden E, de Garmo P. The time and financial impact of training fellows in endoscopy. CORI research project. Clinical outcomes research initiative. Am J Gastroenterol. 2000;95:3129-3132. [Crossref]
11. Desilets DJ, Banerjee S, Barth BA, Kaul V, Kethu SR, Pedrosa MC, et al. Endoscopic simulators. Gastrointest Endosc. 2011;73:861-867. [Crossref]

12. Ziv A, Ben-David S, Ziv M. Simulation based medical education: an opportunity to learn from errors. Med Teach. 2005;27:193-199. [Crossref]
13. Van Sickle KR, Buck L, Willis R, Mangram A, Truitt MS, Shabahang M, et al. A multicenter, simulation- based skills training collaborative using shared GI Mentor II sys- tems: results from the Texas Association of Surgical Skills Labo- ratories (TASSL) flexible endoscopy curriculum. Surg Endosc. 2011;25:2980-2986. [Crossref]
14. Buzink SN, Koch AD, Heemskerk J, Botden SM, Goossens RH, de Ridder H, et al. Acquiring basic endoscopy skills by training on the GI Mentor II. Surg Endosc. 2007;21:1996-2003. [Crossref]
15. Cohen J, Cohen SA, Vora KC, Xue X, Burdick JS, Bank S, et al. Multicenter, randomized, controlled trial of virtual-reality simulator training in acquisition of competency in colonoscopy. Gastrointest Endosc. 2006;64:361-368. [Crossref]
16. Eversbusch A, Grantcharov TP. Learning curves and impact of psycho-motor training on performance in simulated colonoscopy: a randomized trial using a virtual reality endoscopy trainer. Surg Endosc. 2004;18:1514-1518. [Crossref]
17. Barsuk JH, Cohen ER, Feinglass J, McGaghie WC, Wayne DB. Use of simulation-based education to reduce catheter-related bloodstream infections. Arch Intern Med. 2009;169:1420-1423. [Crossref]
18. Cohen ER, Feinglass J, Barsuk JH, Barnard C, O'Donnell A, McGaghie WC, et al. Cost savings from reduced catheter- related bloodstream infection after simulation-based education for residents in a medical inten- sive care unit. Simul Healthc. 2010;5:98-102. [Crossref]
19. Koch AD, Buzink SN, Heemskerk J, Botden MBIS, Veenendaal R, Jakimowicz JJ, et al. Expert and construct validity of the Simbionix GI Mentor II endoscopy simulator for colonoscopy. Surg Endosc. 2008;22:158-162. [Crossref]
20. Piskorz MM, Wonaga A, Bortot L, Linares ME, Araya V, Olmos JI, et al. Impact of a Virtual Endoscopy Training Curriculum in Novice Endoscopists: First Experience in Argentina. Dig Dis Sci. 2020;65:3072-3078. [Crossref]
21. Bar-Meir S. Simbionix simuulator. Gastrointest Endosc Clin N Am. 2006;16:471-478, vii. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.28247

Hamidiye Med J 2022;3(3):184-190

Investigation of the Relationship Between Coronary Artery Disease and Mechanical Hemolysis

Koroner Arter Hastalığı ve Mekanik Hemoliz Arasındaki İlişkinin Araştırılması

Önder Demiröz[1], Burak Öztürkeri[1], Muhammed Bahadır Omar[1], Serhat Mert Tiril[2]

[1]*University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, Clinic of Cardiology, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, Clinic of Internal Medicine, İstanbul, Türkiye*

ABSTRACT

Background: In coronary artery disease (CAD), atherosclerotic plaques cause varying degrees of coronary stenosis. It is thought that these strictures will lead to hemolysis by causing turbulent flow other than ischemia. The aim of our study is to compare the frequency of mechanical hemolysis and the relationship between CAD intensity and the degree of hemolysis in patients diagnosed with CAD by coronary angiography.

Materials and Methods: Ninety-four consecutive patients who were admitted to cardiology outpatient clinics or emergency department and had coronary angiography were included in the study. Fifty-two patients with significant coronary artery stenosis (>70%) (patient group) and 42 patients without significant stenosis (control group) were compared in terms of demographic, clinical, laboratory and hemolysis parameters, including haptoglobin and schistocyte count.

Results: The patient group was significantly older (62.08±9.33 vs. 56.74±10.36, p=0.010) and had a higher percentage of males (78.8% vs. 50.0%, p=0.005). Use of beta blockers and angiotensin converting enzyme inhibitors were also higher in patient group. When compared by haptoglobin and schistocyte presence there were no difference between groups. On further analysis lower age [odds ratio (OR): 0.930, confidence interval (CI): 95%, p=0.027] in total study group and higher triglyceride levels (OR: 1.022, CI: 95%, p=0.009) in acute coronary syndrome subgroup were associated with schistocyte presence.

Conclusion: Hemolysis parameters were found to be the same in patients with significant CAD when compared to the control group. In addition, especially higher triglyceride levels might be associated with subclinical mechanical hemolysis in acute coronary syndrome patients. Furthermore, the relation of schistocyte presence and higher triglyceride levels in ACS patients could be an indicator of a possible inflammatory state.

Keywords: Coronary artery disease, atherosclerosis, hemolysis, triglyceride, schistocyte, haptoglobin

ÖZ

Amaç: Koroner arter hastalığında (KAH), aterosklerotik plaklar değişen derecelerde koroner darlığa neden olur. Bu darlıkların iskemi dışında türbülanslı akıma neden olarak hemolize yol açacağı düşünülmektedir. Çalışmamızın amacı, koroner anjiyografi ile KAH tanısı konulan hastalarda mekanik hemoliz sıklığını ve KAH şiddeti ile hemoliz derecesi arasındaki ilişkiyi karşılaştırmaktır.

Gereç ve Yöntemler: Kardiyoloji polikliniklerine veya acil servise başvuran ve koroner anjiyografi yapılan ardışık 94 hasta çalışmaya dahil edildi. Önemli koroner arter stenozu (>%70) olan 52 hasta (hasta grubu) ve belirgin darlığı olmayan 42 hasta (kontrol grubu) demografik, klinik, laboratuvar ve haptoglobin ve şistosit sayısı dahil hemoliz parametreleri açısından karşılaştırıldı.

Bulgular: Hasta grubu anlamlı olarak daha yaşlıydı (62,08±9,33'e karşı 56,74±10,36, p=0,010) ve erkek yüzdesi daha yüksekti (%78,8'e karşı %50,0, p=0,005). Beta bloker ve anjiyotensin dönüştürücü enzim inhibitörlerinin kullanımı da hasta grubunda daha yüksekti. Haptoglobin ve şistosit varlığı açısından karşılaştırıldığında gruplar arasında fark yoktu. İleri analizde toplam çalışma grubunda daha düşük yaş [olasılık oranı (OO): 0,930, güven aralığı (GA): %95, p=0,027) ve akut koroner sendrom alt grubunda daha yüksek trigliserit düzeyleri (OO: 1,022, GA: %95, p=0,009) şistosit varlığı ile ilişkili bulundu.

Sonuç: Önemli KAH olan hastalarda hemoliz parametreleri kontrol grubu ile karşılaştırıldığında aynı bulundu. Ayrıca akut koroner sendromlu hastalarda özellikle yüksek trigliserit seviyeleri subklinik mekanik hemoliz ile ilişkili olabilir. Öte yandan akut koroner

Address for Correspondence: Önder Demiröz, University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, Clinic of Cardiology, İstanbul, Türkiye
Phone: +90 551 609 44 88 E-mail: onderdmrz1@windowslive.com **ORCID ID:** orcid.org/0000-0002-2401-6109

Received: 04.08.2022 **Accepted:** 17.10.2022

ÖZ

sendrom hastalarında yüksek trigliserit düzeyleri ile şistosit varlığı arasındaki ilişki literatürle uyumlu biçimde enflamatuvar bir durumun habercisi olabilir.

Anahtar Kelimeler: Koroner arter hastalığı, ateroskleroz, hemoliz, trigliserit, şistosit, haptoglobin

Introduction

Coronary artery disease (CAD) is an atherosclerotic disease of inflammatory character that may present with stable, unstable angina or sudden cardiac death (1). It is one of the leading causes of morbidity and mortality worldwide. In the TEKHARF 2010 adult patient follow-up study, the coronary mortality rate was measured as 7.4 per 1.000 in men and 4.1 in women in the general population (2). It is stated that 6 million people in the American society died in 2005 due to causes related to CAD (3). There are several studies on the detection of significant (>50%) stenosis in CAD by acoustic systems. These studies are based on the measurement of the waves created by the post-stenotic turbulent coronary blood flow (4,5).

There are many immunological, infectious, genetic or iatrogenic causes of intravascular hemolytic anemia. In the case of hemolytic anemia, the blood parameters taken into consideration include complete blood count, reticulocyte count, direct and indirect Coombs test, serum lactate dehydrogenase, serum haptoglobin, peripheral blood smear, and bone marrow aspiration and biopsy if necessary (6). Intra-aortic balloon pump implantation (7), cardiopulmonary bypass (8). and mechanical prosthetic heart valves were stated among the causes of mechanical hemolysis. On the other hand, no significant mechanical hemolysis was observed in studies on coronary stents except ticlopidine induced thrombotic thrombocytopenic purpura (9,10,11,12).

In our literature search, no study was found on parameters related to hemolysis in the presence of CAD. We think that CAD may cause significant hemolysis in the presence of turbulent flows in the presence of a significant stenosis. The aim of our study is to compare the angiography data and the degree of stenosis with hemolysis parameters in patients with significant stenosis and to reveal the relationship between them.

Material and Methods

The study protocol received institutional review board approval and all participants provided informed consent in the format required by the University of Health Sciences Türkiye, Ümraniye Training and Research Hospital Ethics Committee (approval number: 60, date: 11.03.2021).

After obtaining the necessary ethics committee approval, a total of 94 patients who had presented to outpatient cardiology polyclinic with proven ischemia through exercise testing/myocardial perfusion scintigraphy or emergency department with acute coronary syndrome (ACS) were included in the study. Informed consent forms were obtained from all patients. After questioning the patients in terms of age, gender, smoking, diabetes, hypertension, hyperlipidemia, and comorbidities, blood samples which were taken for complete blood count (hemogram), serum haptoglobin, peripheral blood smear for schistocyte count were sent to biochemistry and hematology laboratories. After the coronary angiography of the hospitalized patients, these images were evaluated and the degree of stenoses was recorded. The patients were divided into 2 groups according to whether or not they have significant CAD (at least 1 vessel with ≥70% stenosis). Peripheral blood films were examined from blood drawn on the day of admission for the presence of fragmented red blood cells (schistocytes). The average of five 100X (high power) fields was scored on a 0 to 4 scale as follows: 0 for <1% schistocytes, 1+ for 1-2% schistocytes, 2+ for 2-5% schistocytes, 3+ for 5-10% schistocytes, and 4+ for >10% schistocytes. Inclusion criteria for study participants were being between 18-65 years of age and have the necessary decision-making skills and exclusion criteria were that the patient had a history of hemolytic anemia, had a prosthetic heart valve or coronary stent, had an active bleeding lesion, chronic kidney failure, recent transfusion, active infection, genetic blood diseases and previously detected CAD via angiography. The patient and control groups were compared in terms of demographic and laboratory parameters, haptoglobin and schistocyte presence.

Statistical Analysis

Statistical analysis was performed using SPSS 22 for the Windows Evaluation Version statistical package. The normality distribution was evaluated using the Kolmogorov-Smirnov test. Continuous variables were presented as mean ± standard deviation and median (25-75 percentiles). Categorical variables were summarized as frequencies. Differences between the two groups according to continuous variables were determined by the independent samples t-test or Mann-Whitney U test. Categorical variables were

compared by, chi-square or Fisher's Exact test. After the finding the potential determinants of hemolysis, univariate and multivariate regression analyses were performed to find out which determinants were independently related. A p level of <0.05 was accepted as statistically significant with 95% confidence interval and 5% margin of error.

Results

The patient group (n=52) was significantly older than the control group (n=42) (62.08±9.33 vs. 56.74±10.36, p=0.010) and had more males (78.8% vs. 50.0%, p=0.005). The patient group also had more smokers (9.6% vs. 0%, p=0.039), more beta blocker and angiotensin converting enzyme inhibitor usage and less angiotensin receptor blocker. The demographic and clinical information is shown in Table 1.

In terms of laboratory parameters, the patient group had higher blood urea nitrogen (37.50 vs. 33.50, p=0.015) and creatinine levels (0.90 vs. 0.80, p=0.022). The patient group's C-reactive protein levels were also higher (7.75 vs. 3.25, p=0.001) and HDL levels were lower (38.55±8.83 vs. 43.90±36.00, p=0.018). There was no significant difference between groups in terms of haptoglobin or schistocyte count. Laboratory parameters are shown in Table 1.

Table 1. Demographic, clinical information and laboratory parameters

Variables	Significant stenosis+ (>%70) n=52	Significant stenosis- (<%70) n=42	p
Age, years	62.08±9.33	56.74±10.36	**0.010**
Female sex, n (%)	Female: 11 (21.2%)	Female: 21 (50.0%)	**0.005**
Current smoking, n (%)	5 (9.6%)	0 (0%)	**0.039**
Hypertension, n (%)	36 (69.2%)	29 (69.0%)	0.985
Diabetes mellitus, n (%)	14 (26.9%)	11 (26.2%)	0.936
Cerebrovascular disease, n (%)	2 (3.8%)	1 (2.4%)	0.688
COPD, n (%)	10 (19.2%)	9 (21.4%)	0.792
Beta blocker usage, n (%)	52 (100%)	37 (88.1%)	**0.011**
ACE inhibitor usage, n (%)	33 (63.5%)	16 (38.1%)	**0.014**
ARB usage, n (%)	4 (7.7%)	10 (23.8%)	**0.029**
Diagnosis	CCS: 19 (36.5%) USAP: 2 (3.8%) NSTEMI: 26 (50.0%) STEMI: 5 (9.6%)	CCS: 35 (83.3%) USAP: 3 (7.1%) NSTEMI: 3 (7.1%) STEMI: 0 (0%)	N/A
Hemoglobin, (g/dL)	13.17±1.82	13.05±1.68	0.746
WBC, (count/mm^3)	7.850 (6.525-9.800)	7.550 (6.650-9.325)	0.510
Platelet, (count/mm^3)	232,789±65.72	232,534±61.73	0.475
BUN, (mg/dL)	37.50 (28.25-52.00)	33.50 (25.00-37.50)	**0.015**
Creatinine, (mg/dL)	0.90 (0.70-1.00)	0.80 (0.60-0.90)	**0.022**
Na, (mEq/L)	139.00 (137.00-140.75)	139.00 (137.00-141.00)	0.291
K, (mEq/L)	4.20 (3.90-4.40)	4.30 (3.90-4.50)	0.488
Total cholesterol, (mg/dL)	178.76±38.55	179.28±36.92	0.950
LDL, (mg/dL)	108.20±33.95	104.48±82.50	0.590
HDL, (mg/dL)	38.55±8.83	43.90±36.00	**0.018**
Triglyceride, (mg/dL)	151.00 (142.00-207.00)	126.50 (92.25-200.00)	0.110
CRP, (mg/dL)	7.75 (3.30-19.25)	3.25 (0.88-6.50)	**0.001**

*P<0.05 statistically significant. Continuous variables are reported mean ± standard deviation or median (interquartile range). Categorical variables are reported n (%). Differences between groups are calculated by chi-square test for categorical variables, independent variables student t-test and Mann-Whitney U test for continuous variables.
COPD: Chronic obstructive pulmonary disease, ACE: Angiotensin converting enzyme inhibitor, ARB: Angiotensin receptor blocker, CCS: Chronic coronary syndrome, USAP: Unstable angina pectoris, NSTEMI: Non-ST elevation myocardial infarction, STEMI: ST elevation myocardial infarction, WBC: White blood cell, BUN: Blood urea nitrogen, Na: Sodium, K: Potassium, LDL: Low density lipoprotein, HDL: High-density lipoprotein, CRP: C-reactive protein, significant results are marked bold

After angiographic evaluation and PCI/CABG decision if necessary, SYNTAX scores and the level of maximum stenoses were calculated. The patient group had a median SYNTAX score of 13.75 (7.00-21.38) and median maximum stenosis of 95.00% (90.00-100.00), on the other hand the control group had a median SYNTAX score of 0.00% (0.00-0.00) and median maximum stenosis of 10.00% (0.00-30.00).

