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Original Article

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The use of artificial intelligence-supported communication technologies in neurological fields: A case study on brain tumor detection

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ABSTRACT

Objective: The global health system is being shaped by multidisciplinary studies on the diagnosis of diseases and the provision of effective treatment services. Information and communication technologies have been developing laboratory and imaging studies through artificial intelligence-supported systems for the last twenty years. Studies with high accuracy levels in the diagnosis and treatment protocols of diseases make important contributions to making healthy decisions. Artificial intelligence applications have been actively used in the treatment processes of neurological cancer cases in the field of health, as in many fields in recent years. Among these applications, the machine learning model has started to be preferred in the detection of brain tumors because it can provide remarkable results. The main purpose of the study is to provide a supportive analysis for the organization of early diagnosis and rapid treatment in areas such as intracranial pressure, tumor treatment and radiotherapy of patients during intensive care processes.

Materials and Methods: In this study, the method developed by doctors with machine learning Kaggle and developers of samples in the network through an example of an application that was developed through machine learning on brain tumors, brain tumor detection carried on with the validation of the data sets includes four classifications.

Results: The study consists of two different study systems, namely practice and test. Sectional images from 2865 brain magnetic resonance imaging (MRI)and computed tomography (CT) samples were examined as training in the first stage of the application using the convolutional neural network (CNN) model, and the detected tumors were classified. In this context, MRI results were obtained on 2865 samples with 2470 units and 86.23% with tumors, and 395 units and 13.76% no tumors.

Conclusion: In the study, samples with tumors were detected in a 3-month period for brain tumor detection with artificial intelligence and classified typologically. Accordingly, the reliability of the application was proven by providing 98.55% verification on 2865 samples, 3 different tumor types and no tumor data.

Keywords: Tumor detection, MRI, Artificial intelligence, Kaggle, Case study

1. INTRODUCTION

In the 21st century, in which information and communication technologies spread rapidly, there is a tendency to artificial intelligence applications in the treatment processes in the field of health, as in many other disciplines. The use of artificial intelligence in health has started to be used in neurological fields in recent years to reduce the error rate of early diagnosis and treatments. It is seen that the global health system is turning to artificial intelligence solutions for the detection of neurological-based tumors with tools such as deep learning and machine learning to combat complex and difficult data.

Brain tumors turn into possible cancer cases if subjective decisions are made in the pathological and clinical processes of the central nervous system as the activities of the neoplasm group, apart from the surrounding tissues. In the face of the development of brain tumors through mutations and gene fusions, magnetic resonance (MR) and computed tomography (CT) solutions are performed, apart from molecular tests.

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On the other hand, models with a relatively superior level of objective decision, such as artificial intelligence, are preferred for the detection and classification of brain tumors.

Cancerous structures in brain tumors are divided into benign and malignant tumor groups. Benign tumors may not cause harm to the patient's health because they do not carry cancerous cell characteristics. On the contrary, malignant tumors, on the other hand, as cancerous structures, can also spread rapidly to other tissues in the brain. Human being is physiologically directly proportional to the way cells regenerate. When aging and damaged cells turn into malignant tumors in the face of regeneration, this regeneration process is prevented and can cause the production of tumor tissue cells in the process.

Regarding brain tumors, the risk of brain cancer has been increasing on all individuals in recent years. Because it is determined that there has been a rapid increase in this issue at the level of 30% in the last 300 years. Brain tumors can basically be seen in two different ways. It can occur when the tumor grows in intrabrain cells on tissue or spreads from primary areas to the brain, which is observed in other organs [1]. Important research has been carried out in recent years on the study of brain tumors and cancer. This type of cancer has a structure that also limits the ability of other organs to function, which occurs when cells grow uncontrollably [2-4]. Here, within the general structure of the masses; these growths take place on cells such as glial cells, neurons, lymphatic tissue and blood [5]. There are different types of brain tumors. Among them, glioma, medulloblastoma, lymphoma, meningioma, craniopharyngioma, pituitary decubitus come to the fore in particular [6].

Brain tissue and tumor segmentation are being determined through magnetic resonance imaging (MRI) and CT images, which are one of the most applied areas in brain tumor detection in recent years [1,7,8]. One of the various algorithms and sample study areas developed for the detection of brain tumor is seen as MRI. MRI is the most widely used imaging technique in radiology to visually visualize human structure and function. The issue of presenting MRI images with a low margin of error also shapes an important field of study in the field of classification in the field of medicine. Vankdothu and Hameed [9] and Damodharan and Raghavan [10] in their study classify the general characteristics of pathological tissues as well as other segments by proposing a brain tumor detection technique through an alternative cluster and segmentation study for MRI applications.

Various algorithms have been developed for brain tumor classification. Within these algorithms, K-Mean Clustering includes numerical, unsupervised, non-deterministic iterations. Here, while there is at least one element in the clusters, each element is positioned near the center of its cluster [6].

Layered structures are shown as an important justification for providing an orientation to artificial intelligence in the field of brain tumor classification. The fields of artificial intelligence and application forms (such as machine learning and deep learning) have multi-layered structures. Because neural networks located between input and output generally consist of a two-Decker structure. This can also ensure that the percentage of validation on the taught data is increased when different layer and node sales are increased. At this point, different parameters and calculation resources can also be used.

The main purpose of using Graphics Processing Units (GPU)s in machine learning and deep learning applications, particularly in the field of artificial intelligence, is to enhance the performance and accuracy of data analysis, especially in MRI calculations. GPUs play a crucial role in achieving detailed learning principles that involve data labeling with algorithms. Their primary purpose is to act as the deciding factor in data analysis, particularly in critical domains such as health and engineering, where accurate and reliable results are essential for preserving lives and ensuring optimal outcomes. By leveraging GPUs, complex data classifications can be performed efficiently, enabling the processing of large amounts of data through high-scale matrix calculations. This ultimately leads to more qualified and precise results in the field of MRI calculations and other related areas.

2. MATERIALS and METHODS

Previous Studies

It is considered as an effective technique in the recognition of a brain tumor, identification of treatment processes and recovery processes. The brain tumor is experiencing different formation, development and change processes from each other. Volume structures, cross-sectional values and imaging techniques for processing images and image fragments constitute the required data area. In 2009, Sharma developed a new brain tumor segmentation in 2D and 3D and created a computational model that will determine the overall area and volume of the tumor using data sets for surgical planning [11].

On the basis of the imaging performed via the MRI device, the water molecules present in the human tissue, the hydrogen nuclei are spatially encoded and the image is obtained by providing a signal [12,13]. Detecting the tumor region using MRI alone is not enough, and radiologists in particular are trying to achieve diagnostic accuracy with new technological systems such as machine learning in order to determine treatment by measuring the size of the tumor region [4]. In another study, Gopal and Karnan classify groups with and without brain tumors using image processing algorithms on 42 MRI samples. This classification has reached an accuracy rate of 92% with the particle swarm optimization technique [14].

Machine learning represents an important field of study in tumor detection and classification processes. Al-Dahshan and the others et al., emphasizes that within the general field of machine learning there are actions of preprocessing, dimensionality reduction, feature extraction and object selection [15]. The subject of deep learning is one of the important areas of artificial intelligence used in the detection of brain tumors in recent years. Deep learning is an artificial intelligence model that constitutes a subset of machine learning that uses a hierarchical structure based on representative learning. On the basis of the increase in

studies carried out through deep learning in tumor detection, it is effective that GPU can perform calculations on a lot of data without human intervention, since they are in the structure of automation and analytical analysis.

Mohsen et al., conducted on the classification of brain tumors., in a sample study conducted by, normal, glioblastoma, sarcoma and metastatic bronchogenic carcinoma tumors were detected by performing 4 classifications via deep neural networks on 66 brain MRI [16]. MRI samples are often preferred for brain tumor and imaging processes. In these imaging models, various experimental studies can be carried out in order for brain tumor lesions and volumes to move to a stationary level in 2D and 3D. Evaluation of brain tumors through two different application modules as training and testing positively shapes the verification and reliability processes of research.

In addition to research on classification and detection of brain tumor methods, the conversion of analysis times of tests into output is also considered to be another important detail. Dahab et al., the level of the result times of the tests were examined within the scope of the study in which they made two different suggestions by [17,18]. In this study, firstly, based on an integrated set of image processing algorithms, and secondly, based on the structure of probabilistic artificial neural networks developed and implemented via Matlab. MRI images were selected as a test set out of 18 randomly selected samples out of 64 subjects by simulation, while 46 subjects were used for training. It was found that the processing time was shortened by 79% during the measurement process with the Learning Vector Quantization (LVQ)-based probability neural network (PNN) system. Tun Zav et al., based on a Naive Bayesian classification model based on a class and category based on an important taught data set for the detection of brain tumors., in a sample study conducted by 50 MRI images, structures with tumors were examined at a rate of 81.25%, structures without tumors at a rate of 100%, and a verification level of 94% was achieved [19].

While important application methods for brain tumor and artificial intelligence are considered as machine learning and deep learning, models that provide detection, such as the Convolutional Neural Network (CNN) model, can make important contributions in this process. It is also accepted as a recommendation study in clinical studies as a sample study that determines segments by performing tumor detection via CNN with 92.13% accuracy and 7.87% margin of error [18].

Tumor Detection and CNN Model

Among the models used for the detection of brain tumors, there is also CNN method. CNN is a machine learning model from the type of artificial neural networks [20]. In this model, it performs tasks such as classifying, recognizing or predicting data by learning based on input data. CNN's work especially effectively in problems related to images and manage to learn the properties of images.

The detection of brain tumors is a problem with images, which is why a model such as CNN can be used. In particular, CNN can be used for the detection of tumors using MRI of the brain. These images are taken by taking advantage of the magnetic properties of the brain tissue and show the areas where tumors are located [20-23].

The CNN model takes brain MRI images as input and tries to detect whether there are tumors or not. This model can be pre-trained or trained. A pre-trained model can give better results when applied to an unprecedented set of data. However, if the model has not been trained in advance, the model can become more effective for the detection of tumors by learning by training.

In the training process, data sets are used that are known whether the brain MRI images given as input to the model contain tumors. The model is trained by selecting samples from these data sets. During the training, the teaching model learns the features of MRI images and tries to detect the presence of tumors. After the completion of the model training, the model brain can be used for the detection of tumors when applied to MRI images. The model processes the brain MRI images given as input and tries to detect whether there are tumors or not. This detection result is given by the model as a result output. This result can be a dialog box or a number indicating the presence of a tumor.

However, the use of the CNN model for the detection of tumors also brings some disadvantages. For example, the performance of the model may decrease if there is not enough dataset for the training of the model. In addition, the use of the model for the detection of tumors may not fully adapt to the clinical evaluation process and decision-making process of doctors. Therefore, in addition to the use of the CNN model for the detection of tumors, the clinical evaluation process and decision-making process of doctors are also taken into account [24,25]. CNN architecture is generally accepted as a classification, object identification and detection method [26].

The use of the CNN model for the detection of tumors makes it possible to learn the features of brain MRI images. Thanks to this, the model tries to detect the presence of tumors. However, the use of the model for the detection of tumors also entails some disadvantages. For example, the performance of the model may decrease if there is not enough data set for the training of the model. In addition, the use of the model for the detection of tumors may not fully adapt to the clinical evaluation process and decision-making process of doctors. Therefore, in addition to the use of the CNN model for the detection of tumors, the clinical evaluation process and decision-making process of doctors should also be taken into account.

In addition to using the CNN model for the detection of tumors, other machine learning models can also be used. For example, the support vector machine (SVM), a model that is able to learn the characteristics of brain MRI images, can also be used. SVM performs classification by parsing data on a special plane and performs tasks such as classifying, recognizing or predicting data by learning based on input data [27]. In the studies conducted based on the CNN model, Swati et al., conducted a method study to analyze MRI images using a pre-trained CNN model. In this study, five additives provided a level of 94.82% with a

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verification feature [28]. In the study developed from SR-FCM-CNN models based on Fuzzy C-Means model, and in the study conducted on 500 samples selected from the Cancer Imaging Archive, a verification level of 98.33% was achieved through MRI images [29].

Convolutional Neural Network and other machine learning models can be used for the detection of brain tumors. However, it is important that there is enough data set for the training of the model and that the model adapts to the clinical evaluation process and decision-making process of doctors. Therefore, the models used for the detection of brain tumors should be carefully evaluated by doctors.

Methodology

In this study, a two-stage software and application process was determined for the brain tumor classification process. The brain tumor detection software analysis installation usually consists of two main components, an imaging system and a data analysis software. These two components are integrated with each other and used for scanning brain images, detecting tumors and evaluating the characteristics of tumors. Within the scope of the research, an artificial intelligence scheme was determined through the CNN model via the Kaggle data set in the tensorlow library via the python software language and machine learning was performed.

In this context, the installation of the imaging system and the installation of the data analysis software have been provided for the installation. Below is a general installation scheme for these two components:

Installation of the imaging system: this system is usually used to scan images of the brain magnetic resonance imaging (MRI) or computed tomography (CT) imaging techniques, such as with one of works. This system requires a scanning device for scanning brain images and a data processing server for processing scan data. The scanning device generates magnetic field or radiation waves that are used to scan brain images. The data processing server, on the other hand, processes the data generated by the scanning device and creates images.

Installation of data analysis software: This software is used to detect tumors and evaluate their characteristics after scanning brain images. This software can be an application running on a data processing server, or it can be run on a computer. The data analysis software examines the images generated after scanning the brain images and uses a series of algorithms to detect tumors. These algorithms can perform operations such as detecting tumors according to the characteristics of tumors, determining the size and location of tumors.

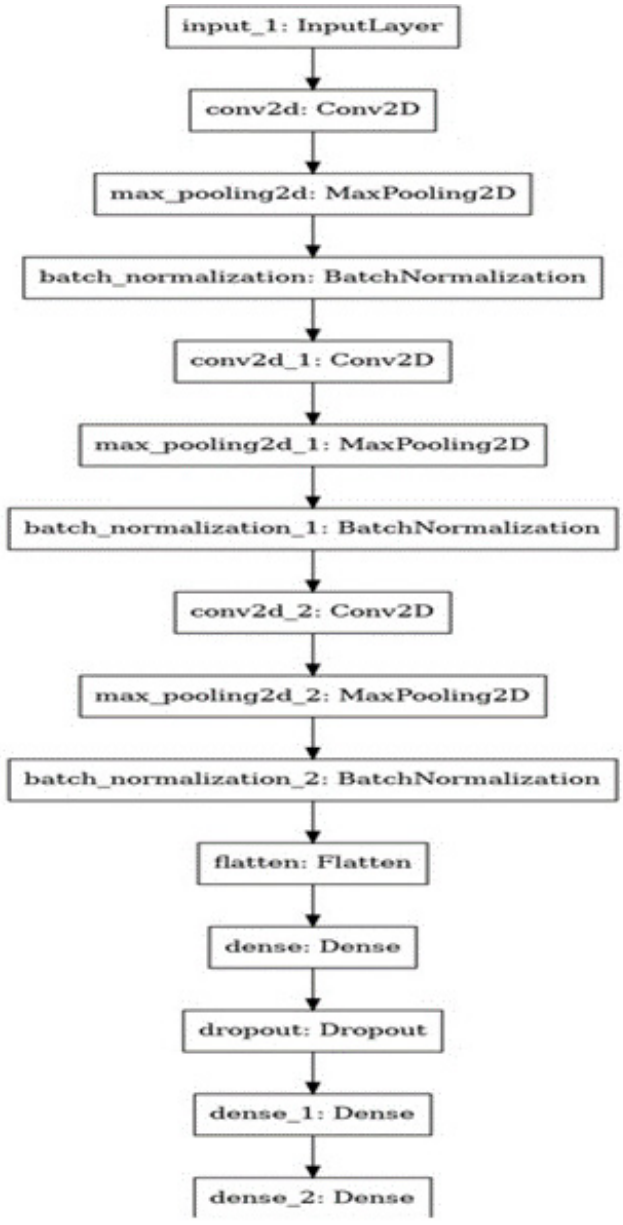

Figure 1. *The stages of machine learning of the CNN model*

As seen in Figure 1 "CNN stages of the machine learning model" that is used for machine learning research in the context of the data set during the processing phase is examined, the density in the layer between the input layer with processes, the identification and classification of taking the picture and RGB codes colors after the decision of the mechanism used in the determination of the correct and incorrect sensitivity is not understood.

In order to analyze a brain tumor detection software and create an installation diagram, first of all, the goals and objectives for which the software will be used have been determined. Not only

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will the software be used to detect brain tumors, or in addition, other brain lesions to detect about how to use the software in accordance with goals and objectives related to the detection and classification of the needed properties were determined. Then, the data set required for the software was collected and various operations were performed on this data set. Here, training and test sets have been created as the two basic sets necessary especially for preprocessing operations on the dataset and making the dataset suitable for learning algorithms.

After this stage, in which training and test sets were created, a suitable learning algorithm was selected for the software. Classification algorithms such as neural networks or support vector machines have been trained on the dataset, increasing its performance in detecting brain tumors. The performance of the trained learning algorithm was evaluated on the test set. At this stage, important metrics such as the accuracy rate of the algorithm and the error detection rate were measured and the performance of the algorithm was evaluated in accordance with these metrics.

```
import numpy as np
import tensorflow as tf
from tensorflow import keras

# Load the brain MRI scan dataset
(x_train, y_train), (x_test, y_test) = keras.datasets.mnist.load_data()

# Reshape the data to have a single channel
x_train = x_train.reshape((x_train.shape[0], 28, 28, 1))
x_test = x_test.reshape((x_test.shape[0], 28, 28, 1))

# Normalize the data
x_train = x_train.astype("float32") / 255
x_test = x_test.astype("float32") / 255

# Create a simple convolutional neural network
model = keras.Sequential([
    keras.layers.Conv2D(32, (3, 3), padding="same", input_shape=(28, 28, 1)),
    keras.layers.MaxPooling2D((2, 2)),
    keras.layers.Conv2D(64, (3, 3), padding="same"),
    keras.layers.MaxPooling2D((2, 2)),
    keras.layers.Flatten(),
    keras.layers.Dense(128, activation="relu"),
    keras.layers.Dense(10, activation="softmax")
])

# Compile the model
model.compile(optimizer="adam",
              loss="sparse_categorical_crossentropy",
              metrics=["accuracy"])

# Train the model
model.fit(x_train, y_train, epochs=10)

# Evaluate the model
model.evaluate(x_test, y_test, verbose=2)
```

```
2833/2865 [============================>.] - ETA: 0s - loss: 0.0103 - accuracy: 0.9971
2835/2865 [============================>.] - ETA: 0s - loss: 0.0103 - accuracy: 0.9971
2837/2865 [============================>.] - ETA: 0s - loss: 0.0104 - accuracy: 0.9971
2839/2865 [============================>.] - ETA: 0s - loss: 0.0103 - accuracy: 0.9971
2841/2865 [============================>.] - ETA: 0s - loss: 0.0103 - accuracy: 0.9971
2843/2865 [============================>.] - ETA: 0s - loss: 0.0103 - accuracy: 0.9971
2845/2865 [============================>.] - ETA: 0s - loss: 0.0103 - accuracy: 0.9971
2847/2865 [============================>.] - ETA: 0s - loss: 0.0103 - accuracy: 0.9971
2849/2865 [============================>.] - ETA: 0s - loss: 0.0103 - accuracy: 0.9971
2851/2865 [============================>.] - ETA: 0s - loss: 0.0103 - accuracy: 0.9971
2863/2865 [============================>.] - ETA: 0s - loss: 0.0103 - accuracy: 0.9971
2865/2865 [==============================] - 44s 24ms/step - loss: 0.0103 - accuracy: 0.9971
2865/2865 - 2s - loss: 0.0758 - accuracy: 0.9855
[Finished in 455.0s]
```

Figure 2. *Artificial intelligence machine learning software code and working section*

In Figure 2, software cross-sectional sample, it was determined that the performance of the learning algorithm trained on the coded data of brain cross-sectional samples with and without tumors was sufficient, and a software installation diagram was created. In this context, the following steps were followed for the process of creating the installation diagram of the brain tumor detection software via artificial intelligence and CNN method.

Firstly, the collection of data through an artificial intelligence-based model, determination of their qualities, classification and verification have been determined as a general study directive. It has been determined which type of brain tumor detection software is. Important information such as the working principle of this software, the necessary hardware and software requirements have been added. The hardware and software systems necessary to run the brain tumor detection software and the application has been run for scanning and testing processes via a computer that can perform high performance according to the working principle and requirements of the software.

In this study, the artificial intelligence-based software is decoded in such a way that while working in a high-performance computer on the GPU, it can also work as a distributed system between the server and a client. In this context, the installation of the hardware and software systems has been completed and the installation and configuration of all the systems necessary for the software to work correctly has been carried out.

At the last stage, after completing the installation of the software for detecting and classifying brain tumors, the software was tested to check whether it was working correctly. This process was carried out to check whether the software is installed and configured correctly and whether it gives the expected results.

In the image in Figure 3 there are images in the size of 240x240 that are used in the training phase for tumor detection and classification on MRI images in the data set. These images have been processed from the machine learning training process onwards and general detection and classification analyses have been carried out until the testing processes.

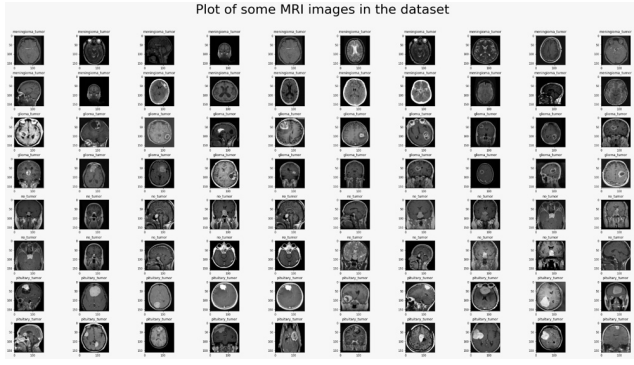

Figure 3. *Tumor detection data classification cross-sectional image*

3. RESULTS

In this study on brain tumor detection using a CNN model trained with an artificial intelligence program, machine learning analysis was conducted. The analysis involved processing a dataset consisting of MRI images and performing detection and classification of tumors. The dataset was divided into training and testing stages, and the process was carried out in two stages. At the first stage of the research, 3 tumor types and

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1 no-tumor structure were detected as a result of the detection and classification processes performed on 2865 MRI. In the second stage of the research, which was determined as a test process, the pituitary tumor was realized as 74 MRI and 18.78%, glioma tumor was realized as 100 MRI and 25.38%, meningioma tumor was realized as 115 MRI and 29.19% and no tumor MRI was realized as 105 MRI and 26.65% on 395 total MRI as seen in Figure 4.

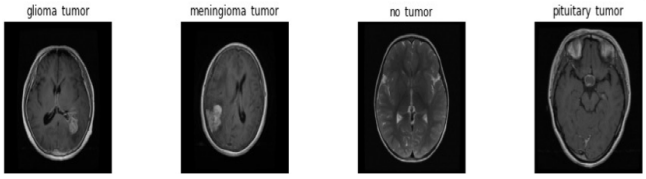

Figure 4. *Tumor detection data validation graphic image*

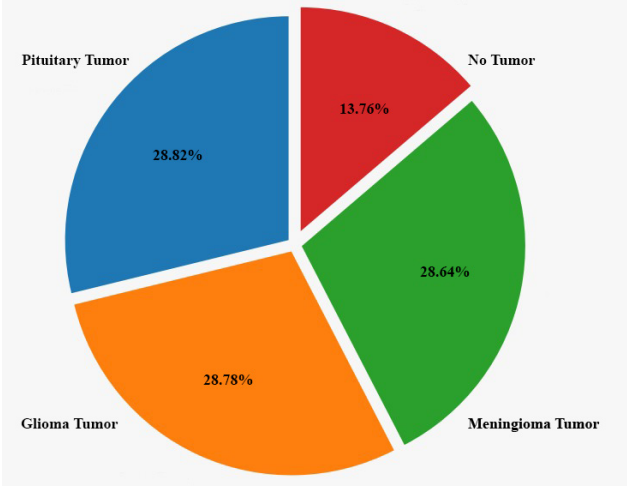

Figure 5. *Percentage distributions of machine learning training tumor detection*

As seen in Figure 5 during the training process, pituitary tumor was 827 MRI and 28.82%, glioma tumor was 826 MRI and 28.78%, meningioma tumor was 822 MRI and 28.64%, and no tumor MRI was 395 MRI and 13.76%.

Table I. *Cross-sectional image of tumor verification and classification*

No	Tumor classification	precision	recall	f1-score	support
0	no_tumor	0.97	1.00	0.99	75
1	glioma_tumor	0.95	0.99	0.97	141
2	meningioma_tumor	1.00	0.94	0.97	139
3	pituitary_tumor	0.99	1.00	0.99	135
	Accuracy	0.98	395		
	Macro avg	0.98	0.98	0.98	395
	Weighted avg	0.98	0.98	0.98	395

As can be seen in the Table I; evaluation of classification results 4 different values emerge. detection of tumored images from 3 with tumored structures and 1 no-tumor structure is observed at the highest value with 98.55% success in terms of sensitivity rate. Among the tumor classification areas, meningioma tumor ranks first as the highest sensitivity rate of 100%.

Another area of research is the "Confusion matrix" measurement. The Confusion matrix is a measurement used to assess the accuracy of the predictions of a given classification model. For example, a classification model attempts to estimate the values of a specific target variable in a data set. According to the actual values of this target variable, it can be evaluated whether the estimates are true or false.

The Confusion matrix is presented in the form of a table for assessing the accuracy of forecasts. This table decodes the relationship between the actual values of the target variable and its estimated values. For example, if the target variable consists of two classes (class A and class B), the confusion matrix will have one row and one column. The rows show the actual values, while the columns show the estimated values. Each cell of this matrix shows a value that intersects with the predicted values of a class of the target variable. For example, if the actual value of a cell is class A and the estimated value is class B, the number of values of class A estimated as B is written in that cell.

Various metrics can be used when evaluating the accuracy of the predictions of the Confusion matrix, classification model. For example, metrics such as accuracy, precision and recall can be calculated. These metrics are calculated based on the data obtained from the Confusion matrix and provide information about the performance of the classification model.

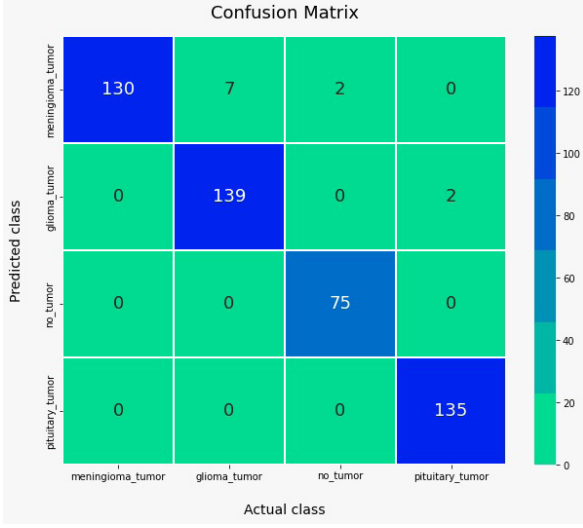

Figure 6. *Cross-sectional image of the tumor detection data Confusion matrix*

Looking at the sectional image Figure 6 as a Confusion matrix; In the model consisting of actual classification values and estimated classification values as two basic variables, meningioma tumor

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130 real 9 is estimated as 139, no-tumor 75, glioma 139 real and 2 deviation values 141, and finally, pituitary 135 real and 2 estimates, out of 137 values, the number of 490 is reached. Since, the findings, which were determined as 99.71% in the training process of the study, were obtained 98.55% accuracy with a loss of 1.16% in the testing process, this research has been comparatively proven with the same value.

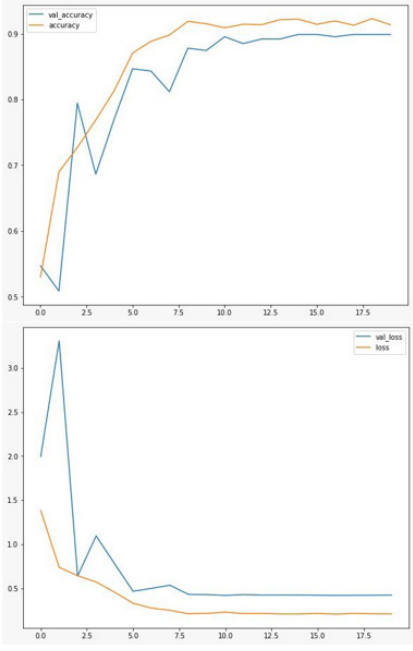

Figure 7. *Estimated value view of CNN model test data*

Using the CNN model, the accuracy loss of the estimates performed on the test data, the training loss (Validation loss) rates indicated in blue and the verification loss rates over time indicated in orange are examined. Accordingly, in Figure 7 the loss of training shows how well the model fits the training data, while the loss of verification shows how well the model fits the new data.

When the data obtained from both graphs are examined, the accuracy values in the first graph are shown in the blue colored unit at the training stage, showing the loss rates on the previously detected tumor status images, while in the orange colored unit, the loss of new test images to the system during verification over time is observed. In this case, it is understood that the accuracy rate is 99.71% and the loss rate is about 1%. In the second graph, it is also understood that the loss levels within the relevant color codes show a rapid decrease based on the loss rates and prove the level of accuracy.

4. DISCUSSION

The global health system, because of the higher percentage of patients per doctor in the health of the employees who have less time to make the decision more accurate diagnostic and treatment protocols possible in the process of the establishment of the possibility of faulty decision-making to reduce risk in the face of developments in the fields of information and communication technologies and engineering considers important to take advantage of. For this reason, qualified solution processes can be provided by combining studies in the field of artificial intelligence with the help of diagnoses made in previous treatments. In recent years, the rapid increase in cancer cases and the low number of trained medical personnel, as well as the high cost of treatment, are factors for the production of alternative solutions. Especially in order to reduce the permanent damage of tumor formations in the brain region, it is important to perform early detection and classification studies in terms of treatment processes.

According to data from previous case studies in the field; Kurup et al. (3064 images from 233 patients) using CNN TensorFlow architecture, they achieved 87% accuracy in the test phase and 92% accuracy in the validation process [30]. Swati et al., studied a method for analyzing MRI images using a pre-trained CNN model. In this study, five additive validation features achieved a 94.82% success rate [28]; Özyurt et al., examined the features of images using the NS-EMFSE method for the detection of brain tumors. They obtained an accuracy rate of 95.62% on 160 MRIs using the MatConvnet library with SVM and KNN classification system [21]. Wozniak et al., proposed a new correlation learning mechanism (CLM) for deep neural network architectures that combines a CNN with a classical architecture. The results show that the CLM model can achieve about 96% accuracy and about 95% precision and recall [31].

Seetha and Raja used the BraTS 2015 dataset consisting of 220 high-grade glioma (HGG) and 54 low-grade glioma (LGG) MR images. They achieved 83.0% accuracy using SVM-based classification and 97.5% accuracy using CNN [32]. Hossain et al. used the BRATS dataset for Brain Tumor Segmentation. They used a total of 207 MRI images, 187 with tumors and 30 without tumors. They achieved 92.42% accuracy using SVM and 97.87% accuracy using CNN [33]. Kachwalla et al. achieved 98% accuracy using the Harvard dataset (66 real human brain MRIs including 22 normal and 44 abnormal images) [34]. Khairandish et al. analyzed 220 brain MRI images in their study. In this context, the accuracy levels were 95% on Deep CNN (DCNN) and 97.5% on the CNN model. The overall accuracy of the hybrid CNN-SVM was calculated as 98.49% [35].

In studies analyzed through the CNN model, which is one of the various classification formats used in tumor detection studies, small-scale data sets are preferred in order to achieve high accuracy. It is thought that various studies in the literature differ significantly from our study for this reason. Because, in order to use large-scale data sets, models with high level of analysis and effective artificial intelligence models should be used.

This study has been prepared to examine the success rate of detection and classification operations using the machine learning method. The research was carried out in a three-month study process in total. As a result of trainings conducted on multiple neural network models, it is observed that the CNN method learns faster, but the transfer model also shows more

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successful results. Against the background of the fact that the results obtained are at the level of 98.55% prediction success rate, there are also contributions of both the method and coding system used and the sample study model based on the visual data sets obtained from the Kaggle library.

In this study model, it is possible to increase the level of success by supporting machine learning through more visual data in order to increase the reliability and accuracy levels. When interpreting the results of a blood test taken as output in the past years, Today, blood test results, if provided along with reference ranges through emerging computing technologies in the near future, especially in cases of cancer in the determination of the test results, artificial intelligence-machine learning methods also aided in the health sector can play a decisive role in the output as it is considered in the light of this study. It is also predicted that in the near future, the health sector, especially by supporting technological innovations and accelerated systems, will be able to shape the common global health model as well as qualified doctor-patient communication. In the research, while 99.71% accuracy was achieved on 2865 MRI images in the training process, 98.55% accuracy was achieved with 1.16% loss in the test process. The performance of our proposed method is considered to be a qualified reference for brain tumor detection and multi-classification studies since high accuracy is obtained as a result of analyzing 2865 MR images.

Compliance with the Ethical Standards

Financial Support: The authors have no relevant financial information to disclose.

Conflict of Interest: The authors have no potential conflicts of interest to disclose.

Authors' Contributions: Both authors contributed 50% equally to this study.

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Wearable technology data-based sleep and chronic disease relationship

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ABSTRACT

Objective: The aim of the study was to examine the cross-sectional relationship between sleep duration and 12 chronic diseases (obesity risk, diabetes, asthma, renal failure, hypertensive diseases, chronic obstructive pulmonary disease, cardiovascular diseases, ischaemic heart disease, pulmonary heart disease, immunodeficiencies and immune system disorders) by transferring the data to the national electronic patient record system through wearable device technology.

Materials and Methods: The data of the study were obtained from the Ministry of Health "Turkey National Personal Health Record System" (The "e-Nabız"). Between 30.03.2023 and 28.05.2023, 315448 data from 27847 people (15167 male) were collected and analysed on the basis of province, rural status, age group, gender and presence of chronic diseases and average, minimum (min.), maximum (max.) sleep duration. Descriptive statistics, chi-square analysis, Independent Samples t-Test, One-way Analysis of Variance and Pearson's correlation coefficient were used.

Results: The max. and average sleep duration were significantly shorter in men. Max. sleep duration decreased while the average sleep duration increased with increasing age. Participants with asthma, chronic renal failure and cerebrovascular diseases had decreased average sleep duration. The frequency of those who slept for 7-8 hours, which is the ideal sleep duration, is lower in all chronic diseases.

Conclusion: Most of the common chronic diseases may affect the sleep duration times and quality, which may further affect the prognosis of these patients.

Keywords: Sleep duration, Wearable technology, Chronic diseases, Cross-sectional study

1. INTRODUCTION

Sleep is essential for life. We spend one third of our lives in sleeping. Moreover, sleep is a very important part of quality of life [1]. There is increasing research evidence of an association between sleep disorders and cognitive dysfunction, occupational and traffic accidents, as well as some chronic diseases, metabolic, cardiovascular and cerebrovascular complications and mortality [2-6]. These results are important both in clinical practice and in the planning and implementation of public health policies for health economics. Questionnaire-based population studies have suggested high prevalence rates of sleep disorders [7-10]. In Turkey in 2015, the prevalence rate of insomnia (men/females) was 15.3% (10.5%/20.2%; P <0.001), high sleep-related breathing disorders was 13.7% (11.1%/20.2%; P <0.001), excessive daytime sleepiness was 5.4% (5.0%/5.7%); P: 0.09), restless legs syndrome was reported as 5.2% (3.0%/7.3%; P <0.001) [11].

The interaction between sleep and diseases is reciprocal. Both sleep disorders cause diseases and diseases cause sleep disorders. The impairment of sleep duration and quality cause decreased cognitive abilities and increased risk of occurrence or the frequency of existing symptoms of many psychiatric (such as panic disorder, major depressive disorder), cardiovascular, neurological (such as frequent seizures in epileptic patients), metabolic (such as diabetes, obesity), rheumatological and infectious diseases [12-15]. Symptoms of diseases such as asthma, coronary artery disease and stroke may occur with sleep disorders. Sleep disorders related to the presence of medical and psychosocial diseases are more common than primary disorders of sleep [16-18].

Studies on sleep disorders are generally based on extensive "questionnaire survey" in the literature. However, with the development of wearable technology, vital data such as sleep,

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walking, respiration can be easily recorded and measured. Turkey National Personal Health Record System (The e-Nabız) was established in Turkey in 2015. Since then, "personal health records" are stored nationally. Patients can manage, share, safely store and process their own health records [19]. In very few countries, health records of the whole population can be kept with a similar system. Therefore, in this study, the data of the study were obtained by patients transferring their "personal health records" to the national database to the extent permitted by wearable technologies.

The aim of the study was to examine the cross-sectional relationship between sleep duration and 12 chronic diseases (obesity risk, diabetes, asthma, renal failure, hypertensive diseases, chronic obstructive pulmonary disease (COPD), cardiovascular diseases, ischaemic heart disease, pulmonary heart disease, immunodeficiencies and immune system disorders) by transferring the data to the national electronic patient record system through wearable device technology. In this study, the relationship between sleep duration and demographic data, province, urban/rural residence status was also investigated. In the literature, the relationship between chronic diseases and sleep has been frequently investigated while studies on rural/urban region distinction are rare.

2. MATERIALS and METHODS

This study was designed in an analytical and cross-sectional model to reveal the relationship between sleep duration and chronic diseases. The data of the study were obtained from the Ministry of Health the e-Nabız system with the permission letter numbered E-26216721-708.99-215526724 between 30.03.2023 and 28.05.2023 on the basis of province, rural status, age group, gender and presence of chronic diseases and average, minimum (min), maximum (max) sleep duration. In this study, 315448 data from 27847 people were collected and analysed. The data were analysed using IBM SPSS Statistics 22.0 package programme. While the outcome variable of the study was considered as sleep duration; gender, age group, region, rural area and chronic diseases were considered as independent variables. While 11 chronic diseases were grouped as "present" and "no", for obesity, the prevalence of the disease obtained from the Ministry of Health e-Nabız system on the basis of province, gender and age groups and calculated per 1000 people according to the population was used. Also, in this study, we focused on chronic disease of those who shared their sleep data. For the analyses, sleep duration was classified into 3 categories as 6 hours or less, 7-8 hours ideal and 9 hours or more excessive based on previous national and international studies [20].

Table I. *Mean sleep duration according to baseline characteristics of the participants*

	Number (n)	Percentage (%)	Min. Sleep Time (min) (mean±SD)	Max. Sleep Time (min) (mean±SD)	Average Sleep Time (min) (mean±SD)
Gender					
Women	12680	45.53	108.89±90.49	692.30±108.7	412.42±40.21
Male	15167	54.47	108.01±87.01	675.03±109.62	396.31±43.53
p			*0.407*	***<0.001***	***<0.001***
Age Groups					
<25 years	13201	47.41	95.12±72.03	703.11±98.80	403.08±34.28
25-39 years	10398	37.34	100.94±81.45	683.69±108.33	400.61±39.67
40-64 years	3984	14.31	162.34±119.41	622.83±117.64	412.85±64.22
>64 years	264	0.95	252.75±129.02	546.59±113.85	412.38±92.91
p			*0.407*	***<0.001***	***<0.001***
Region					
Mediterranean Region	2491	8.9	123.240±93.46	633.56±97.56	402.0±47.28
Eastern Anatolia Region	779	2.8	185.96±118.70	588.58±110.82	400.34±65.99
Aegean Region	4095	14.7	117.15±96.05	671.79±104.98	408.12±46.62
Southeastern Anatolia Region	997	3.6	136.57±97.51	601.34±110.62	385.74±61.24
Central Anatolia Region	5145	18.5	101.25±83.63	683.22±97.79	403.39±96.82
Black Sea Region	1775	6.4	168.81±108.70	593.28±106.99	399.09±62.13
Marmara Region	12565	45.1	89.97±71.57	721.13±98.24	404.71±33.28
p			***<0.001***	***<0.001***	***<0.001***
Ruralisation					
Rural	2549	9.15	172.72±116.02	583.28±109.99	397.40±65.49
Urban	25298	90.85	101.92±82.63	692.93±104.34	404.28±39.76
p			***<0.001***	***<0.001***	***<0.001***
Sleep Time					
≤6 hours	1988	7.14	135.67±75.86	474.23±145.18	304.3±65.11
7-8 hours	25099	90.13	98.74±75.05	700.73±87.55	408.00±20.91
≥9 hours	760	2.73	356.19±142.25	639.55±71.31	519.60±42.0
p			***<0.001***	***<0.001***	***<0.001***

Min: minimum, Max: maximum

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Table II. *Mean sleep duration of participants according to chronic diseases*

	Number (n)	Percentage (%)	Min. Sleep Time (min) (mean±SD)	Max. Sleep Time (min) (mean±SD)	Average Sleep Time (min) (mean±SD)
Asthma					
No	25604	91.95	101.49±80.93	692.23±103.19	404.271±39.28
Present	2243	8.05	187.32±126.28	576.33±122.68	396.57±71.35
p			***<0.001***	***<0.001***	***<0.001***
Kidney failure					
No	27813	99.88	108.21±88.34	683.09±109.36	403.65±42.66
Present	34	0.12	270.14±143.46	516.05±126.21	400.08±110.90
p			***<0.001***	***<0.001***	*0.627*
Diabetes					
No	25295	90.84	100.33±80.13	693.02±103.04	403.76±38.67
Present	2552	9.16	188.47±122.41	582.48±120.76	402.55±71.95
p			***<0.001***	***<0.001***	*0.173*
Hypertensive diseases					
No	27733	99.59	107.72±87.710	683.54±108.93	403.62±42.369
Present	114	0.41	274.71±137.68	524.50±137.74	408.92±105.36
p			***<0.001***	***<0.001***	*0.187*
Hypertension					
No	23719	85.18	95.164±73.315	698.73±98.701	403.51±35.219
Present	4128	14.82	184.51±123.65	591.86±123.43	404.41±72.376
p			***<0.001***	***<0.001***	*0.217*
COPD					
No	27505	98.77	106.48±86.349	684.95±107.70	403.69±41.742
Present	342	1.23	263.67±123.91	517.36±127.57	400.42±95.575
p			***<0.001***	***<0.001***	*0.161*
Chronic renal failure					
No	27649	99.29	107.37±87.366	684.12±108.44	403.71±42.106
Present	198	0.71	253.44±131.87	510.67±123.67	394.71±100.91
p			***<0.001***	***<0.001***	***0.003***
Pulmonary heart diseases					
No	27790	99.80	108.07±88.253	683.22±109.22	403.65±42.647
Present	57	0.20	270.45±113.02	523.08±141.09	403.78±94.282
p			***<0.001***	***<0.001***	*0.981*
Cerebrovascular diseases					
No	27565	98.99	106.91±86.771	684.56±108.01	403.71±41.705
Present	282	1.01	254.46±133.96	519.30±131.88	397.29±104.80
p			***<0.001***	***<0.001***	***0.012***
Immune deficiencies and immune system disorders					
No	27819	99.90	108.24±88.409	683.05±109.42	403.64±42.764
Present	28	0.10	276.32±126.62	522.32±100.85	407.78±78.746
p			***<0.001***	***<0.001***	*0.609*
Ischaemic heart disease					
No	26263	94.31	102.51±82.148	689.25±104.90	403.55±39.420
Present	1584	5.69	206.14±126.64	577.49±129.26	405.25±80.372
p			***<0.001***	***<0.001***	*0.125*
Prevalence of obesity					
r			*-0.160*	*0.249*	*0.087*
p			***<0.001***	***<0.001***	***<0.001***

Min: minimum, Max: maximum; COPD: chronic obstructive pulmonary disease

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Statistical Analysis

The data were analysed using IBM SPSS Statistics 22.0 package programme. Descriptive statistics, chi-square analysis, Independent Samples t-Test, One-Way Analysis of Variance and Pearson's correlation coefficient were used to analyse the data. In order to determine whether the average, min. and max. sleep duration of the participants differed according to gender, age group, rurality, region and presence of chronic disease, Independent Samples t-Test was performed for variables with two groups and One-Way Analysis of Variance was performed for variables with three or more groups. Chi-square analysis was performed to determine the differences in sleep duration categories according to independent variables. Statistical significance level P<0.05 was accepted in the evaluations.

3. RESULTS

The distribution of the participants according to their basic characteristics is shown in Table I. 54.4% of the participants were male, 47.4% were younger than 25 years of age, 45.1% lived in the Marmara region and 90.8% lived in non-rural settlements. When the distribution of the average sleep duration of the participants was analysed, it was determined that 90.1% of them slept for 7-8 hours. It was found that the max. and average sleep duration of males were less while the max. sleep duration decreased and the average sleep duration increased with increasing age. The average sleep duration was the highest in the Aegean and Marmara regions, and the average sleep duration of those who did not live in rural areas was higher.

Figure 1 shows the provinces with the lowest and highest average sleep duration in Turkey. The provinces with the lowest mean sleep duration were Kilis and Erzincan, while the provinces with the highest mean sleep duration were Uşak and Ardahan. In the provinces with the lowest mean sleep duration, the share of those with chronic diseases was higher and their mean sleep duration was shorter.

Table II shows the min., max. and average sleep duration according to chronic diseases. In the presence of asthma, chronic renal failure and cerebrovascular diseases, average sleep duration decreased, max. sleep duration decreased and min. sleep duration increased in all diseases. In parallel, the relationship between the prevalence of obesity and sleep duration showed a low-level positive relationship for average and max. sleep duration and a low-level negative relationship with min. sleep duration.

Table III shows the distribution of sleep duration according to the basic characteristics of the participants. Among women, those younger than 25 years of age, those living in Central Anatolia and Marmara regions, and those who do not live in rural areas, there are more people who sleep at ideal sleep duration.

Table IV shows the distribution of sleep duration of the participants according to their chronic diseases. The frequency of those who slept for 7-8 hours, which is the ideal sleep duration, is lower in all chronic diseases. Especially among those with renal failure, hypertensive diseases, COPD, chronic renal failure and cerebrovascular disease, the number of people who deviate from the ideal sleep time and sleep less is higher.

Table III. *Distribution of sleep duration according to the basic characteristics of the participants*

	SLEEP TIME			
	≤6 hours	**7-8 hours**	**≥9 hours**	**P**
Gender				
Female (n-%)	659 (5.2%)	11610 (91.6%)	411 (3.2%)	***<0.001***
Male (n-%)	1329 (8.8%)	13489 (88.9%)	349 (2.3%)	
Age Groups				
<25 years (n-%)	774 (5.9%)	12230 (92.6%)	197 (1.5%)	***<0.001***
25-39 years (n-%)	695 (6.7%)	9532 (91.7%)	171 (1.6%)	
40-64 years (n-%)	457 (11.5%)	3186 (80%)	341 (8.6%)	
>64 years (n-%)	62 (23.5%)	151 (57.2%)	51 (19.3%)	
Region				
Mediterranean Region (n-%)	229(9.2%)	2183(87.6%)	79(3.2%)	***<0.001***
Eastern Anatolia Region (n-%)	141(18.1%)	592(76%)	46(5.9%)	
Aegean Region (n-%)	299(7.3%)	3648(89.1%)	148(3.6%)	
Southeastern Anatolia Region (n-%)	228(22.9%)	734(73.6%)	35(3.5%)	
Central Anatolia Region (n-%)	307(6%)	4710(91.5%)	128(2.5%)	
Black Sea Region (n-%)	347(19.5%)	1322(74.5%)	106(6%)	
Marmara Region (n-%)	437(3.5%)	11910(94.8%)	218(1.7%)	
Ruralisation				
Rural (n-%)	474(18.6%)	1921 (75.4%)	154 (6%)	***<0.001***
Urban (n-%)	1514 (6%)	23178(91.6%)	606(2.4%)	

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Table IV. *Distribution of sleep duration of participants according to chronic diseases*

	SLEEP TIME			
	≤6 hours	**7-8 hours**	**≥9 hours**	**P**
Asthma				
No (n-%)	1516(5.9%)	23497(91.8%)	591(2.3%)	***<0.001***
Present (n-%)	472(21%)	1602(71.4%)	169(7.5%)	
Kidney failure				
No (n-%)	1980(7.1%)	25079(90.2%)	754(2.7%)	***<0.001***
Present (n-%)	8(23.5%)	20(58.8%)	6(17.6%)	
Diabetes				
No (n-%)	1578(6.2%)	23176(91.6%)	541(2.1%)	***<0.001***
Present (n-%)	410(16.1%)	1923(75.4%)	219(8.6%)	
Hypertensive diseases				
No (n-%)	1961(7.1%)	25038(90.3%)	734(2.6%)	***<0.001***
Present (n-%)	27(23.7%)	61(53.5%)	26(22.8%)	
Hypertension				
No (n-%)	1290(5.4%)	22049(93%)	380(1.6%)	***<0.001***
Present (n-%)	698(16.9%)	3050(73.9%)	380(9.2%)	
COPD				
No (n-%)	1896(6.9%)	24899(90.5%)	710(2.6%)	***<0.001***
Present (n-%)	92(26.9%)	200(58.5%)	50(14.6%)	
Chronic renal failure				
No (n-%)	1926(7%)	24995(90.4%)	728(2.6%)	***<0.001***
Present (n-%)	62(31.3%)	104(52.5%)	32(16.2%)	
Pulmonary heart diseases				
No (n-%)	1976(7.1%)	25062(90.2%)	752(2.7%)	***<0.001***
Present (n-%)	12(21.1%)	37(64.9%)	8(14%)	
Cerebrovascular diseases				
No (n-%)	1916(7%)	24944(90.5%)	705(2.6%)	***<0.001***
Present (n-%)	72(25.5%)	155(55%)	55(19.5%)	
Immune deficiencies and immune system disorders				
No (n-%)	1982(7.1%)	25079(90.2%)	758(2.7%)	***<0.001***
Present (n-%)	6(21.4%)	20(71.4%)	2(7.1%)	
Ischaemic heart disease				
No (n-%)	1702(6.5%)	23995(91.4%)	566(2.2%)	***<0.001***
Present (n-%)	286(18.1%)	1104(69.7%)	194(12.2%)	
Prevalence of obesity (mean ±SD)	4.09±2.71	5.32±2.49	4.64±2.86	***<0.001***

COPD: chronic obstructive pulmonary disease

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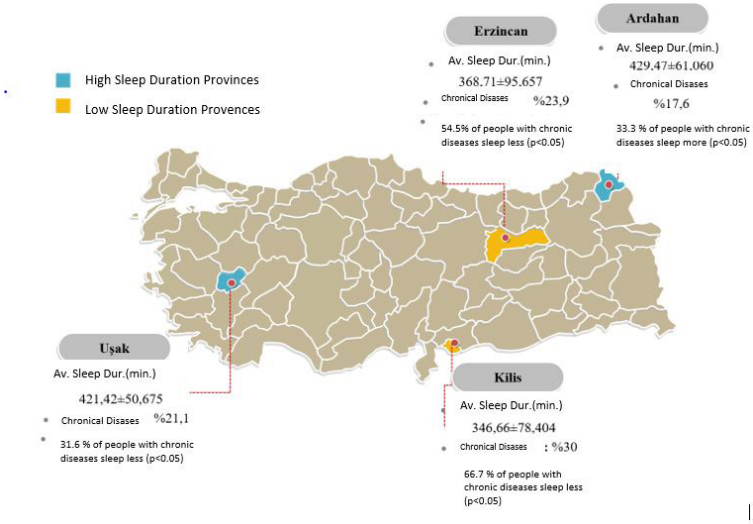

Figure 1. *Provinces with the lowest and highest average sleep duration in Turkey*

4. DISCUSSION

Sleep is one of the basic needs of human beings with physical, social, intellectual and spiritual needs, which must be met in a balanced way in order to be healthy [21].

Recent guidelines have shown promising progress in reducing morbidity and mortality in patients with cardiovascular disease. The importance of lifestyle changes such as balanced diet, physical activity and management of obstructive sleep apnea has been emphasised in the published guidelines [22-24]. However, the importance of having adequate sleep duration has not yet been included in guidelines, whereas this may help patients especially in the prevention of further cardiovascular disease. Indeed, according to national electronic patient registries, statistically significant associations were found between cerebrovascular disease, hypertensive diseases, ischaemic heart disease and min. sleep duration.

In a consensus published in 2015, the American Academy of Sleep Medicine (AASM) and the Sleep Research Society (SRS) defined normal sleep duration as 7 or more hours of sleep per night. However, this consensus also stated that sleeping more than 9 hours per night poses a health risk, but this applies to young adults, individuals who are trying to pay off sleep debt, and those who do not belong to any of the individuals with disease [25]. Among the sampled individuals aged 40-64 years (8.6%), women (5.6%) had a sleep duration of 9 hours or more. The detected individuals should be directed to normal sleep patterns through wearable technologies and warned to increase mobility.

The relationship between obesity prevalence and sleep duration was determined as a low positive relationship for average and max. sleep duration and a low negative relationship with min. sleep duration. In 2020, a study conducted in China found a strong association between excessive sleep duration and short sleep duration [26], while a meta-analysis suggested that short sleep duration was associated with obesity risk, but no such association was found for long sleep duration [27]. Among our findings, the number of people who slept far below the ideal sleep duration was higher among patients with renal failure. While the study by Lu et al. [26], supported our findings, in the study by Chen et al. [27], in 2022, sleep metrics predicted decreased renal function only in individuals with chronic kidney disease at baseline. Among this group, equations for glomerular filtration rate (eGFR) decline was associated with decreased stage N3 sleep (0.32 mL/min/1.73 m2/y per 10% decrease in N3; increased actigraphy napping frequency (beta: – 0.20 [-0.30, – 0.07]); and actigraphy sleep midpoint trajectory in early morning (ref: midnight, beta: – 0.84 [-1.19, – 0.50]). Urinary albumin-to-creatinine ratio increase was associated with high wake bouts trajectory (ref: low, beta: 0.97 [0.28, 1.67]) and increased sleep-related hypoxemia (oxygen saturation time<90% [≥5%], beta: 2.17 [1.26, 3.08]). Sleep metrics—N3 sleep, naps, and midpoint trajectory significantly modified associations between haemoglobin A1C and eGFR decline. According to the study, decreased deep sleep, daytime sleepiness, increased waking bouts, delayed sleep rhythms, and hypoxaemia during the night are associated with longitudinal renal function decline, and the effects are most prominent in individuals with chronic kidney disease [28]. The share of those who sleep 7-8 hours, which is the ideal sleep time, is less among those with all chronic diseases obtained in our study. Among those with hypertensive diseases, COPD and cerebrovascular disease, the number of people who deviated from the ideal sleep time and slept less is higher. Many studies in the literature support our findings [12-17]. In addition, regular sleep has been considered within the scope of preventive health services. The evaluation of regular sleep within the scope of health policies is one of the important conditions to be taken

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into consideration today. The provinces with the lowest mean sleep duration were Kilis and Erzincan, while the provinces with the highest mean sleep duration were Uşak and Ardahan. In the provinces with the lowest mean sleep duration, the share of those with chronic diseases was higher and their mean sleep duration was shorter. Health service providers should evaluate the sleep parameter in disease management with regard to both extremes of too little sleep and too much sleep. Multidisciplinary studies should be carried out to improve the quality of life by contributing to health planning in individuals with chronic diseases who experience sleep deficiency or excess.

Compliance with Ethical Standards

Approval: The data were obtained from the e-Nabız database of the Ministry of Health with the permission letter numbered E-26216721-708.99-215526724.

Conflicts of Interest: The author declares that he has no conflict of interest relevant to this article.

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Author Contribution: SB was responsible from the design, statistical analysis, and drafting of the article. He critically revised the manuscript.

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Heart rate variability of acute ischemic stroke patients according to troponin levels

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ABSTRACT

Objective: Neurogenic myocardial stunning is a type of stress-induced cardiomyopathy thought to be a result of dysregulation of the autonomic nervous system. Heart rate variability (HRV) analysis is a potential method for understanding the underlying mechanisms of autonomic dysfunction in ischemic stroke. The aim of the study was to investigate HRV in stroke patients in accordance with troponin levels.

Patients and Methods: Sixty-six patients (mean age 65 ± 13 years; 39 male) presenting with acute ischemic stroke were consecutively included. High-sensitive cardiac troponin I (hs-cTnI) levels were accepted as elevated when> 0.04 ng/mL. All patients underwent ambulatory electrocardiographic (ECG) monitoring within the first seven days to obtain time-domain and frequency-domain measures of HRV.

Results: Twenty patients (30.3 %) had elevated hs-cTnI. Patients with high troponin levels had significantly lower left ventricular ejection fraction (LVEF), higher ST-segment-T wave changes, and higher N terminal pro-brain natriuretic peptide (NT-proBNP) levels. Low-frequency/high-frequency (LF/HF) value was significantly higher in the troponin-positive group, but other ambulatory ECG monitoring parameters such as SDNN, SDANN, RMSSD, and pNN50 were similar among patients.

Conclusion: Neurogenic myocardial damage presenting with high troponin levels can be seen in ischemic stroke patients and may be associated with sympathetic overactivity.

Keywords: Myocardial stunning, Neurogenic cardiac injury, Troponin

1. INTRODUCTION

Neurogenic stunned myocardium (NSM) is a spectrum of cardiac changes seen after various neurologic events [1]. Cardiac abnormalities seen in NSM include electrocardiographic changes, an increase of cardiac biomarkers such as B-type natriuretic peptide and troponin, arrhythmias, and echocardiographic changes such as wall motion abnormalities with decreased ventricular ejection fraction (LVEF) and diastolic dysfunction [2,3]. Sympathetic overactivity and catecholamine surges that occur after neurological events or dysfunction of the autonomic nerve system after brain injury are the leading underlying mechanisms [4,5]. NSM, a reversible myocardial dysfunction, is mainly caused by dysfunction of the autonomic nervous system [6]. Heart rate variability (HRV) can be used as a biomarker of autonomic dysregulation in patients with ischemic stroke [7].

Heart rate variability refers to beat-to-beat variations in the duration of the RR intervals [8]. It is a non-invasive method used to understand the autonomic regulation of the heart [9]. With HRV it is possible to analyze parasympathetic or sympathetic activity and their response to different conditions. Reduced HRV has been reported in acute ischemic stroke patients compared to healthy controls [10]. Also, autonomic dysfunction has been linked to poor prognoses in ischemic stroke patients [11]. Changes in the autonomic nervous system after ischemic stroke may be associated with stroke recurrence and recovery [7]. Therefore, HRV has an important role in the detection of autonomic dysfunction after stroke.

The aim of our study was to investigate HRV parameters with ambulatory electrocardiographic (ECG) monitoring in patients with neurogenic stunned myocardium.

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2. PATIENTS and METHODS

The investigation conformed to the principles outlined in the Declaration of Helsinki. All participants gave written informed consent. The study was approved by the ethics committee of the Marmara University School of Medicine.

Seventy-eight consecutive patients admitted to the neurology clinics with the diagnosis of acute ischemic stroke were invited to participate in the study. After the exclusion of the 5 patients with artifacts in ambulatory ECG recordings and 7 patients with poor image quality for transthoracic echocardiography the remaining 66 patients were included. The Baseline National Institutes of Health Stroke Scale (NIHSS) of patients were noted at admission. Transient ischemic attacks (TIA) were not included in our study population because the diagnosis was based on subjective neurological evaluation.

A detailed medical history, physical examination, and 12-lead electrocardiography were obtained from all patients. Serum troponin I levels were analyzed by the Siemens ADVIA Centaur hs-cTnI assay (Siemens Healthcare Diagnostics, Deerfield, IL, USA), and a hs-cTnI > 0.04 ng/mL was accepted as elevated in our laboratory. A detailed transthoracic examination was performed using a commercially available system (Epiq 7, Philips Healthcare, Andover, MA, USA) by an experienced cardiologist within the first three days following acute ischemic stroke. Conventional left ventricular echocardiographic parameters were measured according to the standard recommendations and LVEF was calculated using the biplane Simpson method [12].

Heart Rate Variability Assessment

The ECG raw data was recorded in three channels using a miniature ambulatory ECG monitoring device, 'DMS 9800' for 24 hours and were digitalized with a 128-Hz sampling rate. HRV indices were measured with commercially available HRV software (Cardioscan 12.0, DMS, USA). Time domain and frequency domain analyses were performed using this software. For time domain analysis, the standard deviation of all normal-to-normal RR intervals (SDNN) and standard deviation of the mean of all 5-minute segments of normal RR intervals (SDANN) indicate sympathetic activity. As well as the root mean square of the sum of the differences of adjacent normal to normal R-R intervals (RMSSD) and the percentage of interval differences of adjacent normal-to-normal R-R intervals greater than 50 msec (pNN50) indicate the vagal influence on HRV. For frequency domain analysis, total power represents variations between normal-to-normal RR intervals, and low-frequency power (LF: 0.04–0.15 Hz) represents a combination of sympathetic and parasympathetic activity with a slight predominance of the first one. High-frequency power (HF: 0.15–0.40 Hz) indicates vagal activity, while LF/HF represents sympathovagal balance in favor of sympathetic activity [13].

During the recording period, participants were encouraged to continue their daily activities. Five of the ambulatory ECG monitoring revealed more than 2% artifact beats per total beats and the recordings were excluded from the analysis.

Table I. *The characteristics and laboratory parameters of the ischemic stroke patients according to troponin levels*

	Stroke patients with elevated troponin (n= 20)	Stroke patients with normal troponin (n= 46)	P
Age (years)	67.9 ± 15	64.3 ± 13	0.326
Male sex (n – %)	12 (60%)	27 (58.7%)	0.921
BMI (kg/m^2)	26.4 ± 3.9	27 ± 4.5	0.578
NIHSS	5.05 ± 3.3	5.1 ± 2.5	0.889
Hypertension (n – %)	15 (75%)	32 (69.6%)	0.654
Diabetes (n – %)	9 (45%)	20 (43.5%)	0.909
Hyperlipidemia (n – %)	4 (20%)	13 (28.3%)	0.481
Coronary artery disease (n – %)	9 (45%)	10 (21.7%)	0.055
Chronic kidney failure (n – %)	2 (10%)	3 (6.5%)	0.635
Glucose (mg/dL)	136.6 ± 65.3	118.5 ± 49.4	0.283
Creatinine (mg/dL)	1.01 ± 0.35	0.9 ± 0.40	0.061
Total cholesterol (mg/dL)	185 ± 55.4	199.2 ± 44.8	0.277
LDL cholesterol (mg/dL)	114 ± 50.9	127 ± 35.9	0.239
HDL cholesterol (mg/dL)	38.7 ± 10.5	42.4 ± 11.6	0.221
hs-CRP (mg/L)	21.6 ± 30.8	16.2 ± 24.6	0.353
hs-cTnI (ng/mL)	0.45 ± 0.77	0.01 ± 0.007	**<0.001**
NT-proBNP (pg/mL)	3119 ± 6955	1581 ± 3341	**0.036**
ST segment/T wave changes (n – %)	17 (85.2%)	17 (37.8%)	**<0.001**
Beta-blocker usage (n – %)	8 (40%)	10 (21.7%)	0.126

BMI: Body mass index, NIHSS: National Institutes of Health Stroke Scale, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, hs-CRP: High sensitive C reactive protein, hs-cTnI: High sensitive cardiac troponin I, NT-proBNP: N terminal pro-brain natriuretic peptide

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Statistical Analysis

Statistical analyses were performed by statistical software (SPSS 21.0 for Windows, Chicago, IL, USA). Continuous variables were checked for normal distribution by the Kolmogorov-Smirnov test and were expressed as mean ± standard deviation. Where appropriate, differences between continuous variables were tested using the independent sample Student's t-test or Mann–Whitney U test. Categorical data were presented as numbers or percentages and the Chi-square test was used for the comparison of categorical variables. A value of $P<0.05$ was considered statistically significant.