On further analysis, the total population was evaluated whether there are other potential factors affecting schistocyte presence. After statistical analyses, lower age (54.39±6.54 vs. 60.95±10.42, p=0.012) was associated with schistocyte presence and higher triglyceride levels also showed a tendency to be significantly associated with schistocyte presence (234.00 vs. 139.00, p=0.011). The factor analyses regarding total population are given in Table 2. When multiple logistic regression analysis was performed, lower age was found to be independently associated with schistocyte presence [odds ratio (OR): 0.930, confidence interval (CI): 95%, p=0.027]. Regression analysis of the complete study group is given in Table 3.

When only ACS patients were analyzed, schistocyte + group (n=7) was showing a tendency to be younger (55.14±5.05 vs. 63.03±10.63, p=0.065) and had significantly higher triglyceride levels (188.00 vs. 132.00, p=0.024). The factors associated with schistocyte presence in ACS patients were shown in Table 4. Univariate and multivariate regression analyses established triglyceride level as an independent predictor of schistocyte presence (OR: 1.022, CI: 95%, p=0.009). Regression analysis is given in Table 5.

Table 2. Factors affecting schistocyte presence-complete study group

Variables	Schistocyte+ (n=18)	Schistocyte- (n=76)	p
Age (years)	54.39±6.54	60.94±10.42	**0.013**
Female sex, n (%)	7 (38.9%)	26 (34.2%)	0.803
Current smoking, n (%)	0 (0.0%)	5 (17%)	0.263
Hypertension, n (%)	11 (61.1%)	54 (71.1%)	0.412
Diabetes mellitus, n (%)	3 (16.7%)	22 (28.9%)	0.289
Cerebrovascular disease, n (%)	1 (5.6%)	2 (2.6%)	0.526
COPD, n (%)	2 (11.1%)	17 (22.4%)	0.285
Beta blocker usage, n (%)	17 (94.4%)	72 (94.7%)	0.960
ACE inhibitor usage, n (%)	7 (38.9%)	42 (55.3%)	0.211
ARB usage, n (%)	4 (22.2%)	10 (13.2%)	0.331
Hemoglobin, (g/dL)	13.38±1.37	13.06±1.83	0.428
WBC, count/mm^3	8.100 (6.850-9.900)	7.750 (6.525-9.550)	0.634
Platelet, count/mm^3	203,000 (174,750-271,500)	235,000 (196,500-264,250)	0.337
BUN, mg/dL	36.50 (29.50-46.25)	35.00 (28.00-43.00)	0.690
Creatinine, mg/dL	0.80 (0.68-1.00)	0.80 (0.70-0.96)	0.782
Na, (mEq/L)	138.50 (136.75-140.00)	139.00 (137.00-141.00)	0.299
K, (mEq/L)	4.30 (3.98-4.43)	4.20 (3.90-4.50)	0.696
Triglyceride (mg/dL)	234.50 (133.50-281.25)	139.00 (97.00-197.00)	**0.011**
Total cholesterol (mg/dL)	193.50±35.28	175.89±38.31	0.094
LDL cholesterol (mg/dL)	113.75±29.21	105.03±33.06	0.332
HDL cholesterol (mg/dL)	41.81±14.40	40.71±9.95	0.712
CRP, (mg/dL)	5.90 (2.00-9.00)	5.00 (1.60-15.00)	0.509
Haptoglobin (mg/dL)	2.40 (1.30-142.00)	2.30 (1.35-143.00)	0.814
SYNTAX score	0.00 (0.00-12.25)	7.00 (0.00-16.00)	0.224

*P<0.05 statistically significant. Continuous variables are reported mean ± standard deviation or median (interquartile range). Differences between groups are calculated by independent variables student t-test and Mann-Whitney U test for continuous variables.
COPD: Chronic obstructive pulmonary disease, ACE: Angiotensin converting enzyme inhibitor, ARB: Angiotensin receptor blocker, WBC: White blood cell, BUN: Blood urea nitrogen, Na: Sodium, K: Potassium, LDL: Low density lipoprotein, HDL: High-density lipoprotein, CRP: C-reactive protein, SYNTAX: Synergy between percutaneous coronary intervention with taxus and cardiac surgery, significant results are marked bold

Table 3. Regression analysis of the potential indicating factors on the presence of schistocytes in total study group

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	OR (95% CI)	p
Age	0.932 (0.879-0.987)	**0.016**	0.930(0.873-0.992)	0.027
Triglyceride	1.006 (1.000-1.013)	**0.038**	1.006(0.999-1.012)	0.080

OR: Odds ratio, CI: Confidence interval, significant results are marked bold

Table 4. Factors affecting schistocyte presence in patients with acute coronary syndrome

Variables	Schistocyte+ (n=7)	Schistocyte- (n=32)	p
Age (years)	55.14±5.05	63.03±10.63	0.065
Female sex, n (%)	1 (14.3%)	5 (15.6%)	0.929
Current smoking, n (%)	0 (0.0%)	3 (9.4%)	0.399
Hypertension, n (%)	6 (85.7%)	21 (65.6%)	0.297
Diabetes mellitus, n (%)	1 (14.3%)	7 (21.9%)	0.657
Cerebrovascular disease, n (%)	1 (14.3%)	1 (3.1%)	0.225
COPD, n (%)	0 (0%)	5 (15.6%)	0.263
Beta blocker usage, n (%)	7 (100%)	32 (100%)	N/A
ACE inhibitor usage, n (%)	5 (71.4%)	18 (56.3%)	0.460
ARB usage, n (%)	1 (14.3%)	4 (12.5%)	0.898
Hemoglobin, (g/dL)	13.39±0.97	13.35±1.72	0.962
WBC, count/mm^3	9.800 (8.700-9.900)	7.850 (6.250-9.975)	0.151
Platelet, count/mm^3	175,000 (168,000-260,000)	236,500 (194,250-256,000)	0.186
BUN, mg/dL	39.00 (34.00-49.00)	36.50 (26.50-43.50)	0.761
Creatinine, mg/dL	0.90 (0.70-1.20)	0.90 (0.73-0.98)	0.654
Na, (mEq/L)	137.00 (134.00-139.00)	139.00 (137.25-140.75)	0.089
K, (mEq/L)	4.30 (4.00-4.40)	4.20 (3.83-4.40)	0.707
Triglyceride (mg/dL)	250.00 (193.00-290.00)	132.00 (64.95-181.00)	**0.001**
Total cholesterol (mg/dL)	193.43±43.42	171.00±37.99	0.177
LDL cholesterol (mg/dL)	117.71±33.89	105.97±32.30	0.394
HDL cholesterol (mg/dL)	37.43±8.34	38.84±8.43	0.691
CRP, (mg/dL)	7.40 (3.50-11.00)	11.00 (4.30-39.25)	0.458
Haptoglobin (mg/dL)	2.35 (1.25-52.98)	2.40 (1.59-172.50)	0.717
SYNTAX score	13.00 (6.25-21.38)	12.00 (7.00-27.50)	0.929

*P<0.05 statistically significant. Continuous variables are reported mean ± standard deviation or median (interquartile range). Differences between groups are calculated by independent variables student t-test and Mann-Whitney U test for continuous variables.
COPD: Chronic obstructive pulmonary disease, ACE: Angiotensin converting enzyme inhibitor, ARB: Angiotensin receptor blocker, WBC: White blood cell, BUN: Blood urea nitrogen, Na: Sodium, K: Potassium, LDL: Low density lipoprotein, HDL: High-density lipoprotein, CRP: C-reactive protein, SYNTAX: Synergy between percutaneous coronary intervention with taxus and cardiac surgery, significant results are marked bold.

Table 5. Regression analysis of the potential factors on the presence of schistocytes in acute coronary syndrome patients

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	OR (95% CI)	p
Age	0.918 (0.834-1.010)	0.078	-	-
Sodium	0.728 (0.525-1.011)	**0.050**	0.736 (0.508-1.067)	0.105
Triglyceride	1.022 (1.006-1.037)	**0.006**	1.022 (1.005-1.039)	**0.009**

OR: Odds ratio, CI: Confidence interval, significant results are marked bold

Discussion

We found that clinically significant coronary stenosis was not associated with significant mechanical erythrocyte breakdown. This finding persisted when the population was divided and compared according to the diagnosis, cigarette smoking, comorbidities and SYNTAX scores. On the other hand, we found that lower age and higher triglyceride levels were associated with schistocyte presence in the total population and ACS population, respectively.

The effect of lower age on the presence of mechanical hemolysis may be explained by a relatively higher physical activity resulting in muscular compression of erythrocytes. Demirci and Gün (13) suggested that younger individuals that have regular exercise might have a higher rate of mechanical hemolysis because of the breakdown of erythrocytes when they cross minor capillaries with a high speed, the compression resulting from muscle contraction (14) and also in foot soles while walking (15). It is also reported that despite hemolysis is frequent in young, erythropoiesis from bone marrow is more effective than older individuals, protecting these patients from hemolytic anemia. In our study, we also found out that even though hemolysis was more frequent in younger patients, hemoglobin levels were not significantly different, which is explanatory to this phenomenon.

Another finding of this study was the association of higher triglyceride with schistocyte presence in ACS patients. When a literature search is made, one of the rare studies that has a similar finding of high triglyceride in hemolysis was made by Koseoglu et al. (16). The mechanism might be associated with a higher inflammatory or oxidative status resulting in hemolysis. In an older study by Druml et al. (17), it is suggested that increased triglyceride levels which accompany massive hemolysis may be caused by circulatory shock, released cytokines or catecholamine release. On another note, it was shown that in microangiopathic hemolytic anemia, triglyceride levels were also higher (18). Since metabolic stress factors are known to be causing acute triglyceride increase, it may be accompanied with hemolysis.

Our study found that stable CAD was not a causative factor in mechanical hemolysis, unlike mechanical valve prostheses or assist devices. Furthermore, in ACS, triglyceride levels might be an indicator to hemodynamic alteration which ends up with erythrocyte destruction. To our knowledge, this is the first study in literature studying a possible relationship between CAD and its possible relationship with hemolysis.

Study Limitations

Since this was a single center study, the sample size was unfortunately relatively small. These findings therefore should be further evaluated through extensive multicenter researches with bigger sample sizes.

Conclusion

The most important finding of our study is that it highlights hypertriglyceridemia as a possible marker of increased erythrocyte destruction in ACS patients. This matter should be further verified with bigger sample sizes and prospective analyses of patient prognoses.

Ethics

Ethics Committee Approval: The study protocol received institutional review board approval and all participants provided informed consent in the format required by the University of Health Sciences Türkiye, Ümraniye Training and Research Hospital Ethics Committee (approval number: 60, date: 11.03.2021).

Informed Consent: Informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ö.D., S.M.T., Concept: Ö.D., Design: Ö.D., B.Ö., Data Collection or Processing: Ö.D., B.Ö., M.B.O., S.M.T., Analysis or Interpretation: Ö.D., B.Ö., M.B.O., S.M.T., Literature Search: Ö.D., M.B.O., Writing: Ö.D., B.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Ross R. Atherosclerosis-An inflammatory disease. N Engl J Med. 1999;340:115-126. [Crossref]
2. Onat A, Murat SN, Ciçek G, Ayhan E, Ornek E, Kaya H, et al. [Regional distribution of all-cause mortality and coronary disease incidence in Turkey: findings of Turkish Adult Risk Factor survey 2010]. Turk Kardiyol Dern Ars. 2011;39:263-268. [Crossref]
3. Malakar AK, Choudhury D, Halder B, Paul P, Uddin A, Chakraborty S. A review on coronary artery disease, its risk factors, and therapeutics. J Cell Physiol. 2019;234:16812-16823. [Crossref]
4. Lee D, Yoo S, Kim DB. A wide range gate data acquisition for diagnosing coronary artery disease. Echocardiography. 2019;36:1467-1474. [Crossref]
5. Winther S, Schmidt SE, Holm NR, Toft E, Struijk JJ, Bøtker HE, Bøttcher M. Diagnosing coronary artery disease by sound analysis from coronary stenosis induced turbulent blood flow: diagnostic performance in patients with stable angina pectoris. Int J Cardiovasc Imaging. 2016;32:235-245. [Crossref]

6. Broadway-Duren JB, Klaassen H. Anemias. Crit Care Nurs Clin North Am. 2013;25:411-426, v. [Crossref]
7. Sibbald M, Džavík V. Severe hemolysis associated with use of the Impella LP 2.5 mechanical assist device. Catheter Cardiovasc Interv. 2012;80:840-884. [Crossref]
8. Chumakova SP, Urazova OI, Novitskiĭ VV, Shipulin VM, Mal'tseva IV, Khokhlov OA, et al. [Factors of intravascular hemolysis in cardiosurgical patients after cardiopulmonary bypass procedures]. Vestn Ross Akad Med Nauk. 2012;15-19. [Crossref]
9. Mansoor S, Roman A, Weinstein R. Intravascular stents do not cause microangiopathic hemolysis or thrombotic microangiopathy. J Clin Apher. 1999;14:130-134. [Crossref]
10. Amin S, Chandrasekaran V. Coronary stent-induced schistocytes and ticlopidine-induced thrombocytopenia mimicking thrombotic thrombocytopenic purpura (TTP). Journal of Clinical Apheresis. 1997;12:38a.
11. Steinhubl SR, Tan WA, Foody JM, Topol EJ. Incidence and clinical course of thrombotic thrombocytopenic purpura due to ticlopidine following coronary stenting. EPISTENT Investigators. Evaluation of Platelet IIb/IIIa Inhibitor for Stenting. JAMA. 1999;281:806-810. [Crossref]
12. Chemnitz JM, Schulz A, Salameh A, Scheid C, Müller R, Draube A, et al. [Thrombotic thrombocytopenic purpura (Moschkowitz-syndrome) caused by ticlopidine]. Med Klin (Munich). 2000;95:96-100. [Crossref]
13. Demirci Ş, Gün C. Adipoz Doku ve Adipoz Dokudan Salınan Bazı Proteinler. MAKÜ Sag Bil Enst Derg. 2017;5:155-179. [Crossref]
14. Linderkamp O, Friederichs E, Meiselman HJ. Mechanical and geometrical properties of density-separated neonatal and adult erythrocytes. Pediatr Res. 1993;34:688-693. [Crossref]
15. Kurz ER. March hemoglobinuria. Brooklyn Hosp J. 1948;6:91-94. [Crossref]
16. Koseoglu M, Hur A, Atay A, Cuhadar S. Effects of hemolysis interferences on routine biochemistry parameters. Biochem Med (Zagreb). 2011;21:79-85. [Crossref]
17. Druml W, Grimm G, Laggner AN, Schneeweiss B, Lenz K. Hyperlipidemia in acute hemolysis. Klin Wochenschr. 1991;69:426-429. [Crossref]
18. Whitaker AN, Bunce I, Knowles BR, Richardson ER. Hyperlipidemia in experimental disseminated intravascular coagulation. J Trauma. 1974;14:116-126. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.29290

Hamidiye Med J 2022;3(3):191-196

Effect of Different Psychoactive Substances on Hematological Parameters of Dependents in Türkiye

Türkiye'de Farklı Psikoaktif Maddelerin Bağımlılarda Hematolojik Parametreler Üzerine Etkisi

Dilek Beker Şanlı[1], Rabia Bilici[2]

[1]*University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, Clinic of Biochemistry, İstanbul, Türkiye*

[2]*Erenköy Mental Health and Neurology Training and Research Hospital, Clinic of Psychiatry, İstanbul, Türkiye*

ABSTRACT

Background: In this study we aimed to define the effects of psychoactive substances on hematological parameters.