3. RESULTS

Sixty-six acute ischemic stroke patients (mean age: 65 ± 13 years; 39 male) were consecutively included in the study. Twenty patients (30.3%) had elevated hs-cTnI. The general characteristics and laboratory parameters of the patients according to troponin levels are shown in Table I. Although, there were not any significant differences in the general characteristics of the patients, the stroke patients with elevated hs-cTnI had significantly higher NT-proBNP and higher frequency of ST segment/T wave changes compared to those with normal hs-cTnI. There were no significant differences in the NIHSS scores and beta-blocker usage among patients with different troponin levels. Although, the frequency of coronary artery disease was numerically higher in the troponin-positive group, this difference could not reach statistical significance.

Table II. *The conventional transthoracic echocardiographic measures and ambulatory ECG monitoring parameters of the ischemic stroke patients according to troponin levels*

	Stroke patients with elevated troponin (n= 20)	Stroke patients with normal troponin (n= 46)	P
Left atrium (mm)	47.5 ± 10.6	40.6 ± 7.3	**0.020**
LAVI (mL/m²)	31.3 ± 10.9	23.6 ± 10.1	**0.013**
LVEDD (mm)	50.4 ± 9.5	46.8 ± 6.2	0.217
LVESD (mm)	34.2 ± 10.7	31.2 ± 7.5	0.499
IVS (mm)	13.6 ± 2.4	12.5 ± 2.1	0.255
PW (mm)	10.8 ± 1.3	10.3 ± 1.4	0.178
LVEF (%)	50.3 ± 13.5	58.4 ± 8.9	**0.028**
E/e'	10.9 ± 5.4	9 ± 3.1	0.422
AES (n)	952 ± 1298	1780 ± 3225	0.409
SDNN	102.4 ± 48.9	95.5 ± 34.5	0.515
SDANN	122.2 ± 90.2	87.4 ± 45.4	0.163
RMSSD	45.9 ± 71.6	34.5 ± 29	0.989
pNN50	11.03 ± 18.3	11.8 ± 19	0.784
LF/HF	2.5 ± 1.1	1.7 ± 1.04	**0.011**

LAVI: Left atrial volume index, LVEDD: Left ventricular end-diastolic diameter, LVESD: Left ventricular end-systolic diameter, IVS: Interventricular septum thickness, PW: Posterior wall thickness, LVEF: left ventricular ejection fraction, E/e': the ratio of early diastolic mitral inflow velocity to early diastolic mitral annulus velocity, AES: Atrial extrasystole, SDNN: standard deviation of all normal-to-normal RR intervals, SDANN: standard deviation of the average NN intervals for each 5 min segments, RMSSD: root mean square of differences of adjacent normal-to-normal RR intervals, pNN50: percentage of successive RR intervals that differ by more than 50 msec, LF/HF: Low frequency/high frequency

The conventional transthoracic echocardiographic parameters and ambulatory ECG monitoring parameters of the patients are listed in Table II. The troponin-positive group had significantly larger left atriums and lower LVEF values compared to patients with normal troponin levels. LF/HF value was significantly higher in the troponin-positive group, but other ambulatory ECG monitoring parameters such as SDNN, SDANN, RMSSD, and pNN50 were similar among patients.

4. DISCUSSION

In our study, we evaluated the HRV in neurogenic stunned myocardium. We found sympathetic overactivity in patients with high troponin levels, which is an important result in terms of demonstrating the presence of autonomic dysregulation in these patients.

Heart rate variability, the beat-to-beat change in heart rate, provides information about the autonomic nervous system function [9]. HRV parameters are altered in different stages of ischemic stroke and have a prognostic value [7]. Autonomic dysregulation seen after acute ischemic stroke, is associated with non-neurological deaths [7]. Changes in autonomic functions due to cardiac reasons or infection in the acute phase of stroke reduce HRV [7,14]. In these patients, time domain measures of HRV such as SDNN, SDANN, and RMSSD are lower, and frequency domain measures exhibit low-frequency predominance [7]. The HF part of the HRV shows parasympathetic activity and the LF part shows sympathetic activity. In our study, we could not find the difference between the groups in terms of time domain measures, but we found an increased LF/HF ratio indicating sympathetic overactivity. Approximately 60% of stroke cases have an increased LF/HF ratio indicating higher sympathetic activity [15]. There are conflicting findings as to which of the right and left hemisphere involvement has more sympathetic overactivity [16,17]. Due to the small number of patients in our study, we could not show the relationship between stroke localization and sympathetic overactivity.

Neurogenic stunned myocardium is diagnosed with elevated troponin levels, ECG changes, and decreased LVEF values. In our study, we found that while the frequency of coronary artery disease was similar between the groups, the LVEF values were significantly lower and the frequency of ST segment-T wave changes was higher in the troponin-positive group. One of the important features of NSM is reversible left ventricular dysfunction, but we could not demonstrate the reversibility in LVEF value since our study was not a follow-up study.

Neurogenic stunned myocardium is defined as one of the stress-related cardiomyopathies, and the underlying mechanism is not clearly known [18]. The mechanisms that may be the cause of myocardial dysfunction in acute stroke are sympathetic overactivity, local catecholamine release from nerve endings in the myocardium, neurohormonal activation, and autonomic nervous system dysregulation [1,19,20]. Depending on the acute trigger, the dominant sympathetic response can be of two types, neural and adrenal [21]. The neural type is catecholamine

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release from local nerve endings in the myocardium, while the adrenal type is increased catecholamine release in blood circulation [21]. Unopposed sympathetic overactivity is thought to cause dysfunction as a result of uncontrolled inflammation in the myocardium [3,6]. On the other hand, sympathetic overstimulation and hypercatecholamine can also cause reversible myocardial damage through coronary spasm, endothelial dysfunction, and microcirculation involvement [22].

A high NIHSS score was found to be correlated with autonomic dysfunction characterized by decreased parasympathetic tone and it was suggested that it can be used in risk stratification of cardiovascular events [23]. Also, in another study reduced HRV is associated with stroke severity [24]. Although, we found increased sympathetic activity in patients with high troponin levels in our study, we could not show any difference between the groups in terms of NIHSS.

Heart rate variability parameters change after an ischemic stroke and decreased parasympathetic activity and unopposed sympathetic activation may persist until the chronic phase of the stroke [25]. HRV analysis in patients with ischemic stroke can be used as a prognostic tool to understand the pathophysiological mechanisms of autonomic disorders common after stroke [7]. With future research on HRV, we can better understand the heart and brain interactions and obtain better prognostic value.

Study Limitations

The first limitation of our study was the lack of a consensus definition of NSM. In our study, we used elevated troponin levels as a sign of NSM as most studies defined NSM based on data from troponin, ECG, and transthoracic echocardiogram. The second limitation was a small sample size, and the study was a single-center study. Another limitation of the study was that we did not repeat transthoracic echocardiography to evaluate the reversibility of LV dysfunction.

Conclusion

In acute ischemic stroke, troponin levels may be elevated, which may be accompanied by a decrease in LVEF and ST segment-T wave changes and these may be a sign of NSM. Although, the pathophysiology of NSM is not fully understood, autonomic dysfunction characterized by sympathetic overactivation may play an important role. Therefore, HRV analysis in ischemic stroke patients is a valuable approach to understand the underlying pathophysiological mechanisms of NSM.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the ethics committee of the Marmara University School of Medicine on 11.02.2022 (approval number: 09.2022.282). The investigation conformed to the principles outlined in the Declaration of Helsinki. All participants gave written informed consent.

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Conflict of Interest: None declared.

Authors Contributions: CI: Concept and design, IM: Supervision, CI and ZD: Data collection and/or processing, analysis and/or interpretation, CI: Literature search and writing, CI, ZD and IM:Critical review. All authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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Predictors of outcomes in patients with candidemia in an Intensive Care Unit

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ABSTRACT

Objective: Candidemia is a life-threatening infection that causes high mortality rates in intensive care units (ICUs). This study aims to evaluate predictors of the outcome of patients with candidemia in an ICU.

Patients and Methods: This observational, retrospective study included patients with *Candida* bloodstream infection (BSI) in ICUs between 6 years of the episode. A binary logistic regression analysis was conducted to inspect the association with mortality.

Results: The median age of 74 patients was 68.5, and 53.8% were men. *C. parapsilosis* was the most frequently isolated fungal species. The 30-day mortality rate was 50%. In the logistic regression model the Acute Physiology and Chronic Health Evaluation (APACHE) II score, positive blood culture on the seventh day, inotropes needed on the day of blood culture positivity, and ventilator-associated pneumonia (VAP) were significant risk factors for the outcome of patients. There was no difference in mortality between an early start of antifungal treatment or central venous catheter removal time.

Conclusion: A shift to *C. parapsilosis* is observed in this study. Host-related factors such as APACHE II score, need for mechanical ventilation or need for inotropes affect mortality more than early treatment and source control in patients with *Candida* BSI.

Keywords: *Candida*, Candidemia, Intensive Care Unit, Mortality, Outcome

1. INTRODUCTION

Candidemia is a bloodstream infection (BSI) caused by the yeast Candida. This infection is the fourth most common cause of healthcare-associated BSI, especially in patients admitted to intensive care units (ICU) [1]. It is estimated that the number of candidemia cases is nearly 25000 per year worldwide, and the attributable mortality rate rises by 30%-40%, especially in ICU patients [2]. Candidemia causes increased hospital costs and deaths in ICU patients. In these high-risk groups, the development of septic shock associated with candidemia, insufficient source control, and delay in antifungal treatment for more than 24 hours raise the mortality to 100% [3].

The most commonly isolated species of *Candida* BSI are *C. albicans*, *C. glabrata*, and *C. parapsilosis*. The *C. albicans* strain is predominant worldwide. However, some institutions have recently reported an increase in non-*albicans* strains in bloodstream isolates [4]. This increase in non-*albicans* strains, often resistant to antifungal drugs, has been a growing trend in all hospitals worldwide in recent years [4].

Several factors are associated with candidiasis, such as the use of broad-spectrum antibiotics, total parenteral nutrition (TPN), immunosuppressive status, recent abdominal surgery, and the presence of a central venous catheter (CVC) [5]. Early initiation of appropriate and adequate antifungal therapy in patients with *Candida* BSI is associated with a 50% reduction in mortality [6]. It has been reported in previous studies that early removal of a CVC leads to source control and betters outcomes for ICU patients with candidemia [3,7]. In recent years, some experts have shown that host-related factors might have a more crucial effect on the mortality of patients than delayed treatment or early catheter removal [8-10].

The present study aims to evaluate the epidemiologic data of our hospital, changes in the distribution of *Candida* strains over the years, and risk factors associated with the survival of patients with candidemia in the ICU.

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2. PATIENTS and METHODS

This was a retrospective, single-center, observational study conducted on adult hospitalized ICU patients with Candida BSI between April 2014 and January 2021. The ethics committee of our hospital approved this study on June 17, 2021 (approval no. B10.1.TKH.4.34.H.GP.0.01/197).

The following medical data of patients were collected from the hospital database: age, sex, comorbidities, and predisposing risk factors on the admission day to the ICU. *Candida* species (spp.) growth in at least one of the blood culture bottles in patients with clinical signs were defined as candidemia. If the blood culture specimen yielded a *Candida* spp. after 30 days in the same patient, it was considered a new infection. Early antifungal therapy was defined as the start of effective antifungal treatment within 48 hours after the first blood culture was drawn.

Ventilator-associated pneumonia (VAP) was defined as a new or progressive pulmonary infiltrate after at least two days of invasive mechanical ventilation (IMV) with clinical signs of infection. Prior corticosteroid use was defined as the use of a prednisolone 15 mg/day for more than two weeks.

Patients who were < 18 years of age, those transferred to another hospital within 30 days, and those who died before blood culture positivity were excluded from the study. Additionally, patients who developed candidemia before or within the first 48 hours of ICU admission were excluded. Because coronavirus disease 2019 (COVID-19) infection may be a confounding factor, ten patients who were monitored in intensive care units for COVID-19, developed candidemia, and died in 2019 were omitted from the study (Figure 1). The primary outcome was the 30-day survival of patients after culture positivity.

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (VITEK MS [bioMérieux, France]), was used to isolate *Candida* species from blood culture bottles. Antifungal susceptibility testing was performed with a commercial kit based on colorimetric microdilution (Sensititre YeastOne antibiotic susceptibility test colorimetric plate [TREK Diagnostic Systems, Cleveland, OH, USA]) for some of the isolates, and an automated system (VITEK 2; bioMérieux, France) for the other isolates. The detected minimum inhibitory concentration values were interpreted by the Clinical and Laboratory Standards Institute guidelines [11]. The roll-plate semiquantitative method was used for catheter tips.

Statistical analysis

Statistical analysis results were presented as mean ± standard deviation and median (interquartile range [IQR]) for quantitative data and frequency (percent) for categorical data. The Shapiro-Wilk test was used to test whether data followed a normal distribution. The Mann-Whitney U test was used to compare non-normally distributed data, and the independent two-sample t-test was used to compare normally distributed data. Chi-square and Fisher's Exact tests were used to compare categorical variables for mortality. Risk factors affecting survival were analyzed by binary logistic regression analysis. P values < 0.05 was taken as statistically significant. IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY) was used for statistical analysis.

3. RESULTS

Seventy-four patients with candidemia, admitted to the ICU over six years, were included in the study. The median age of the participants was 68 years (IQR 23-95), and 59.4% were men. Of 74 patients, 31% needed inotrope infusion, and 54% required IMV during their ICU stay. The median Acute Physiology and Chronic Health Evaluation (APACHE) II score was 23.6 (8.7) during admission to the ICU. The median duration of a hospital stay was 32 days (IQR 2-64), and an ICU stay was 26 days (IQR 3-135) before blood culture positivity. In our patients, predisposing factors for candidemia were the use of TPN (71.6%), intravenous corticosteroids (35.1%), proton pump inhibitors (100%), and carbapenem (37%). The rate of abdominal surgery within one month was 28.3%. Thirty-eight (51.3%) of the patients had two or more comorbidities. The most common comorbidities were chronic cardiovascular disease (48.6%), neurological disease (35.1%), malignancy (32.4%), and diabetes mellitus (32.4%). Ninety-seven percent of patients had a CVC on the day of blood culture positivity (72/74). The CVC could not be removed after candidemia in 13 patients who died due to poor clinical conditions and lack of new vascular access. Table I shows the demographic and clinical characteristics of the patients.

The mortality rate was 50%. In univariate analyses, an increased mortality rate was associated with a higher C-reactive protein (CRP) level (P = 0.016), concomitant VAP (P= 0.01), higher APACHE II score (P= 0.003), need for IMV (P = 0.008), need for inotropes (P< 0.001), CVC removal at any time (P< 0.001), and positive blood culture on the seventh day despite starting antifungal treatment (P < 0.001). The days before CVC removal, CVC removal within 48 hours, starting early antifungal therapy (within 48 hours), or empirical treatment with fluconazole were not associated with mortality (Table I).

In the logistic regression analysis, the APACHE II score (odds ratio [OR] 1.135, 95% confidence intervals [CI] 1.028, 1.253), positive blood culture on the seventh day (OR 0.024, 95% CI 0.002, 0.237), need for inotropes at the onset of candidemia (OR 5.937, 95% CI 1.201, 29.35), and concomitant VAP (OR 9.798, 95% CI 1.644, 58.413) were strongly associated with mortality (Table II).

Nine different *Candida* species were identified in 74 candidemia episodes. *C. parapsilosis* (39.1%) was the most common spp. The distribution of species is given in Figure 2. The *albicans*/non-*albicans* ratio has changed over the six years of the study. The prevalence of *C. albicans* accounted for 50% until 2019. Over the last two years, an increasing trend of non-*albicans* spp. was observed, and *C. parapsilosis* has been the predominant isolate. Figure 3 shows the distribution of strains over the years.

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Table I. *Demographic and clinical characteristics of patients with candidemia: 30-day mortality*

Characteristics (N=728)	Total (N=74)	Survived (N= 37)	Non-survived (N= 37)	P value
Male gender, n (%)	44 (59.4)	23 (52.3)	21 (47.7)	.636*
Age (years), (median [IQR])	68.5 (57-84)	66 (48-79)	73 (58-85)	.078▲
≥65, n (%)	41 (55.4)	19 (46.3)	22 (53.7)	.483*
Days of hospital stay, (median [IQR])	32 (2-164)	33 (6-137)	31 (2-164)	.697▲
Days of ICU stay, (median [IQR])	26 (3-135)	28 (5-135)	24 (3-128)	.465▲
Comorbidities, n (%)				
≥2	38 (51.3)	16 (42.1)	22 (57.9)	.163*
Malignancy	24 (32.4)	11 (45.8)	13 (54.2)	.619*
Diabetes mellitus, n (%)	24 (32.4)	11 (45.8)	13 (54.2)	.619*
Neurologic disease, n (%)	26 (35.1)	16 (61.5)	10 (38.5)	.144*
Cardiovascular disease, n (%)	36 (48.6)	15 (41.7)	21 (58.3)	.163*
Renal disease, n (%)	4 (5.4)	1 (25)	3 (75)	.615**
Respiratory disease	10 (7.4)	4 (40)	6 (60)	.496*
Hepatic disease, n (%)	3 (4)	2 (66.7)	1 (33.3)	1.000**
Predisposing factors, (n, %)				
Total parental nutrition, n (%)	53 (71.6)	27 (50.9)	26 (49.1)	.797*
IV Corticosteroids, n (%)	26 (35.1)	13 (50)	13 (50)	1.000*
Abdominal surgery within 1 month, n (%)	21 (28.3)	11 (52.4)	10 (47.6)	.797*
Carbapenem use, n (%)	50 (37)	24 (48)	26 (52)	.619*
CRP level, (median [IQR])	11.2 (8.08-17)	9.2 (7.9-16.2)	13.3 (10-19)	.016*
VAP, n (%)	31 (41.8)	15 (36.6)	26 (63.4)	.010*
Apache II score	23.6 (8.7)	20.6 (8.7)	26.5 (7.8)	.003†
Characteristics after enrollment				
Candida spp.				
Non-*albicans*	47 (63.5)	25 (53.2)	22 (46.8)	.469*
C. albicans	27 (36.5)	12 (44.4)	15 (55.6)	
IMV, n (%)	47 (63.5)	18 (38.3)	29 (61.7)	.008*
Inotropes need at the onset of candidemia, n (%)	32 (43.2)	8 (25)	24 (75)	
CVC removal at any time, n (%)	13 (17.5)	0 (0)	13 (100)	
Days before CVC removal (median [IQR])	3 (1-5)	3 (1-5)	3.5 (2-5)	.423▲
Days before antifungal therapy, (median [IQR])	3 (1-4)	3 (2-4)	2 (1-4)	.204▲
Early antifungal therapy, n (%)	46 (60.5)	26 (44.4)	20 (55.6)	.352*
Empirical treatment with fluconazole	36 (48.6)	10 (25.6)	26 (41.9)	.096*
Positive blood culture on the seventh day, n (%)	18 (24.3)	2 (11.1)	16 (88.9)	
Days before sterile blood culture, (median [IQR])	4 (2-7)	4 (3-6.5)	3 (2-7)	.183▲

**Chi-square test, **Fisher's Exact test, ▲ Mann-Whitney U test, †independent two-sample t-test, VAP, Ventilator-associated pneumonia; IMV, Invasive mechanical ventilation; CVC, central venous catheter; ICU: Internal Care Unit*

Table II. *Multivariate logistic regression analysis of risk factors for 30-day mortality*

	OR (95% CI)	P value
Number of comorbidities	0.987 (0.366 – 2.659)	.979
Malignancy	1.237 (0.237 – 6.458)	.801
Days of ICU stay	1.008 (0.985 – 1.032)	.504
Apache II score	1.135 (1.028 – 1.253)	.012
Empirical antifungal therapy within 48 hours	0.378 (0.08 – 1.79)	.220
Positive blood culture on seventh day	0.024 (0.002 – 0.237)	.001
Inotrope need at the onset of candidemia, n (%)	5.937 (1.201 – 29.35)	.029
Chronic cardiovascular disease, n (%)	1.789 (0.195 – 16.428)	.607
Abdominal surgery within 1 month, n (%)	2.104 (0.367 – 12.053)	.404
VAP, n (%)	9.798 (1.644 – 58.413)	.012
Empirical treatment with fluconazole	0.314 (0.062 – 1.584)	.161

VAP, Ventilator associated pneumonia; ICU: Internal Care Unit

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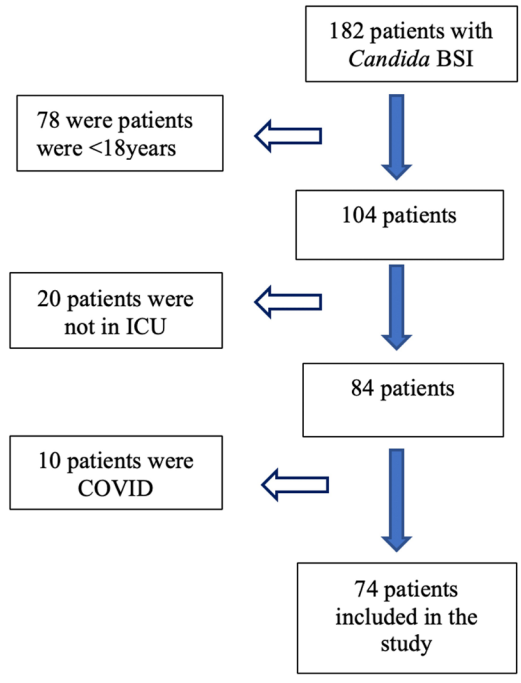

Figure 1. *Patients' inclusion diagram*

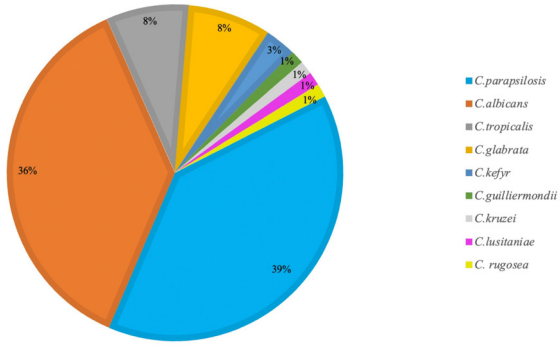

Figure 2. *Candida species distribution*

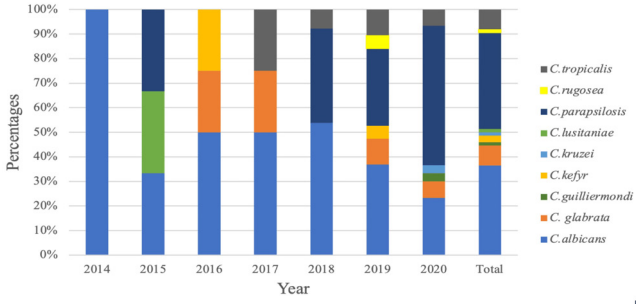

Figure 3. *Distribution of Candida species over the years*

4. DISCUSSION

A higher APACHE II score, positive blood culture on the seventh day after initiation of antifungal treatment, need for inotropes at the onset of candidemia, and a concomitant diagnosis of VAP were identified as risk factors for mortality. A high mortality rate was observed in the patients. *C. parapsilosis* was a commonly isolated species during the study period.

Mazzanti et al., showed that the mortality rate associated with candidemia was higher in ICU than in non-ICU patients (41% vs. 23%) [12]. Some investigators have reported that the overall mortality rates increase by up to 50% in critically ill patients and up to 70% in patients with septic shock [3,9]. The overall 30-day mortality rate was 50% in this study. Other investigators have reported similar mortality rates in ICU patients (52.7% and 51.2%) [12,13]. On the other hand, Tigen et al., report a mortality rate of 83.3%, and Karacaer et al., report 78% in ICU patients from studies in Turkey [14,15]. These differences may be associated with the different rates of comorbid illnesses, predisposing factors, patients' clinical conditions, and therapeutic approaches between centers.

Previous studies demonstrate that timely catheter withdrawal positively affects the outcome of patients with candidemia [3,7,16]. However, some authors mention that early CVC removal is not associated with a better outcome [9,10]. A nine-year study from Italy determined that host-related factors have a more significant effect than early removal of CVC on the outcome of ICU patients with candidemia [12]. Additionally, a randomized controlled study did not find any association between CVC withdrawal within 24 or 48 hours and the survival of patients [10]. In this study, an increased mortality rate was associated with CVC removal at any time during candidemia in the univariate analysis (P< 0.001) but was not significant in the multivariate analysis. Kutluay et al., show that the removal of CVC at any time after candidemia was associated with decreased mortality rates [17]. Furthermore, no association is found between CVC removal time and mortality. Since CVC is the source of infection, removal is an essential component of the approach to treating *Candida* infections. Future studies are needed to analyze the optimal time for the removal of the CVC.

In addition to the removal of the CVC, early intervention with appropriate antifungal therapy was generally associated with a better overall outcome. However, in this study, no association was found between starting empirical antifungal therapy within 48 hours and mortality, which is consistent with the findings of Nucci et al. [10]. Current studies have demonstrated that patients with *Candida* BSI are more critically ill than patients with bacteremia [18,19]. Recently published studies suggest that host-related factors significantly affect patients' survival with candidemia [8,9]. In this study, the APACHE II score at diagnosis, underlying diseases, organ support therapies like IMV, and vasopressor use were the most common predisposing conditions associated with candidemia, which might be associated with mortality [9,10]. Other studies show that septic shock is an independent factor for the outcome [12,20]. The authors find that host-related factors, such as the Apache II score,

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inotropes need on the day of candidemia, and a concomitant diagnosis of VAP, are independent risk factors for the outcome. In other studies from Turkey, a higher APACHE II score or SOFA score is associated with mortality [15,17]. The findings of this study show that host-related factors have a major influence on 30-day mortality rather than early treatment.

Follow-up blood cultures were obtained every other day to establish clearance of *Candida* spp. from the bloodstream. A positive blood culture with *Candida* spp. on the seventh day of antifungal treatment was associated with the worst outcome. A multicenter study conducted in South Korea found that patients with a positive blood culture with *Candida* spp. on the seventh day of hospitalization had nearly five times greater mortality risk [21]. The sensitivity of blood culture for diagnosing candidiasis is almost 50%. The persistence of candidemia seems to affect the outcome adversely.

Although, C. *albicans* remains the most isolated species worldwide, the incidence of non-*albicans* species has increased. The distribution of *Candida* species varies over time and geography. The use of fluconazole might affect the geographic differences in species distribution. In a 20-year surveillance study in which 20,788 *Candida* isolates were examined, *C. albicans* (46.9%) was the most isolated strain, and *C. glabrata* (18.7%) was the most common in the non-*albicans* species in all regions, except Latin America [22]. The surveillance studies demonstrated *C. parapsilosis* dominated non-*albicans* species in Latin America and Brazil (26.5% and 24.1%, respectively) [22,23]. A multicenter study from Colombia showed a change in epidemiology, with *C. parapsilosis* (38.5%) being the most identified isolate from 2008 to 2021 [24]. Similarly, *C. parapsilosis* became the predominant species during the last two years of this study. However, *C. albicans* was still the most commonly identified isolate, including in ICUs in most of the studies [14,15,17].

In this study, most patients had a CVC (98%). The association between *C. parapsilosis* and CVC-associated *Candida* BSI has been demonstrated in US hospitals [25]. The fact that most patients had a CVC may explain the rising trend of the *C. parapsilosis* strain in our ICUs. However, the different results in species distribution may be related to the hospital environment, the use of carbapenems or fluconazole, and the ICU settings of countries.

There are some limitations to this study. It was a single-center, retrospective, observational study. Second, only ICU patients were evaluated in this study.

Conclusions

Prospective multicenter randomized trials involving the outcome of a greater number of cases with *Candida* BSI are warranted. A higher APACHE II score, positive blood culture on the seventh day of follow-up, inotrope need at the onset of candidemia, and a concomitant diagnosis of VAP are independent risk factors for mortality in patients with *Candida* BSI. *C. parapsilosis* was the leading non-*albicans Candida* species in this study. It is crucial to closely monitor risk factors, especially host-related factors that significantly affect ICU patients' outcomes with *Candida* BSI.

Compliance with Ethical Standards

Ethical approval: Ethical approval was obtained from Umraniye Training and Research Hospital, Clinical Research Ethics Committee (approval no: B10.1.TKH.4.34.H.GP.0.01/197).

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Conflict of interest: The authors declare that they have no conflict of interest.

Authors Contributions: ASO: Concept and design, LNA and MA: Collecting data, BES and MA: Analysis and interpretation. All authors read and approved the final version of the manusctipt.

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Congenital *cytomegalovirus* infection cases and follow-up findings in Antalya, Turkey

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ABSTRACT

Objective: To investigate the presence of congenital *cytomegalovirus* (cCMV) infection and the *CMV*-DNA virus in the newborns who applied for newborn hearing screening test (NHST) and *CMV*-DNA viruria with physical, mental-motor development and hearing status of c*CMV* cases in the second year of age.

Patients and Methods: *CMV*-DNA was investigated in 1150 newborns' oral swabs (0-21 days) by polymerase chain reaction kit and urine of patients with positive *CMV*-DNA in saliva. Transient Evoked Otoacoustic Emission test was performed for NHST.

Results: CMV-DNA was posititve in saliva of 38 (3.3%) newborns and urine of 10 out of 37 newborns. The prevalence of c*CMV* was 0.87% (95% CI=0.697-1.042). All newborns passed the NHST. In newborns with c*CMV*:jaundice in 60% (6/10), low birthweight in 40% (4/10), small for gestational age in 50% (5/10) of them. Jaundice was the most significant variable (P<0.001, OR:23.411, 95% CI=5.772-94.960) and bilirubin levels were slightly elevated. In the second year of 8 cases, *CMV*-DNA viruria was detected in all of them and sensorineural hearing loss was detected in one infant.

Conclusion: The c*CMV* infection rate is 0.87% in a population with high maternal seropositivity.When diagnosing c*CMV*, saliva may give false-positive results and urine should be tested. Bilirubin levels may not be as high as expected in c*CMV* cases in highly seroimmune populations and sequelaes may occur in the following years.

Keywords: Congenital CMV infection, High seroprevalence population, Urine, Saliva, CMV-DNA PCR

1. INTRODUCTION

Cytomegalovirus (*CMV*) is the most common cause of congenital infections worldwide [1]. To date, the factors associated with intrauterine transmission of *CMV* and the occurrence of disease in the fetal, neonatal or infantile period are not fully defined. However, in both the United States and other developed countries, congenital *CMV* (c*CMV*) is known to be a leading cause of neurologic disease and sensorineural hearing loss (SNHL) in children [1,2]. Furthermore, intrauterine *CMV* transmission to the fetus has been demonstrated in *CMV* seropositive pregnancies, and no difference has been found between primary and nonprimary maternal *CMV* infections in the severity and prognosis of symptomatic c*CMV* infection [3]. In recent years, the incidence of c*CMV* infection has been associated with the epidemiological characteristics of each community. It has been reported that c*CMV* infection rates were higher in populations with high seroprevalence [4].