Materials and Methods: Three hundred-nineteen dependents, 82 control subjects were involved in this study. Diagnostic and statistical manual of mental disorders-IV criteria is used to determine patient group. We obtained the complete blood count and toxicology parameters in all cases and controls. Study participants with additional chronic diseases are not included.

Results: Changes in hematological parameters according to the urine toxicology results were evaluated for statistical significance. There were statistically significant differences in red blood cell (RBC), mean corpuscular value (MCV), hemoglobin (HGB), hematocrit (HCT), medians between dependent group and control group ($p<0.05$). We found a statistically significant difference in RBC, MCV, HGB and HCT levels between the opium dependent group and control group ($p<0.05$). The difference was also significant between HGB, neutrophil (NEU)%, MCH, RDW levels of cannabinoid dependent group compared to control group ($p<0.05$). We found a statistically significant difference in monocyte%, lymphocyte%, NEU%, eosinophil% levels between benzodiazepine group and control group ($p<0.05$). Whereas in ethyl glucuronide group significant difference observed only in neutrophile count ($p<0.05$).

Conclusion: Monitoring hematological parameters in psychoactive substance dependents can be used to confirm need for special treatment programs.

Keywords: Hematological parameters, psychoactive substance, urine toxicology

ÖZ

Amaç: Bu çalışmada farklı psikoaktif maddelerin hematolojik parametreler üzerine etkisini araştırmak amaçlanmıştır.

Gereç ve Yöntemler: Çalışmada, 319 bağımlı ve 82 kontrol yer almıştır. Hasta grubu ruhsal bozuklukların tanısal ve istatistiksel el kitabı-5 kriterlerine göre belirlenmiştir. Tüm hastalar ve kontroller hematolojik parametreler açısından ve idrar toksikoloji parametreleri açısından test edilmiştir. Diyabet, kanser, metabolik bozukluk ve benzeri tanısı olan olgular ve kontroller çalışma dışı bırakılmıştır.

Bulgular: İdrar toksikoloji sonuçları ve hematolojik parametreler arasındaki ilişki istatistiksel olarak değerlendirilmiştir. Kırmızı kan hücresi (RBC), ortalama korpüsküler değer (MCV), hemoglobin (HGB), hematokrit (HCT) değerleri açısından, bağımlı grubu ve kontrol grubu medyanları arasında istatistiksel olarak anlamlı fark bulunmuştur ($p<0,05$). Bu çalışmada, opiat bağımlı grup ve kontrol grubu arasında RBC, MCV, HGB, HCT düzeyleri açısından istatisksel anlamlı fark bulunmuştur ($p<0,05$). Cannabinoid bağımlılarının yer aldığı grupta, HGB, nötrofil (NEU)%, MCH, RDW düzeylerinde istatistiksel olarak kontrol grubuna göre farklılık görülmüştür ($p<0,05$). Benzodiazepin kullanıcıları ile kontrol grubu karşılaştırıldığında, monosit%, lenfosit%, NEU%, eozinofilik% düzeylerinde istatistiksel olarak anlamlı fark görülmüştür ($p<0,05$). Etil glucuronid grubunda ise, sadece NEU sayısında kontrol grubu ile karşılaştırıldığında istatistiksel anlamda fark izlenmiştir ($p<0,05$).

Sonuç: Psikoaktif madde bağımlılarında, yoğun takip ve tedavi programlarına olan ihtiyacın belirlenmesinde, hematolojik parametreler yol gösterici olabilir.

Anahtar Kelimeler: Hematolojik parametreler, psikoaktif madde, idrar toksikoloji

Address for Correspondence: Dilek Beker Şanlı, University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, Clinic of Biochemistry, İstanbul, Türkiye
Phone: +90 533 232 35 02 E-mail: sanlidilek@yahoo.com **ORCID ID:** orcid.org/0000-0002-2110-2888
Received: 26.07.2022 **Accepted:** 27.10.2022

Introduction

The effects of psychoactive substances on different neuroendocrine and physiologic functions have been reported (1). Many psychoactive drugs have a high addiction potential by usually affecting the central nervous system (CNS). In clinical practice psychoactive drugs also used for therapeutic purposes like anesthesia and analgesia to relieve pain (2).

The psychoactive substance abuse appears to be a world-wide problem. Since large majority of addicts have used different types of psychoactive drugs concurrently defining the effects of only one type of substance is difficult (3).

The immunomodulatory effects of psychoactive substances have been demonstrated in animal and human studies (4,5,6). Changes in the immunologic functions of dependents, return to normal if withdrawal period was longer than 2 years (7). Previous reports on animals showed that not all opioids have similar immunosuppressive effects. Morphine can alter natural and adaptive immune functions but buprenorphine produces strong analgesia without compromising immune function (8).

It has been demonstrated that psychoactive substance dependence like opium can be associated with increases in erythrocyte sedimentation rate, white cell count, monocytes (MON) and platelets (9). Richards et al. (10) suggested that amphetamines may be related with idiopathic leukocytosis. This may be attributable to the unique pharmacologic, neuroendocrine and immunomodulatory properties of these substances.

Previous studies have documented the effect of different psychoactive drugs on many chronic diseases like; coronary artery disease, diabetes mellitus, thyroid disorders (11,12,13). Cocaine may likely to increase the risk of acute myocardial infarction by altering blood viscosity due to cocaine induced erythrocytosis and increase in Von Willebrant factor (14,15). Cocaine use is associated with elevated hemoglobin (HGB) and hematocrit (HCT) levels. Cocaine abuse induces constriction of blood vessels in the spleen and cause altered hematologic parameters. Contribution of bone marrow to cocaine induced erythrocytosis may be negligible due to the lack of reticulocytosis (16).

Urine analysis is the usually preferred method for monitoring psychoactive substance abuse. The concentrations of psychoactive substances are higher in urine compared to blood. The frequency of testing and definition of reference intervals important to determine psychoactive substance abuse (17).

There is not enough data in previous literature about effects of psychoactive drugs on hematologic parameters (18).

In this study we aimed to evaluate and compare, the effects of different psychoactive drugs on hematological parameters.

Material and Methods

This study was conducted at the Erenköy Mental Health and Neurology Training and Research Hospital. The hospital has an AMATEM Clinic for treatment of substance use disorders. In our study we evaluated retrospectively the data of 319 patients and 82 controls admitted to hospital. Diagnostic and statistical manual of mental disorders-IV criteria is used to determine the patient group. Hospital admissions for routine control with negative urine toxicology results and without any accompanying chronic disease selected as control group.

Before starting treatment first urine and blood samples are taken from every patient who admitted to the AMATEM Clinic. Study participants with additional chronic diseases like diabetes, cancer, metabolic disorders etc. are excluded from the study. In urine samples; heroin, cannabinoids, cocaine, benzodiazepines, opiates, buprenorphine, amphetamines, ecstasy and ethyl glucuronide levels determined by HITACHI Automatic Analyzer (hitachi high-technologies corporation, roche diagnostics) using an enzyme immunoassay method (Microgenics CEDIA, Fremont, CA, USA for urine toxicology). Hematological values [red blood cell (RBC), white blood cell (WBC), HGB, HCT, Mean corpuscular values (MCV, MCH, MCHC), red blood cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV), platecrit (PCT), platelet distribution width (PDW), monocyte% (MON%), lymphocyte% (LYM%), neutrophil% (NEU%), basophile% (BAS%) eosinophil% (EOS%), monocyte (MON), lymohocyte (LYM), neutrophil (NEU), basophile (BAS) and eosinophil (EOS) count] were tested by Cell-Dyn 3700 Hematology analyzers (Abott Diagnostics).

The study was approved by the Local Ethical Committee of Erenköy Mental Health and Neurology Training and Research Hospital (no: 12/1, date: 03.02.2014).

Statistical Analysis

We used SPSS IBM 20.0 software for statistical analysis. We accepted a p-value less than 0.05 ($p<0.05$) as statistically significant.

Results

Totally 319 patients (312 men and 7 women) and 82 controls (77 men and 5 women) were included in the study. The median age of participants of study group were 26 (23-34) and 27.5 (22-37) years (Table 1).

We evaluated the changes in hematologic parameters of the substance dependent group and control groups (Table 2). There were statistically significant differences between RBC, MCV, HGB, HCT medians in the patient and control groups (p<0.05).

Table 1. Demographic data of subjects involved in this study

Subjects	Dependent group	Control group
	n (%)	n (%)
Gender		
Male	312 (97.8)	77 (93.9)
Female	7 (2.2)	5 (6.1)
Age		
<20	33 (10.3)	16 (19.5)
≥20 - <30	177 (55.5)	30 (36.6)
≥30 - <40	61 (19.1)	23 (28.0)
≥40	48 (15.0)	13 (15.9)
Age (median quartiles)	26 (23-34)	27.5 (22-37)

A total of 92 of the patients tested positive for only opium (cut-off 300 ng/mL). We compared the hematological parameters of opium dependent group and control groups (Table 3). When the data evaluated RBC, MCV, HGB and HCT levels of the opium dependent group was found significantly lower than control group (p<0.05).

We found 9 of the patients test results positive for only cannabinoids (cut-off 50 ng/mL). The difference was significant for HGB, NEU%, MCH, RDW parameters of the cannabinoid dependent group compared to control group (p>0.05).

The consumption of only benzodiazepine was detected in 13 of all addicts (cut-off >300 ng/mL). Compared to control group, benzodiazepine dependents showed significant differences in MON%, LYM%, NEU%, EOS% levels (p<0.05).

Positive test results for only ethyl glucuronide observed in 7 patients (cut-off >500 ng/mL). We found significant difference in only NEU count of ethyl glucuronide group compared to control group (p<0.05).

Table 2. Comparison of hematological parameters in dependent and control groups

Tests	Dependent group median (quartiles)*	Control group median (quartiles)*	p
PLT (K/µ)	217 (256-298)	243 (216-278)	0.312
RBC (M/µL)	4.8 (4.51-5.10)	4.95 (4.70-5.20)	0.010
WBC (K/µ)	8.10 (6.8-9.4)	8.10 (6.41-9.17)	0.609
MCV (fL)	90.4 (88.3-92.8)	91.7 (89.28-94.10)	0.014
HGB (g/dL)	14.4 (13.5-15.1)	15.0 (14.20-15.53)	<0.001
HCT (%)	43.2 (40.7-45.8)	45.10 (42.90-47.65)	<0.001
MON%	6.7 (5.12-8.16)	6.56 (5.36-8.30)	0.996
LYM%	33.5 (26.6-39.9)	31.95 (27.35-37.23)	0.213
NEU%	55.2 (48.8-63.9)	58.20 (52.0-62.98)	0.164
BAS %	0.8 (0.5-1.05)	0.80 (0.45-1.20)	0.589
EOS%	2.3 (1.4-3.45)	1.88 (1.15-2.81)	0.088
MON (K/µ)	0.5 (0.4-0.7)	0.50 (0.34-0.70)	0.745
LYM (K/µ)	2.61 (2.0-3.2)	2.60 (2.06-2.90)	0.249
NEU (K/µ)	4.5 (3.3-5.62)	4.56 (3.49-5.32)	0.647
BAS (K/µ)	0.03 (0.10-0.10)	0.074 (0.038-0.100)	0.615
EOS (K/µ)	0.2 (0.1-0.3)	0.136 (0.100-0.201)	0.110
MCH (pg)	30.0 (28.9-31.0)	30.15 (29.3-31.3)	0.144
MCHC (g/dL)	33.2 (32.7-33.6)	33.0 (32.5-33.7)	0.215
RDW (%)	14.9 (14.2-15.7)	14.90 (14.47-15.50)	0.667
PDW (%)	17.3 (16.8-18.0)	17.50 (16.68-18.0)	0.659
MPV (%)	7.6 (6.9-8.4)	7.54 (6.99-8.46)	0.711
PCT (%)	0.200 (0.188-0.217)	0.20 (0.18-0.21)	0.168

* 25 and 75 percentiles,
p-value less than 0.05 (p<0.05) were accepted as statistically significant, PLT: Platelets, RBC: Red blood cell, WBC: White blood cell, MCV: Mean corpuscular values, HGB: Hemoglobin, HCT: Hematocrit, MON: Monocyte, LYM: Lymphocyte, NEU: Neutrophil, BAS: Basophile, EOS: Eosinophil, RDW: Red blood cell distribution width, PDW: Platelet distribution width, PCT: Platecrit

Table 3. Comparison of hematology parameters in all groups according to medians

	Control group (n=82) median (quartiles)	Opiate group (n=92) median (quartiles)	Cannabinoid group (n=9) median (quartiles)	Benzodiazepine group (n=13) median (quartiles)	Glucuronide group (n=8) median (quartiles)
PLT (K/µ)	243 (216-278)	259 (223-310)	228 (191-258)	249 (196-286)	268 (201-286)
RBC (M/µL)	4.95 (4.70-5.20)	4.8 (4.50-5.08)*	5.00 (4.85-5.25)	5.00 (4.65-5.30)	4.65 (4.05-5.10)
WBC (K/µ)	8.10 (6.41-9.17)	8.15 (7.07-9.48)	8.30 (6.20-8.65)	8.40 (7.30-9.15)	6.45 (6.23-7.63)
MCV (fL)	91.7 (89.28-94.10)	90.0 (86.52-91.78)**	95.10 (91.55-96.45)	93.20 (90.75-94.90)	94.40 (90.85-96.75)
HGB (g/dL)	15.0 (14.20-15.53)	14.4 (13.53-14.90)***	15.90 (14.75-16.70)*	15.00 (14.35-16.45)	14.90 (13.05-15.48)
BAS %	0.80 (0.45-1.20)	0.80 (0.51-1.00)	1.10 (0.75-1.20)	0.90 (0.75-1.10)	1.05 (0.83-1.58)
MON %	6.56 (5.36-8.30)	6.75 (5.60-7.98)	8.40 (5.95-9.90)	7.30 (6.95-9.75)*	7.90 (6.80-8.95)
LYM %	31.95 (27.35-37.23)	34.00 (27.55-38.78)	39.60 (27.10-45.90)	35.20 (33.60-39.55)*	38.95 (28.48-42.13)
NEU %	58.20 (52.0-62.98)	54.95 (50.03-63.03)	45.80 (40.20-60.40)*	51.90 (49.00-54.55)**	46.50 (44.30-61.18)
MON (K/µ)	0.50 (0.34-0.70)	0.56 (0.415-0.70)	0.60 (0.50-0.85)	0.70 (0.55-0.80)**	0.50 (0.50-0.68)
LYM (K/µ)	2.60 (2.06-2.90)	2.77 (2.01-3.30)	3.00 (2.10-3.45)	2.90 (2.65-3.45)*	2.30 (2.13-2.58)
PDW (%)	17.50 (16.68-18.0)	17.30 (16.80-17.98)	18.00 (16.95-18.55)	17.70 (16.95-18.30)	17.30 (16.28-18.0)
MPV (%)	7.54 (6.99-8.46)	7.30 (6.80-8.28)	7.50 (6.80-8.10)	7.60 (6.60-8.50)	7.55 (6.13-8.75)
PCT (%)	0.20 (0.18-0.21)	0.20 (0.20-0.20)	0.20 (0.15-0.20)	0.20 (0.12-0.20)	0.20 (0.13-0.20)
MCHC (g/dL)	33.0 (32.5-33.7)	33.40 (32.90-33.90)**	33.20 (33.05-33.95)	33.30 (32.55-33.55)	33.15 (33.0-33.88)
MCH (pg)	30.15 (29.3-31.3)	30.05 (28.65-30.98)	31.70 (30.40-32.75)*	30.70 (29.95-31.25)	31.75 (29.50-32.10)
EOS %	1.88 (1.15-2.81)	2.35 (1.50-3.38)	2.90 (1.10-4.45)	3.40 (1.88-3.95)*	2.35 (1.60-4.68)
BAS (K/µ)	0.074 (0.038-0.100)	0.100 (0.047-0.100)	0.10 (0.10-0.10)	0.10 (0.10-0.10)*	0.10 (0.10-0.10)
EOS (K/µ)	0.136 (0.100-0.201)	0.200 (0.100-0.300)	0.200 (0.050-0.350)	0.300 (0.200-0.350)*	0.15 (0.10-0.28)
NEU (K/µ)	4.56 (3.49-5.32)	4.55 (3.33-5.68)	3.60 (2.55-5.05)	4.40 (3.50-5.05)	2.90 (2.50-4.65)*
RDW (%)	14.90 (14.47-15.50)	14.70 (14.00-15.58)	14.40 (13.55-14.95)*	14.80 (13.95-15.05)	14.60 (14.05-16.45)
HCT (%)	45.10 (42.90-47.65)	43.0 (40.60-44.68)***	46.40 (44.60-50.25)	45.90 (42.85-50.05)	44.55 (39.23-45.80)

Significantly different from control group *p<0.05, **p<0.01, ***p<0.001, PLT: Platelets, RBC: Red blood cell, WBC: White blood cell, MCV: Mean corpuscular values, HGB: Hemoglobin, HCT: Hematocrit, MON: Monocyte, LYM: Lymphocyte, NEU: Neutrophil, BAS: Basophile, EOS: Eosinophil, RDW: Red blood cell distribution width, PDW: Platelet distribution width, PCT: Platecrit

Since other patients test results were positive for more than one drug they were not included in statistical analysis.

Discussion

Clinical studies have demonstrated that psychoactive substance addiction could affect immune function. Previous studies have shown that some psychoactive substances can influence the function of natural killer cells, T-cells, neutrophil, macrophages and affect the secretion of immunoregulatory cytokines (4,5,6) Stress also causes impairments in immune system functions (19). Psychoactive substance dependence is a long-lived stress condition. Both substance abuse and stress induced by these psychoactive drugs together cause the reinforcement of immunosuppressive effects (20). Decreased LYM numbers and changes in cytokine levels in psychoactive substance dependents would influence susceptibility to infection, inflammation or cancer (21). Studies have documented that both natural and endogenous cannabinoid compounds regulate resistance to bacterial, viral and protozoan infections (22). Increased rate of infections in addicts could be related with immunosuppression. Findings of previous studies showed that number of LYM decreased significantly in some opium and heroin addicts (23).

Deficits in immune function in psychoactive substance addicts might be due to direct effect of psychoactive drugs on receptors of immune cells or by a central mechanism through receptors in the CNS. The effects of some psychoactive substances on CNS could be related with hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system. HPA axis activation cause release of glucocorticoids and increase peripheral release of dopamine, epinephrine and norepinephrine from the adrenal medulla. Norepinephrine and glucocorticoids act as immunosuppressors by decreasing the number of LYMs (24,25,26).

Psychoactive substance users usually spend their money for drugs and have increased risk of poor nutrition (27). Some hematology parameters are affected by nutritional factors (28). Psychoactive substances are known to cause hematological abnormalities. Finding of previous studies showed that addiction caused changes in RBC counts, HCT and HGB values in human (29). The current study demonstrated that RBC, MCV, HCT, HGB levels significantly decreased in opium dependent group ($p<0.05$). Since psychoactive substance users usually came from lower socio-economic groups, nutritional problems may contribute to appearance of anemia in dependents. It is important to increase awareness about different physiologic complications associated with psychoactive substance use.

Opioids exert their effects through specific receptors and four different opioid receptor was previously defined (30). A new opioid receptor named as zeta with different functions have been identified. This receptor usually named as opioid growth factor receptor (OGFr) (31). Results of previous studies demonstrated that OGFr inhibits cell proliferation in normal cells and act as negative growth factor. Since OGFr receptors are also located in kidney tissue (32), there might be a negative regulatory action through erythropoetin production to decrease cellular proliferation on bone marrow and this may explain decreased HGB levels opioid dependents.

Previous studies reported that opioid addiction can cause pancytopenia; decreased number of erythrocytes, leucocytes and platelets by bone marrow suppression. Together with decreased RBC, WBC, LYMs, PLT, HCT, HGB levels, significantly increased EOS count reported in opioid addiction (29). In this study we didn't found statistically significant difference between WBC, PLT and EOS counts in opium dependent and control groups ($p>0.05$). We found significant increase in EOS and basophile count only in benzodiazepine dependent group ($p<0.05$). This may be due to individual differences in susceptibility.

Cannabinoids are the most typical component of marijuana plant. Oseni et al. (21) indicated that hematological values did not show a significant change in marijuana smokers compared to control group. In our study we found a significant increase in HGB and MCH levels in cannabinoid dependent group ($p<0.05$). On the other hand, neutrophil% significantly decreased compared to control group ($p<0.05$). Atwood and Mackie (33) demonstrated that two types of cannabinoid receptors are found; type one receptors mainly located in the brain and type two receptors are located in the immune system and hematopoietic cells. Cannabinoids can reduce cell mediated immune response and inflammation (33). Murikinati et al. (34) showed that stimulation of type two cannabinoid receptors decreased ischemic damage and caused a decreased number of the neutrophils in the ischemic brain.

Buprenorphine can also be abused by psychoactive substance users since these drugs are cheap and easily available. In a study carried out in buprenorphine treated mice it was shown that LYM and MON counts decreased together with severe leucopenia, NEU count increased and blood HCT levels decreased. Periodic monitoring of hematological parameters recommended in human buprenorphine abusers (35). In our study buprenorphine abusers were using concurrently more than one type of psychoactive substance so we couldn't determine the effects of buprenorphine on hematological parameters. On the other hand, in benzodiazepine group (13 of all addicts tested positive for only benzodiazepine) we found significant increase in MON, LYM, EOS, basophile count, MON%, LYM%, EOS% and a significant decrease in NEU% ($p<0.05$). Therefore, periodic monitoring of blood parameters in abusers of the drug may be important.

Heroin is a semi synthetic opioid commonly used. ElHamady HS Nabil et al. (29) demonstrated that RBC, WBC, LYM, PLT count, HCT, HGB value decreased and EOS number significantly increased in heroin dependent group compared to controls. In our study, we found a significant decrease in RBC, MCV, HGB, HCT values but other parameters not significantly changed in opium addicts compared to controls ($p>0.05$). In previous studies, both increase and decrease in LYM numbers were demonstrated (23). In our study, we excluded accompanying disorders which may contribute to changes in LYM numbers or other parameters.

Conclusion

In this study, we evaluate the potential effects of many psychoactive substances like heroin, cannabinoids, cocaine, benzodiazepine, opiate, buprenorphine, amphetamine, ecstasy, ethyl glucuronide on hematological parameters. In conclusion, we suggest that monitoring hematological parameters in psychoactive substance dependents can be used to confirm need for special treatment programs. Early detection of physiologic health problems related with substance abuse may decrease the burden over health care resources.

Acknowledgements

Profound thanks to all laboratory technicians and clinicians of the AMATEM Clinic of Erenköy Mental Health and Neurology Training and Research Hospital.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethical Committee of Erenköy Mental Health and Neurology Training and Research Hospital (no: 12/1, date: 03.02.2014).

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.B., Concept: D.B.Ş., Design: D.B.Ş., Data Collection or Processing: R.B., Analysis or Interpretation: D.B.Ş., Literature Search: D.B.Ş., Writing: D.B.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Katz N, Mazer NA. The impact of opioids on the endocrine system. Clin J Pain. 2009;25:170-175. [Crossref]
2. Kreek MJ, Bart G, Lilly C, Laforge KS, Nielsen DA. Pharmacogenetics and human molecular genetics of opiate and cocaine addictions and their treatments. Pharmacol Rev. 2005;57:1-26. [Crossref]
3. Musshoff F, Madea B. Driving under the influence of amphetamine-like drugs. J Forensic Sci. 2012;57:413-419. [Crossref]
4. Saurer TB, Carrigan KA, Ijames SG, Lysle DT. Suppression of natural killer cell activity by morphine is mediated by the nucleus accumbens shell. J Neuroimmunol. 2006;73:3-11. [Crossref]
5. Saurer TB, Ijames SG, Carrigan KA, Lysle DT. Neuroimmune mechanisms of opioid- mediated conditioned immunomodulation. Brain Behav Immun. 2008;22:89-97. [Crossref]
6. Baldwin GC, Roth MD, Tashkin DP. Acute and chronic effects of cocaine on the immune system and the possible link to AIDS. J Neuroimmunol. 1998;15,83:133-138. [Crossref]
7. Govitrapong P, Suttitum T, Kotchabhakdi N, Uneklabh T. Alterations of immune functions in heroin addicts and heroin withdrawal subjects. J Pharmacol Exp Ther. 1998;286:883-889. [Crossref]
8. Sacerdote P. Opioids and the immune system. Palliat Med. 2006;20(Suppl 1):9-15. [Crossref]
9. Reece AS. Relative and age-dependent stimulation of soluble and cellular immunity in opiate dependence. J Addict Med. 2012;6:10-17. [Crossref]
10. Richards JR, Farias VF, Clingan CS. Association of leukocytosis with amphetamine and cocaine use. The ScientificWorldJournal. 2014;2014:207651. [Crossref]
11. Najafi M, Sheikhvatan M, Ataie-Jafari A. Effects of opium use among coronary artery disease patients in Iran. J Subst Use Misuse. 2010;45:2579-2581. [Crossref]
12. Azod L, Rashidi M, Afkhami-Ardekani M, Kiani G, Khoshkam F. Effect of opium addiction on diabetes. Am J Drug Alcohol Abuse. 2008;34:383-388. [Crossref]
13. Gozasht MH, Mohammadzadeh E, Divsalar K, Shokoohi M. The effect of opium addiction on thyroid function tests. J Diabetes Metab Disord. 2014;13:5. [Crossref]
14. Hollander JE, Hoffman RS, Burstein JL, Shih RD, Thode HC. Cocaine-associated myocardial infarction: mortality and complications. Arch Intern Med. 1995;155:1081-1086. [Crossref]
15. Arthur J, Siegel MD, Michelle B, Sholar BA, Jack H, Mendelson MD, et al. Cocaine-induced erithrocytosis and increase in von willebrant factor. Arch Intern Med. 1999;159:1925-1929. [Crossref]
16. Weber JE, Larkin GL, Boe CT, Fras A, Kalaria AS, Maio RF, et al. Effect of cocaine use on bone marrow- mediated erythropoiesis. Acad Emerg Med. 2003;10:705-708. [Crossref]
17. Taracha E, Habrat B, Chmielewska K, Baran-Furga H. Excretion profile of opiates in dependent patients in relation to route of administration and type of drug measured in urine with immunoassay. J Anal Toxicol. 2005;29:15-21. [Crossref]
18. Mami S, Eghbali M, Cheraghi J, Mami F, Salati AP, Bayaz Dasht JJ. Effect of Opium Addiction on Some Hematological Parameters in Rabbit. World Journal of Zoology. 2011;6:246-248. [Crossref]
19. Novio S, Nunez-Iglesia MJ, Freire-Garabal M. Psychoactive drugs against effects of stress in infectious and non-infectious viral diseases. Science against microbial pathogens: Communicating current research and technological advances. Formatex, Spain, 2011. [Crossref]
20. Fox HC, D'Sa C, Kimmerling A, Siedlarz KM, Tuit KL, Stowe R, et al. Immune system inflammation in cocaine dependent individuals: implications for medications development. Hum Psychopharmacol. 2012;27:156-166. [Crossref]
21. Oseni BS, Togun VA, Taimo OF. Effect of marijuana smoking on some hematological parameters of smokers. World Journal of Medical Sciences. 2006;1:82-85. [Crossref]
22. Massi P, Vaccani A, Parolaro D. Cannabinoids, immune system and cytokine network. Curr Pharm Des. 2006;12:3135-3146. [Crossref]
23. Haghpanah T, Afarinesh M, Divsalar K. A review on hematological factors in opioid- dependent people (opium and heroin) after the withdrawal period. Addict Health. 2010;2:9-16. [Crossref]
24. Vallejo R, Leon-Casasola O, Benyamin R. Opioid therapy and immunosuppression: a review. Am J Ther. 2004;11:354-365. [Crossref]
25. Budd K. The immune system and opioimmunotoxicity. Review in Analgesia. 2004;8:1-10. [Crossref]
26. Fecho K, Maslonek KA, Dykstra LA, Lysle DT. Assesment of the involvement of central nervous system and peripheral opioid receptors in the immunomodulatory effects of acute morphine treatment in rats. J Pharmacol Exp Ther. 1996;276:626-636. [Crossref]
27. Asgary S, Sarrafzadegan N, Naderi GA, Rozbehani R. Effect of opium addiction on new and traditional cardiovascular risk factors:do duration of addiction and route of administration matter? Lipids Health Dis. 2008;7:42. [Crossref]
28. Díaz-Flores Estévez JF, Díaz-Flores Estévez F, Hernández Calzadilla C, Rodríguez Rodríguez EM, Díaz Romero C, Serra-Majem L. Application of linear discriminant analysis to the biochemical and haematological differentiation of opiate addicts from healthy subjects: a case-control study. Eur J Clin Nutr. 2004;58:449-455. [Crossref]
29. ElHamady HS Nabil, Nasher T, Eddin R, AlMadi A. Some Biochemical and hematological parameters on some cases of dependence amaong Yemenies. EJHM. 2006;23:226-230. [Crossref]
30. Corbett AD, Henderson G, Mc Knight AT, Paterson SJ. 75 years of opioid research:the exciting but vain quest for the Holy Grail. Br J Pharmacol. 2006;147(Suppl 1):S153-162. [Crossref]
31. Zagan IS, Verderame MF, Mc Laughlin PJ. The biology of the opioid growth factor receptor (OGFr). Brain Res Brain Res Rev. 2002;38:351-376. [Crossref]
32. Cheng F, Mc Laughlin PJ, Verderame MF, Zagon IS. The OGF-OGFr axis utilizes the p16 INK4a and p21WAF1/CIP1 pathways to restrict normal cell proliferation. Molecular biology of the cell. Mol Biol Cell. 2008;20:319-327. [Crossref]
33. Atwood BK, Mackie K. CB2: a cannabinoid receptor with an identity crisis. Br J Pharmacol. 2010;160:467-479. [Crossref]
34. Murikinati S, Jütter E, Keinert T, Ridder DA, Muhammad S, Waibler Z, et al. Activation of cannabinoid 2 receptors protects against cerebral ischemia by inhibiting neutrophil recruitment. FASEB J. 2010;24:788-798. [Crossref]
35. Banerjee D, Sarkar NK. Hematological changes in buprenorphine-treated mice. Folia Biol (Krakow). 1997;45:157-162. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.36035