Various data are available from developed countries on the prevalence of c*CMV* infection and its impact on newborns [4]. The prevalence of c*CMV* infection is approximately 0.6% – 0.7% in developed countries with low maternal seroprevalence [1]. However, these specific data are limited to developing countries [4]. Data from developed countries where *CMV* seroprevalence is low or moderate may not correlate with data from developing countries with high seroprevalence. Therefore, it seems necessary to investigate the health problems caused by c*CMV* infection in developing countries. When the data from different study groups are analyzed, Turkey is among the countries with

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a high seroprevalence of *CMV* that ranges between 93.6% and 100% [5-8]. Despite the high *CMV* seroprevalence, the prevalence of c*CMV* infection was reported to be 1.91% and 0.2% in two different studies from different geographical areas of Turkey [9,10].

The effects of c*CMV* infection in newborns are observed in a broad clinical spectrum; more than one symptom and/or anomalies affecting the central nervous system may occur during birth or lead to long term late-onset sequelae. The most common long-term sequela is SNHL [11]. In the long-term, hearing loss may follow a progressive or fluctuating course [12]. Psychomotor and cognitive impairments are observed in most symptomatic cases and visual impairments occur in almost half of the symptomatic cases [11].

This study aimed to investigate the prevalence of c*CMV* infection in newborns admitted for newborn hearing screening test (NHST) and the presence of *CMV*-DNA viruria with physical, mental-motor development and hearing status of c*CMV* cases in the second year of age.

2. PATIENTS and METHODS

Newborn period

Between January 2013 and May 2014, a total of 1150 (561 girls and 589 boys) newborns (age range: 0 – 21 days) were enrolled in the study who were admitted to Akdeniz University Medical Faculty, Otolaryngology Clinic for NHST. A NHST is mandatory during the newborn period (0 – 28 days) in Turkey. The order of admission was considered in the selection of the study group. In addition, including newborns into the study in the first three weeks of life was a criterion. Parents were informed about the study and written informed consent was obtained. Subsequently, saliva samples were collected from the newborns after NHST. The characteristics of the newborns (gestational week, birthweight, hyperbilirubinemia, NHST results, and cranial anomalies such as microcephaly/ hydrocephalus) were recorded.

Newborn Hearing Screening Test (NHST)

The NHST was performed in all newborns. For this purpose, a test of Transient Evoked Otoacoustic Emission (TEOAE) (Accuscreen, Madsen, Denmark) was performed [13]. In those who failed the TEOAE test, the Auditory Brainstem Response (ABR) test (Acuscreen, Madsen, Denmark) was performed [13]. The ABR test is only applied to newborns who failed the TEOAE test in our country. In addition, the ABR test was performed immediately based on TEOAE test results at the second-year admission of c*CMV* cases as part of the screening programme.

Laboratory tests

Saliva samples from the newborns were collected with sterile swabs (Copan Diagnostics, Italy), placed in sterile plastic tubes containing viral transport medium (Copan Diagnostics, Italy) and delivered to the laboratory. The median day of saliva collection was 1 day (range: 0 – 21 days) for all newborns. *CMV*-DNA extraction was done with a commercial kit (High Pure Viral Nucleic Acid Kit, Roche Diagnostics, Germany) and *CMV*-DNA was analyzed in the saliva samples using real-time quantitative polymerase chain reaction (RT-qPCR) method (LightMix Human *Cytomegalovirus*, TIB MOLBIOL, Germany). Subsequently, urine samples of newborns whose saliva was positive for *CMV*-DNA were tested for the presence of *CMV*-DNA using the same method. Urine samples were collected within the first three weeks of life. Data of total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), complete blood count (CBC) and clinical findings of newborns diagnosed with c*CMV* infection were retrospectively analyzed.

Definitions of patients

The c*CMV* infection was diagnosed when *CMV*-DNA was detected in the newborn's urine. At least one of the following physical findings; cranial anomaly (microcephaly/ hydrocephaly), neurologic findings (lethargy/hypotonia, seizures) or radiologic findings such as chorioretinitis, intracranial calcification, intrauterine growth retardation, hepatomegaly, splenomegaly or laboratory findings of a direct bilirubin level higher than 2 mg/dL, ALT, AST or GGT levels at least twice the average and thrombocytopenia (<100000 mm^3/ mL) were considered evidence of symptomatic infection [14]. A birthweight of less than 2500 grams was diagnosed as low birthweight (LBW) irrespective of the week of gestation. Small for gestational age (SGA) was defined as birthweight below the 10^{th} percentile of the corresponding gestational week.

Evaluation of cCMV cases in their second year of life

The children diagnosed with c*CMV* infection were recalled in their second year of life to assess their physical, mental-motor development and hearing status. In our study, it was decided to follow-up on the c*CMV* cases at the age of two for research purposes. The complete audiological screening was performed and physical development was evaluated in c*CMV* cases at their admission in the second year of life following postpartum screening. *CMV*-DNA was measured by RT-qPCR (COBAS AmpliPrep/COBAS TaqMan, Roche Diagnostics) in urine samples. In addition, CBC and liver function tests were analyzed. Hearing status was determined in all infants using the TEOAE test, followed by an ABR test for those who failed the TEOAE test.

Statistical analysis

Data were analyzed in SPSS for Windows 23.0. Pearson chi-square test, Fisher's exact test, and Mann – Whitney *U* test were used to compare the results. Logistic regression was used to predict the relations between independent variables and binary logistic regression for dependent variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to measure associations. $P < 0.05$ was considered significant.

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3. RESULTS

Newborn period

CMV-DNA was detected in the saliva samples of 38 (n = 1150, 3.31%) newborns. Urine samples were collected from 37 newborns and 10 were positive for *CMV*-DNA. The prevalence of c*CMV* infection was 0.87% (95% CI = 0.697 – 1.042) in our study group. The median day of saliva collection was 4 days (range: 0 – 18 days) in *CMV*-DNA detected newborns. The urine sample was collected within a few days after the saliva sample collection. The median day of urine collection was 6 days (range: 1 – 20 days) in newborns with c*CMV*. The characteristics of the newborns are summarized in Table I.

A total of 6 (60%) newborns were defined as symptomatic c*CMV* infected: Two with SGA, two with LBW and SGA, one with LBW, SGA and microcephaly, and one newborn with elevated GGT (three times higher than average), ALT/AST (two times higher than average) levels and thrombocytopenia (48000/mm3). Six infants had jaundice, but direct bilirubin levels were lower than 2 mg/dL. At birth, jaundice was 60% (6/10) and 5% (55/1140) (P < 0.001), LBW was 40% (4/10) and 12.5% (143/1140) (P = 0.029), and SGA was 50% (5/10) and 17% (194/1140) (P= 0.018) in newborns with and without c*CMV* infection, respectively. In multivariate analysis, the presence of jaundice was the most significant variable (P< 0.001, OR:23.411, 95% CI = 5.772 – 94.960) (Table II).

All newborns with c*CMV* infection passed the initial NHST. No significant relationship was found between sex, gestational week, presence of microcephaly/hydrocephalus, NHST results and c*CMV* infection. Two fetuses with c*CMV* infection were found to have oligohydramnios during pregnancy. Postnatal ophthalmologic examination of the children who participated in the study revealed no pathologic findings.

Main findings in children with cCMV

Only eight families agreed to be included in the screening program. Thus, 8 out of 10 c*CMV* cases were screened for c*CMV*-related complications in the second year of life. *CMV*-DNA was detected in the urine of eight c*CMV* cases. Case 9 had moderate mental-motor growth retardation and strabismus, enlargement of the lateral ventricles and mineralization of brain tissue on magnetic resonance imaging. Case 1 did not pass the TEOAE test in the left ear when she was admitted for screening at age two, although she had passed the initial hearing screening test with TEOAE for both ears in the newborn period. Then an ABR test was performed, which revealed a SNHL of 90 dB in the left ear. Laboratory screening revealed that four infants had slightly elevated AST levels (Table III).

Table I. *The characteristics of newborns with cCMV infection*

Case No	Sex	Birthweight (g)	Gestational week	LBW	SGA	Urine *CMV*-DNA	Jaundice	Total Bilirubin (mg/dL)[a]	Direct Bilirubin (mg/dL)[b]	ALT (U/L)[c]	AST (U/L)[d]	GGT (U/L)[e]	Microcephaly	Hearing Loss	Symptom
1	F	2100	40	+	+	+	+	4.61	0.26	N	N	N	-	-	+
2	F	3000	40	-	+	+	-	NA	NA	NA	NA	NA	-	-	+
3	F	3000	40	-	+	+	+	12.38	0.48	N	N	N	-	-	+
4	M	3580	39	-	-	+	+	13.94	0.39	N	N	N	-	-	-
5	M	3900	39	-	-	+	-	NA	NA	NA	NA	NA	-	-	-
6	M	4170	40	-	-	+	-	NA	NA	NA	NA	NA	-	-	-
7	M	1406	29	+	-	+	+	5.28	0.25	N	N	N	-	-	-
8	M	1744	34	+	+	+	+	13.90	0.39	N	N	N	-	-	+
9	M	1340	32	+	+	+	-	NA	NA	NA	NA	NA	+	-	+
10	F	3310	38	-	-	+	+	17.80	0.80	44	59	135	-	-	+[#]

cCMV: Congenital Cytomegalovirus, F: Female, LBW: Low birthweight, M: Male, N: Normal, NA: Not applied, SGA: Small for gestational age, aTotal bilirubin reference range: 0,1 – 1,2 mg/dL, bDirect bilirubin reference range: 0,0 – 0,2 mg/dL, cALT reference range: 0 – 33 U/L, dAST reference range: 0 – 32 U/L, eGGT reference range: 5 – 40 U/L, # with thrombocytopenia

Table II. *The relation of cCMV infection with presence of LBW, SGA, and jaundice*

	n (%)*	Odds Ratio and 95% Confidence Interval	Multivariate analysis	Univariate analysis, n(%)
LBW	4 (40)	OR: 0.880, 95% CI (0.118 – 6.554)	P = .901	P< .001, 143 (12.5)
SGA	5 (50)	OR: 3.295, 95% CI (0.669 – 16.219)	P = .143	P = .029, 194 (17)
Jaundice	6 (60)	OR: 23.411, 95% CI (5.772 – 94.960)	P < .001	P= .018, 55 (5)

cCMV: Congenital Cytomegalovirus, LBW: Low birthweight, SGA: Small for gestational age
** Distribution of cCMV cases according to symptoms.*

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Table III. *The characteristics of children aged two with cCMV infection*

Case No	Urine *CMV*-DNA		Hearing Loss		Mental Motor Retardation		Growth Retardation		ALT* (U/L)		AST# (U/L)	
	Newborn	2nd year	Newborn	2nd year	Newborn	2nd year	Newborn	2nd year	Newborn	2nd year	Newborn	2nd year
1	+	+	-	+	-	-	-	-	N	N	N	42
2	+	+	-	-	-	-	-	-	NA	N	NA	35
4	+	+	-	-	-	-	-	-	N	N	N	N
5	+	+	-	-	-	-	-	-	NA	N	NA	N
7	+	+	-	-	-	-	-	-	N	N	N	44
8	+	+	-	-	-	-	-	-	N	N	N	39
9	+	+	-	-	–	+	–	+	NA	N	NA	N
10	+	+	-	-	-	-	-	-	44	NA	59	NA

cCMV: Congenital Cytomegalovirus, N: Normal, NA: Not applied
*Case no 3 and 6 did not apply for 2nd year follow-up *ALT reference range: 0 – 33 U/L,*
#AST reference range: 0 – 32 U/L

4. DISCUSSION

The prevalence of c*CMV* infection was found to be 0.87% in our study group, which is the largest study group in Turkey to investigate c*CMV* prevalence. There are only two studies on the prevalence of c*CMV* infection in our country and very different results were found. In the first study, Şahiner et al., reported that the prevalence of c*CMV* infection in 944 newborns with *CMV*-DNA PCR in saliva was 1.91% [9]. This study reported that the *CMV*-DNA PCR test in urine was negative in five out of 18 infants who had positive *CMV*-DNA PCR test in saliva. In 2017, the European Expert Statement on Congenital *CMV* Diagnosis and Management reported that a single negative *CMV*-DNA result in urine is sufficient to rule out c*CMV* infection [14]. When the study of Şahiner et al., was evaluated according to this criterion, the prevalence of c*CMV* infection was 1.38% [9]. The rate was higher compared to our results. In the second study, Zeytinoglu et al., found that the prevalence of c*CMV* infection was 0.2% in 1000 newborns [10]. This rate seems low for a population with high seroprevalence and is lower than the rate we found. These differences could be due to the different methods used. To clarify, unlike the phenol-chloroform extraction and in-house PCR assay used by Şahiner et al.[9], standardized commercial kits were used in our study. The saliva collection method in Zeytinoglu et al.'s study differed from ours, and they did not use a standardized commercial kit for *CMV*-DNA isolation [10]. In addition, altering of the *CMV* seropositivity of mothers according to different ethnicities, races, and socioeconomic statuses in the same geography can also affect the epidemiology of c*CMV* infection [15]. In countries with high *CMV* seroprevalence, the prevalence of c*CMV* infection ranges from 0.65% to 5.4%; 0.89% in Mexico, 1.19% in Brazil, 0.7% in Israel, 0.7% in China, 5.4% in Gambia, 2.1% in India, and 0.65% in Iran [16-22].

In this study, *CMV*-DNA was not detected in the urine of 27 (73%) of the 37 newborns whose saliva was positive for *CMV*-DNA. The absence of *CMV* in the urine sample excludes the diagnosis of c*CMV* infection [14]. Previous studies have shown that *CMV*-DNA testing in saliva is as sensitive and specific as *CMV*-DNA testing in urine and that the saliva sample is easier to collect than the urine sample [23]. However, *CMV* also occurs in breast milk and causes postnatal *CMV* transmission [24]. In our country, breastfeeding rates are reported to be 96% – 97.4%[25,26]. Since, breastfeeding rates and early initiation of breastfeeding are high in our country, *CMV*-DNA can be detected in saliva without the presence of congenital infection. Koyano et al., collected urine and saliva samples from newborns whose mothers had positive *CMV*-DNA in their milk immediately before and within 30 minutes after breastfeeding [27]. *CMV*-DNA was found positive only in saliva samples collected after breastfeeding. In our study, breastfeeding status was not recorded when the saliva samples were collected. However, since 73% of the newborns with *CMV* positive saliva did not have *CMV* in their urine, it can be assumed that these positive saliva results are related to breastfeeding, considering the breastfeeding rates in our country. Therefore, urine should be tested for accurate diagnosis of c*CMV* infection in populations with high breastfeeding rates. Koyano et al., also recommended urine sample testing in populations where breastfeeding is common [27]. In addition to specimen selection, it has been reported that the collection of urine specimens on filter paper is sufficient to detect *CMV*-DNA [28].

Microcephaly, thrombocytopenia, SGA and jaundice (direct bilirubin> 2 mg/dL) are the symptoms described in association with symptomatic c*CMV* infection in a recent report [14]. In this study, the rate of symptomatic c*CMV* infection was 60% of newborns with c*CMV* infection. Jaundice in newborns was statistically associated with c*CMV* infection; however, the direct bilirubin level did not exceed 2 mg/dL in any of them. For this reason, jaundice was not considered as the sole symptom. It was reported that about 10% – 15% of infants with c*CMV* had very mild and nonspecific symptoms such as jaundice, hepatosplenomegaly, and LBW. Therefore, *CMV* has not been investigated in these groups of newborns [29]. Immunity in seroimmune mothers may alleviate symptoms in the baby. Fowler et al., reported that the presence of *CMV* antibodies

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before pregnancy protected the fetus from *CMV* infection and reduced the severity of symptoms and sequelae even if they did not protect it [30]. Lilleri et al., showed an early and robust neutralizing antibody response to the gH/gL/pUL128-130-131 pentameric complex in pregnant women who did not transmit *CMV* infection to their babies [31]. In addition, it was reported that the proliferative CD4 T-cell response to the pp65 antigen of the virus was high in pregnant women who did not transmit *CMV* infection to their babies [32]. According to these results, the degree of *CMV* specific maternal immunity may also play a role in the different c*CMV* prevalence rates in the same geography. In our study, maternal serology was not investigated. However, *CMV* seroprevalence is 93.6% in society and 97.4% in fertile women in our city, Antalya [5]. In studies conducted with pregnant women in different cities of our country, the rate of *CMV* seropositivity ranged from 98.3% to 100% [6-8]. Considering these data, all mothers of almost all babies with c*CMV* infection can be considered *CMV* seroimmune.

Although, eight newborns diagnosed with c*CMV* infection passed the NHST, left ear SNHL was detected in one child by ABR test during the screening at two years of age. In this case, symptoms at birth were nonspecific and at first glance did not suggest c*CMV* infection; however, hearing loss developed in the following years. In children, bilateral SNHL is caused by *CMV* in second place after genetic defects in the United States [2]. In our country, the prevalence of deafness in infants with probable c*CMV* infection is reported to be 4.08% [33]. Grosse et al., reported that about 14% of children with c*CMV* infection have permanent SNHL and about half of them are asymptomatic at birth and hearing loss develops later, so these children passed NHST after birth [2]. Fowler et al., reported that 43% of infants with c*CMV* infection who had hearing loss in infancy could not be [34] detected by the NHST. A systematic review by Fletcher et al., reported that 9% – 68% of babies with c*CMV* infection had late-onset hearing loss [35]. The results of these studies also support our findings. When hearing loss related to c*CMV* infection is diagnosed at an early stage, it is reported that the effect can be reduced by antiviral treatment, cochlear implantation and speech therapy [1]. Therefore, asymptomatic newborns with c*CMV* infection should be screened for late sequelae such as hearing loss. Currently, no country has a routine screening program for the diagnosis of c*CMV* infection. It is argued that screening should be targeted at all newborns or those who do not pass the NHST. According to the results of our study and the studies mentioned above, the selection of babies who do not pass the NHST is not sufficient for the diagnosis of c*CMV* infection because the NHST is not able to identify newborns with c*CMV* infection, as found in our study and screening should be applied for all newborns [2,34,35].

In c*CMV* infection, neurological sequelae such as microcephaly, intracranial calcifications, ventricular dilatation and cortical atrophy are encountered [11]. In our study, one patient who had microcephaly in the neonatal period was found to have mental-motor developmental retardation and growth retardation in the second year of follow-up (Case 9). In case of neurological involvement or severe regional organ damage in symptomatic c*CMV* cases, treatment protocols with antiviral agents should be considered, taking into account the possible side effects [14]. However, there was no case receiving antiviral treatment in our study. While it is known that antiviral treatment can improve hearing and neurologic development in the long-term in symptomatic cases, there is no evidence that it prevents the occurrence of late-onset disease in asymptomatic cases of c*CMV* [14].

The main limitation of our study is that the screening program covered only a single time point at two years of age and was not performed for all newborns with c*CMV*.

In children of pregnant women with nonprimary *CMV* infection, c*CMV* infection may present with nonspecific findings and therefore be overlooked after delivery. However, as observed in children of pregnant women with primary *CMV* infection, sequelae may occur in the following years. In our study, bilirubin levels were slightly elevated in c*CMV* cases. This finding may be overlooked in the diagnosis of c*CMV* infection. For this reason, it would be beneficial to implement screening programs in highly seroimmune populations.

Conclusion

This is the first study in our country in which infants with c*CMV* infection were followed-up and their laboratory and clinical findings were recorded in the following years and remarkable results were obtained. The prevalence of c*CMV* infection was 0.87% in a population with high maternal *CMV* seroprevalence. All newborns diagnosed with c*CMV* passed the NHST, and the target of c*CMV* screening should be all newborns. When diagnosing c*CMV* infection, the urine sample should be tested, as saliva samples may give false positive results. However, since it is difficult to collect a urine sample, saliva can be tested first and urine can be examined in those who are positive for *CMV*-DNA. Since, hearing loss develops after two years in a child with nonspecific symptoms at birth, it should be kept in mind that symptoms during birth may be mild in newborns of *CMV* seropositive mothers. More comprehensive and long-term studies can guide c*CMV* infection.

Compliance with Ethical Standards

Ethical approval

This study was approved by the Akdeniz University Ethics Committee (June 19, 2012, Approval Number 208) and conducted following the guidelines of the 1964 Helsinki Declaration. Parents were informed about the study and written informed consent was obtained.

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Conflict of interest

No potential conflict of interest was reported by the authors

Authors Contributions

ZES: Data curation, Formal analysis and Writing-Original draft preparation, BOP: Formal analysis, Visualization, Writing-Original draft preparation, DC: Conceptualization, Methodology, Data curation and Writing-Reviewing and Editing, MT, ABT, NO and ME: Investigation, Data curation, IS, RCS, DM and GO: Formal analysis, Writing – Reviewing and Editing.All authors approved the final version of the manuscript.

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The role of radiocontrast agents in the pulsed radiofrequency treatment of lumbar dorsal root ganglion

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ABSTRACT

Objective: The aim of this study was to investigate the effect of contrast agent use on procedure time and accuracy in pulsed radiofrequency treatment of the lumbar dorsal root ganglion.

Patients and Methods: Patients aged 23–79 years with lumbar radicular pain due to disc herniation for at least 3 months were randomized into two groups of 35 patients each. Patients in both groups underwent fluoroscopy-guided pulsed radiofrequency treatment of the dorsal root ganglion at the level of the L5 foramen. In the radiocontrast group, unlike the control group, the location of the ganglion was determined by administering the contrast agent before the radiofrequency treatment.

Results: Procedure time in the radiocontrast group was significantly longer than in the control group ($P< 0.05$). In 50 cases ganglion was detected in the extraforaminal or intraforaminal location, the excitation of the ganglion in the range of 0.4–0.6 V was significantly higher in the radiocontrast group (95.8%) than in the control group (69.2%) ($P< 0.05$).

Conclusion: The use of radiocontrast material in pulsed radiofrequency application on the dorsal root ganglion prolongs the procedure time. However, for ganglia that cannot be detected by stimulation, contrast injection is useful on procedural accuracy.

Keywords: Dorsal root ganglion, Pulsed radiofrequency therapy, Contrast agent, Lumbar radicular pain

1. INTRODUCTION

Low back pain is a leading cause of disability, with a lifetime prevalence of 40%–70% [1]. Although, there are many causes of low back pain, radicular pain secondary to lumbar disc herniation is one of the most common pathologies [2, 3]. Pulsed radiofrequency (pRF) therapy applied to the dorsal root ganglion (DRG) is an alternative interventional modality in the treatment of lumbar radicular pain not responding to conservative methods and epidural injection treatments [4].

The DRG contains the cell bodies of primary sensory neurons, which transmit sensory information to the spinal cord. The modulation of sensory processing, its role in pain development, and its anatomical accessibility make the DRG an important target for interventional pain management [5]. The pRF technique developed by Sluijter in 1998 prevents tissue damage by ensuring that the temperature does not rise above 42 °C [6]. The electrical field generated by pRF alters the cellular activity in DRG neurons, reduces nociceptive transmission by polarizing cell membranes, and contributes to analgesia [7]. In order to achieve these effects, it has been suggested that the electrode be placed 1–2 cm peripheral to the DRG [8]. However, although, these are minimally invasive procedures, complications can occur. The duration and accuracy of the procedure are important to reduce the radiation dose and the risk of complications and to improve treatment success.

In lumbar dorsal root ganglion pRF applications, when the targeted point is reached, the position is verified by providing motor and sensory stimuli, followed by pRF application [9]. However, because the DRG is not always located in the same place, time is often wasted searching for it with stimulation, and sometimes the ganglion cannot be found. To prevent these issues, the location of the ganglion can be determined primarily by injecting a contrast agent into the spinal nerve

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root and epidural area while using intermittent fluoroscopic imaging [8]. The aim of this study was to investigate the effect of using a contrast agent for procedure time and accuracy in pRF treatments of lumbar DRG.

2. PATIENTS and METHODS

Study Design

The present study was designed as a prospective randomized controlled trial and was approved by the Harran University, School of Medicine, Clinical Researches Ethics Committee (Date: 18.01.2021, No: 21.02.29). The study included patients between the ages of 18–80 years with lumbar radicular pain not responding to conservative treatment for at least 3 months. The reason for radicular pain was L5 nerve root compression due to disc herniation. Patients with L5 nerve root compression due to causes other than disc herniation, the presence of spondylolisthesis, transitional vertebrae, active infection, bleeding diathesis, renal insufficiency, and pregnants were excluded from the study. A total of 70 patients who applied to pain outpatient clinics between March 2021 and December 2022 were included in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki. All patients were informed about the nature of the study and written informed consent was obtained.

Patients were randomized into two groups of 35 patients each. Patients in both groups underwent fluoroscopy-guided pRF treatment of the DRG at the level of nerve root compression due to disc herniation (L5 foramen level). All procedures were performed by a pain specialist with 5 years of experience in interventional pain management. Unlike the control group, a contrast material was administered before the intervention and the location of the DRG was initially determined in the radiocontrast group. In the control group, the location of the DRG was determined by sensory and motor stimuli, as detailed below. After the procedure was terminated, the location of the DRG was also determined in the control group via contrast injection.

Interventional Procedure

Patients were placed in the operating room in the prone position, the skin was sterilized, and a pillow was placed under the abdomen to correct the lumbar lordosis. The vertebral endplates were flattened by angling the C-arm in the cephalic or caudal direction. The needle entry site was then determined by adjusting the scope to the ipsilateral oblique position. The needle insertion site was chosen based on intraforaminal localization, which is the most common location of DRG. After achieving skin and subcutaneous anesthesia, a 22-G radiofrequency (RF) hybrid cannula was advanced toward the target under coaxial imaging. When the targeted point (the dorsocranial part of the intervertebral foramen) was reached, contrast material was injected in the radiocontrast group, C-arm was switched to the anteroposterior (AP) position, and the electrode was directed toward the area where the ganglion was stained on the epidurogram. In the control group, DRG location was confirmed by providing motor and sensory stimuli without the contrast agent at this stage. For this purpose, sensory stimulation was given at 50 Hz and motor stimulation at 2 Hz. Attempts were made to provide stimuli at levels higher than 0.4 V to avoid intraganglionic localization. Paresthesia below 0.6 V was considered close to the DRG [9]. Within this range, the ganglion was searched in four quadrants of the foramen, namely ventrocranial, dorsocranial, ventroinferior, and dorsoinferior, without deviating from the intraforaminal (IF) direction. If paresthesia was not achieved, the electrode was directed toward the extraforaminal (EF) region. If the ganglion could not be stimulated, a response up to 1 V was sought with 0.1 V increments, first in the IF and then in the EF region. After the stimulus was provided, motor stimulation was given by increasing the volts at which paresthesia was achieved with sensory stimulation by up to twofold. No response confirmed that the location was far enough from the motor nerve. After the position was confirmed following the stimulations, pRF was applied at 2 Hz over 6 minutes. Meanwhile, the temperature was not allowed to rise above 42 ^{0}C. In the control group, after the PRF procedure was completed, a contrast agent was administered and the location of the DRG was determined.

A stopwatch was started when the needle was inserted into the skin and stopped when the position of the DRG was confirmed by stimulation, and the time was recorded. Whether the ganglion could be distinguished by a contrast agent was noted. If so, the location of the DRG was classified by drawing a line from the medial and lateral borders of the pedicle. Accordingly, intraspinal (IS), intraforaminal (IF), and extraforaminal (EF) location of the ganglion based on the location of its center was recorded (Figure 1A-B). In addition, after the location of the DRG was confirmed by stimulation, the quadrant of the intervertebral foramen where the needle tip was located was identified on lateral imaging. Cases in which DRG localization could not be confirmed after stimulation were recorded separately.

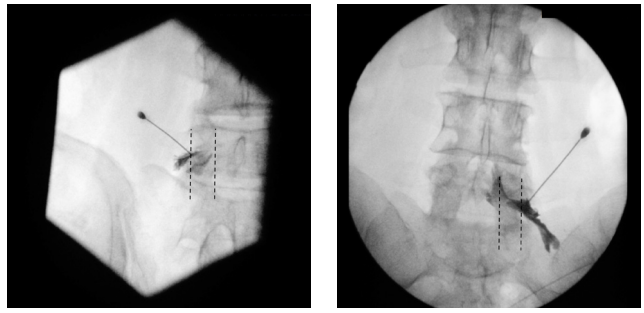

Figure 1. *The dorsal root ganglion is classified by drawing dotted lines from the medial and lateral borders of the pedicle.* ***A)*** *Intraforaminal location of the ganglion.* ***B)*** *Ekstraforaminal location of the ganglion.*

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Statistical Analysis

The IBM SPSS Statistics 22 program was used for statistical analyses. The conformity of variables to a normal distribution was evaluated by the Kolmogorov–Smirnov and Shapiro–Wilks tests. In addition to descriptive statistical methods (mean, standard deviation, and frequency), the Student's t-test was used to compare normally distributed quantitative variables between the groups and the Mann–Whitney U test was used to compare non-normally distributed variables between two groups. The chi-square test, Fisher's exact test, and continuity (Yates's) correction were used to compare qualitative variables. $P < 0.05$ was accepted as statistically significant in all analyses.

3. RESULTS

The present study was conducted with 70 patients, of whom 36 (52.4%) were males and 34 (48.6%) were females. The patients were aged between 23 and 79 years. There was no statistically significant difference between the groups in terms of age, gender, and educational level ($P > 0.05$) (Table I). The parameters evaluated in the study are presented in Table II.

Table I. *Evaluation of the groups in terms of demographic characteristics*

	Radiocontrast	Control	*Total*	p
Age $_{\text{Mean} \pm \text{SD}}$	44.74 ± 13.54	45.66 ± 12.7	*45.2 ± 1.04*	[1]0.772
Gender $_{\text{n (\%)}}$				
Male	16 (45.7%)	20 (57.1%)	*36 (51.4%)*	[2]0.473
Female	19 (54.3%)	15 (42.9%)	*34 (48.6%)*	
Educational Status $_{\text{n (\%)}}$				
Illiterate	9 (25.7%)	5 (14.3%)	*14 (20%)*	[3]0.384
Literate	5 (14.3%)	4 (11.4%)	*9 (12.9%)*	
Primary education	11 (31.4%)	10 (28.6%)	*21 (30%)*	
High School	9 (25.7%)	11 (31.4%)	*20 (28.6%)*	
University	1 (2.9%)	5 (14.3%)	*6 (8.6%)*	

[1]*Student's t-test* [2]*Continuity (Yates's) correction* [3]*Chi-square test*

Table II. *Distributions of procedural parameters*

		n	%
*pRF-treated dorsal root ganglion	Right L5	37	52.9
	Left L5	33	47.1
Level and localization of disc herniation causing L5 root compression	L4–L5 (paracentral)	57	81.4
	L4–L5 and L5–S1 (pc and ef)	9	12.9
	L5–S1 (extraforaminal)	4	5.7
Excitability of **DRG in the range 0.4–0.6 V	Yes	47	67.1
	No	23	32.9
Voltage at which DRG can be stimulated (n = 61)	0.4	5	7.1
	0.5	18	25.7
	0.6	24	34.3
	0.7	4	5.7
	0.8	4	5.7
	0.9	6	8.6
Cases where DRG cannot be stimulated below 1 V	Extraforaminal (control group)	2	2.9
	Intraforaminal (control group)	1	1.4
	Intraspinal (control group)	1	1.4
	Intraspinal (radiocontrast group)	2	2.9
	Unidentified	3	4.3
Recognition of DRG localization by contrast agent injection	Yes	62	88.6
	No	8	11.4
DRG Localization (According to contrast agent staining)	Extraforaminal	12	17.1
	Intraforaminal	38	54.3
	Intraspinal	12	17.1
	Unidentified	8	11.4
Localization of the needle tip relative to the foramen on lateral fluoroscopic imaging when DRG position was confirmed by stimulation (n = 61)	Dorsocranial	46	75.4
	Ventrocranial	15	24.6

**pRF: pulsed radiofrequency; **DRG: Dorsal root ganglion*

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The procedure time was significantly longer in the radiocontrast group than in the control group (P= 0.000; P < 0.05). The excitation rate of DRG at 0.4–0.6 V was 74.3% in the radiocontrast group and 60% in the control group, and the difference between the groups was not statistically significant (P > 0.05) (Table III).