Hamidiye Med J 2022;3(3):197-204

Anaplastic Thyroid Carcinoma, Evaluation of Clinical, Histopathological, and Immunohistochemical Features

Anaplastik Tiroid Karsinomlarının Klinik, Histopatolojik ve İmmünohistokimyasal Açıdan Değerlendirilmesi

Tuğba Taşkın Türkmenoğlu, Demet Yılmazer

University of Health Sciences Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Clinic of Pathology, Ankara, Türkiye

ABSTRACT

Background: Anaplastic thyroid carcinoma (ATC) is the most lethal but also the rarest thyroid cancer. Pathologic diagnosis can be challenging in this clinically and morphologically aggressive tumor. The main objective of this study is to evaluate the contribution of immunohistochemical (IHC) markers, especially epithelial markers, commonly used in the diagnosis of ATC. We also aimed to uncover ATC-related demographic data and determine its frequency among thyroid carcinomas.

Materials and Methods: A retrospective analysis was used to identify 13 cases of ATC in the pathology department of our institution. In addition to demographic data, all cases were evaluated for a history of multinodular goiter (MNG), the predominant pattern, the presence of concomitant differentiated/poorly differentiated thyroid carcinoma, invasion of surrounding structures, lymph node metastasis (LNM), and distant metastasis. The intensity of staining and percentage of tumor cells with TTF-1, thyroglobulin, PAX8, CAM5.2, vimentin, p53, Ki-67, CEA, and calcitonin antibodies were interpreted by IHC studies.

Results: The incidence of ATC was 0.77% (13/1.678). MNG was present in 46% of cases. LNM was observed in 62% and distant metastases in 46% of cases. Sixty-nine percent of patients died of the disease and the median survival was 5.7 months. An associated component of differentiated/poorly differentiated thyroid carcinoma was noted in 62% of the cases. The epithelioid pattern was the most common histologic subtype. On IHC analysis, the positivity rate was 54% for CAM5.2, 38% for TTF-1 and PAX8, and 31% for thyroglobulin.

Conclusion: In this study, we have shown demographic, clinical and histopathological data related to ATC. Because this tumor can have different morphologies, IHC studies are crucial for diagnosis, especially in small biopsies. We recommend the use of TTF-1, thyroglobulin, and CAM 5.2 in addition to PAX8 to confirm the diagnosis of ATC.

Keywords: Anaplastic thyroid carcinoma, immunohistochemistry, TTF-1, PAX8, thyroglobulin

ÖZ

Amaç: Anaplastik tiroid karsinomu (ATK) oldukça ölümcül ancak aynı zamanda en nadir görülen tiroid kanseridir. Klinik ve morfolojik açıdan agresif olan bu tümör patolojik açıdan tanı güçlüğü oluşturabilmektedir. Bu çalışmada amacımız ATK tanısında sık kullanılan başta epitelyal olmak üzere immünohistokimyasal (İHK) belirleyicilerin tanı üzerine olan katkısını değerlendirmektir. Ayrıca ATK ilişkili demografik verileri ortaya koymak ve tiroid kanserleri içinde görülme sıklığını belirlemeyi de hedefledik.

Gereç ve Yöntemler: Retrospektif özellikteki çalışmamızda kurumumuz patoloji bölümünde ATK tanısı konulan 13 olgu saptandı. Olgular demografik verileri yanı sıra multinodüler guatr öyküsü, tümörün baskın paterni, eşlik eden iyi/az diferansiye tiroid karsinomu varlığı, çevre yapılara invazyon, lenf nodu metastazı ve uzak metastaz açısından değerlendirildi. İHK çalışması ile TTF-1, tiroglobulin, PAX8, CAM5.2, vimentin, p53, Ki-67, CEA ve kalsitonin antikorlarının boyanma yaygınlığı ve yoğunluğu araştırıldı.

Bulgular: ATK'nın sıklığı %0,77 (13/1,678) olarak saptandı. Olguların %46'sında multinodüler guatr mevcuttu. %62'sinde lenf nodu metastazı, %46'sında uzak metastaz gözlendi. %69 olguda hastalığa bağlı ölüm geliştiği izlenmiş olup ortalama yaşam süresi 5,7 ay olarak bulundu. Eşlik eden diferansiye/az diferansiye tiroid kanseri %62 oranında saptandı. Histolojik alt tipler içinde en sık epiteloid patern izlendi. İHK çalışmalarda CAM5.2 ile %54, TTF-1 ve PAX8 ile %38, tiroglobulin ile %31 oranında pozitiflik saptandı.

Address for Correspondence: Tuğba Taşkın Türkmenoğlu, University of Health Sciences Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Clinic of Pathology, Ankara, Türkiye

Phone: +90 505 370 65 84 E-mail: tugbataskin78@hotmail.com **ORCID ID:** orcid.org/0000-0001-6314-2928

Received: 10.03.2022 **Accepted:** 31.10.2022

ÖZ

Sonuç: Çalışmamızda ATK ile ilişkili demografik, klinik ve histopatolojik veriler sunuldu. Bu tümör farklı morfolojiler gösterebileceği için İHK'sal çalışmalar özellikle küçük biyopsilerde tanıyı destelemekte oldukça faydalıdır. ATK tanısında PAX8 yanı sıra TTF-1, tiroglobulin ve CAM 5.2 kullanılmasını öneriyoruz.

Anahtar Kelimeler: Anaplastik tiroid karsinomu, immünohistokimya, TTF-1, PAX8, tiroglobulin

Introduction

Anaplastic thyroid carcinoma (ATC) is a rare but very aggressive tumor of the thyroid. It accounts for approximately 1-2% of all thyroid cancers. It is more common in older ages and demonstrates female predominance. ATC often arises in the setting of an abnormal thyroid gland (1,2). Although the etiology is unknown, the frequent coexistence of differentiated thyroid carcinoma (DTC)/poorly differentiated thyroid carcinoma (PDTC) areas in ATC suggests that high-grade/anaplastic transformation is involved in the etiology of these tumors. There are also publications showing that de novo development is also in question (2,3). Consistent with its aggressive behavior, ATC has a significantly higher mutation load than papillary thyroid carcinoma (PTC) and PDTC (2). TP53 and CTNNB1 mutations are detected at a high rate in ATC, which are 70-80% and 60-70% respectively. RAS (40-50%) and BRAF (10-15%) mutations may be also detected in ATC (4). Clinically, it presents with a rapidly growing mass in the neck. Other common symptoms are hoarseness, dysphagia, and vocal cord paralysis. In a majority of the cases, lymph node involvement and distant organ metastases are present at the time of diagnosis. The prognosis is dismal, with a mortality rate of more than 90% (1,2,5,6).

ATC is composed of undifferentiated cells that can be recognized as thyroid follicle cell origin by immunohistochemically (IHC) or ultrastructurally. According to the World Health Organization Classification of Endocrine Organ Tumors, it contains three main histological patterns: Sarcomatoid, giant cell, and epithelioid. Less frequently, paucicellular, angiomatoid, rhabdoid, lymphoepithelioma-like, and small cell variants have also been described (1,6).

IHC studies have an important role in the diagnosis of ATC and are commonly used to establish thyroid cell origin, especially in small biopsy materials. TTF-1, thyroglobulin, and PAX8 antibodies are frequently used for this purpose. The immunoreactivity of TTF-1 and thyroglobulin in ATC is controversial and generally not detected in most cases (2,7). PAX8 expression is maintained in approximately half of the ATC cases (6). Positive staining of cytokeratins, which is frequently used to support epithelial origin, supports the diagnosis of ATC, but negative staining does not exclude this diagnosis. The use of IHC is valuable in differentiating from other undifferentiated/anaplastic tumors such as metastatic carcinoma, lymphoma, malignant melanoma, and sarcoma (5,6).

In this study, we aimed to i) determine the frequency of ATC among thyroid carcinomas, ii) identify demographic data associated with this tumor, iii) evaluate the contribution of IHC markers to the diagnosis, iv) reveal possible different staining patterns of IHC markers in various histological patterns, and v) determine the presence of other thyroid tumors that may accompany.

Material and Methods

A total of 1.678 resected thyroid carcinomas were identified between January 2014 and April 2020 at the pathology clinic of our hospital. In the retrospective analysis, 13 ATC cases were found. Clinical, radiological, surgical, and pathology data were obtained using the electronic medical information system of our hospital. Resection materials were fixed in 10% buffered formaldehyde, 3.5 µ-thick sections were obtained after routine tissue processing and were stained with hematoxylin-eosin.

This retrospective study was approved by the Ethics Committee of University of Health Sciences Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital (88/11, 20.05.2020), and was carried out by the principles of the Declaration of Helsinki. In addition to demographic data such as age and gender, the patients were evaluated in terms of multinodular goiter (MNG) history, histological subtype, presence of accompanying DTC/PDTC, invasion of surrounding structures, lymph node metastasis, distant metastasis, and presence of BRAF mutation.

IHC staining was performed using a fully automated immunostaining device (Ventana Benchmark XT, Roche Diagnostics, USA). To block endogenous biotin activity, Ultraview Universal DAB Detection kit (Ventana Medical Systems, Roche Diagnostics, USA) was used. The antibodies applied in the study were TTF-1 [Ventana, 8G7G3/1, monoclonal, ready-to-use (RTU)], thyroglobulin (Cellmarque, 2H11+6E1, RTU), PAX8 (Cellmarque, MRQ-50, RTU), Cam 5.2 (Ventana, RTU), vimentin (Ventana, RTU), p53 (Ventana, Bp53-11, RTU), Ki-67 (Ventana, 30-8, RTU), CEA

(Cellmarque, monoclonal, RTU), and calcitonin (Cellmarque, SP17, RTU). Staining intensity (0: None staining, +1: Weak staining, +2: Moderate staining, +3: Strong staining) and percentage of positive cells for IHC markers were evaluated considering different components in the tumor. The immunohistochemical studies were not financially supported by any institution or organization.

BRAF mutation analysis was conducted in 4 cases from formalin-fixed paraffin-embedded tissue blocks, which were collected from our pathology medical database. Mutational status for BRAF was done by direct sequencing method. Exon 15 was checked for V600E, V600A, V600D, and V600K mutations.

Statistical Analysis

Statistical analysis was performed with the SPSS 25.0 package programme (SPSS Inc, Chicago, IL, USA). Only descriptive statistics were used to analyse the data. Mean, standard deviation, and range were used for continuous data analysis, whereas frequency and percentages were used for categorical variables.

Results

Clinico-pathological Features

The frequency of ATC in total thyroid cancers was 0.77% (13/1678). Among 13 cases, 5 were total thyroidectomy, 3 were excisional biopsy, 1 was lobectomy, and 4 were incisional biopsies. The mean age was 66.15 (range 47-78, standard deviation 11,39), and the female to male ratio was 1.17. Clinical and descriptive characteristics are shown in the table (Table 1).

Clinical examination revealed MNG in 46% (6/13) of cases. One case, who had MNG, was diagnosed with PTC 4 years ago and treated with radioactive iodine. At presentation, 62% (8/13) of cases had lymph node metastases, and 46% (6/13) had distant metastases. All patients with distant metastases had lung metastases. Follow-up data were available for all patients. Sixty-nine percent (9/13) of patients died of disease (DOD). Median overall survival was 5.7 months (range, 1 month-23 month). A history of DTC/PDTC and/or concurrent DTC/PDTC was found in 62% (8/13) of patients, including PTC (6/13), PDTC (1/13), and PTC+PDTC (1/13).

On histologic examination, more than one histologic subtype was found in 92% (12/13) of cases. Epithelioid subtype was the most common pattern, followed by sarcomatoid/spindle cell and giant cell patterns, respectively (Figure 1). In 38% (5/13) of cases, accompanying giant cell areas were detected as a minor component (<5%). Focal or diffuse necrosis, marked pleomorphism, and high mitotic rate (typical and atypical) were observed in all cases. Vascular invasion was observed in 69% (9/13) of cases, perineural invasion in 54% (7/13), and invasion into the surrounding muscle tissue in 38% (5/13) (Figure 2). Skin invasion was noted in 15% (2/13) and invasion of the submandibular gland in 7.7% (1/13) of all cases. In four cases, with the clinical request, BRAF status was evaluated by Sanger sequencing. No BRAF mutation was detected.