In 50 cases the DRG was found to be in IF or EF location based on contrast staining, the rate of excitation of the DRG in the range of 0.4–0.6 V was significantly higher in the radiocontrast group (95.8%) than in the control group (69.2%) (P = 0.024; pP< 0.05) (Table IV).

Table III. *Evaluation of groups in terms of procedure times and excitability of DRG at 0.4–0.6 V (n = 70)*

		Radiocontrast	Control	p
Procedure time (sec) Mean±SD (median)		265.97 ± 103.29 (245.5)	168.24 ± 65.38 (160)	[1]0.000*
Excitability of DRG at 0.4–0.6 V	Yes	26 (74.3%)	21 (60%)	[3]0.309
	No	9 (25.7%)	14 (40%)	

*[1]Mann–Whitney U Test [2]Fisher's Exact Test [3] Continuity (Yates's) correction *P < 0.05*

Table IV. *Comparison of groups in terms of excitability rates of dorsal root ganglion at 0.4–0.6 V in patients with intraforaminal and extraforaminal localization (n = 50)*

Dorsal root ganglion excitation at 0.4-0.6 V	Radiocontrast n (%)	Control n (%)	P
Yes	23 (95.8%)	18 (69.2%)	0.024*
No.	1 (4.2%)	8 (30.8%)	

*Fisher's Exact Test *P< 0.05*

4. DISCUSSION

The lumbar DRG can be radiologically divided into three locations [8, 10-12]. In addition to the medial border of the vertebral pedicle, the locations of the ganglion can be defined by vertical lines drawn from the central [8, 10] or lateral border [11, 12] of the pedicle. There are radiologic and cadaveric studies investigating the location of the L5 DRG in the literature [8, 10, 12-15]. These studies reported IS, IF, and EF localization of L5 DRG as 66.7%–94.3%, 0%–33.3%, and 0%–19.2%, respectively [8, 10, 12-15]. In the present study, IS, IF, and EF localization rates after contrast injection were 17.1%, 54.3%, and 17.1%, respectively. In 11.4% of the cases, the ganglion could not be identified based on the spread of the contrast agent (Table II). In the present study, IF localization was found at a slightly lower rate compared to that in the previously mentioned studies. Nevertheless, our location findings are consistent with other studies. In addition, the inability to distinguish the ganglion in some cases after contrast staining may be responsible for this to some extent. Because the aforementioned studies were mostly MRIs and cadaveric studies or unidentified ganglions were excluded from those studies, this disadvantage was not observed in them [10, 12-15].

In the lower lumbar region, the DRG is localized in the foramen, below and just exterior to the vertebral pedicle [8]. In cases where we were able to confirm the location of the DRG with sensory and motor stimulation, we found that the ganglion was located in the upper part of the foramen. DRG was located in the posterior part of the foramen in 24.6% of cases and in the anterior part of the foramen in 75.6% of cases (Table II). It has been reported in the literature that the ganglion moves toward the anterior of the foramen as it approaches the inferior lumbar area [8].

Paresthesia with sensory stimulation at 0.4–0.6 V was considered ideal proximity to the DRG in the present study. Accordingly, the ganglion could be appropriately stimulated in 47 patients (67.1%). In patients with no response in the ideal range, the ganglion was located intraspinally in 10 patients and it could not be distinguished with a contrast agent in 4 patients. In five cases, the ganglion was located EF and these patients were in the control group. In the control group, we believe that the lack of contrast agent injection before pRF application resulted in an inability to direct the needle toward the EF area. In cases with IF and EF DRG localization, a significant difference was found in favor of the radiocontrast group in terms of ganglion excitability in the ideal voltage range (P< 0.05) (Table IV). No paresthesia response was obtained in four patients despite stimulation in the appropriate range and the electrode being in the location identified by the contrast agent. In these patients, this barrier was overcome below 1 V when the stimulus voltage was increased. We believe that this may be due to altered nociception caused by neuropathy or chronic pain. The patients were not questioned about additional diseases that could cause polyneuropathy and this could be a limitation of the study.

The stimulation voltage was increased in cases with no response in the ideal range (0.4–0.6 V). Paresthesia occurred below 1 V in 14 patients. As a result, 87.1% of all patients responded to sensory stimuli below 1 V. In the present study, the ganglia of nine patients could not be stimulated below 1 V. When these cases were analyzed, it was observed that the ganglion could not be differentiated even with a contrast agent in three patients. In three of the other six cases, the DRG was located intraspinally, which is probably why we could not get close enough to the ganglion. The remaining three patients were in the control group, and prior injection of a contrast agent could have served as a guide for appropriate stimulation in these patients.

When all cases were considered, the procedure time was significantly longer in the radiocontrast group than in the control group (Table III). In addition, no difference was found between the groups regarding the ganglion's excitability in 0.4–0.6 V (Table III). However, when intraspinal cases were excluded, there was a significant difference in ganglion excitability in favor of the radiocontrast group (Table IV). The ganglion could not be stimulated in the ideal voltage range (0.4–0.6 V) in 10 of the 12 cases with IS localization (83.3%). In conclusion, IS localization of DRG appears to be the most difficult challenge in pRF applications.

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One patient in each group developed a vasovagal reaction during the procedure, but pulse and blood pressure were controlled with atropine 0.5 mg/iv. Although, prolonged procedure time is a factor that increases the risk of complications, in the present study, the longer procedure time in the radiocontrast group (Table III) did not create a difference in terms of complications.

In a cadaveric study, Silverstein et al., determined the position of the DRG using MR imaging before dissection [16]. Subsequently, they determined the anatomical location by dissection. Accordingly, MR imaging and anatomical evaluation were 86.3% compatible. In the present study, the DRG localization assumed after contrast injection and confirmed after stimulation coincides with 82.1%. Because this is not a cadaveric study, the inability to confirm the anatomical location is one of the limitations of the study. Nevertheless, the data obtained, leads to certain conclusions. In 46 of 70 patients, radiological and stimulation localization were consistent, whereas in 10 patients, they were not. The DRG could not be identified by contrast injection in 5 of the 70 patients and could not be stimulated below 1 V in 6 patients; these patients were not included in the analysis. In three cases, the ganglion could neither be stimulated nor differentiated with a contrast agent. All 10 patients with discordance between contrast agent and stimulation-mediated localization were in the control group. In this group of patients, it is possible to position the needle closer to the DRG and achieve stimulation with a lower voltage if the radiofrequency needle is directed to the target after contrast agent injection.

The present study examined the L5 foramen level, where pRF applications are most commonly performed. The fact that other lumbar foraminal levels were not evaluated can be considered another limitation of the study.

Conclusion

The use of radiocontrast agents in pRF applications for the DRG prolongs the procedure time. In addition, ganglion location can be determined using sensory and motor stimulations without the use of radiocontrast in most cases. Therefore, we do not routinely recommend contrast agents in these procedures. However, contrast injection is helpful regarding procedural accuracy for ganglia that cannot be detected by stimulation, especially in cases with EF localization. It can also detect IS localization, thereby revealing why the ganglion is not stimulated in the ideal voltage range and preventing prolonged procedures. In conclusion, the use of contrast agents in pRF treatment for lumbar DRG should be considered as an adjunct modality, but not the primary component of the procedure.

Compliance with the Ethical Standards

Ethics Committee Approval: The present study was approved by the Harran University, School of Medicine, Clinical Researches Ethics Committee (Date: 18.01.2021, Approval No: 21.02.29). The study was conducted in accordance with the principles of the Declaration of Helsinki. All patients were informed about the nature of the study and written informed consent was obtained.

Conflict of interest: The authors declare that they have no conflict of interest.

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The relationship between smartphone and computer games and anger in adolescents

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ABSTRACT

Objective: In this study, it was aimed to determine the relationship between the causes and duration of gaming and types of games and levels of anger among Turkish adolescents studying at high schools.

Materials and Methods: The study was conducted using a cross-sectional descriptive design with adolescents enrolled in high schools in an Eastern Turkish province. A total of 819 adolescent students aged 13-18 were included in the study. Data were collected online through Google Forms, utilizing both the "Sociodemographic Form" and the "Adolescent Anger Rating Scale."

Results: The mean age of the adolescents was 15.52±1.29. Gender, academic performance, daily sleep duration, and daily walking distance were not found to be associated with anger levels. Additionally, there was no significant correlation between anger scores and the status of computer and smartphone usage. However, individuals who used smartphones for 3 hours or more for purposes such as gaming, entertainment, chatting, messaging, and socializing exhibited higher anger scores.

Conclusion: Academic grades, the duration of smartphone usage, computer and smartphone use for gaming/entertainment, chatting, messaging, and socializing were found to be associated with anger. Specifically, extended periods of playing war, fighting, and similar games on both computers and smartphones were correlated with higher levels of anger. It would be advantageous for parents to monitor and regulate the content of the games their adolescents play, observe any behavioral differences, and take necessary precautions.

Keywords: Adolescent, Anger, Computers, Smartphone

1. INTRODUCTION

Adolescence is an important stage of rapid physical growth and mental development [1]. Feelings and thoughts can change rapidly during this period. The adolescent's effort to accept their selves and the problems they experience with their family or environment can cause stress, anger, fear, and anxiety [2]. Adolescents sometimes are engaged in activities that they believe will make them feel happy to reduce their increased stress level. Games, which are among these activities, have shown an increasing trend in recent years [3]. Studies have reported that the highest number of game users worldwide are in Asia. Only 17% of adolescents in China [4] and 19.9% of adolescents in England [5] play games at an addictive level.

Although, games are a popular recreational activity for adolescents, their uncontrolled and prolonged use may lead to physical and mental problems. Various studies have produced different results when examining the effects of gaming on adolescents. Adolescents' addiction to games can lead to confusion between the real and virtual worlds, a decline in problem-solving abilities [6], and increased distraction [7]. A cross-sectional study found a correlation between excessive gaming during adolescence and certain psychiatric disorders, such as depression [8]. Another study reported that excessive gaming is associated with reduced sleep quality and duration, increased suicidal ideation and suicide attempts, compromised academic performance, and behavioral problems [9].

In a study conducted in Norway, it was determined that adolescents who play games have high levels of anger, depression, loneliness and aggression [10]. In another study conducted with Turkish adolescents, it was found that increased playtime is associated with impulsive behaviors, aggression

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and psychological disorders [11]. On the other hand, recent studies emphasize the positive effects of games [12]. For example, Annetta et al., argued that some game-based education programs make the theoretical knowledge permanent in adolescents. Games are effective in motivating learners [13]. In addition, reports were published that games are beneficial for cognitive functions [1]. The results of some studies showed that gamers have faster response times in processes that require rapid response [14]. The results of a meta-analysis study showed that games positively support visual perception [15].

Anger and aggression are among the most widely discussed topics in relation to gaming. According to Anderson and Bushman, "an individual directs anger and aggression to another person, often with the intent to harm" [16]. Previous studies have established a connection between anger, aggression, and playing games [17]. Kim et al., reported that anger and aggression were associated with game addiction [18]. Another study indicated a significant relationship between game addiction and anger and aggressiveness [19]. A study examined the relationship between anger behavior and game preference [20]. Lemmens et al., reported that adolescent males prefered to play games involving anger and violence [21]. Similarly, Griffiths stated that children with anger issues are interested in violent games [22]. The mechanism underlying this relationship is based on the assumption that anger and aggression lead to excessive gaming. This situation has been explained in different ways: According to the first perspective, players select a specific type of game that aligns with their pre-existing characteristics, such as anger and aggression [23]. The second viewpoint suggests that as individuals play games and earn high points and rewards, their anger behavior intensifies. During this process, anger and aggressive behaviors may become more "purposeful." [24].

This study was designed to fill some gaps in the literature. In this study, it was aimed to determine the relationship between the causes of gaming, time spend playing games, and game types and anger levels of Turkish adolescents studying in high schools.

2. MATERIALS and METHODS

Type of Study

The study was designed as a cross-sectional-descriptive type.

Population and Sample

The study was conducted with adolescents attending high school in a province in Eastern Turkey. There are a total of 22 high schools and 11652 students aged 13-18 years in the province where the study was conducted. A total of 628 individuals would be sufficient with a 99% confidence interval and a 5 percent margin of error in the sample calculation made with OpenEPI. However, the research initially involved 628 participants; data collection continued beyond this number with additional volunteers who expressed interest in participating in the survey, ultimately reaching a total of 819 individuals.

Inclusion Criteria

-Being between the ages of 13-18,

-Agreeing to participate in the study voluntarily.

Exclusion Criteria

-Having a physical disability that cannot answer the questions,

-Not playing games.

Application Data Collection

Data were collected online with Google Forms. The prepared questionnaire link was sent to the adolescents via WhatsApp. Confidentiality of the survey data is ensured. The forms are standardized to be answered only once from each phone/tablet/computer. The responses to the questionnaire were reviewed daily. A sociodemographic form and "Adolescent Anger Rating Scale" were used to collect data from adolescents.

Data Collection Tools

Sociodemographic Form

It consists of questions prepared to determine the characteristics such as age, gender, family income, family type, academic degree and success, presence of chronic disease, psychiatric disease, average sleep time in the last month, games played and playing time [1,3,13].

Adolescent Anger Rating Scale

The Adolescent Anger Rating Scale was developed by McKinnie Burney and Kromrey in 2001 for adolescents aged 11-19 years [25]. Aslan and Sevinçler-Togan conducted a validity and reliability study of the scale in an adolescent population in Turkey [26]. The scale, which is a four-point Likert type, consists of 41 questions. 4-point response scale ranges from hardly ever to very often. Scores are reported for total anger and for three subscales measuring aspects of the adolescent's typical anger response pattern: "reactive anger", "instrumental anger", "anger control" sub-dimensions and total anger scores are calculated in the scale:

Reactive anger (20 items) is an angry, sudden reaction to an event that creates fear, threat, or a negative emotion.

Instrumental anger (8 items) is a negative emotion that triggers a delayed response that results in a planned, willing revenge behavior.

Anger control (13 items) is a cognitive and behavioral method used to resolve the anger response.

Statistical Analysis

While the data were reported as percentage, arithmetic mean, standard deviation, minimum and maximum values, independent groups t-test, one-way analysis of variance and Pearson's correlation analysis were performed. SPSS 23 software was used in the analysis. The normality distribution of the data

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was checked with the Shapiro-Wilk's test. A significance level of "P<0.05" was considered statistically significant in the study.

Ethical Statement

Ethical approval was obtained from Bingöl University Non-Interventional Research Ethics Committee before starting the study (approval number: 28.11.2022-E.86417). After ethical approval was obtained, institutional permissions were obtained through the governorship for the research to be conducted in educational institutions (10.01.2023-92001). Adolescents were informed about the purpose of the study and their consent was obtained.

3. RESULTS

Among the adolescents, 52.02% were female, 32.47% were in their second year, 55.43% reported good academic achievement, 86.69% slept for 6-9 hours a day, and 49.08% walked between 0-2000 meters daily.

In the study, the relationship between demographic results and anger was determined. The mean age of the adolescents participating in the study was 15.52±1.29. The gender, academic achievement, daily sleep duration, and daily walking distance of the adolescents were not related to the anger scores of the adolescent (Table I). Our study found that the academic grades of the adolescents were associated with anger reactive (P=.036), anger control (P=.019) and anger total scores (P=.033), and the anger mean scores of the adolescents who were in the last year of high school were found to be high (Table I).

In the study, the relationship between the duration and purpose of computer and smartphone usage by adolescents and their levels of anger was evaluated. Factors such as "Computer and smartphone usage status", "Duration of computer usage", and "Duration of computer and smartphone usage for information purposes" were assessed, but no associations were found with the anger scores of adolescents, as indicated in Table II.

Higher scores in the "anger reactive", "anger control", and "anger total" categories were observed among adolescents who used their smartphones for 3 hours or more, and these differences were found to be statistically significant, as demonstrated in Table II. Furthermore, higher scores in the anger sub-dimensions and total scores were also noted among adolescents who used phones and computers for 3 hours or more for activities such as gaming, entertainment, chatting, messaging, and socializing, and these differences were similarly found to be statistically significant (Table II).

In the study, an examination was conducted on the relationship between the type and duration of video game usage and the anger scores of adolescents who played games on computers and smartphones. It was observed that all anger sub-dimensions and the total anger scores of adolescents who played war, fighting, racing, and competitive games on their computer or smartphone for 3 hours or more were found to be significantly higher (Table III). On the other hand, the "anger reactive", "anger control", and "total anger" scores of adolescents who played sports games on the computer or smartphone for 0-2 hours were lower compared to those who either did not play this game at all or played it for 3 hours or more, and this difference was found to be statistically significant (Table III). Additionally, it was noted that there was no significant change in the anger scores of adolescents who played games such as puzzles and calm games, even as their playing time was increased (Table III).

Table I. *Relationship between adolescent demographic features and AARS (n=819)*

	Reactive Anger		Instrumental Anger		Anger Control		Anger Total	
	Mean±SD	**Test(p)**	**Mean±SD**	**Test(p)**	**Mean±SD**	**Test(p)**	**Mean±SD**	**Test(p)**
Gender*								
Male (n=393)	41.92±7.86	.277(.782)	17.16±3.57	.741(.459)	25.95±4.91	.068(.945)	85.04±16.02	.281(.779)
Female (n=426)	41.77±7.05		16.98±3.26		25.97±4.49		84.74±14.39	
Age: Mean±SD: 15.52±1.29, Range: 13-18, N=819								
Psychiatric Disorders*								
No (n=779)	41.90±7.40	.956(.339)	17.08±3.39	.613(.540)	26.00±4.67	1.122(.262)	85.00±15.09	.954(.340)
Yes (n=40)	40.75±8.39		16.75±3.81		25.15±5.11		82.65±17.01	
Family Type**								
Nuclear family (n=717)	42.09±7.37	5.032(.081)	17.17±3.37	3.868(.145)	26.10±4.69	5.032(.081)	85.37±15.05	4.939(.085)
Extended family (grandmother, grandfather, etc.) (n=78)	40.15±8.27		16.35±3.85		24.98±4.92		81.50±16.69	
Fragmented of the family (parents divorced) (n=24)	40.00±6.26		16.33±2.98		24.79±3.69		81.12±12.54	
Family Income***								
Low (n=253)	41.61±7.59	1.283(.278)	16.98±3.43	1.058(.348)	25.81±4.83	1.519(.220)	84.41±15.47	1.365(.256)
Medium (n=465)	41.73±7.48		17.01±3.42		25.87±4.66		84.63±15.21	
High (n=101)	42.95±6.89		17.53±3.31		26.72±4.46		87.20±14.24	
Academic Grade (High School)***								
1st year (n=160)	41.61±6.59	**2.865(.036)**	16.91±3.07	2.154(.092)	25.83±4.06	**3.35(.019)**	84.36±13.35	**2.92(.033)**
2nd year (n=266)	41.48±7.46		16.86±3.37		25.80±4.74		84.15±15.20	
3rd year (n=213)	41.28±6.71		16.97±3.28		25.46±4.02		83.72±13.60	
4th year (n=180)	43.26±8.75		17.63±3.84		26.90±5.70		87.80±17.97	
Academic Success***								
Bad (n=47)	39.95±8.61	1.741(.176)	16.12±3.86	1.950(.143)	25.06±5.52	1.438(.238)	81.14±17.55	1.641(.194)
Moderate (n=318)	41.79±6.98		17.16±3.24		25.81±4.39		84.77±14.21	
Good (n=454)	42.07±7.63		17.10±3.47		26.16±4.80		85.34±15.56	
Duration of Sleep (per Day)***								
2-5 hours (n=36)	42.52±9.26	.335(.715)	17.11±3.78	.338(.714)	25.80±5.52	.628(.534)	86.45±19.07	.429(.651)
6-9 hours (n=710)	41.79±7.18		17.38±3.98		25.91±4.49		84.74±14.62	
10 hours or more (n=73)	41.61±8.56		17.03±3.33		26.54±6.07		84.52±17.54	
Daily Walking Distance *								
0-2000 mt (n=402)	41.96±8.02	.041(.960)	17.19±3.59	.297(.743)	25.98±4.66	.089(.915)	85.20±16.37	.057(.945)
2001-5000 mt (n=235)	41.87±7.28		17.13±3.40		25.85±4.54		84.86±14.75	
5000 mt or more (n=182)	41.78±7.30		16.98±3.34		26.04±5.09		84.75±14.91	

**t test, **Kruskal Wallis Test, ***One-Way ANOVA, AARS: Adolescent Anger Rating Scale.*

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Table II. *The relationship of the duration and purpose of computer and smartphone use with AARS*

	Reactive Anger		Instrumental Anger		Anger Control		Anger Total	
	Mean±SD	Test(p)	Mean±SD	Test(p)	Mean±SD	Test(p)	Mean±SD	Test(P)
Do You Use a Computer?*								
Yes (n=251)	42.31±7.33	1.096(.273)	17.25±3.41	.931(.352)	26.23±4.63	1.016(.310)	85.81±14.97	1.062(.289)
No (n=558)	41.69±7.55		17.01±3.44		25.87±4.75		84.58±15.37	
How Long Do You Use the Computer per Day?***								
Never or very little (n=529)	41.58±7.51	2.736(.065)	16.93±3.46	2.344(.097)	25.83±4.69	2.540(.079)	84.34±15.29	2.681(.069)
0-2 hours (n=123)	41.38±7.56		17.00±3.32		25.56±4.93		83.95±15.47	
3 hours or more (n=167)	43.04±7.07		17.58±3.28		26.67±4.51		87.29±14.46	
Do You Use a Smartphone?*								
Yes (n=656)	41.78±7.33	.477(.633)	17.02±3.34	.821(.412)	25.96±4.67	.075(.940)	84.78±14.95	.396(.692)
No (n=163)	42.09±7.92		17.26±3.69		25.93±4.80		85.30±16.11	
How Long Do You Use the Smartphone per Day?***								
Never or very little (n=163)	41.73±6.77	**5.788(.003)**	17.04±3.17	2.887(.056)	25.81±4.25	**9.767(.000)**	84.59±13.79	**6.368(.002)**
0-2 hours (n=451)	41.40±8.14		16.90±3.67		25.71±5.03		84.02±16.53	
3 hours or more (n=205)	45.21±9.42		18.15±4.16		28.74±6.26		92.11±19.49	
I Use a Computer for Gaming and Entertainment (per Day)***								
Never (n=689)	41.63±7.46	**4.737(.009)**	16.97±3.44	**3.263(.039)**	25.81±4.64	**6.057(.002)**	84.42±15.18	**4.986(.007)**
0-2 hours (n=86)	41.88±6.16		17.19±2.84		25.95±4.11		85.03±12.61	
3 hours or more (n=44)	45.18±8.81		18.31±3.82		28.34±5.96		91.84±18.24	
I Use a Computer for Chatting, Messaging and Socializing (per Day)***								
Never (n=710)	41.60±7.37	**5.036(.007)**	16.95±3.39	**5.795(.003)**	25.80±4.60	**4.311(.014)**	84.36±15.00	**5.176(.006)**
0-2 hours (n=66)	42.27±6.71		17.19±2.92		26.50±4.63		85.96±13.76	
3 hours or more (n=43)	45.25±8.95		18.76±4.01		27.83±5.90		91.86±18.48	
I Use a Computer for Information and Research Purposes (per Day)***								
Never (n=524)	42.00±7.75	.355(.701)	17.19±3.56	.979(.376)	25.99±4.89	.177(.838)	85.19±15.82	.345(.708)
0-2 hours (n=159)	41.45±6.80		16.86±3.17		25.77±4.23		84.09±13.84	
3 hours or more (n=136)	41.70±7.01		16.83±3.08		26.07±4.47		84.61±14.20	
I Use a Smartphone for Gaming and Entertainment (per Day)***								
Never (n=501)	41.26±7.22	**11.888(.000)**	16.79±3.35	**12.295(.000)**	25.60±4.43	**10.504(.000)**	83.66±14.63	**12.150(.000)**
0-2 hours (n=175)	41.29±7.47		16.82±3.42		25.66±4.75		83.78±15.29	
3 hours or more (n=143)	44.57±7.65		18.34±3.37		27.58±5.19		90.49±15.79	
I Use a Smartphone for Chatting, Messaging and Socializing (per Day)***								
Never (n=384)	41.20±8.00	**11.643(.000)**	16.67±3.58	**15.306(.000)**	25.30±3.80	**8.312(.000)**	83.59±16.28	**11.778(.000)**
0-2 hours (n=212)	40.88±6.35		16.67±3.02		25.71±5.04		82.87±12.80	
3 hours or more (n=223)	43.86±7.07		18.13±3.25		27.01±4.69		89.01±14.59	
I Use the Smartphone for Information and Research Purposes (per Day)***								
Never (n=336)	42.35±8.30	1.458(.233)	17.36±3.78	2.094(.124)	26.25±5.25	1.205(.300)	85.96±16.96	1.576(.207)
0-2 hours (n=238)	41.31±6.51		16.81±2.97		25.64±4.11		83.77±13.22	
3 hours or more (n=245)	41.66±7.05		16.93±3.27		25.87±4.41		84.47±14.33	

**t test, ***One-Way ANOVA, AARS: Adolescent Anger Rating Scale.*

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Table III. *Relationship of gaming purposes on computer and smartphone with AARS*

	Reactive Anger		Instrumental Anger		Anger Control		Anger Total	
	Mean±SD	Test(p)	Mean±SD	Test(p)	Mean±SD	Test(p)	Mean±SD	Test(p)
I Play War, Fight etc. Games on My Smartphone or Computer (per Day)* **								
Never (n=325)	41.41±7.16	**7.690(.000)**	16.80±3.38	**9.820(.000)**	25.73±4.38	**5.767(.000)**	83.95±14.57	**7.880(.000)**
0-2 hours (n=240)	40.87±7.68		16.62±3.46		25.42±4.80		82.92±15.55	
3 hours or more (n=254)	43.32±7.39		17.84±3.30		26.76±4.89		87.93±15.20	
I Play Sports Games on My Smartphone or Computer (per Day)* **								
Never (n=474)	42.05±7.10	**3.063(.047)**	17.20±3.33	**3.429(.033)**	25.97±4.40	2.535(.080)	85.23±14.44	**3.021(.049)**
0-2 hours (n=226)	40.90±7.92		16.58±3.41		25.53±5.14		83.02±16.14	
3 hours or more (n=119)	42.84±7.75		17.46±3.65		26.73±4.88		87.03±15.92	
I Play Racing and Competitive Games on My Smartphone or Computer (per Day)* **								
Never (n=491)	41.73±6.90	**4.377(.013)**	17.05±3.28	**3.362(.035)**	25.80±4.23	**5.940(.003)**	84.59±14.02	**4.754(.009)**
0-2 hours (n=223)	41.19±7.65		16.76±3.33		25.63±4.88		83.59±15.54	
3 hours or more (n=105)	43.75±9.08		17.80±4.07		27.41±5.98		88.98±18.72	
I Play Puzzles and Calm Games on My Smartphone or Computer (per Day)* **								
Never (n=415)	42.27±7.25	1.706(.182)	17.29±3.39	1.891(.152)	26.13±4.57	1.134(.322)	85.70±14.79	1.568(.209)
0-2 hours (n=278)	41.20±7.72		16.79±3.47		25.61±4.86		83.62±15.73	
3 hours or more (n=126)	41.85±7.44		16.95±3.34		26.16±4.75		84.97±15.18	

****One-Way ANOVA, AARS: Adolescent Anger Rating Scale.*

4. DISCUSSION

The results of the study conducted to determine the relationship between reasons of playing games, the duration of playing games and the types of games and anger levels of adolescents were discussed in this section.

The causes of anger in adolescence are usually of social origin. These are reasons such as problems with parents, criticism, academic failure. In a study conducted with Indian adolescents, the prevalence of anger was determined to vary between 17.7-66.5%. It was reported in the study that, as the academic degree increases, physical and passive aggression also increase [27]. Furthermore, the "anger reactive," "instrumental anger," and "anger total" mean scores of high school senior students were found to be high. Additionally, the mean scores for "anger reactive," "anger instrumental," and "anger total" in males were observed to be higher. Studies conducted by Sharma and Marimuthu, Mohammad Hossein et al., reported that physical aggression is more common in boys, while verbal aggression is more common in girls [28,29]. Gudlaugsdottir et al. stated that boys express their anger by fighting and shouting, whereas girls express their anger by crying and shouting [30]. In this study, although the "anger reactive", "instrumental anger" and "anger total" mean scores of males were higher, this difference was not statistically significant. In addition, the higher level of anger related to academic degree in this study was found to be consistent with the literature [27-30].

Sleep problems are commonly encountered in adolescents. When left untreated, they can give rise to various psychological and somatic problems in the future [31]. Negative behaviors like anger have been observed in adolescents with sleep disorders. It has been found that sleep quality is more closely associated with feelings of anger than the average amount of sleep. Mood can be affected, and anger can be provoked in adolescents due to poor sleep quality [32]. Donoghue and Meltzer stated that insomnia or reduced sleep duration cause behavioral problems such as irritability and anger [33]. In this study, although, the mean scores of "anger reactive", "anger instrumental" and "anger total" were lower in adolescents who slept 10 hours or more per day, this difference was not statistically significant. It is well-established that sleep patterns influence factors such as circadian rhythm and mood regulation. It is thought that prolonged sleep duration may reduce irritability and anger.

The most common form of exercise is considered to be walking. Many bodily and spiritual processes are improved by walking [34]. Beneficial psychological effects have been indicated by numerous studies when it comes to walking and exercise [35,36]. Nevertheless, there is no clarity regarding the optimum duration for these exercises [37]. A significant reduction in negative emotions was reported by Sakuragi and Sugiyama after walking for one hour daily over a four-week period [38]. In this study, although, the mean scores of "anger reactive", "anger instrumental" and "anger total" were found to be lower in adolescents whose daily walking distance was 5000 meters or more, this difference was not statistically significant. These results suggest that walking may induce relaxation, which in turn may contribute to the potential reduction of anger.

Studies have reported that the using smartphones in stressful times make it easier for individuals to cope with difficult situations. However, the beneficial effect only occurs when this interaction is minimal [39]. Excessive and uncontrolled use can cause many harmful mental problems, especially addiction. Long-term and excessive smartphone use was reported to be associated with depressive symptoms, high anxiety, anger, and

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stress. In this study, "anger reactive", "anger control" and "anger total" mean scores were found to be higher in those with a smartphone usage time of 3 hours or more.

According to a study conducted in the USA, phone or computer games are used by 8% of children and adolescents between the ages of 8-18 [40]. It is stated that an average weekly playing time of 16.4 hours on the computer is reported for males, while females spend 9.2 hours [41]. In the Netherlands, a cross-sectional study reported that online games are played by 3% of school-age children aged 13-16 [42]. In this study, it was found that the "anger total" and all sub-dimension averages were higher in those who used computers and phones extensively for games and entertainment. This finding suggests that users see games as an escape to make up for the lack in their real lives or to get away from their problems. In addition, it is thought that excessive use, which continues with the increase in the time spent in the virtual environment, may cause an increase in anger [39].

The internet, computers, and mobile phones are used by adolescents almost everywhere [43]. Widespread use is caused by activities such as chatting and messaging in social networks. On the other hand, sometimes arguments, anger, and negative behaviors may be encountered during conversations with other people or through social networks. [44]. This study showed that "anger total" and all sub-dimension averages were higher in those who used computers and phones for a long time for chatting, messaging, and socializing. It is easier to express anger on online platforms, and it is possible to receive more likes through other interactions. This is believed to be the reason why adolescents with long-term computer or smartphone use tend to have higher anger scores.

Gaming has become a popular activity in recent years, especially among adolescents. Violent games can increase anger and increase the tendency to engage in aggressive behavior towards others [45]. Some adolescents may play such games because their anger levels are high. Result of a study on violent games have shown that children may be at higher risk [46]. It is known that many children use games for emotional regulation to relax, forget problems or feel less alone [47]. Playing violent games to deal with anger can have positive or negative consequences for children. This study detected that adolescents who play war, fighting, etc. games on the phone or computer for a long time have higher mean scores of anger. It is believed that the violent content in games exacerbates the anger among adolescents.

Not all games have the same content, but most games contain competitive elements. Therefore, aggressive behavior and anger may occur in players. Jerabeck and Ferguson reported that the content of the games and the style of playing games such as cooperative or solo play are effective on anger [48]. This study indicated that adolescents who play racing and competitive games on the phone or computer for a long time have higher "anger reactive", "anger instrumental" and "anger total" averages. The competitive environment in racing games is believed to provoke anger in adolescents.

Limitations

The study possesses several limitations that warrant consideration. Firstly, the participants were exclusively limited to high school adolescents within a specific province in Eastern Turkey. Consequently, it is crucial to conduct studies encompassing samples from various regions of Turkey and different age groups to ensure broader generalizability. Furthermore, it was not feasible to establish baseline anger levels prior to the introduction of computer and mobile phone usage. All assessments relied on self-reported data, thus precluding the establishment of a definitive cause-effect relationship. Additionally, a significant limitation stems from the collection of phone and computer usage information solely from a single source. The lack of access to parents' perspectives represents a notable constraint on the study's comprehensiveness. Moreover, the use of self-filled forms instead of face-to-face interviews introduces an element that may undermine the reliability of the gathered information. Finally, it is important to acknowledge that while there may exist other variables that influence anger levels, the assessment conducted through the questionnaire did not account for these potential psychopathologies.

Conclusion

This study determined that the increase in the academic degree, the increase in the duration of smartphone use, the use of computers and phones for gaming/entertainment, chat, messaging, and socializing increase anger. In addition, playing violent war, fighting, etc. games for a long time on the computer and phone increases anger, was found. It is known that aggressive behaviors occur during childhood and adolescence and do not change much after they occur. Such feelings of anger can lead to increased aggressive behavior. For this reason, attitudes and behaviors that may lead to various mismatches or crimes in adulthood should be prevented in adolescents. Adolescents playing games that may cause anger may increase their tendency to violence. Long-term use of technology can lead to problems such as addiction, poor problem-solving skills, or difficulty in regulating emotions. Therefore, it may be useful for families to monitor the content, duration and type of games children play.

Compliance with Ethical Standards

Ethical approval: Ethical approval was obtained from Bingöl University Non-Interventional Research Ethics Committee before starting the study (approval number: 28.11.2022-E.86417). After ethical approval was obtained, institutional permissions were obtained through the governorship for the research to be conducted in educational institutions (10.01.2023-92001). Adolescents were informed about the purpose of the study and their consent was obtained.

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Authors Contributions: ST and AS: Study idea (concept) and design, ST: Data collection / literature review, ST and AS:

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Data analysis and interpretation, ST and AS: Preparation of the article, ST and AS: Approval of the final version to be published.

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Relationship between COVID-19 and antimicrobial resistance

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ABSTRACT

Objective: Bacterial and fungal infections, antimicrobial resistance (AMR) results of bacterial agents, and the effect of the pandemic on AMR were evaluated in hospitalized COVID-19 patients. In addition, the detected AMR rates were compared with the AMR rates of the pre-pandemic period.

Patients and Methods: The isolates grown in respiratory and blood samples of adult patients hospitalized with the diagnosis of COVID-19 between March 2020 and December 2020 were evaluated retrospectively. The same data in hospitalized patients before the pandemic, between March and December 2019, were evaluated retrospectively.

Results: A total of 724 samples were included in the study. The superinfection rate was found to be 15.3%. The most frequently isolated microorganisms are; *Acinetobacter baumannii* (34.4%), *Staphylococcus aureus* (10.8%), *Klebsiella pneumoniae* (9.7%) and *Pseudomonas aeruginosa* (7.3%). The lowest resistance rates in Klebsiella pneumoniae isolates were found for aminoglycosides, in *Acinetobacter baumannii* isolates were found for trimethoprim-sulfamethoxazole, in *Pseudomonas aeruginosa* isolates were found for amikacin. When pre-pandemic and pandemic AMR rates were compared; a significant increase in amikacin resistance was detected only in *Klebsiella pneumoniae* isolates during the pandemic period (P:0.049).

Conclusion: The data we have presented may help clinicians in the selection of antimicrobials for empirical therapy by revealing the effect of the pandemic on AMR.

Keywords: Antimicrobial resistance, Bacterial infection, COVID-19, Fungal infection

1. INTRODUCTION

The new coronavirus, named severe acute respiratory syndrome-coronavirus-2 (SARS-COV-2), was declared a pandemic on 11 March 2020 by the World Health Organization (WHO) [1]. While more than 32.7 million people were affected by the disease, more than 1 million died [2]. However, the main problem that has been overlooked is the antimicrobial resistance (AMR) that will emerge after the pandemic [3].

The relationship between SARS-CoV-2 and bacterial infections can be summarized in three scenarios; 1-Secondary SARS-CoV-2 infection following bacterial infection or colonization, 2-Combined viral and/or bacterial pneumonia, 3 – Bacterial superinfection following SARS-CoV-2 infection. These scenarios vary due to the complex time-dependent interactions between virus, host, and bacteria [4]. Bacterial infections in patients infected with SARS-CoV-2 have been reported to cause an increase in disease severity, resource use, and deaths [5]. However, the rate of bacterial or fungal superinfection in patients with coronavirus disease 2019 (COVID-19) is unclear [6]. It has been reported that 25-70% of severe COVID-19 patients have symptoms of sepsis. Therefore, it is very difficult to exclude the diagnosis of bacterial superinfection with symptoms, physical examination findings, imaging and laboratory results. This has led to the widespread use of antimicrobials during the pandemic period [4]. Widespread antimicrobial use is predicted to increase AMR rates in the coming years [5]. There is also limited data on the results of antibiotic susceptibility tests of bacteria in bacterial superinfection [6]. In this article, bacterial and fungal infections in hospitalized patients infected with COVID-19 and AMR results were evaluated. In addition, the detected AMR rates were compared with the AMR rates of the pre-pandemic period.

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2. PATIENTS and METHODS

Study design

This retrospective study was conducted in Izmir Katip Celebi University Ataturk Training and Research Hospital with an 1149-bed capacity, having six ICUs. All adult patients hospitalized with the diagnosis of COVID-19 between March and December 2020 were included in the study.

Sputum, tracheal aspirate, and blood cultures of these patients were evaluated. In addition, the microorganisms reproduced in respiratory and blood samples of patients hospitalized between March and December 2019 before the pandemic, and antibiotic susceptibility test results were evaluated retrospectively. The study was approved by Izmir Katip Celebi University non-interventional clinical research ethics committee (Decision/protocol number: 930, Approval date: 17.09.2020).

Data collection

The data were collected from the hospital's medical records using an electronic database. These data included the patient's gender, age, admitting department, microorganism that caused the infection, and antibiotic susceptibility test results of bacterial agents.

Microbiologic methods

Blood samples were incubated in the automated blood culture system (BACTEC FX, BD, USA). Passages were made on Eosin-Methylene Blue agar (Becton Dickinson, USA), 5% sheep blood agar (Becton Dickinson, USA) and chocolate agar media (Becton Dickinson, USA) from vials with bacteria detected in Gram stain made from bottles that gave a positive signal during incubation. Agar plates were incubated at 37˚C for 24-48 hours. Vials with yeast or hyphae detected in Gram stain made from bottles were additionally passaged to sabouraud dextrose agar (Becton Dickinson, USA) in duplicate and incubated at room temperature and 37°C. Only one growth was taken into account from the repetitive growth of the same microorganism in more than one blood sample of the same patient.

In sputum samples, sputum quality was evaluated initially. Samples that have mucus in macroscopic examination and Bartlett score above zero were included in the study [7]. In addition, dominant microorganisms have been investigated in Gram staining. In more than one respiratory tract sample of the same patient, only one growth from repeated growths of the same bacteria showing the same AMR pattern was taken into account. Sputum and tracheal aspirate samples were planted in 5% sheep blood agar, chocolate agar media, Eosin Methylene Blue agar, and incubated at 37˚C for 18-24 hours. In addition, samples with yeast or hyphae in Gram staining were passaged to sabouraud dextrose agar in duplicate and incubated at room temperature and 37°C.

Strains found to be bacteria in Gram staining were evaluated by colony morphology and biochemical tests (oxidase, coagulase, and catalase test, three sugar iron test, urea hydrolysis test, Indole, Methyl Red, citrate test). Identification and antibiotic susceptibility test of isolated bacteria were done using an automated system (Phoenix, BD, USA). The results of the antibiotic susceptibility test were evaluated according to the European Committee on Antimicrobial Susceptibility Testing criteria [8]. Antimicrobials that were found to be moderately sensitive as a result of antimicrobial susceptibility tests were accepted as resistant.

Strains found to be yeast in Gram staining were evaluated by colony morphology, germ tube test, urease test, and automated system (PhoenixTM 100-yeast ID, BD, USA). Automated system was used for definition of all types. Conventional methods were used for the verification of the automated system. If it was impossible to define the species with conventional methods, they were defined only with the automated system.

Colony structure and colony color of mold-growing colonies on Saboraud dextrose agar were examined macroscopically. Preparations were prepared using lactophenol cotton blue for microscopic examination. For identification, details such as the number of sterigmata, vesicle structure, odd-even phyalite, and color, structure, and location of conidiophores were examined.

Definitions

Bloodstream infection was defined as the growth of a non-skin flora commensal from ≥ 1 blood culture in a patient with systemic signs of infection. To distinguish bloodstream infection from contamination caused by a common skin colonizer such as coagulase-negative staphylococci or *Corynebacterium*, we required ≥2 blood cultures drawn from different sites. Results that were considered as contamination were excluded from the study.

Statistical analysis

SPSS version 22.0 (IBM Corp., USA) package program was used for statistical analysis. For descriptive analyses, mean ± standard deviation, number and percentage distributions were calculated. Pearson's chi-square test was used for statistical analysis of categorical variables. Fisher's exact test was used for analyzing variables among the groups. A value of $\mathbf{P}< 0.05$ was considered to be significant.

3. RESULTS

A total of 4662 (3750 blood, 912 respiratory) samples from 4461 patients were analyzed retrospectively. Growth was detected in 1779 samples from 1727 patients. Growths considered as contamination were excluded from the study. Only one growth was evaluated from the repetitive growth of the same bacteria in the same patient. A total of 724 samples from 681 patients, including 381 (52.6%) respiratory samples and 343 (47.4%) blood samples, were included in the study. Of these samples, 610 (84.3%) were from patients hospitalized in ICU (Table I). In our hospital, the superinfection rate was found to be 15.3% (681/4461) in patients diagnosed with COVID-19.

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Table I. *Distribution of isolated agents*

	Respiratory samples			Blood samples			TOTAL
	Standard ward n (%)	ICU n (%)	Total n (%)	Standard ward n (%)	ICU n (%)	Total n (%)	n (%)
Acinetobacter baumannii	8 (1.1)	171 (23.6)	179 (24.7)	4 (0.6)	66 (9.1)	70 (9.7)	249 (34.4)
Klebsiella pneumoniae	6 (0.8)	28 (3.9)	34 (4.7)	6 (0.8)	30 (4.1)	36 (5)	70 (9.7)
Pseudomonas aeruginosa	6 (0.8)	38 (5.2)	44 (6)	2 (0.3)	7 (1)	9 (1.2)	53 (7.3)
Escherichia coli	2 (0.3)	10 (1.4)	12 (1.7)	6 (0.8)	6 (0.8)	12 (1.7)	24 (3.3)
Stenotrophomonas maltophilia	0	11 (1.5)	11 (1.5)	0	1 (0.1)	1 (0.1)	12 (1.7)
Enterobacter aerogenes	2 (0.3)	8 (1.1)	10 (1.4)	0	0	0	10 (1.4)
Serratia marcescens	3 (0.4)	5 (0.7)	8 (1.1)	0	0	0	8 (1.1)
Proteus mirabilis	1 (0.1)	3 (0.4)	4 (0.5)	1 (0.1)	1 (0.1)	2 (0.3)	6 (0.8)
Haemophilus influenzae	0	4 (0.6)	4 (0.6)	0	0	0	4 (0.6)
Staphylococcus aureus	3 (0.4)	26 (3.6)	29 (4)	31 (4.3)	18 (2.5)	49 (6.8)	78 (10.8)
Enterococcus faecium	1 (0.1)	3 (0.4)	4 (0.5)	10 (1.4)	23 (3.2)	33 (4.6)	37 (5.1)
Enterococcus faecalis	0	0	0	6 (0.8)	23 (3.2)	29 (4)	29 (4)
Streptococcus spp.	0	1 (0.1)	1 (0.1)	7 (1)	12 (1.7)	19 (2.6)	20 (2.8)
Streptococcus pneumoniae	0	9 (1.2)	9 (1.2)	0	10 (1.4)	10 (1.4)	19 (2.6)
Candida albicans	0	9 (1.2)	9 (1.2)	0	8 (1.1)	8 (1.1)	17 (2.3)
Candida glabrata	0	0	0	0	1 (0.1)	1 (0.1)	1 (0.1)
Candida parapsilosis	0	0	0	0	24 (3.3)	24 (3.3)	24 (3.3)
Candida trophicalis	0	1 (0.1)	1 (0.1)	0	1 (0.1)	1 (0.1)	2 (0.3)
Asgergillus fumigatus	0	3 (0.4)	3 (0.4)	0	0	0	3 (0.4)
Asgergillus flavus	0	3 (0.4)	3 (0.4)	0	0	0	3 (0.4)
Asgergillus niger	0	1 (0.1)	1 (0.1)	0	0	0	1 (0.1)
Other	5 (0.7)	10 (1.4)	15 (2.1)	4 (0.6)	35 (4.8)	39 (5.4)	54 (7.5)
TOTAL	37 (5.1)	344 (47.5)	381 (52.6)	77 (10.6)	266 (36.7)	343 (47.4)	724

ICU: Intensive care units, CNS: Coagulase negative staphylococcus. Other: In this group; Citrobacter koseri, Enterobacter cloacae, Klebsiella oxytoca, Pseudomonas putita, Pseudomonas fluorescens, Serratia odorifera, Streptococcus vestibularis, Hafnia alvei, Delftia acidovorans, Achromobacter spp. is located.

When all samples were evaluated, the most frequently isolated microorganisms were; *Acinetobacter baumannii* 34.4%, *Staphylococcus aureus* 10.8%, *Klebsiella pneumoniae* 9.7%, *Pseudomonas aeruginosa* 7.3% and *Enterococcus faecium* (5.1%). The most isolated bacteria were *Acinetobacter baumannii* (24.7%), *Pseudomonas aeruginosa* (6%), *Klebsiella pneumoniae* (4.7%), *Staphylococcus aureus* (4%) and *Escherichia coli* (1.7%) in respiratory samples. The most isolated bacteria were *Acinetobacter baumannii* (9.7%), *Staphylococcus aureus* (6.8%), *Klebsiella pneumoniae* (5%), *Enterococcus faecium* (4.6%) and *Enterococcus faecalis* (4%) in blood samples. In addition, fungal growth was detected in 51 samples. Of these, 34 were blood samples and 17 were respiratory samples. The most isolated fungi were *Candida parapsilosis* (3.3%) and *Candida albicans* (2.3%) (Table I).

Amikacin, daptomycin, vancomycin, teicoplanin and linezolid resistance were not detected in *Staphylococcus aureus* isolates. The first three antimicrobials with the highest resistance rate were; erythromycin (15.4%), clindamycin (12.8%), and gentamicin (10.3%). In *Klebsiella pneumoniae* isolates, the lowest resistance rate was found in aminoglycosides (amikacin 24.3%, gentamicin 17.1%), while the first two antimicrobials with the highest resistance rate were cephalosporins (ceftazidime 82.9%, cefuroxime 82.1%, ceftriaxone 81.4%, cefepime 75.4%) and quinolones (ciprofloxacin 78.6%, levofloxacin 77.1%). While the lowest resistance rate was found in trimethoprim-sulfamethoxazole (TMP/SMX) (68.7%) in *Acinetobacter baumannii* isolates, resistance rates were quite higher in the other antimicrobials. The first two antimicrobials with the lowest resistance rate in *Pseudomonas aeruginosa* isolates were; cephalosporins (ceftazidime 30.6%, cefepime 28.9%) and amikacin (22.6%). The highest resistance rate in these isolates was found in carbapenems (imipenem 60.4%, meropenem 60.4%) (Table II).

When the resistance rates of patients in standard wards and ICU were compared for *Staphylococcus aureus* isolates; the resistance rates of fosfomycin, fusidic acid and gentamicin were higher among patients in standard wards, and the resistance rates of clindamycin, erythromycin, tetracycline and oxacillin were higher patients in ICU. In *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates, antibiotic resistance was found to be higher among the patients in ICU than the patients in standard wards.

When the AMR rates detected in the year 2019 before the pandemic were compared with our findings based on more than 10% change; amikacin and TMP/SMX have increased in

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Acinetobacter baumanni isolates, and gentamicin, amikacin, ciprofloxacin and carbapenem resistance were increased in *Pseudomonas aeruginosa* isolates [9]. In addition, erythromycin resistance in *Staphylococcus aureus* isolates and gentamicin and TMP/SMX resistance rates in *Klebsiella pneumoniae* isolates were decreased. However, a significant increase in amikacin resistance was detected only in *Klebsiella pneumoniae* isolates during the pandemic period (P:0.049).

Table II. *Distribution of AMR rates of isolated bacteria*

	Staphylococcus aureus					*Klebsiella pneumoniae*					*Acinetobacter baumannii*					*Pseudomonas aeruginosa*				
	PP n: 78			PPP n:189		PP n: 70			PPP n:490		PP n: 249			PPP n:446		PP n: 53			PPP n:227	
	SW	**ICU**	**Total**	**Total**	***P**	**SW**	**ICU**	**Total**	**Total**	***P**	**SW**	**ICU**	**Total**	**Total**	***P**	**SW**	**ICU**	**Total**	**Total**	***P**
GEN	8.9	1.4	10.3	6.9	>0.05	2.9	14.2	17.1	35.3	>0.05	4	93.2	97.2	88.3	>0.05	2	33.8	35.8	13.7	>0.05
AMK	0	0	0	3	NA	4.3	20	24.3	19.4	**0.049**	2.8	92.4	95.2	83.2	>0.05	1.9	20.7	22.6	10.1	>0.05
CLI	2.6	10.2	12.8	20.6	>0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ERY	3.8	11.6	15.4	27	>0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DAP	0	0	0	0	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FA	5.1	2.6	7.7	6.3	>0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CIP	2.6	2.5	5.1	4.8	>0.05	14.3	64.3	78.6	79.6	>0.05	4.8	93.6	98.4	91	>0.05	2	43.3	45.3	24.2	>0.05
LVX	2.6	2.5	5.1	4.8	>0.05	12.9	64.2	77.1	-	NA	3.2	93.2	96.4	-	NA	6	53.2	59.2	-	NA
MXF	2.6	2.5	5.1	0	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OXA	3.8	10.3	14.1	21.2	>0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TE	2.6	6.4	9	18	>0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VA	0	0	0	0	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TEC	0	0	0	0	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TMP/SMX	1.3	1.3	2.6	3.2	>0.05	7.1	40	47.1	60.4	>0.05	2.4	66.3	68.7	52.5	>0.05	-	-	-	-	-
LZD	0	0	0	0	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FOF	3.8	0	3.8	3.7	>0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AMC	-	-	-	-	-	14.3	57.1	71.4	78.2	>0.05	-	-	-	-	-	-	-	-	-	-
SAM	-	-	-	-	-	12.9	62.1	75	-	NA	-	-	-	-	-	-	-	-	-	-
CAZ	-	-	-	-	-	14.3	68.6	82.9	79.2	>0.05	-	-	-	-	-	7.5	23.1	30.6	25.6	>0.05
CRO	-	-	-	-	-	14.3	67.1	81.4	78.8	>0.05	-	-	-	-	-	-	-	-	-	-
CXM	-	-	-	-	-	12.9	69.2	82.1	81.6	>0.05	-	-	-	-	-	-	-	-	-	-
FEP	-	-	-	-	-	10	65.4	75.4	79.2	>0.05	-	-	-	-	-	3.8	25.1	28.9	27.3	>0.05
TPZ	-	-	-	-	-	12.9	61.4	74.3	76.5	>0.05	-	-	-	-	-	7.5	26.5	34	27.8	>0.05
ETP	-	-	-	-	-	12.9	55.7	68.6	68.6	>0.05	-	-	-	-	-	-	-	-	-	-
IPM	-	-	-	-	-	12.9	54.2	67.1	61.4	>0.05	2.8	95.6	96.4	92.8	>0.05	6	54.4	60.4	44.1	>0.05
MEM	-	-	-	-	-	11.4	47.2	58.6	61.4	>0.05	2.8	95.6	96.4	91.9	>0.05	7.5	52.9	60.4	46.3	>0.05

*SW: Standard ward, ICU: Intensive care units, PP: Pandemic period; During the period in March 2020 and December 2020, PPP: Pre-pandemic period; During the period in March-December 2019, *P: It was obtained by comparing the total resistance rates of the pandemic and prepandemic period. NA: Not available, GEN: Gentamicin, AMK: Amikacin, CLI: Clindamycin, ERY: Erythromycin, DAP: Daptomycin, FA: Fusidic acid, CIP: Ciprofloxacin, LVX: Levofloxacin, MXF: Moxifloxacin, OXA: Oxacillin, TE: Tetracycline, VA: Vancomycin, TEC: Teicoplanin, TMP/SMX: Trimethoprim-Sulfamethoxazole, LZD: Linezolid, FOF: Fosfomycin, AMC: Amoxicillin-clavulanate, SAM: Ampicillin-sulbactam, CAZ: Ceftazidime, CRO: Ceftriaxone, CXM: Cefuroxime, FEP: Cefepime, TPZ: Piperacillin-tazobactam, ETP: Ertapenem, IPM: Imipenem, MEM: Meropenem*

4. DISCUSSION

Although, there is limited clinical experience with patients hospitalized for COVID-19, many critical decisions had to be made during the implementation of the treatment. One of these decisions is antimicrobial therapy [9]. Superinfection may increase antibacterial intolerance, inhibit the host's immune system, and thus adversely affect the prognosis of the disease [10]. The rate of superinfection in patients with a diagnosis of COVID-19 has been reported between 6.9% and 15% in various studies [5,11-15]. These rates have been reported as 5.1% to 38.9% in China and 4.8% to 27.4% in Western countries, especially in patients hospitalized in the ICU [12]. In our study, the superinfection rate was found to be 15.3%, which was consistent with the literature.

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Bacterial infections take the first place among the superinfection agents in patients infected with COVID-19. The most isolated bacteria in these patients are; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* [9,16,17]. In our study, we detected *Acinetobacter baumannii*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* as the superinfection agents in COVID-19 patients, respectively, which was also consistent with the literature.

The superinfection rate in patients with COVID-19 infection followed in ICUs has been reported as 13.5-44% [18-20]. Bacterial or fungal pneumonia is the most common infection in these patients, while bloodstream and urinary tract infections have also been reported. The most isolated microorganisms are *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Serratia marcescens*, *Aspergillus* spp. and *Candida* spp [6]. In our study, in accordance with the literature, the most isolated microorganisms in ICU hospitalized patients were *Acinetobacter baumannii*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively. Zamora-Cintas et al., reported that the most isolated microorganisms were *Candida albicans* and *Enterococcus faecalis* in blood samples, and *Candida albicans* and *Pseudomonas aeruginosa* in respiratory samples in patients infected with COVID-19 in the ICU [21].

In our study, the most isolated microorganisms in intensive care patients were; *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in blood samples, and *Acinetobacter baumannii* and *Klebsiella pneumoniae* in respiratory samples. These discordant results are thought to be due to regional differences and different treatment protocols.

It is thought that risk factors such as ICU admission, corticosteroid therapy, intubation/mechanical ventilation, underlying respiratory diseases and cytokine storm may be responsible for the increase in invasive fungal infections in COVID-19 patients [22]. While Chen et al., reported positive fungal culture from respiratory samples in 5% of 99 patients, Du et al., reported fungal infection in 10.6% of 85 COVID-19 cases [23,24]. Silva et al,. reported 25.5% *Candida* spp., and 1.4% *Aspergillus* spp. in 212 patients with COVID-19 [17]. In our study, *Candida* spp. were isolated in 6%, and *Aspergillus* spp. in 0.9% of the patients. The discordance in these results is thought to be due to differences in study populations, treatment protocols, and geographical location.

Antibacterial selection and duration of treatment are very important in respiratory bacterial or fungal infections. Many antimicrobials used in therapy have the potential to cause prolongation of the QT wave on the electrocardiogram and cardiac arrest, raising concerns about antimicrobial choice [12]. Inflammatory markers can be used to support antimicrobial decisions. However, since the data obtained with these tests may have been affected by COVID-19, it may be insufficient to diagnose bacterial infection. In addition, routine microbiological examinations with long-term patient contact may have been decreased due to the fear of getting sick by health professionals. In this instance, studies reporting AMR rates guide the selection of empirical antimicrobials. WHO has recommended that antimicrobial selection in empirical antimicrobial therapy should be based on individual and local epidemiological data [9]. In our study, resistance to amikacin, daptomycin, vancomycin, teicoplanin, and linezolid was not found in *Staphylococcus aureus* isolates. *Klebsiella pneumoniae* isolates were more sensitive to aminoglycosides, *Acinetobacter baumanni* isolates to TMP/SMX, and *Pseudomonas aeruginosa* isolates were more sensitive to 3rd and 4th generation cephalosporins and amikacin. In addition, as expected, AMR rates were found to be higher in patients hospitalized in ICU compared to patients hospitalized in other wards. It is very important for antimicrobial stewardship programs to focus on the determination of antimicrobials to be used in empirical treatment, but, studies on the AMR patterns of bacterial agents that cause infections in patients infected with COVID-19 are very limited in the literature. For this reason, we believe the data we presented in our study will make a significant contribution to the literature.

It is very difficult to exclude bacterial or fungal infections in severe COVID-19 patients only with symptoms, signs, radiological abnormalities and blood tests [6]. Although, the superinfection rate is 6.9-15% in patients infected with COVID-19, it has been reported that 58-100% of these patients received antimicrobial therapy [5,6,11,12,14,15,25]. Due to the inconsistency between the initiation of empirical antimicrobials and the rates of bacterial infection, WHO recommended that empirical antimicrobial therapy should be initiated only in severe COVID-19 patients [9]. The widespread use of antimicrobials during the pandemic period may increase resistance rates in the following years. It is expected that there will be approximately 10 million deaths worldwide in the next 30 years due to AMR in the post-COVID-19 period compared to the pre-COVID-19 period [3]. Therefore potential management interventions that support the reduction of antimicrobial prescribing in the pandemic should be evaluated urgently. Nori et al., compared the antimicrobial susceptibility results with the antimicrobial susceptibility data of the same period of 2019 before the pandemic and reported that the susceptibility to cephalosporin, ciprofloxacin, and meropenem in *Klebsiella pneumoniae* isolates decreased by more than 10% [9]. In our study, when the AMR rates detected in the same period of 2019 before the pandemic was compared with the AMR rates during the pandemic period; it was determined that the resistance rates of gentamicin, amikacin, ciprofloxacin and carbapenem in *Acinetobacter baumanni* isolates, amikacin and TMP/SMX in *Pseudomonas aeruginosa* isolates were increased by more than 10%. In addition, in our study, it was found that amikacin resistance increased significantly in *Klebsiella pneumoniae* isolates during the pandemic period. Although, it is predicted that the increasing use of antimicrobials in the pandemic will increase AMR rates in forthcoming years, studies on this subject are very limited. Our results will shed light on the limited information on this subject. In the future, the effect of the pandemic on AMR rate will be better understood with multicenter studies involving a large patient population.

The two major limitations of our study are; the study was a single-center study and the COVID-19 pandemic period was limited to the year 2020.

In conclusion; despite the high rate of empirical broad-spectrum antibiotics being prescribed in patients with respiratory tract infection with a diagnosis of SARS-CoV-2, the data supporting the association of symptoms with bacterial/fungal infection is quite low. A general evidence base for developing antimicrobial management strategies is required to prevent the undesirable consequences of antimicrobials prescribed during the COVID-19 pandemic, both on the individual and the community. This evidence can only be provided by prospective clinical and laboratory studies focusing on antimicrobial therapy. The results of the relevant study will guide researchers in future comprehensive studies on the antimicrobials to be selected in empirical antimicrobial therapy and the impact of the pandemic on AMR rate.

Compliance with the Ethical Standards

Ethics Committee Approval: The study was approved by Izmir Katip Celebi University non-interventional clinical research ethics committee (Decision/protocol number: 930, Approval date: 17.09.2020).

Conflict of Interest: The authors declare that they have no conflict of interest relevant to this article.

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Authors Contribution: TM: Planning, data collection, analysis, article writing, SK: Planning, data collection, article writing, FBB and EO: Data collection, analysis, BOP: Analysis, article writing, AAG and SGY: Planning, data collection. All authors read and approved the final version of the article.

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Diagnostic utility of the systemic immune-inflammatory index in preterm neonates with late-onset sepsis

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ABSTRACT

Objective: To assess if systemic immune-inflammatory index (SII) has a diagnostic role for late-onset sepsis (LOS) in premature neonates.

Patients and Methods: A single-center retrospective observational study including preterm infants with culture-proven LOS and controls was conducted between January 2017 and December 2022. SII was derived using complete blood count values acquired at the beginning of and three to five days before LOS. SII was compared between the LOS group and controls.

Results: A total of 144 infants were included in the study. The SII values of the LOS group were found to be significantly increased in comparison to the control group [376.74 (11.11 – 15170) vs. 235.24 (46.83 – 1214.38) (median, min-max), P =0.018]. The SII values significantly increased when pre-sepsis and LOS values were compared [200.6 (0 – 1295.78) vs. 328.28 (0 – 14678, P<0.001]. As determined using the receiver operating characteristic analysis, the area under the curve for SII was 0.621 (44.4% sensitivity, 83.3% specificity, 72.7% positive predictive value, and 60% negative predictive value) for predicting LOS.

Conclusion: Although, further research is required, SII may be used with other biomarkers to identify LOS in preterm infants and may constitute a readily accessible additional diagnostic parameter.

Keywords: Late-onset sepsis, Systemic immune-inflammatory index, Neonate, Biomarker

1. INTRODUCTION

Neonatal sepsis is described as an immune system dysregulation induced by bacterial, viral, or fungal bloodstream infection accompanied by hemodynamic abnormalities [1,2].

Neonatal sepsis is classified as early or late onset sepsis (LOS), depending on the onset time. LOS occurs after 72 hours of life, according to the pathophysiology and etiology of the responsible microorganisms [3].

Despite advances in clinical management and laboratory diagnosis methods, LOS remains the major reason for morbidity and mortality in preterm neonates receiving care in neonatal intensive care units (NICUs) [4]. LOS is a common consequence of extreme prematurity due to low immunoglobulin concentrations and poor neutrophil function, which makes preterm infants more vulnerable to infection [2]. Contact with hospital personnel, family members, contaminated equipment, nutritional sources, increased hospital stay, a necessity for invasive interventions, central venous access, and use of broad-spectrum antibiotics are multiple factors that put preterm neonates at increased risk for LOS. Infants with extremely low birth weight are reported to have an incidence of LOS ranging from 12.2% to 24.4% [4].