Table 1. Demographics and clinicopathological features of anaplastic thyroid carcinoma

Case	Age	Sex	History of MNG	Procedure type	Distant metastasis	Lymph node metastasis	Survival, months	Predominant pattern	ATC associated with DTC/PDTC
1	54	F	-	L	-	-	DOD, 3 months	Epithelioid	PTC
2	71	M	+	EB	-	+	DOD, 4 months	Sarcomatoid	PTC
3	75	F	-	IB	-	-	DOD, 11 months	Epithelioid	-
4	76	F	+	IB	Lung	-	DOD, 4 months	Sarcomatoid	-
5	66	F	+	EB	Lung, liver, surrenal	-	DOD, 1 month	Sarcomatoid	PDTC
6	76	F	-	TT	-	+	Alive, 13 months	Sarcomatoid	-
7	56	M	+	TT	-	+	DOD, 14 months	Epithelioid	PTC+PDTC
8	48	M	+	TT	-	+	Alive, 11 months	Sarcomatoid	PTC
9	63	M	-	TT	Lung	-	DOD, 3 months	Rhabdoid	PTC
10	47	M	-	IB	Lung	+	Alive, 12 months	Epithelioid	-
11	78	F	-	IB	Lung, liver	+	DOD, 1 month	Epithelioid	-
12	73	M	+	EB	-	+	DOD, 23 months	Epithelioid	PTC
13	77	F	-	TT	Lung	+	DOD, 1 month	Epithelioid	PTC

M: Male, F: Female, MNG: Multinodular goiter, L: Lobectomy, EB: Excisional biopsy, IB: Incisional biopsy, TT: Total thyroidectomy, DOD: Death of disease, ATC: Anaplastic thyroid carcinoma, DTC: Differentiated thyroid carcinoma, PTC: Papillary thyroid carcinoma, PDTC: Poorly differentiated thyroid carcinoma

Immunohistochemical Findings

Of the antibodies used to support thyroid cell origin, 38% (5/13) were positive for TTF-1 and 31% (4/13) for thyroglobulin. Staining with TTF-1 was often higher than 10% and staining intensity was weak to moderate. In all cases where immunoreactivity with thyroglobulin was detected, the percentage of staining was less than 5% and weak. PAX8 was positive in 38% (5/13) of cases. In PAX8 positive cases, all showed more than 10% and usually more than half of the tumor cells were stained. Fifty-four % (7/13) of cases were positive with CAM5.2. An epithelioid component was present in cases that positive staining with TTF-1, PAX8, and CAM5.2 was detected (Figure 3). The results of immunostaining with epithelial markers are shown in the table (Table 2).

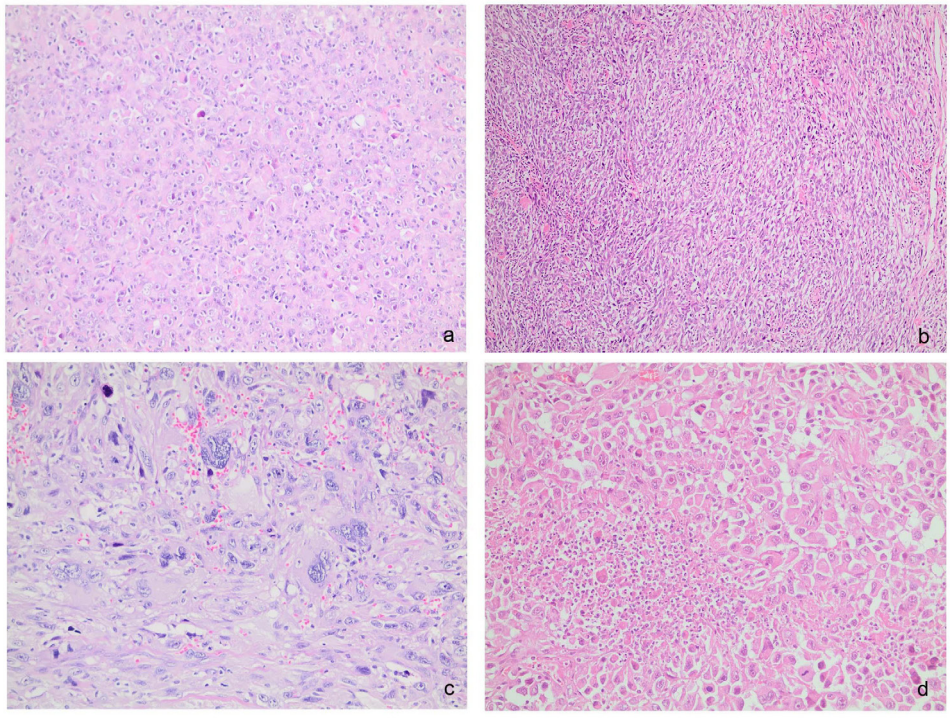

Figure 1. Histologic features of anaplastic thyroid carcinoma. Anaplastic thyroid carcinoma may show **a)** epithelioid, **b)** sarcomatoid, **c)** giant cell-rich, **d)** rhabdoid features (hematoxylin and eosin, X100, a-d)

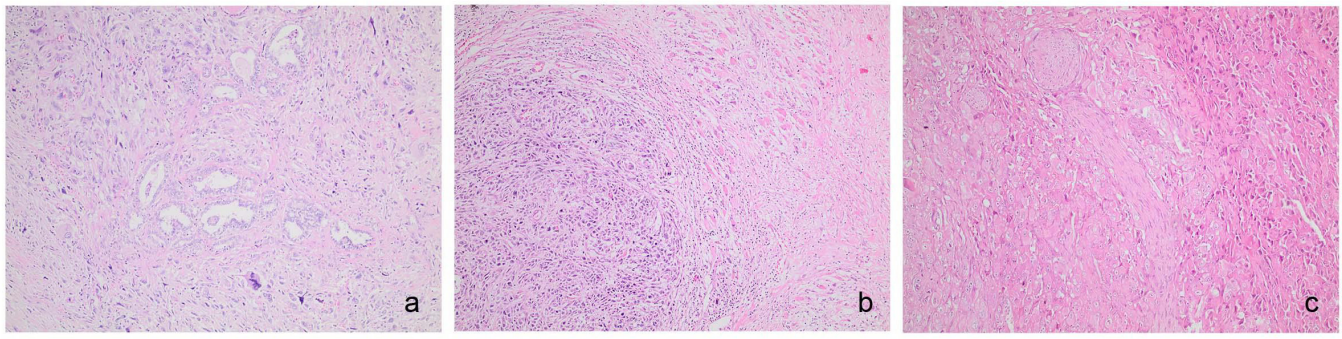

Figure 2. Anaplastic thyroid carcinoma having areas of **a)** concurrent papillary thyroid carcinoma, **b)** striated muscle invasion, **c)** extensive perineural invasion (hematoxylin and eosin, X100, a-c)

Strong immunostaining with p53, which varied between 35-100%, and diffuse strong immunostaining with vimentin were observed in all (100%) cases. The Ki-67 proliferation index was high, ranging from 20% to 90%. No immunoreaction was detected with calcitonin. In 7.7% of cases (1/13), CEA showed weak and patchy/focal immunostaining. Immunohistochemical staining features of vimentin, p53, CEA, calcitonin, and Ki-67 are provided in the table (Table 3).

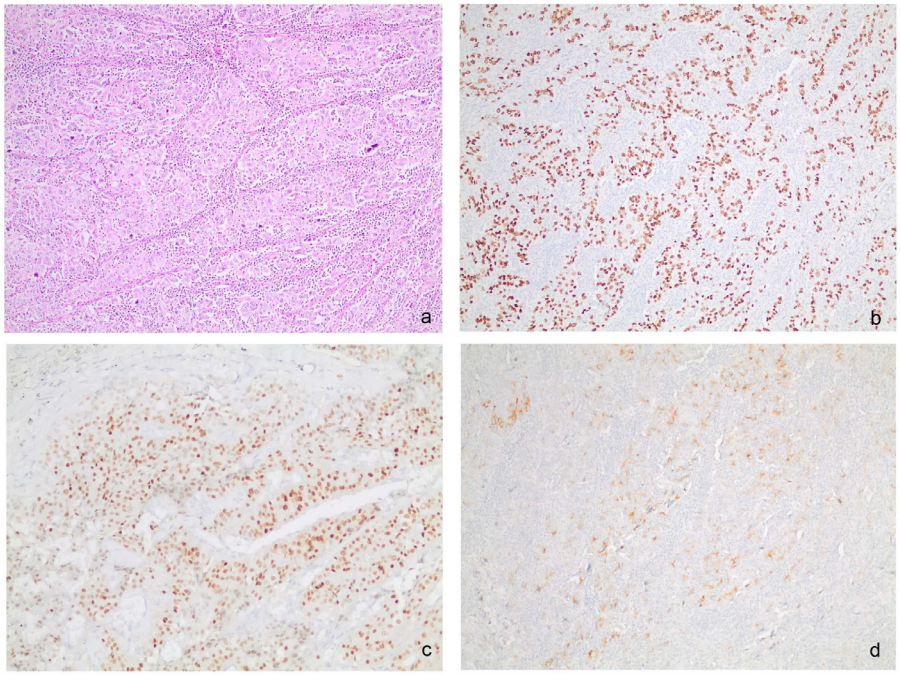

Figure 3. a) Immunprofile of epithelioid anaplastic carcinoma, **b)** PAX8 positivity, diffuse and strong, **c)** TTF-1 positivity, less pronounced and moderate, **d)** Thyroglobulin, focal and weak staining (X200 a, c, d; X100 b)

Table 2. Immunohistochemical profile of anaplastic thyroid carcinoma with TTF-1, PAX8, thyroglobulin, and CAM5.2

Case number	1	2	3	4	5	6	7	8	9	10	11	12	13
TTF-1	+2	0	0	0	0	0	+1	0	0	+3	0	+2	+3
PAX8	+2	0	+3	0	0	0	+2	0	0	0	0	+3	+3
Thyroglobulin	0	0	0	0	0	0	+1	+1	+1	0	+1	+1	0
CAM 5.2	+2	0	+2	0	0	+1	+2	0	+1	0	+2	+3	0

0: Negative staining, +1: Immunostaining in 10%≤ of tumor cells, +2: Immunostaining in 11-50% tumor cells, +3: Immunostaining in 50%> tumor cells

Table 3. Immunohistochemical profile of anaplastic thyroid carcinoma with vimentin, p53, CEA, calcitonin and Ki-67 antibodies

Case number	1	2	3	4	5	6	7	8	9	10	11	12	13
Vimentin	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3
p53	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3
CEA	0	0	0	0	0	0	+1	0	0	0	0	0	0
Calcitonin	0	0	0	0	0	0	0	0	0	0	0	0	0
Ki-67	55%	50%	20%	80%	75%	80%	35%	25%	50%	20%	85%	90%	70%

For vimentin, p53, CEA, and calcitonin staining intensity; for Ki-67 percentage of tumor cells are provided. 0: Negative staining, +1: Immunostaining in 10%≤ of tumor cells, +2: Immunostaining in 11-50% tumor cells, +3: Immunostaining in 50%> tumor cells

Discussion

ATC is a rare thyroid carcinoma that has a very aggressive clinical course and high mortality. The incidence of ATC varies from 0.5% to 5% (1,5,8). In our retrospective study, we found the incidence of ATC among thyroid carcinomas to be 0.77% (13/1.678).

The morphologic spectrum varies from case to case and may show different morphologies even within the same tumor. There are three main histologic patterns: epithelioid, sarcomatoid/spindle-shaped, and giant cell. These patterns usually coexist (1,7,9,10,11,12). Rarely, rhabdoid, paucicellular, lymphoepithelioma-like, angiomatoid, and small cell variants may also be observed. Common features of ATC include marked nuclear pleomorphism, necrosis, high proliferation rate, and invasiveness. In the present study, we observed at least two histological subtypes together in 12 of 13 (92%) cases. The predominant pattern was epithelioid, followed by sarcomatoid and giant cell patterns. One case had a purely epithelioid morphology. Focal or diffuse necrosis, marked pleomorphism, and high mitotic activity were present in all cases, also vascular invasion was a common finding.

ATC may be associated with or have a history of DTC and PDTC (3,7,12,13). This may suggest dedifferentiation of the existing differentiated/poorly differentiated tumor component. It has been postulated that it may arise de novo, and this situation is more common in younger patients (<50 years) (1). In our series, accompanying PTC was observed in 6 (46%) cases, PDTC in 1 case (7.7%), and coexistence of PTC and PDTC in 1 case (7.7%).

The differential diagnosis of ATC can be quite broad, as there are different morphological appearances even within the same tumor. To support the diagnosis of ATC, it is very important to demonstrate the differentiation of the follicular cells of the thyroid gland. In the absence of evidence of thyroid differentiation, the presence of an accompanying DTC component, localization of the tumor primarily within the thyroid gland, and exclusion of metastasis or direct invasion of the thyroid gland from elsewhere are critical to the diagnosis of ATC (14). IHC can be helpful in differentiating ATC from other aggressive/high-grade malignancies, such as sarcomas, melanomas, lymphomas, and carcinomas which may be difficult to distinguish on a morphologic basis alone, especially in small biopsies where the surrounding thyroid tissue cannot be observed.

The tumor cells of ATC have lost the biological properties or functions of follicular thyroid cells (1). The immunohistochemical antibodies TTF-1, thyroglobulin, and PAX8 are commonly used to demonstrate thyroid origin. PAX8 is a transcription factor expressed early in thyroid development and is a very specific marker for primitive thyroidal differentiation (5). It has been reported to be the most sensitive IHC marker for the diagnosis of ATC (7,9,12). PAX8 is also expressed in kidney and Müllerian epithelium (7,14,15). TTF-1 is a transcription factor and plays a very important role in thyroid organogenesis (16). In addition to the thyroid, it is also expressed in the lung and diencephalon (1). Thyroglobulin serves as a substrate for T4 and T3 synthesis and is produced and secreted only by thyroid cells.

TTF-1, thyroglobulin, and PAX8 antibodies are commonly used to detect the thyroid origin of the tumor in ATC. TTF-1 expression in ATC has been reported to be 0-41% (8,12,15,16,17). PAX8 expression is observed more frequently than TTF-1, and immunostaining with PAX8 ranges from 36-79% (7,8,11,12,14,16). The expression of thyroglobulin in ATC is controversial, and among the other IHC markers of thyroid lineage, the lowest expression is observed with thyroglobulin (1,5,7,8,11,12,18). Although thyroglobulin positivity ranges from 0-50%, it has been reported that staining is often weak (1). In our study, we found 38% positivity for PAX8 and TTF-1 and 31% for thyroglobulin, but the staining for thyroglobulin was focal (less than 5%) and weak. We observed higher positivity for TTF-1 and especially for thyroglobulin compared with the literature. This result may be attributed to the relatively frequent presence of an epithelioid component in the ATC cases in our study. In the study of Nonaka et al. (16), 3 of 5 TTF-1 positive cases of ATC were squamoid, 1 case and epithelioid, 2 cases. It is also suggested that PAX8 expression was positively correlated with the presence of an epithelial pattern, also lower PAX8 expression rates were observed in tumors with a sarcomatoid pattern (7). The presence of a DTC component was positively correlated with PAX8 expression (7). Similarly, Bishop et al. (14) described that ATC cases with a well-differentiated component were more likely associated with PAX8 expression.