Each laboratory test has yet to be identified as having high enough sensitivity and specificity for the early recognition of LOS. The gold standard for LOS diagnosis is appropriately collected cultures (blood, urine, cerebrospinal fluid, etc.). In over 99% of patients, the causative bacterium may be identified within 36 hours [5]. Immediate commencement of therapy is vital and lifesaving. On the other hand, overdiagnosis is associated with the harmful effects of neonatal antibiotic exposure [6]. C-reactive protein (CRP), procalcitonin (PCT), as well as the neutrophil-to-lymphocyte ratio (NLR), and the platelet-to-lymphocyte ratio (PLR), are considered to be crucial markers in diagnosing neonatal sepsis [7-9]. Several parameters from a complete blood count (CBC) – peripheral lymphocyte, neutrophil, and platelet counts, are used to derive the systemic immune-inflammatory index (SII). Both inflammation and

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ischemia are reported to lead to an increase in SII [10]. The purpose of this study was to evaluate, if any, the role that SII plays in assessing LOS in preterm infants when there is a clinical suspicion that it may be present. We are unaware of any previous study on the predictive efficacy of SII in diagnosing LOS in premature infants.

2. PATIENTS and METHODS

Study design, ethical considerations, and participants

This retrospective analysis involving preterm neonates with culture-proven sepsis and controls was undertaken between January 2017 and December 2022 in the NICU of Marmara University Research and Training Hospital in Istanbul, Turkey. The Marmara University School of Medicine's Clinical Studies Ethics Committee approved the study (Date: 01.06.2023, No: 09.2023.105).

Seven hundred fifty-five of the 2268 infants that were followed up in the NICU were born prematurely. Of these, 110 preterm infants <37w gestational age (14.5%) had culture-proven LOS. The study group was comprised of preterm infants ≤35w gestation with culture-proven sepsis that occurred after 72 hours of life (n=101, 13.3%). The control group was comprised of premature children born in our hospital during the same period of time, which matched the inclusion criteria and were free of infection. Control SII was calculated at day 19, corresponding to the LOS group's median first sepsis day. In cases of several LOS episodes, only the initial incident was included. The following exclusion criteria were applied: (1) incomplete laboratory data, (2) any episode of early-onset sepsis, (3) preterm infants with a gestational age >35 weeks, (4) major congenital and chromosomal anomalies, (5) hypoxic-ischemic encephalopathy, and (6) cyanotic heart disease. Following these criteria, 29 patients were excluded; consequently, the study consisted of 72 (9.5%) infants (Figure 1).

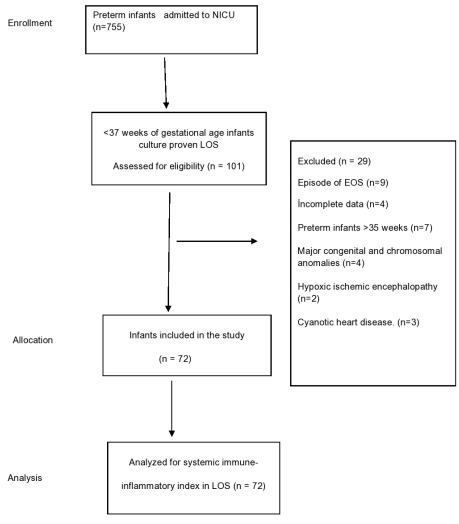

Figure 1. *Flow chart for selection of eligible infants in the study*

Data collection and definitions

Data were extracted from patient records. The demographic, perinatal, and neonatal characteristics of patients were analyzed. A positive blood culture was required to define LOS. Two positive blood cultures were required to diagnose sepsis caused by coagulase-negative staphylococci. Blood culture isolates were analyzed using a fully automated BACTEC method – the BACT/ALERT 3D system (bioMerieux, SA, France).

For all patients, white blood cells (WBC) (/mm3), absolute neutrophil count (ANC) (/mm3), lymphocyte count (/mm3), platelet count (103/mm3), CRP (mg/L) and PCT ((g/L) obtained at the beginning of the LOS episode (6h before or 24h after blood culture samples were obtained when clinical findings were present) and three to five days before sepsis (pre-sepsis) were collected. Hematologic indices that were calculated using this data were: NLR (neutrophil count/lymphocyte count), PLR (platelet count/lymphocyte count), and SII (neutrophil count x platelet count/lymphocyte count).

Neonatal morbidities were analyzed. Severe intraventricular hemorrhage (IVH) included stages ≥ III, classified according to Volpe's cranial ultrasound classification [11]. Bronchopulmonary dysplasia (BPD) was defined according to oxygen and ventilator support requirements at 36w of gestation [12]. Severe retinopathy of prematurity (ROP) included standardized international criteria stages ≥3 or any stage requiring cryotherapy or laser photocoagulation [13]. Bell criteria were used for severe necrotizing enterocolitis (NEC) [14].

Outcomes

The primary outcome was the diagnostic value of SII in preterm infants with LOS. Secondary outcomes were duration of ventilation, BPD, NEC, ROP, IVH, hospitalization days, and mortality.

Statistical analysis

IBM SPSS Statistics for Windows was utilized for statistical analysis (IBM Corp. Released 2017, Version 25.0. Armonk, NY, USA). The sample size was determined with G*Power version 3.1.9.7 (Franz Faul, Germany). Power analysis using alpha = 0.05, effect size = 0.419, and power = 0.80 revealed a required sample size of 144 participants for the two groups. The Pearson's chi-square test was used to compare categorical variables expressed as n (percent). Continuous variables were reported using mean ± standard deviation (SD) or medians and ranges (min-max). The normality of data was evaluated using the Kolmogorov-Smirnov test. Continuous variables were compared using the t-test or the Mann-Whitney U test on independent samples. The Spearman correlation analysis assessed the relationship between continuous variables. For intragroup comparisons, the Wilcoxon Test was utilized. Using receiver operating characteristic (ROC) analysis, the SII values used to predict LOS were investigated. For the appropriate cut-off value, the sensitivity and specificity with positive likelihood ratio (+LR) and negative likelihood ratio (-LR) ratios were computed. The cut-off values exhibited

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the greatest sensitivity. A P-value of <0.05 was considered to be statistically significant.

3. RESULTS

The study included 72 preterm neonates that had culture-proven sepsis. On the other hand, the control group was made up of 72 preterm infants who were infection free. Table I shows the demographic and laboratory parameters of the study population. The LOS and the control group were similar concerning gestational age (P=0.052), gender (P=0.739), and IVH (P=0.286). When LOS and control groups were compared, vaginal delivery (P=0.006), presence of venous catheter (P=0.006), ventilator-associated pneumonia (VAP) (P<0.001), duration of ventilation (P<0.013), days on parenteral nutrition (P<0.001), hospitalization (P<0.001), NEC (P<0.001), ROP (P<0.010), and mortality (P <0.001) were observed to be statistically significantly higher in the LOS group. Birth weight (P<0.001) and Apgar scores were significantly lower at the first minute (P=0.002) and fifth minute (P=0.001), respectively, in the LOS group when compared to the control group.

When laboratory parameters were compared, median serum levels of CRP (p<0.001), PCT (p<0.001), WBC (p=0.01), ANC (p<0.001), NLR (p<0.001), SII (p=0.018) were significantly higher in the LOS group whereas, median lymphocyte count (p<0.028), and platelet count (p=0.001) were lower. However, the median PLR (p=0.979) was similar between groups.

Gram-positive microorganisms led to more LOS when compared to (53/77, 68.8%) gram-negative microorganisms [50/72 (69.4%) vs. 20/72 (27.8%)] and fungus (2/72, 2.8%). *Coagulase-negative staphylococci* were the most commonly observed gram-positive organisms that caused LOS (n=31, 43.1%), followed by *Staphylococcus aureus* (n=13, 18.1%), other gram-positive organisms (n=6, 8.3%), *Stenotrophomonas maltophilia* (n=5, 6.9%), *Klebsiella spp.* (n=4, 5.6%), *Enterobacter spp.* (n=3, 4.2%), other gram-negative organisms (n=8,11.2%), and fungus (n=2, %2.8).

The laboratory data of the study population three to five days before sepsis (pre-sepsis), and when sepsis was diagnosed (sepsis) have been summarized in Table II. Lymphocyte count, platelet count, and PLR were similar at pre-sepsis and sepsis measurements. On the other hand, when pre-sepsis values were compared to sepsis values, WBC, PNL, CRP, PCT, NLR, and SII were found to be higher in sepsis (p=0.003, p<0.001, p<0.001, p<0.001, p<0.001 and p<0.001 respectively).

ROC revealed that SII, CRP, PCT, and NLR are predictive when defining culture-proven LOS among preterm infants (Figure 2). The AUC of SII, CRP, PCT, and NLR was 0.621 (0.526-0.716) (44.4% sensitivity, 83.3% specificity), 0.948 (0.912-0.984) (83.3% sensitivity, 97.3% specificity), 0.909 (0.845-0.973) (88.2% sensitivity, 100.0% specificity), and 0.697 (0.609-0.785) (43% sensitivity, 91.6% specificity) respectively (Table3).

SII level was found to be significantly lower in preterm neonates with BPD [511.15 (10.3-14678)] vs. [246.09 (0-4107.3) p=0.038]. SII values were similar for patients with IVH [560.36 (10.33-14678)] vs. [258.57 (0-5474) p=0.104], ROP [387 (5.77-14678)] vs. [270 (0-6549.7) p=0.631], NEC [314.15 (0-14678)] vs. [381.6(10.3-5474) p=0.626], and when mortality was compared [242.1 (0-1534.74)] vs. 381.6 (5.77-14678) p=0.231].

Additionally, no correlation was found between SII and CRP levels. PCT demonstrated a weak positive correlation with SII levels (r=0.464, P<0.001). NLR was highly correlated with SII levels (r=0861, P<0.001).

Table I. *Demographic and laboratory parameters of the study population*

	Late-onset sepsis group (n=72)	Control group (n=72)	P
**GA[a] at birth, week	27 (22-35)	28 (23-35)	0.052
**BW[b], g	947 (315-3460)	1452 (515-2660)	**<0.001**
*Gender, male	34 (47.2)	36(50)	0.739
*Vaginal delivery	28 (38.9)	13 (18.06)	**0.006**
**Apgar 1	5 (0-9)	6 (2-9)	**0.002**
**Apgar 5	7 (3-10)	8 (2-10)	**0.001**
*Presence of venous catheter	53 (73.6)	37 (51.3)	**0.006**
*VAP[c]	21 (29.2)	4 (5.7)	**<0.001**
*BPD[d]	40 (55.5)	20 (27.7)	**<0.001**
*IVH[e]	16 (22.22)	11 (15.28)	0.286[3]
*NEC	43 (59.72)	14 (19.72)	**<0.001**[3]
*ROP[g]	33 (45.83)	10 (14.08)	**<0.001**[3]
**Ventilation days	17 (0-200)	8 (0-118)	**<0.013**
**Parenteral nutrition days	20 (0-220)	10 (3-120)	**<0.001**
**Hospitalization days	84 (3-373)	45 (0-142)	**<0.001**
*Mortality	11(15.3)	0 (0)	**<0.001**
**WBC (/mm3)[h]	12400 (1100-79600)	10000 (1200-20300)	**0.01**
**ANC (/mm3)[i]	7200 (100-67600)	3450 (600-12800)	**<0.001**
**Lym count (/mm3)[j]	3200 (500-15500)	4050 (1200-11800)	**<0.028**
**Plt count (10^3/mm^3)[k]	165 (15-686)	226 (66-456)	**0.001**
**NLR [m]	1.8 (0.08-41)	0.73 (0.16-5)	**<0.001**
**PLR [n]	60 (10-740)	70 (20-200)	0.979
**Crp (mg/L)[o]	41.7 (0.60-245)	1.59 (0.04-10.8)	**<0.001**
**Pct (µg/L) [r]	3 (0.07-100)	0.3 (0.10-2.0)	**<0.001**
**SII [s]	376.74 (11.11-15170)	235.24 (46.83-1214.38)	**0.018**

**Values are given as percentage, **Values are given as median (min–max), [a]GA gestational age, [b]BW birth weight, C/S cesarean section, [c]VAP ventilator – associated pneumonia, [d]BPD bronchopulmonary dysplasia, [e]IVH intraventricular hemorrhage, [f]NEC necrotizing enterocolitis, [g]ROP retinopathy of prematurity, [h]WBC: White blood cell; [i]ANC:Absolute neutrophil count; [j]Lym: Lymphocyte count ; [k]Plt: Platelet count; [m]NLR: Neutrophil-to – lymphocyte ratio; [n]PLR: Platelet-to-lymphocyte ratio; [o]CRP: C reactive protein,; [r]Pct : Procalcitonin; [s]SII: Systemic immune-inflammatory index*

Table II. *The laboratory data prior to sepsis and at the time of the diagnosis of sepsis.*

	Pre-sepsis	Sepsis	P
WBC[a]	10300 (3400-24700)	12350 (1100-79600)	**0.003***
PNL[b]	4000 (600-10700)	7200 (100-67600)	**<0.001***
LENFO[c]	2900 (400-8500)	3150 (500-15500)	0.402*
PLT[d]	175 (54-249)	158.5 (15-686)	1.000*
CRP[e]	3.11 (0.5-11)	40.17 (0.6-245)	**<0.001***
PCT[f]	0.3 (0.05-5)	3 (0.07-100)	**<0.001***
SII[g]	200.6 (0-1295.78)	328.28 (0-14678)	**<0.001***
NLR[h]	1.08 (0-5.44)	1.92 (0-41)	**<0.001***
PLR[i]	60 (0-260)	60 (10-720)	0.236*

[a]WBC: White blood cell; [b]PNL:Neutrophil count; [c]Lym: Lymphocyte count ; [d]Plt: Platelet count; [h]NLR: Neutrophil-to – lymphocyte ratio; [i]PLR: Platelet-to-lymphocyte ratio; [e]CRP: C reactive protein,; [f]Pct : Procalcitonin; [g]SII: Systemic immune-inflammatory index
**Wilcoxon test*

Table III. *Accuracy of biomarkers for prediction of late-onset sepsis*

TEST	AUC (%95CI)	Cut-off	p	Sensitivity	Specificity	PPV	NPV
SII[a]	0.621 (0.526-0.716)	>450	**0.014**	44.4	83.3	72.7	60.0
CRP[b]	0.948 (0.912-0.984)	>9.5	**<0.001**	83.3	97.2	96.7	85.3
PCT[c]	0.909 (0.845-0.973)	>0.62	**<0.001**	88.2	100.0	100.0	89.8
NLR[d]	0.697 (0.609-0.785)	>2.8	**<0.001**	43.0	91.6	83.7	61.6
PLR[e]	0.484 (0.384-0.584)		0.742				

[a]SII: Systemic immune-inflammatory index; [b]CRP: C reactive protein,; [c]Pct : Procalcitonin; [d]NLR: Neutrophil-to – lymphocyte ratio; [e]PLR: Platelet-to-lymphocyte ratio; AUC: Area under the curve; PPD: Positive predictive value; NPV: Negative predictive value

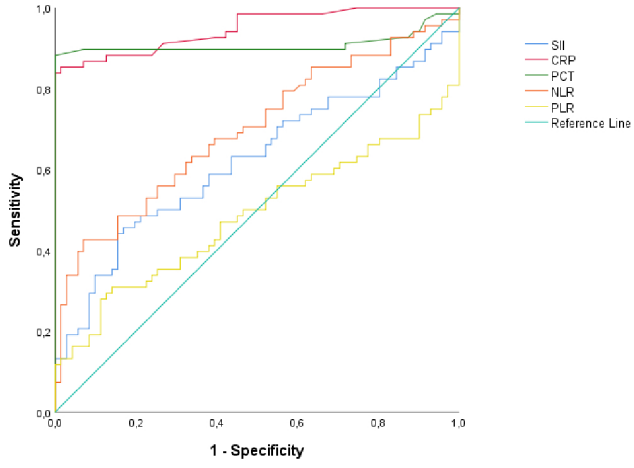

Figure 2. *ROC curves for SII, CRP, PCT, NLR and PLR. SII: Systemic immune-inflammatory index, CRP: C reactive protein, PCT : Procalcitonin, NLR: Neutrophil-to – lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio.*

4. DISCUSSION

In this retrospective observational study, the predictive value of SII in the early diagnosis of LOS in preterm infants was investigated. CRP, PCT, NLR, PLR, and SII are the most frequently utilized biomarkers used for diagnosing neonatal sepsis, and they were compared in this study.

Neonatal sepsis remains the leading reason for mortality and morbidity among NICU patients [15], especially in developing countries [16]. Additionally, premature neonates are more prone to infectious diseases. Neonatal sepsis increases as gestational age decrease, and the risk of death in the preterm population has been reported as 120-fold more than in the term population (17). Humoral and cellular deficiencies arising from an immature innate immune system, dysbiotic gut microbiota, lack of total enteral nutrition, requirement for central venous catheters, invasive operations, prolonged hospitalization, and broad-spectrum antibiotics are risk factors for LOS in premature infants [4,18]. Similar to reports in the literature, prolonged hospitalization, the requirement for ventilation devices, central venous access, and long time spent on total parenteral nutrition before switching to complete enteral feeding were the numerous risk factors for developing LOS in preterm infants in our study. *Coagulase-negative staphylococci*, which cause neonatal sepsis in preterm infants associated with the duration of central line exposure and parenteral nutrition [19], were predominant in our group of preterm infants with LOS.

Sepsis manifests in various ways, depending on the region, gestational age, severity, and causative agent. Although blood culture remains the gold standard for diagnosing sepsis [20], it requires at least 48h, and the bacterium is identified in

only 60-80% of neonates with sepsis [21]. On the other hand, delayed diagnosis and treatment may lead to further morbidity and mortality in these patients [17]. Researchers have been encouraged to investigate novel indicators due to the lack of an early diagnostic marker that can provide definitive conclusions in diagnosing neonatal sepsis. No laboratory indicators have been demonstrated to have sufficient sensitivity and specificity in diagnosing sepsis in preterm newborns [22]. CRP is currently the most commonly utilized first-line biomarker in diagnosing neonatal sepsis. It does, however, have certain limitations. First, it elevates in non-sepsis circumstances such as NEC, hypoxia, shock, IVH, meconium aspiration, and surgery [8,17]. Second, levels of CRP start to elevate 10-12 hours following infection onset. Thus, if the test is administered early in the course, it may remain the same. Thirdly, CRP can respond differently in early-onset and LOS in preterm and full-term newborns, with values rising by 0.40 mg/L [17]. A meta-analysis of 10 trials reported CRP's median sensitivity, and specificity to be 70% and 89%, respectively [23]. CRP's median sensitivity and specificity were found to be higher in our study: 83.3% and 97.2%, respectively.

According to a meta-analysis, the diagnostic accuracy of PCT was shown to be greater in neonates with LOS than in those with early-onset sepsis [24]. PCT begins to be released two hours after stimulation and peaks between 12 and 24 hours, with a half-life of approximately 24 hours [25]. It has been reported that PCT has a median sensitivity and specificity of 85% and 54%, respectively; however, there is very little information regarding LOS [26]. Our study's median PCT sensitivity and specificity were 88.2% and 100%, respectively.

Since, neutrophil numbers increase in sepsis while lymphocyte counts drop, NLR has been deemed a promising biomarker. However, it is recommended to be used in conjunction with CRP to evaluate sepsis [8]. Although, varied cut-off values, sensitivity, and specificity rates have been published in the literature, we determined that preterm infants with LOS had a cut-off value of 2.8, with sensitivity and specificity rates of 43.0% and 91.6%, respectively.

Although, PLR has been advocated in diagnosing early-onset sepsis, a newly published meta-analysis found insufficient trials demonstrating its diagnostic value in sepsis [9, 27, 28]. In our study, there was no significant rise in PLR during LOS. This may be due to the decrease in platelets and lymphocyte counts during the sepsis episode.

SII is an index derived from platelet, lymphocyte, and neutrophil counts that reflects the systemic immune-inflammation status. Its role in the clinical outcomes of patients with coronary artery disease and several types of cancer has previously been examined [29, 30]. At the same time, Ceran et al., demonstrated that SII might predict hypoxic-ischemic encephalopathy in neonates [31]. Based on the available data, the cut-off range of SII is 200 to 1375. According to a recent study by Aydogan et al., SII may be used with other biomarkers to diagnose sepsis in neonates with congenital heart disease using a cut-off value of 517.19, with a sensitivity of 70.5% and a specificity of 70.2% [10]. In a study on newborns with urinary tract infections and renal involvement, SII's cut-off value, sensitivity, and specificity were determined to be 217.2, 60.8%, and 60.8%, respectively [32]. In our study, we have demonstrated that a cut-off value of 450 for SII has a sensitivity of 44.4% and a specificity of 83.3%, PPD of 72.7%, and NPD of 60% for diagnosing LOS. Sadly, this is relatively low compared to CRP and PCT. Hence, it can be considered close to NLR.

Moreover, we found no significant relationship between SII and IVH, NEC, ROP, and mortality. However, SII levels were found to be lower in preterm infants with BPD. Since, inflammation is a major risk factor in BPD pathogenesis, NLR was shown to be an early predictor of BPD in 72h [33]. In contrast, different theories of BPD etiology and disease drivers other than LOS may explain our findings [34].

According to our findings, SII levels increased significantly in premature newborns with LOS. We are unaware of any previous investigation that has evaluated SII levels in conjunction with well-known septic biomarkers such as CRP, PCT, NLR, and PLR in preterm infants with LOS. SII can be calculated, at no additional cost, from a CBC and is simple to use in clinical settings. In contrast to CRP and PCT, its diagnostic value is relatively poor. This research has several limitations. In the first place, it was a retrospective study conducted at a single center, and in the second place, we could not measure the SII at multiple periods.

Conclusion

Herein, we have demonstrated that SII increases in premature newborns with LOS. However, compared to CRP and PCT, the diagnostic value is relatively low. Prospective studies are required to determine if the diagnostic value increases if more biomarkers are included. Further research with larger sample sizes may fill in the gaps in our understanding of its involvement in LOS.

Compliance with Ethical Standards

Ethical approval: The Marmara University, School of Medicine Clinical Research Ethics Committee approved the project (Date: 01.06.2023, No: 09.2023.105).

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

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Delayed surgical treatment of geriatric hip fractures increases the need for intensive care unit, morbidity and mortality rates

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ABSTRACT

Objective: The aim of this study was to present the intensive care admission, morbidity and mortality rates of older adults with hip fractures who could not be operated in the first 48 hours after admission, which is the recommended time in literature.

Patients and Methods: Patients aged >60 years of age with a hip fracture who were operated on in our clinic between January 2012 and June 2021 were identified. The patients were evaluated in 3 groups according to preoperative waiting time, as Group 1: 2-10 days, Group 2: 11-20 days, and Group 3: ≥21 days.

Results: Mortality within 1 year was found to be 20% in Group 1, 31% in Group 2, and 50% in Group 3 (P=0.001). Preoperative waiting time did not affect complications related to surgery infection (P=0.890), implant failure (P=0.129)]but surgeons had to deal with decubitus ulcer (P=0.016) and urinary tract infection (P=0.001). Patients with a long preoperative waiting time required preoperative intensive care (P=0.003).

Conclusion: The study results demonstrate that as the preoperative waiting period increases, the mortality rate also increases, the need for intensive care before and after the operation increases, and there is increased morbidity due to a long hospital stay.

Keywords: Hip fracture, Preoperative waiting time, Intensive care unit

1. INTRODUCTION

Hip fracture is an increasing public health problem within aging populations [1]. Worldwide, hip fractures occur in 18% of females and 6% of males [1]. Assuming no change in the age – and gender-specific incidence, it is estimated that the number of hip fractures will approximately double to 2.6 million by 2025, and to 4.5 million by 2050 [2]. Although, the incidence of hip fracture in developed countries has reached a plateau with prevention of the risk factors of the disease, it is still increasing globally [3, 4].This increasing incidence can make health services inadequate from diagnosis to treatment and treatment delays may occur.

In Turkey and many other countries of the world, health services cannot be accessed quickly and effectively. Although, some authors have stated that a delay in surgery is not a quality indicator [5], this can cumulatively lead to high rates of delayed treatment in hospitals. Due to the increasing number of people living in metropolises, it is important to develop and implement national and international treatment protocols to be able to provide the quality of treatment recommended in the literature. Tuzun et al., reported that the incidence of hip fracture has increased in the last 20 years in Turkey. By 2035, 64,000 hip fractures per year are expected [4]. Doruk et al., reported 17 years ago that mortality increased in patients with preoperative waiting time exceeding 5 days, but these data do not seem to be sufficient to establish a national follow-up plan [6]. Although, the data were established on the basis of clinics, health authorities and managers have not focussed on this subject. In addition, the prolongation of the preoperative waiting period constitutes a financial burden for healthcare units [7].

All over the world, there is an effort to treat these patients quickly and effectively, to provide a long life and to re-integrate patients into society [8]. There are clear treatment protocols in the

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literature from the moment of diagnosis to the end of treatment, but this clarity may not always be true for populations who experience disruptions in the delivery of healthcare services. The main focus of previous studies has been morbidity and mortality, because regardless of how fractures are treated, these 2 parameters are the focus of treatment [9].

Timing matters in hip fracture surgery: patients operated on within 48 hours are known to have better outcomes [6, 7, 10-16]. However, when controlled for age, American Society of Anesthesiologists (ASA) score, gender, and medical comorbidities, some authors have reported that the increases in 90-day and 1-year mortality associated with surgical delay were not significantly different from the rates of patients treated early [17-19]. Although, there is an effort to treat these patients quickly in Turkey and in many countries, the hospital and medical conditions have not reached complete maturity. The aim of this study was to present the morbidity and mortality rates of older adults with hip fractures who could not be operated in the first 48 hours after admission, which is the recommended time in literature.

2. PATIENTS and METHODS

Approval for this study was granted by the Institutional Ethics Committee (09.2021.923). All study procedures were applied in accordance with the principles outlined in the Declaration of Helsinki.

Patients were identified from the hospital database, who were aged > 60 years and underwent surgery for a proximal femoral fracture or femoral neck fracture in our clinic between January 2012 and July 2021.

The patients were separated into 3 groups according to the preoperative waiting time. Group 1 comprised patients operated between 2 and 10 days, Group 2, patients operated between 11 and 20 days, and Group 3, patients operated after 20 days.

The preoperative preparation information and surgical notes of the patients were obtained from the hospital registry system and the notes of the surgeons. Mortality data of the patients were obtained from the national death notification system. Significant differences were investigated between the groups in respect of mortality at 1 month, 3 months, and 1 year.

Study exclusion criteria were defined as patients who were operated in the first 48 hours, pathological fractures, subtrochanteric fractures, and patients without adequate preoperative and postoperative data documentation.

Two patients were excluded because sufficient data were not available (Figure 1 Flowchart).

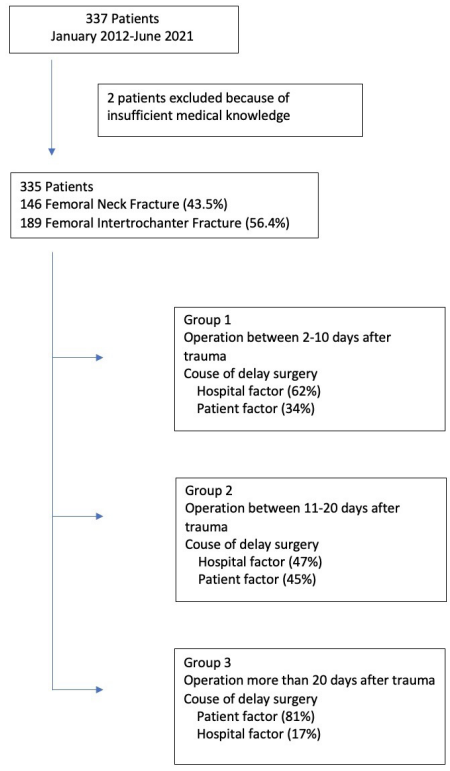

Figure 1. *Patient selection and the reasons for the delay of the operation.*

The patient demographic information (age, gender), preoperative waiting times (days) and ASA scores were retrieved from the patient registration system. The ASA score was preferred because it is fast, easy, and has good predictive value [20, 21]. The operation technique used (open reduction internal fixation (ORIF), closed reduction internal fixation (CRIF), arthroplasty (A)) and anesthesia technique (general anesthesia or regional anesthesia (including spinal, combined spinal, epidural)) were recorded.

It was recorded whether the patients were followed up in the postoperative orthopedic ward or in the anesthesia and reanimation intensive care unit (ICU). The number of days that patients were followed up in anesthesia and reanimation ICU before being transferred to the orthopedic ward was documented.

Postoperative infection and implant failure rates were documented.

Mortality status was identified using hospital records and/or by interviewing the patient's family. A systematic search for death certificates at the National Statistical Office was conducted for patients lost to follow-up.

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Routine follow-up visits were scheduled for 6 weeks, 3, 6, 9 and 12 months, and every year thereafter. Patients unable to attend follow-up evaluations were interviewed by telephone.

Preoperative waiting time was analyzed with all other variables and its effect on the variables was investigated.

This study was conducted in a trauma centre, which is managed by the surgeons and nurses who visit and care for the patients twice a day, and geriatricians when necessary. The operations were performed by 5 surgeons and 1 mentor surgeon who participated in the study.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS version 25.0 software (Statistical Package for the Social Sciences, Chicago, IL, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the normal distribution as initial analyses. Categorical variables were stated as number (n) and percentage (%), and numerical variables as mean and standard deviation values. Chi-square test statistics were used to compare categorical data between groups. Parametric data in two independent groups were compared using the Student's t-test and the Mann-Whitney U test was applied to non-parametric data. The Kruskal-Wallis test was applied to non-parametric data in comparisons of more than two groups. Kaplan Meier analysis and the log-rank test were used for survival analysis. Independent variables that had a statistically significant effect on 1 year mortality in univariate logistic regression analysis were included in the multivariate regression analysis. The results were evaluated within a 95% confidence interval and a value of P< 0.05 was accepted as statistically significant.

3. RESULTS

A total of 335 patients were evaluated, comprising 133 (39.7%) males and 202 (60.3%) females with a mean age of 78.8±9 years (60-102, min-max). The fractures were determined as 146 (43.6%) femoral neck fractures, and 189 (56.4%) femoral intertrochanteric fractures. Arthroplasty was performed in 167 (49.9%) cases, closed reduction internal fixation in 132 (39.4%) and open reduction internal fixation in 36 (10.7%) (Table I).

Preoperative waiting time ranged from 48 hours to 49 days (13.3±7.4 Mean±SD). The ASA variable of 335 patients ranged from 1-4 (2.7±0.8 Mean±SD). The ASA variable was Group 1> Group 2> Group 3 (P=0.001) (Table I).

Implant failure requiring revision surgery developed in 6 patients (1.8%), and prosthesis and implant infection requiring revision surgery in 4 patients (1.2%). There was no significant difference between the groups in terms of implant failure and infection (P>0.05). Decubitus ulcer developed in 43 patients (12.8%), 53 patients (15.8%) received treatment for urinary infection, and there was a significant difference between the groups (P=0.016, P=0.001) (Table II).

Preoperatively, 13 (3.9%) patients needed the ICU, with an increased risk from Group 1 to Group 3 (P=0.003).