In three cases, we could not detect positive immunostaining with PAX8, TTF-1, CAM5.2, and thyroglobulin. In these cases, radiological localization of the tumor in the epicenter of the thyroid, presence of PTC and PDTC in two cases, detection of higher uptake values with positron emission tomography at the thyroid mass compared to distant metastases in two cases were the facts that led us to the ATC diagnosis, despite negative immunostaining with epithelial and thyroid lineage markers. In a multicenter study of 360 ATC cases by Xu et al. (12), no immunostaining was observed in 25% of 225 cases in which immunohistochemical studies of cytokeratin were performed. This result suggests that epithelial markers may not be found in a proportion of ATC cases.

Female to male ratio in ATC varies from 1.2:1 to 3.83:1 (12,16,17,19). In our series, we found female to male ratio as 1.16:1, which is consistent with the previous studies. The mean age of patients was 66.15 in our study; this also supports the literature (12,16,17,19). We found the median survival of patients 5.7 months. Only for 3 patients the survival was over 12 months; which were 13 months alive, 14 months DOD, and 23 months DOD. In the series of Deeken-Draisey et al. (8) only 20% of affected patients survived for 1 year after initial diagnosis.

Regional lymph node metastases are common, and it is estimated that more than half of the patients have distant metastases at the time of diagnosis (1,5,19). Distant metastases to the lung, bone, and brain are most common (1,2,16). We observed regional lymph node and distant metastases in 62% of our cases, with lung being the most common site for distant metastases.

Although some studies claim that the histological subtype of ATC does not alter prognosis (11,13), others reported that epithelioid morphology and the presence of differentiated thyroid cancer are associated with a better outcome, whereas rhabdoid morphology is associated with a poor prognosis (10). Hirokowa et al. (10) suggested that the incidence of epithelial growth was higher in long term survival patients (longer than 1 year) than short-term survival patients (survival of less than 3 months), which they suggested this finding may be associated with more indolent course. They also stated that ATC cases with papillary carcinomas showing squamous cell carcinoma component may have long-term survival. Of our cases with longer survival (≥12 months), 3 had more than one histologic pattern, and only one had pure epithelioid morphology. Of the two patients with rhabdoid morphology, one died within three months and the other was alive for 12 months with lung metastases. Although our results suggest that the predominant pattern in the tumor does not influence survival, we do not have a sufficient number of cases to determine the impact of histologic subtype on survival.

Study Limitations

The major limitations of this study were the relatively few sample number and for some cases type of procedure being incisional biopsy. For particularly small biopsy samples, we could not exclude the possibility of heterogeneous immunostaining, especially when all epithelial markers were negative. Another limitation is that we could only test BRAF mutation status in four cases

Conclusion

ATC is a rare but very aggressive tumor originating from the follicular epithelial cells of the thyroid gland. In our tertiary care and treatment center, we found that the incidence of ATC among thyroid cancers 0.77%. A history of differentiated/PDTC and/or concurrent differentiated/PDTC may support the diagnosis of ATC. The diagnostic process is sometimes challenging, especially with small biopsies, and may require the use of a broad immunohistochemical panel, as morphology may vary, immune profile is variable, and a large number of malignancies are part of the differential diagnosis. Previous studies claim that PAX8 is the most useful immunohistochemical marker for detecting the origin of thyroid cell origin in ATC. Although the positivity of TTF-1 and thyroglobulin are reported to be low in the literature, we found similar positivity for PAX8 and TTF-1, and lower positivity for thyroglobulin. We observed that TTF-1 and thyroglobulin staining was more common with tumors with a predominant epithelioid component. Our results suggest that in addition to PAX8; TTF-1, thyroglobulin, and CAM 5.2 may be useful in supporting ATC diagnosis.

Ethics

Ethics Committee Approval: This retrospective study was approved by the Ethics Committee of University of Health Sciences Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital (88/11, 20.05.2020), and was carried out by the principles of the Declaration of Helsinki.

Informed Consent: Informed consent is not required because this is a retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.T.T., D.Y., Concept: T.T.T., D.Y., Design: T.T.T., D.Y., Data Collection or Processing: T.T.T., D.Y., Analysis or Interpretation: T.T.T., D.Y., Literature Search: T.T.T., D.Y., Writing: T.T.T., D.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Molinaro E, Romei C, Biagini A, Sabini E, Agate L, Mazzeo S, et al. Anaplastic thyroid carcinoma: from clinicopathology to genetics and advanced therapies. Nat Rev Endocrinol. 2017;13:644-660. [Crossref]
2. Yang J, Barletta JA. Anaplastic thyroid carcinoma. Semin Diagn Pathol. 2020;37:248-256. [Crossref]
3. Eloy C, Ferreira L, Salgado C, Soares P, Sobrinho-Simões M. Poorly Differentiated and Undifferentiated Thyroid Carcinomas. Turk Patoloji Derg. 2015;31(Suppl 1):48-59. [Crossref]
4. Düzkale Teker N. Tiroid Kanserlerinde Genetik Yaklaşım. In: Sezer A, Sümbül AT, Sedef M, Özdemir Y, editors. Baş Boyun ve Santral Sinir Sistemi Tümörleri, Ankara: Akademisyen Kitabevi; 2019;249-254. [Crossref]

5. Talbott I, Wakely PE Jr. Undifferentiated (anaplastic) thyroid carcinoma: Practical immunohistochemistry and cytologic look-alikes. Semin Diagn Pathol. 2015;32:305-310. [Crossref]
6. Lloyd RV, Osamura RY, Klöppel G, Rosai, editors. WHO Classification of Tumours of Endocrine Organs. 4th ed. Lyon: IARC; 2017: pp. 104-106. [Crossref]
7. Lai WA, Hang JF, Liu CY, Bai Y, Liu Z, Gu H, et al. PAX8 expression in anaplastic thyroid carcinoma is less than those reported in early studies: a multi-institutional study of 182 cases using the monoclonal antibody MRQ-50. Virchows Arch. 2020;476:431-437. [Crossref]
8. Deeken-Draisey A, Yang GY, Gao J, Alexiev BA. Anaplastic thyroid carcinoma: an epidemiologic, histologic, immunohistochemical, and molecular single-institution study. Hum Pathol. 2018;82:140-148. [Crossref]
9. Higgins SE, Barletta JA. Applications of Immunohistochemistry to Endocrine Pathology. Adv Anat Pathol. 2018;25:413-429. [Crossref]
10. Hirokawa M, Sugitani I, Kakudo K, Sakamoto A, Higashiyama T, Sugino K, et al. Histopathological analysis of anaplastic thyroid carcinoma cases with long-term survival: A report from the Anaplastic Thyroid Carcinoma Research Consortium of Japan. Endocr J. 2016;63:441-447. [Crossref]
11. Ragazzi M, Ciarrocchi A, Sancisi V, Gandolfi G, Bisagni A, Piana S. Update on Anaplastic Thyroid Carcinoma: Morphological, Molecular, and Genetic Features of the Most Aggressive Thyroid Cancer. Int J Endocrinol. 2014;2014:790834. [Crossref]
12. Xu B, Fuchs T, Dogan S, Landa I, Katabi N, Fagin JA, et al. Dissecting Anaplastic Thyroid Carcinoma: A Comprehensive Clinical, Histologic, Immunophenotypic, and Molecular Study of 360 Cases. Thyroid. 2020;30:1505-1517. [Crossref]
13. Chintakuntlawar AV, Foote RL, Kasperbauer JL, Bible KC. Diagnosis and Management of Anaplastic Thyroid Cancer. Endocrinol Metab Clin North Am. 2019;48:269-284. [Crossref]
14. Bishop JA, Sharma R, Westra WH. PAX8 immunostaining of anaplastic thyroid carcinoma: a reliable means of discerning thyroid origin for undifferentiated tumors of the head and neck. Hum Pathol. 2011;42:1873-1877. [Crossref]
15. Suzuki A, Hirokawa M, Takada N, Higuchi M, Yamao N, Kuma S, et al. Diagnostic significance of PAX8 in thyroid squamous cell carcinoma. Endocr J. 2015;62:991-995. [Crossref]
16. Nonaka D, Tang Y, Chiriboga L, Rivera M, Ghossein R. Diagnostic utility of thyroid transcription factors Pax8 and TTF-2 (FoxE1) in thyroid epithelial neoplasms. Mod Pathol. 2008;21:192-200. [Crossref]
17. Oishi N, Kondo T, Ebina A, Sato Y, Akaishi J, Hino R, et al. Molecular alterations of coexisting thyroid papillary carcinoma and anaplastic carcinoma: identification of TERT mutation as an independent risk factor for transformation. Mod Pathol. 2017;30:1527-1537. [Crossref]
18. Miettinen M, Franssila KO. Variable expression of keratins and nearly uniform lack of thyroid transcription factor 1 in thyroid anaplastic carcinoma. Hum Pathol. 2000;31:1139-1145. [Crossref]
19. Karaman N, Yılmaz KB, Özaslan C, Altınok M. Anaplastic Thyroid Carcinoma: Clinical Features, Prognostic Factors and Treatment Outcome. Acta Oncologica Turcica. 2009;42:1-7. [Crossref]

CASE REPORT

DOI: 10.4274/hamidiyemedj.galenos.2022.95967

Hamidiye Med J 2022;3(3):205-208

An Unusual Initial Manifestation of Juvenile Systemic Lupus Erythematosus: Chronic Autoimmune (Spontaneous) Urticaria

Jüvenil Sistemik Lupus Eritematozusun Nadir Bir Başlangıç Bulgusu: Kronik Otoimmün (Spontan) Ürtiker

Fatma Gül Demirkan, Özlem Akgün, Vafa Guliyeva, Nuray Aktay Ayaz

İstanbul University, İstanbul School of Medicine, Department of Pediatric Rheumatology, İstanbul, Türkiye

ABSTRACT

Itchy urticarial lesions frequently affect patients and causing considerable distress and impact on daily activities and quality of life. Although chronic autoimmune urticaria (CAU) has been reported as one of the initial manifestations of adult-onset systemic lupus erythematosus (SLE), it is not a common symptom that springs to mind in juvenile systemic lupus erythematosus (jSLE). Here, we report the case of a girl with jSLE suffering from CAU. A 17-year-old girl was referred to the pediatric rheumatology outpatient clinic for CAU lasting 120 days. During her examinations in dermatology and pediatric gastroenterology clinics, antinuclear antibodies were also positive (1:640 dense fine-spotted pattern) and there was a decrease in complement levels. As proteinuria was 4.5 g/day, kidney biopsy was performed and it was compatible with class-IV lupus nephritis. This was followed sequentially by the diagnosis of SLE. The skin lesions disappeared after treatment with methylprednisolone and hydroxychloroquine. She also received cyclophosphamide monthly for renal involvement. CAU is very rare and may be the first sign of lupus, especially associated with the presence of autoantibodies. This case highlights the importance of evaluation for connective tissue diseases such as pediatric lupus, when investigating the etiology of CAU. Early diagnosis and proper treatment is pivotal to prevent morbidities.

Keywords: Urticaria, juvenile systemic lupus erythematosus, hypocomplementemia

ÖZ

Kaşıntılı ürtikeryal döküntüler kişilerin yaşam kalitesini ve günlük aktivitelerini belirgin şekilde etkileyebilmektedir. Kronik otoimmün ürtiker (KOÜ), erişkin başlangıçlı sistemik lupus eritematozusun (SLE) ilk belirtilerinden biri olarak bildirilmiş olmasına rağmen, juvenil sistemik lupus eritematozusta (jSLE) akla gelen yaygın bir semptom değildir. Burada, KOÜ etiyolojisinde jSLE tanısı alan bir kız olguyu sunuyoruz. On yedi yaşında bir kız çocuğu KOÜ (120 gün süren) nedeniyle çocuk romatoloji polikliniğine yönlendirildi. Dermatoloji ve pediyatrik gastroenteroloji kliniklerindeki tetkikleri sırasında antinükleer antikorları da pozitifti (1:640 yoğun ince benekli patern). Hastanın başvurusunda kompleman seviyelerinde azalma vardı. Proteinüri 4,5 g/gün olduğu için böbrek biyopsisi yapıldı ve sınıf IV lupus nefriti ile uyumlu bulundu. Mevcut bulgularla hasta SLE tanısı aldı. Metilprednizolon ve hidroksiklorokin ile tedaviden sonra deri lezyonları kayboldu. Ayrıca böbrek tutulumu için aylık siklofosfamid tedavisi almaktadır. KOÜ oldukça nadirdir ve özellikle otoantikorların varlığı ile ilişkili lupusun ilk belirtisi olabilir. Bu olgu, KOÜ etiyolojisi araştırılırken pediyatrik lupus gibi bağ dokusu hastalıklarının değerlendirilmesinin önemini vurgulamaktadır. Morbiditeleri önlemek için erken tanı ve uygun tedavi çok önemlidir.

Anahtar Kelimeler: Ürtiker, juvenil sistemik lupus eritematozus, hipokomplementemi

Introduction

Hives (medical nomenclature as *urticaria*) is a common critical symptom leading to hospital *admissions worldwide*. The prevalence of urticarial lesions that occur at one or more times during a lifetime is estimated to be over 10% of children. Based on its duration, urticaria is classified as acute or chronic (1). Chronic urticaria is further subclassified as spontaneous (CSU) and inducible. CSU is defined as the occurrence of wheals, angioedema (AE), or both for >6 weeks (1). It is substantial to perform optimal diagnostic workup to reveal the underlying causes of CSU accurately

Address for Correspondence: Nuray Aktay Ayaz, İstanbul University, İstanbul School of Medicine, Department of Pediatric Rheumatology, İstanbul, Türkiye
Phone: +90 532 621 22 14 E-mail: nurayaktay@gmail.com **ORCID ID:** orcid.org/0000-0003-3594-7387
Received: 17.08.2022 **Accepted:** 04.10.2022

for achieving the best management of the patient. CSU may have a link with autoimmune disorders like autoimmune thyroiditis, yet the relationship with juvenile systemic lupus erythematosus (jSLE) has not been clarified (2).

jSLE is a multisystem disease affecting several organs including mucocutaneous tissues. It is characterized by the loss of immune tolerance to self-antigens and dysregulation of autoantibody production (3). Investigation of cutaneous manifestations revealed that the skin involvement including CSU is pretty much among the patients with SLE. Although the prevalence of CSU-like rash is reported as 4.5-12% in jSLE, there is still a lack of information on the spectrum and prevalence of CSU in jSLE and vice versa (2,4).

Recent literature has reported that neutrophilic urticaria or urticaria with predominantly neutrophilic infiltrates may be a predictor for SLE. In addition, based on the similarities of immunoglobulin (Ig) G and IgE mediated autoreactivity, there are reports linking inflammation and autoimmunity related to the pathogenesis of CSU and SLE (5).

Herein, we aimed to report an unusual case of onset of jSLE complicated with nephritis, emphasizing that jSLE can be a predictable diagnosis in the presence of CSU. Informed consent is obtained from the parents of the patient.

Case Report

In October 2021, a 17 year-old girl presented with recurrent attacks of widespread urticaria accompanied by moderate pruritus lasting for two months. Urticarial plaques were variable in size, without AE or vasculitis. The case was evaluated in a dermatology outpatient clinic and she was given antihistaminic (cetirizine, 10 mg/daily) therapy for about 1 month. Although a partial response was achieved, hives continued with relapses and remissions. No other allergic or autoimmune conditions were reported. There was no rheumatic or allergic conditions in her family history. Her medical history revealed that she had widespread joint pain and myalgia for 6 months and had photosensitivity aggravated by sunlight exposure.

In the physical examination, there was abrasion in her nasal mucosa and movements of the right hand was painful. Other system examinations were normal. Her urticarial rashes are demonstrated in Figure 1A and 1B. Her laboratory exams including complete blood count (white blood cell: $6.7x10^9$/L, platelet: $326x10^9$/L), renal function tests, and urinalysis were normal. Although C-reactive protein was in the normal range (4.19 mg/L), erythrocyte sedimentation rate was abnormally high (61 mm/h). Complement levels also displayed abnormality as follows: C3c was 63.6 mg/dL (80-200) and C4 was 3.4 mg/dL (10-40).

Pathologic examination of a punch-biopsy from hives revealed neutrophilic dermatoses. Immunological tests were as follows: Antinuclear antibodies 1:1250 (dense fine speckled pattern), anti-Sm and anti-RNP antibodies positive; however, negative for anti-double stranded DNA, anti-Ro, anti-La, anti-cardiolipin and anti-phospholipid antibodies. Abdominal ultrasonography and echocardiographic evaluation were normal.

Diagnosis of CSU due to jSLE was established and hydroxychloroquine treatment with a dosage of 200 mg/daily was initiated. Her uveal examination was normal and Schirmer test was negative. The systemic lupus erythematosus disease activity index (6) score was 18 (high activity) at the time of diagnosis.

As 24-hour urinalysis revealed 4.5 gr/day proteinuria, kidney biopsy was performed, revealing class IV-G disease (diffuse proliferative nephritis). According to agreed decision of the pediatric nephrology and rheumatology departments, pulse metilprednisolon (1 gr/daily) therapy for 3 consecutive days and intravenous cyclophosphamide (750 mg/m^2) was initiated as induction treatment. She is under prednisone (1 mg/kg/day), hydroxychloroquine (5 mg/kg/day), and cyclophosphamide (750 mg/m^2/monthly) treatment and ultraviolet-light protection by sunscreen.

Discussion

The cutaneous features of SLE are classified as LE-specific and LE-non-specific lesions based on histopathological findings according to the Gilliam and Sontheimer (7) classification. Malar rash and discoid rash are specific skin lesions while Raynaud's phenomenon, cutaneous vasculitis, alopecia, and urticaria are nonspecific lesions. Urticaria may present as the first complaint before the classical form of jSLE emerges (8). The two most common manifestations

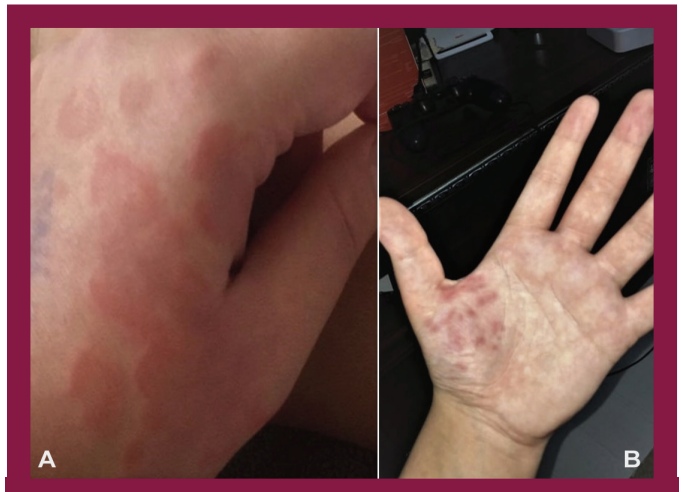

Figure 1A, B. Urticarial vasculitis. Scattered, violaceous, edematous plaques on the upper extremities

of urticarial rash in SLE are CSU and urticarial vasculitis (9). Although urticarial vasculitis resembles CSU it differs in many aspects; lasting longer (>24 h), accompanying by residual pigmentation, burning of the skin rather than itching, and systemic symptoms such as fever, arthralgia and abdominal pain (9). Hypocomplementaemia can present in 18-32% of urticarial vasculitis and indicates a more severe disease (5). CSU is frequently characterized by relapses and remissions, and in several cases clinical features of CSU and jSLE may almost overlap.

In 2018 a nationwide population-based study in Taiwan evaluated the risk of jSLE with a prior clinical diagnosis of urticaria (4). A significant association was found between clinically diagnosed urticaria and jSLE, with a stronger risk associated with more frequent episodes of urticaria (≥3 visits, odds ratio: 2.33, 95% confidence interval 1.91-2.84). In a study by Spadoni et al. (10), during 27 consecutive years, 2 (0.7%) of 271 jSLE patients had chronic, autoimmune urticaria as the first manifestation. Two of them (0.7%) had chronic and painless autoimmune urticaria as the first manifestation of jSLE. In our case, urticarial rashes with flares and remissions were precursors of jSLE. Patients with CSU or urticarial vasculitis may be both harbingers of jSLE. Clinicians should be aware that urticaria may be an early manifestation of jSLE, even in the absence of SLE-specific serologic markers. Although CSU is usually associated with anti-thyroid antibodies in the literature (11,12), thyroid function tests were normal and anti-thyroid antibodies were absent in our case. Drugs used in the treatment of lupus can also trigger CSU. However, in a large cohort study of 852 childhood onset systemic lupus erythematosus by Ferriani et al. (9), none of the patients received lupus treatment at the onset of CSU. They indicated that CSU may be linked to active jSLE, with a predominance of mucocutaneous and musculoskeletal involvement as in our case.

This case report reinforces the importance of a rigorous follow-up of children and adolescents with autoimmune urticaria due to the possibility of concomitant connective tissue diseases. jSLE is one of the pathologies that the clinician should consider, particularly in the differential diagnosis of CSU in children (Figure 2) (1).

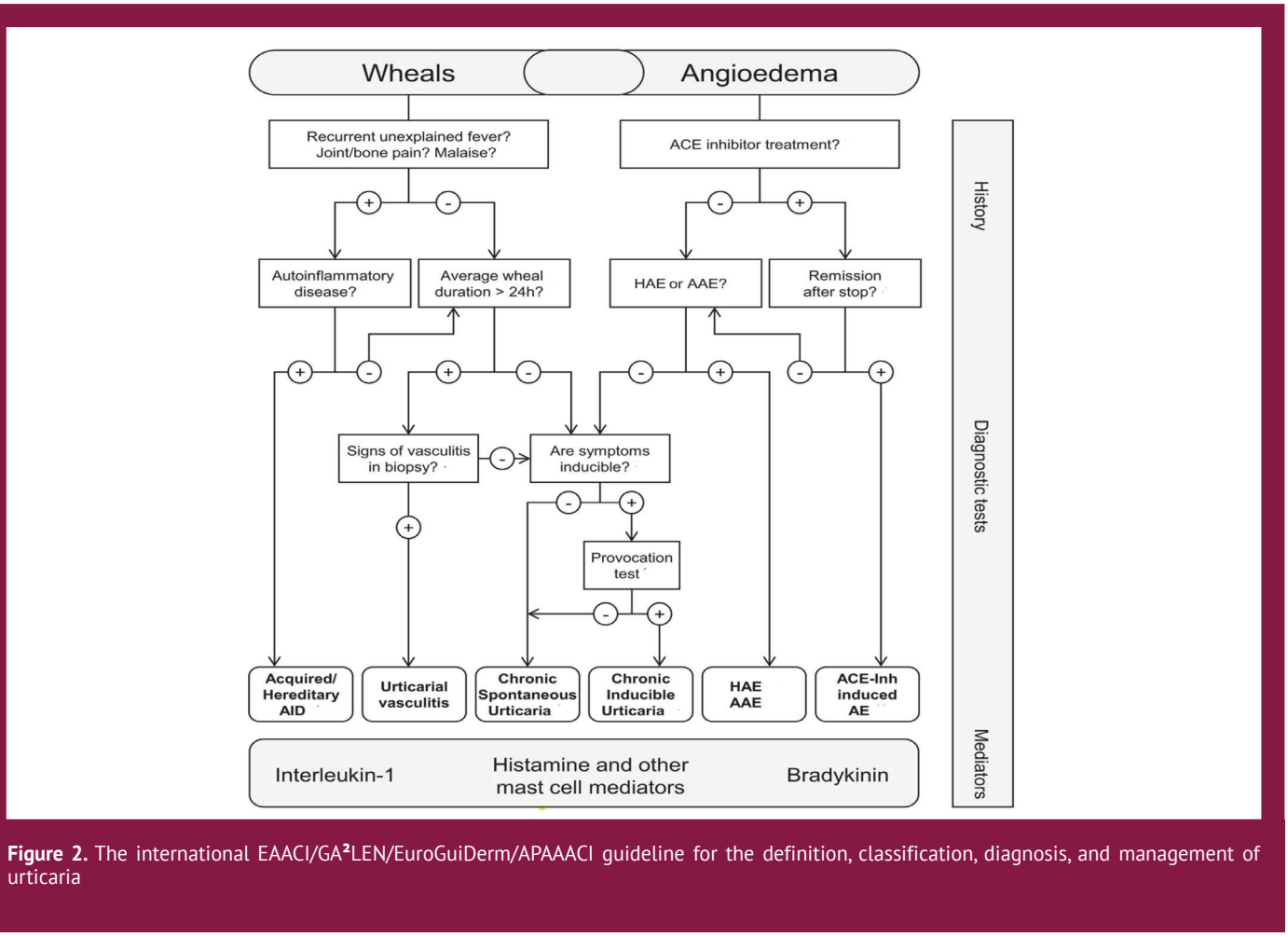

Figure 2. The international EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria

Ethics

Informed Consent: Informed consent was received from the family.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.G.D., Ö.A., V.G., N.A.A., Concept: F.G.D., Ö.A., V.G., N.A.A., Design: F.G.D., Ö.A., V.G., N.A.A., Data Collection or Processing: F.G.D., Ö.A., V.G., N.A.A., Analysis or Interpretation: F.G.D., Ö.A., V.G., N.A.A., Literature Search: F.G.D., Ö.A., V.G., N.A.A., Writing: F.G.D., Ö.A., V.G., N.A.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Zuberbier T, Abdul Latiff AH, Abuzakouk M, Aquilina S, Asero R, Baker D, et al. The international EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. Allergy. 2022;77:734-766. [Crossref]
2. Kolkhir P, Pogorelov D, Olisova O, Maurer M. Comorbidity and pathogenic links of chronic spontaneous urticaria and systemic lupus erythematosus--a systematic review. Clin Exp Allergy. 2016;46:275-287. [Crossref]
3. Smith EMD, Lythgoe H, Midgley A, Beresford MW, Hedrich CM. Juvenile-onset systemic lupus erythematosus: Update on clinical presentation, pathophysiology and treatment options. Clin Immunol. 2019;209:108274. [Crossref]
4. Lin CH, Hung PH, Hu HY, Chung CJ, Chen TH, Hung KY. Clinically diagnosed urticaria and risk of systemic lupus erythematosus in children: A nationwide population-based case-control study. Pediatr Allergy Immunol. 2018;29:732-739. [Crossref]
5. Saeb-Lima M, Charli-Joseph Y, Rodríguez-Acosta ED, Domínguez-Cherit J. Autoimmunity-related neutrophilic dermatosis: a newly described entity that is not exclusive of systemic lupus erythematosus. Am J Dermatopathol. 2013;35:655-660. [Crossref]
6. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol. 2002;29:288-291. [Crossref]
7. Gilliam JN, Sontheimer RD. Skin manifestations of SLE. Clin Rheum Dis. 1982;8:207-218. [Crossref]
8. Charras A, Smith E, Hedrich CM. Systemic Lupus Erythematosus in Children and Young People. Curr Rheumatol Rep. 2021;23:20. [Crossref]
9. Ferriani MP, Silva MF, Pereira RM, Terreri MT, Saad Magalhães C, Bonfá E, et al. Chronic Spontaneous Urticaria: A Survey of 852 Cases of Childhood-Onset Systemic Lupus Erythematosus. Int Arch Allergy Immunol. 2015;167:186-192. [Crossref]
10. Spadoni M, Jacob C, Aikawa N, Jesus A, Fomin A, Silva C. Chronic autoimmune urticaria as the first manifestation of juvenile systemic lupus erythematosus. Lupus. 2011;20:763-736. [Crossref]
11. DeAmicis T, Mofid MZ, Cohen B, Nousari HC. Hypocomplementemic urticarial vasculitis: report of a 12-year-old girl with systemic lupus erythematosus. J Am Acad Dermatol. 2002;47(Suppl 5):S273-274. [Crossref]
12. Erdem Y, Altunay I, Ozkur E, Sivaz O. The Etiological Evaluation of Patients with Chronic Urticaria. Sisli Etfal Hastan Tip Bul. 2020;54:424-427. [Crossref]

2022 REFEREE INDEX

Abdulbaki Kumbasar
Abdülkadir Bilir
Abdullah Baysan
Ahmet Akyol
Ahmet Emin Erbaycu
Ahmet Öz
Ali Çetin
Ali Karaman
Amra Adroviç Yıldız
Aslı Gül Akgül Temel
Ayşe Filiz Arpaçağ Koşar
Aytaç Çetinkaya
Bala Başak Öven
Begümhan Turhan
Bekir Tuğcu
Birsen Sahip
Burcu Yılmaz
Bülent Barış Güven
Cantürk Taşçı
Coşkun Çeltik
Cüneyt Ardic
Çağatay Tezel
Dilaver Demirel
Dilaver Taş
Ebru Kale
Emrah Ermiş
Emre Ata
Erdem Çevik
Ersin Demirer
Esra Canan Kelten Talu
Esra Hayriye Ataoğlu
Ethem Ünal
Fedi Ercan
Fevziye Kabukçuoğlu
Güneş Şenol
Güzin Zeren Öztürk
Halim Ulugöl
Halime Hanım Pençe
Halit Çınarka
Hasan Hüseyin Eker
Hasan Kamburoğlu
Hüseyin Çetin
Işıl Kaya Büyükbayram
İlhami Ünlüoğlu
İlhan Bahşi
İlknur Aktaş
Macit Koldaş
Mehmet Çınar
Mehmet Enes Sözen
Mehmet Gül
Mehmet Zahit Çıracı
Memet Taşkın Eğici
Mesut Tığlıoğlu
Mine Daggez
Muhammed Keskin
Murat Sönmez
Mustafa Altınbaş
Mustafa Çörtük
Mustafa Öztürk
Nesrin Öcal
Okcan Basat
Özkan Adıgüzel
Özlem Altuntaş Aydın
Sabahattin Destek
Sami Eksert
Seda Çetinkaya Karabekir
Serap Aktaş Yıldırım
Serap Üçler
Serdar Altınay
Sevil Alkan
Sıdıka Şeyma Özkanlı
Sibel Bektaş
Şule Ayla
Şükrü Çetin
Yakup Arslan
Yusuf Kayar

2022 AUTHOR INDEX

2022 AUTHOR INDEX

2022 SUBJECT INDEX

2022 SUBJECT INDEX