Table I. *Patients' demographics and preoperative data*

		Groups						
		2 to 10 days		11 to 20 days		>20 days		
		Number	Mean ±SD	Number	Mean ±SD	Number	Mean ±SD	P value
Age (years)		146	77.91±9.3	122	79.99±9.23	67	78.72±7.87	0.156[1]
ASA		146	2.59±0.88	122	2.85±0.83	67	3.03±0.89	0.001[1]
		Number	%	Number	%	Number	%	P value
Gender	Male	56	38.40%	46	37.70%	31	46.30%	0.468[2]
	Female	90	61.60%	76	62.30%	36	53.70%	
Type of injury	FNFx	63	43.20%	54	44.30%	29	43.30%	0.982[2]
	ITFFx	83	56.80%	68	55.70%	38	56.70%	
Type of surgery	ORIF	12	8.20%	18	14.80%	6	9.00%	0.267[2]
	Hemiarthroplasty	69	47.30%	61	50.00%	37	55.20%	
	CRIF	65	44.50%	43	35.20%	24	35.80%	
Type of anesthesia	General	107	73.30%	94	77.00%	51	76.10%	0.763[2]
	Spinal	39	26.70%	28	23.00%	16	23.90%	

[1] Kruskal Wallis [2] Pearson Chi-square

FNFc: Femoral Neck Fracture
ITFFc: Intertrochanteric Femoral Fractures
ORIF: Open reduction internal fixation
CRIF: Close reduction internal fixation
ASA: American Society of Anesthesiologists

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Table II. *Postoperative complications*

		Groups						
		2 to 10 days		11 to 20 days		>20 days		
		Number	%	Number	%	Number	%	P value
Wound infection	no	2	1.40%	1	0.80%	1	1.50%	
	yes	144	98.60%	121	99.20%	66	98.50%	0.890[2]
	total	146	100.00%	122	100.00%	67	100.00%	
Implant fail	no	141	96.60%	121	99.20%	67	100.00%	
	yes	5	3.40%	1	0.80%	0	0.00%	0.129[2]
	total	146	100.00%	122	100.00%	67	100.00%	
Decubitus ulcer	no	134	91.80%	106	86.90%	52	77.60%	
	yes	12	8.20%	16	13.10%	15	22.40%	**0.016[2]**
	total	146	100.00%	122	100.00%	67	100.00%	
Urinary infection	no	138	94.50%	97	79.50%	47	70.10%	
	yes	8	5.50%	25	20.50%	20	29.90%	**0.001[2]**
	total	146	100.00%	122	100.00%	67	100.00%	
	[1] Kruskal Wallis [2] Pearson chi-square							

Table III. *Intensive Care Unit hospitalization rates*

		Groups						
		2 to 10 days		11 to 20 days		>20 days		
		Number	%	Number	%	Number	%	P value
Preop ICU	no	145	99.30%	117	95.90%	60	89.60%	
	yes	1	0.70%	5	4.10%	7	10.40%	**0.003[2]**
	total	146	100.00%	122	100.00%	67	100.00%	
Postop ICU	no	107	73.30%	60	49.20%	31	46.30%	
	yes	39	26.70%	62	50.80%	36	53.70%	**0.001[2]**
	total	146	100.00%	122	100.00%	67	100.00%	
		Number	Mean ±SD	Number	Mean ±SD	Number	Mean ±SD	P value
Days of stay in ICU		38	2.1±1.7	56	2.1±2	29	1.7±1.3	0.279[1]
	[1] Kruskal Wallis [2] Pearson Ki-Kare							

ICU: Intensive care unit

Postoperatively, 137 (40.9%) patients were followed up in the ICU, and 198 (59.1%) patients were followed up in the orthopedics and traumatology ward. There was an increased risk from Group 1 to Group 3 for the need for postoperative intensive care follow-up (P=0.010). The median length of stay in the postoperative ICU was 5 days (1-11 days, min-max). There was no difference between the groups in respect of ICU stay (P= 0.279) (Table III).

The 1st month, 3rd month, and 1st year mortality rates of the whole group were 9%, 16%, and 30% respectively, and there was a

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significant difference between the groups in all 3 periods. For the 1st month, the mortality of Group 1 patients was 3.4%, and 19% for Group 3 patients. The risk increased significantly from Group 1 to Group 3 (P=0.001). The 1-year mortality rate was 20.5% for Group 1 patients, and 50.7% for Group 3 patients, showing a significantly increased risk from Group 1 to Group 3 (P=0.001). The median time from operation to death was 412 days for Group 1, 409 days for Group 2, and 206 days for Group 3 (P=0.037) (Table IV).

When the equality of survival distributions for the different groups was examined with the Log Rank (Mantel-Cox) test, there was a significant difference between the groups (P=0.001).

In the Kaplan-Meier Survival analysis, the mean estimated survival time was found to be 1774 days in Group 1, 1304 days in Group 2, and 958 days in Group 3. As the waiting time increased, so the estimated survival time decreased (Figure 2).

In the univariate logistic regression analysis, independent variables that had a statistically significant effect on 1-year

Table IV. *Postoperative survival rates and overall survival*

		Groups						
		2 to 10 days		11to 20 days		>20 days		
		Number	%	Number	%	Number	%	P value
Mortality 1st month	live	141	96.60%	110	90.20%	54	80.60%	**0.001**[2]
	dead	5	3.40%	12	9.80%	13	19.40%	
	total	146	100.00%	122	100.00%	67	100.00%	
Mortality 3rd month	live	133	91.10%	103	84.40%	45	67.20%	**0.001**[2]
	dead	13	8.90%	19	15.60%	22	32.80%	
	total	146	100.00%	122	100.00%	67	100.00%	
Mortality 1st year	live	116	79.50%	84	68.90%	33	49.30%	**0.001**[2]
	dead	30	20.50%	38	31.10%	34	50.70%	
	total	146	100.00%	122	100.00%	67	100.00%	
Mortality	no	86	58.90%	42	34.40%	17	25.40%	**0.001**[2]
	yes	60	41.10%	80	65.60%	50	74.60%	
	total	146	100.00%	122	100.00%	67	100.00%	
		Number	Mean ±SD	Number	Mean ±SD	Number	Mean ±SD	P value
Days until postop death		60	551.2±555	80	520.6±487.9	50	367.3±499.2	**0.037**[1]

[1] Kruskal Wallis test [2] Pearson chi-squared test

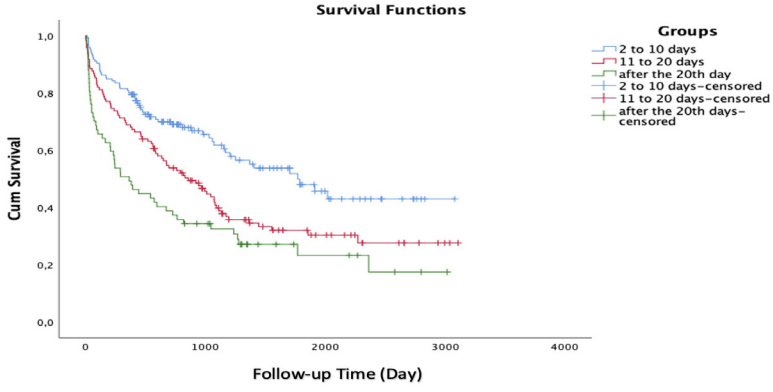

Figure 2. *The cumulative survival distribution of the groups according to preoperative waiting times as operations performed at 2-10 days, between 11-20 days and after 20 days.*

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mortality were included in the multivariate binary logistic regression analysis and the model was found to be significant (P=0.001). The model explained 10% of the variance in the dependent variable. The independent variables of preoperative waiting time and age were determined to significantly change the dependent variable of mortality. For preoperative waiting time, the OR was 1.068 and for age, the OR was 1.051 (Table V).

Table V. *Factors affecting 1st year mortality logistic regression*

Factors Affecting 1st Year Mortality Logistic Regression								
	B	S.E.	Wald	df	p	OR	95% G.A OR	
							Lower	Highest
Preoperative waiting time	0.065	0.017	14.684	1	0.001	1.068	1.032	1.104
Age	0.049	0.016	9.366	1	0.002	1.051	1.018	1.084
ASA	0.241	0.156	2.383	1	0.123	1.273	0.937	1.729
Constant	-6.38	1.285	24.637	1	0.001	0.002		

4. DISCUSSION

The main focus of this study was to determine the effect of surgical delay on patient morbidity, mortality, and ICU stay. The study results showed that the 1-year mortality rate was 20.5% if the operation was performed after the first 48 hours, 31.1% after the first 10 days, and 50.7% after the first 20 days. Preoperative waiting time did not affect complications related to surgery (such as infection and implant failure) but in the longer time-frame, decubitus wounds may develop. If the operation could not be performed in the first 10 days, 1 of every 2 patients required the postoperative ICU.

There are many factors in surgical delay, including the late presentation of patients, unavailability of operating theatres, delays in health system payments, examinations, and doctor shortage. These can differ depending on which health authority the clinic is affiliated with. Chow et al., reported that the most common reasons for delayed surgery were prolonged medical review or stabilization of the patient [12]. Seigmenth et al., and Cha Y-H et al., stated that the reason for delay was always because of hospital-related causes such as unavailability of an operating theatre, surgeon, anaesthetist or theatre staff [13, 22]. Drugs that should be discontinued before the surgery on the request of the anesthesiologist, such as anti-aggregant and anticoagulant drugs (clopidogrel, dipyridamole, acetylsalicylic, etc.) as they can increase the risk of morbidity and mortality, and tests requested to evaluate the cardiac status (transthoracic echocardiography, etc.) may also prolong the waiting period [23, 24]. However, these examinations can be applied rapidly and effectively, such as bedside echocardiography. The critical point is that other branches consulted should evaluate the patient quickly and only request the necessary and effective examinations. Preoperative waiting time is a modifiable risk factor, unlike other preoperative indicators (high ASA, male gender, pre-fracture mobility, advanced age, cognitive impairment) [25]. A complete analysis of these factors could not be performed in the current study as there were multiple reasons for delay in all the patients.

Vidán et al., reported urinary infection in 15% of patients who could not be operated on for the first 5 days [26]. Johnstone et al., reported urinary infection in 48% of all patients at 48 hours postoperatively [27]. Urinary infections are more likely to occur in patients waiting longer than 48 hours, and cephalosporin prophylaxis for surgery does not reduce this risk [27, 28]. Nursing quality is decisive in urinary tract infection because inadequate skin care is an important risk factor for urinary tract infection [29]. In our clinic, importance is given to skin care with 2 nurses and 3 patient care personnel who deal with hip fractures. Another risk is that if urinary tract infection is not treated adequately, it may cause infection of the implant or endoprosthesis used in fracture stabilization [28]. The lack of difference in implant infection between the groups in the current series is perhaps due to the short-to-mid-term results of the patients. It is not known whether the infection rates would have increased if the patients in Group 3 had lived longer. It can be recommended that further studies are conducted with larger patient series to investigate the issue of urinary tract infection in hip fracture patients.

Rai et al., emphasized that early surgery reduces the risk of pressure ulcers [19]. The rate of pressure ulcers in the current study Group 1 patients was 7.6-12%, similar to data in previous studies [19, 30, 31]. The higher rate of pressure ulcers in Groups 2 and 3 than in the literature may be associated with increased mortality. As an independent risk factor, pressure ulcers have been reported to increase the 6-month mortality rate by > 2-fold [30]. The need for debridement surgery due to pressure sores in only 1 of the current study patients can be attributed to the daily visit of the wound care service in our hospital. Pressure ulcer care is important because pressure ulcers are associated with an increased length of hospital stay, higher costs, shorter life expectancy, and worse quality of life [32].

To the best of our knowledge, this is the first study to have presented a correlation between preoperative need for intensive care and preoperative waiting time in hip fractures. Low molecular weight heparin (LMWH) treatment was applied to all the current study patients, and 13 patients with a greater oxygen requirement could not tolerate room air and were applied with a mask with a reservoir due to massive embolism. The need for postoperative intensive care was different between the groups. Eschbach et al., reported the 1st year mortality of patients who had never been admitted to the ICU as 15%, and the 1st year mortality of the patients admitted to the ICU for more than 3 days was 59%. As an independent risk factor in hip fractures, hospitalization for more than 3 days has been shown to increase 1-year mortality [33, 34]. In the current series, the average ICU stay of any group was not more than 3 days. However, the high rate of ICU admission in Group 3 patients may be another reason for high mortality. Hasan et al., reported a similar rate of postoperative intensive care requirement (6%) of patients who were operated on and not operated on in the first 48 hours, and that the risk factor for intensive care was prosthetic surgery [35].

However, this rate was different from that of the current study at < 1 in 4 of the Group 1 patients.

In the literature, the 1st year mortality has been reported to range from 9.5-20.4% in patients operated on in the first 48 hours, and 14.5-32.5% in patients who could not be operated on in the first 48 hours [11-13, 16, 36]. The 1st year mortality rate exceeding 50% in the current study Group 3 patients is above the rates reported in literature. Leer-Salvesen et al., stated that while there was no significant mortality change observed in patients who were operated on in the first 48 hours, there was an increase in mortality with a waiting time of more than 48 hours [16]. However, that study excluded patients with preoperative waiting time of ≥4 days. There are no data of patients with operations performed in the 3rd week or later with current treatment opportunities.

Maheshwari et al., reported a 1-year mortality rate of 22% in a patient group operated on within an average of 30 hours and it was stated that each 10-hour delay increased mortality by 5% [11]. These data overlap only with the Group 1 patients in the current study. The 1st year mortality in the Group 3 patients was >50%. Early surgery seems to provide a survival benefit in comparison with later intervention.

Cha Y-H et al., reported that the 1-year mortality rate was 21.2% when the delay was due to patient-related factors, and 12.6% when it was due to hospital-related factors. In the current study, the risk factors for most of Group 3 were patient-related factors [13].

A higher ASA score has been shown to be significantly correlated with late operation and high mortality [11-13, 23, 26, 37]. The current study results support these previous studies as Group 3 patients had the highest mean ASA scores. As stated by previous authors, most patients are ASA 2-3 but when the ASA value increases by 1 unit, mortality rates increase 2-fold, and mortality increases 1.5-fold for every 10-year increase in age [14, 16]. However, the current study results showed that the independent variables of preoperative waiting time and age were determined to significantly change the dependent variable of mortality.

There are many variables that can predict post-fracture mortality, most of which are not modifiable risk factors, such as high ASA, limited walking capacity before fracture, male gender, advanced age, and renal disease. However, operating on patients as soon as possible, reducing the length of hospital stay, and enabling early mobilization are modifiable risk factors [12]. The main reasons for the delay in the operations of the current study Group 1 and Group 2 patients were hospital-related (unavailability of operating theatres, etc.). This would seem to be able to be resolved with a national follow-up plan.

Limitations of this study can be said to be the retrospective, single-centre design with a low number of patients with high rates of comorbidity, who were not randomized and with no control group. However, the strength of this study is that very few articles in the last 10 years have directly reported the mortality rates of patients who could not be operated on within 48 hours after a hip fracture.

Conclusion

The results of this study have shown that as the preoperative waiting period increases, so the mortality rate of the patients increases, together with an increased need for intensive care before and after the operation, and morbidity due to a longer hospital stay.

Compliance with Ethical Standards

Ethics Committee Approval: This study was approved by the institutional ethics committee (Marmara University Medical School, Ethics Committee for Clinical Research: 09.2021-923).

Conflict of Interest: The authors declare that they have no conflicts of interest.

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

Authors Contributions: YS: Study design, data analysis, writing the article, YS and ODT: Data collection, data analysis, TO and BE: Study design, writing the article, BE, OS and TO: Supervision. All authors read the article and approved the final version of the article.

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Compressive external bracing in pectus carinatum : Results of the first 100 patients

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ABSTRACT

Objective: Pectus carinatum is the second most common chest wall deformity causing psychological problems. There has been a growing interest in the conservative treatment of the disease in recent years. Here, we present results of the first 100 patients who were treated with compressive external bracing therapy.

Patients and Methods: A total of one hundred patients who were treated with compressive external bracing between 2017-2023 were reviewed from database. Parameters recorded include demographics, type of the deformity, duration of the treatment time, complications, and patient satisfaction.

Results: Among 100 patients 88 were male (88%) and 12 were female (12%) and the mean age was 13.3 years (3-19). 76 patients (76%) had a symmetric pectus carinatum and 24 patients (24%) had asymmetric. 23 (23%) patients had scoliosis, 9 (9%) had kyphosis, 1 (1%) had Poland Syndrome and 1 (1%) patient had Marfan Syndrome as accompanying anomalies. Quality of Life Questionnaire revealed 94.8 % satisfaction in patients who completed the treatment.

Conclusion: Compressive external bracing is associated with satisfactory results in the treatment of pectus carinatum. This non-surgical intervention enables us to treat more patients in younger ages with lesser comorbidity than surgical intervention.

Keywords : Pectus carinatum, Chest wall deformities, Pectus excavatum, Orthosis, Compressive external bracing

1. INTRODUCTION

Pectus carinatum (PC) or pigeon chest deformity is characterized by convex protrusion of anterior chest wall along with costal cartilages and sternum [1-3]. It is the second most common chest wall deformity following pectus excavatum (PE) [4,5]. The prevalence of the deformity is between 0.3%-0.7% with a male predominancy [6]. Although, it is less common than PE, it is harder for patients to cover it with clothes and accessories. Etiology of the deformity is unclear, but it is very likely to have a genetic inheritance by having 25% positive family history of the patients [7]. PC becomes prominent with puberty and causes cosmetic and psychosocial problems [8,9]. Although, there are studies investigating the cardiopulmonary impact of the deformity on these patients, no affect has been detected so far [10]. Therefore, patients with PC mainly suffer from cosmetic issues, resulting in a reduced self-image and a lower quality of life compared with control patients without this deformity. Until recently, the mainstay of treatment for PC has been surgical. The most well-known technique is the Ravitch procedure and its modifications, consisting of subperichondreal resection of cartilage and reconstruction of the sternum [11]. A more recent surgical technique for the correction of PC is the Abramson procedure in which a steel bar is placed subcutaneously over the sternum for correction [12]. Abramson procedure has gained popularity in the last decade as being minimally invasive. Although, non-surgical brace treatment has been applied to the patients since 1970s, results were unsatisfactory due to compliance and technical problems until last decade. Similar results with surgical treatment options have been reported after the improvement of compressive external bracing (CEB) in the last decade. There has been guidelines and treatment algorithms reported according to the flexibility of the chest wall. Here,

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we report our experience with the first 100 patients who were treated with CEB.

2. PATIENTS and METHODS

One hundred patients who were treated with CEB between January 2017 and June 2023 were reviewed retrospectively from database. All data regarding pressure for initial correction (PIC) measurement, demographics, type of deformity, comorbidities, duration of the treatment, complications, Quality of Life Questionnaire scores and follow-up have been recorded. Patients were followed up in outpatient clinics for 3 years. Additionally, pre-treatment, and post-treatment pictures of the patients were taken for the archive in every encounter with them. Furthermore, all the patients were administered the Quality of Life Questionnaire after the completion of the CEB program. None of the patients were excluded during the study. Satisfactory result was defined as desired optimum correction following CEB that was verified by the results of Quality-of-Life Questionnaire after completing the CEB treatment.

Compressive External Bracing Protocol

We used the same type of custom-made brace for all the patients (G-pad Pectus Braces, Istanbul). Figure-1 demonstrates the brace and its application. We used a pressure measuring device (G-Pressure, Istanbul, Turkey) which permits the clinician to measure the pressure needed for optimum correction (Figure 2). This pressure was measured at the first consultation by using the measurement device on the thorax of a patient standing with the back against a wall. The pressure required to redress the chest into its normal position is called the pressure for initial correction (PIC) and is measured in pounds per square inch (psi). We used a PIC of 10 psi as a cutoff point for a thorax suitable for the CEB. Patients with a PIC above 10 psi had a more rigid thorax and would probably benefit only from surgical treatment options [13]. Patients were selected for brace treatment if the initial PIC measurement was 10 psi or below. For every patient the mean pressure of initial correction was measured by our team. We only ordered standard chest x-rays in the first outpatient clinic appointment. No other diagnostic imaging was done unless necessary. We did not use any indices for categorizing patients. The patients with PC, who did not receive brace treatment or patients with a PIC above 10 psi, were not enrolled into the CEB program. Therefore, they were treated with Abramson procedure. We performed modified Ravitch surgery only for chondromanubrial type of PC patients which is also named as pectus arcuatum [14]. Brace patients were advised to wear the brace as often as possible during day and night except during showering, bathing, or sports. Patients were seen at our outpatient clinic initially every 4 to 6 weeks for checking on the compliance of the patient and progress of the deformity after CEB. When we reached to a desired correction, we gradually decreased the wearing time (Maintenance Phase). We followed a maintanence phase pattern as decreasing the wearing time to only 8 hours at night, once every 2 nights, 2 nights a week and 1 night a week. After completing this phase, we only recommended them to wear the brace if the deformity starts to reoccur. This phase lasts 2 to 22 months. If there was no improvement or insufficient improvement of the deformity after 6 to 12 months of treatment, we stop CEB and recommend Abramson procedure for optimum correction. Figure 2 shows the application of specific designed custom-made compressive external bracing.

Figure 1. *Images demonstrate the use of measurement device for identifying the pressure for initial correction (PIC) . This pressure is measured when the patient standing with the back against a wall.*

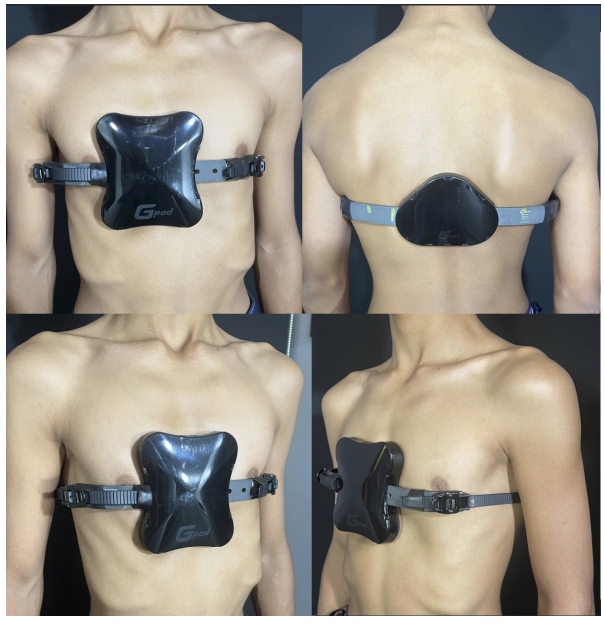

Figure 2. *Images demostrate the application of compressive external brace. Brace is adjusted according to the location of the deformity and size of the toracic cavity.*

Statistical Analysis

Statistical analyses were performed using statistical software (SPSS, version 25.0 for Windows; SPSS, Chicago, Illinois, United States). Discrete random variables were presented as percentage and continuos random variables were presented as mean and range (max-min values).

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3. RESULTS

A total of 100 patients were included for compressive external bracing. Of them, 88 were male (88%) and 12 were female (12%) and the mean age was 13.3 years (range, from 3 to 19 years). 76 patients (76%) had a symmetric PC and 24 patients (24%) had asymmetric. Among them, 24 patients (24%) had a positive family history for PC or PE. Table I shows details of the patient characteristics. Although, majority of patients complained a variety of psychosocial issues because of their thoracic deformity, there were also several physical issues.

We had 52 patients (52 %) in the active phase of treatment, while 24 (24 %) were in the maintenance phase. In addition to that, we had 24 patients (24%) who had completed treatment after a mean treatment time of 16 months (range, 9 to 28 months). No recurrences were detected in the whole series, and we did not have any patients who abandoned or had problems with compliance. Although younger patients had tendency to not to wear as planned, they got better results in a shorter time than the older patients. 23 (23%) patients had scoliosis, 9 (9%) had kyphosis, 1 (1%) had Poland Syndrome and 1 (1%) patient had Marfan Syndrome as accompanying anomalies. The patient with Marfan Syndrome had a history of right thoracotomy due to aortic coarctation operation. Additionally, 46 (46%) of the patients had flaring ribs bilaterally which were treated with specially designed rib bandages along with CEB if they are younger than 16 years old. If bandages did not help in correcting the rib flares, we recommended custom-made rib flare braces as we did in the adult population. For patients who were older than 16 years we did not use bandages and preferred custom-made rib flare braces initially. Figure 3 demonstrates pre – and post-treatment pictures of some of our patients.

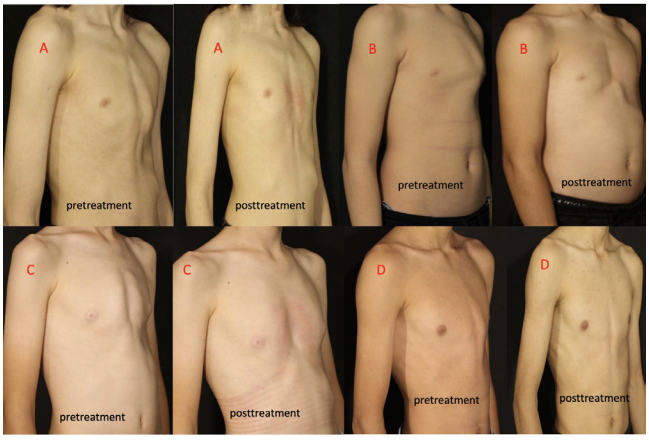

Figure 3. *Pre and post-treatment images of some of the patients. A. A 13-year-old male patient before and after application of CEB B. An 11 -year-old male patient before and after application of CEB C. A 12-year-old male patient before and after application of CEB D. A 16-year-old male patient before and after application of CEB.*
Abbreviations : *CEB ; compressive external bracing*

Four patients (4%) experienced skin lesions as adverse events from application of CEB. We discontinued the CEB treatment until the complete resolution of the skin lesions and restarted application with lower pressures to avoid the same complication. Quality-of-Life Questionnaire revealed 97% patient satisfaction.

Table I. *Characteristics of the patients*[a]

Number of the patients (n)	100
Age (yr.)	13.3 (3-19)
Sex (n)	
Female	12 (12%)
Male	88 (88%)
Type of the deformity (n)	
Symmetrical	76 (76%)
Asymmetrical	24 (24%)
Comorbidities (n)	
Scoliosis	23 (23%)
Kyphosis	9 (9%)
Poland Syndrome	1 (1%)
Marfan Syndrome	1 (1%)
Rib Flare	36 (36%)
Past Surgical History (n)	
Aorta coarctation	1 (1%)
Psychosocial symptoms	
Ashamed of appearance	72 (72%)
Cosmetic issues	46 (46%)
Anxiety	34 (34%)
Problems in social life	21 (21%)
Symptoms	
Exercise intolerance	24 (24%)
Exertional SOB	21 (21%)
Palpitation	15 (15%)
Exertional chest pain	9 (9%)

[a] *The values are presented as a number (the percentage of variables) or the mean value (range). Abbreviations: mo, month; SOB, shortness of breath ; yr, year.*

4. DISCUSSION

There have been some studies about the brace treatment application for the correction of PC. Although, there are multiple types of bracings and techniques, most of them have similar successful results except the difference in costs of the brace of choice [15-18]. Even though, bracing has been used since 1970s in the treatment of PC, the routine use of it became popular in the last decade due to the report of the successful results from various centers [16,17]. This led to the establishment and development of new concepts and protocols in the management of non-surgical treatment of PC. Another reason for the delay in the use of CEB was the successful results from the surgical treatment. Several studies exist in the literature presenting the satisfactory outcomes after Abramson procedure [19-21] It has been preferred by pectus physicians in PC treatment for years by being minimally invasive and having less morbidity rates. Nevertheless, this preference has changed in the last decade. Several studies with various techniques from different centers all over the world proved that CEB is as successful as surgical

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treatment. Results from the first 100 patients of our program revealed 97% of patient satisfaction without any recurrence or major complication. Martinez-Ferro and colleagues reported their series of 208 patients by applying dynamic compression system which the pressure applies can be objectively adjusted by the physician. They presented excellent results with high patient satisfactions [13]. Fraser and friends published their data on 249 patients who had treated with classic CEB and reported 98% concordance and 94% satisfaction. They highlighted the compliance of the patient to the bracing program as the key factor in the whole process [22]. Dekonenko et al., reported outcomes of 460 PC patients with dynamic compression system with a high satisfaction rate in compliant patients [23]. On the other hand, Moon and his colleagues published their work including 320 PC patients. They reported their results with classical CEB treatment with high satisfaction rate (87.4%). In addition to that, they also proved the long-term success of the CEB [24]. Our series with 97% patient satisfaction match with the existing data published by pioneer reference centers all over the world. Although, we did not experience any recurrences in our patient series who completed the CEB program, it remains unknown whether protrusion of the chest may recur in the long run. This success of non-surgical option also made an increase in the numbers of patients admitting to the outpatient clinics by offering a non-invasive option for the correction. Addition to that, CEB enabled us to treat patients at earlier ages before causing psychosocial problems and symptoms.

During exercise even though majority of the centers take 7.5 psi as the cutoff value for starting CEB, few exceptional examples exist. Cohee et al., published their series including a patient with a PIC above 9 PSI who had an excellent result after a total treatment time of 16 months [25]. Lopez and colleagues reported 2 patients with a PIC of 14 psi who were improving under treatment [26]. In our study, we included 2 patients with a PIC above 10 psi and one of them completed the CEB program with satisfaction and the other one was in the retainer mode without any problems. These data show that there will be more studies in the future extending the cutoff value in the decision making of CEB application.

Some PC patients have severe costal flaring which is the protrusion of the lower costal arches. Unfortunately, CEB or DCS cannot correct those accompanying deformities. We recommend application of specially designed costal flaring braces for the correction of them. We have experienced satisfactory result with this non-surgical approach. In contrary, some centers recommend surgical correction of those defomities [27]. Additionally, no data exists in the literature about the results of CEB in patients with Ehlers-Danlos syndrome. We believe there will be studies in the future for the treatment of PC with CEB including patients with Ehlers-Danlos syndrome to draw a conclusion about the outcomes.

Limitations

This study has some limitations. This study shows the results of a single center with a specific type of CEB program and brace. We need to design a multicenter study with the same program especially including centers from all over the world. Addition to that, we need to wait to see the long-term results to assess whether protrusion of the chest will recur after brace treatment is discontinued. This will be an important factor before we can recommend brace therapy as the treatment of choice in patients with PC and a flexible chest.

Conclusion

In conclusion, compressive external bracing provides satisfactory results in compliant patients. It enables us to treat more patients in younger ages with lesser comorbidity than surgical option.

Compressive external bracing is an effective non-surgical and safe option in the treatment of PC patients and can be applied without problems in experienced centers.

Compliance with Ethical Standards

Ethical approval: The study was approved by the Marmara University, School of Medicine Ethical Board (14.07.2023 – 07.2023.1139).

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Conflict of Interest: Author has nothing to declare.

Authors contributions: NOE: Data acquisition, data analysis, data interpretation, manuscript preparation and revisions.

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Original Article

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Monitoring tissue perfusion during extracorporeal circulation with laser speckle contrast imaging

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ABSTRACT

Objective: The laser speckle contrast imaging (LSCI) system is a method to evaluate microcirculation. The primary aim of our study is to evaluate the relationship between LSCI and perfusion markers in coronary artery bypass grafting (CABG). Our second aim is to investigate the relationship between LSCI and extubation time in the intensive care unit.

Patients and Methods: Fifteen patients aged 43-80 years who will undergo on-pump CABG were included in the prospective study. Mean arterial pressure (mmHg), heart rate (min-1), PO2 (mmHg), PCO2 (mmHg) and lactate (mmol/L) levels were measured pre-induction, post-induction, 10th minute of the extracorporeal circulation, post-crossclamp, and post-operatively. At the same time points, LSCI values from the skin were measured and recorded. The intubation times of the patients were also recorded.

Results: There was no significant change in systemic tissue perfusion markers (P>0.05). LSCI perfusion values decreased significantly from induction and remained low until the end of surgery (P<0.05). The perfusion value (98±11 PU) of the patients who were intubated for less than 8 hours was better than the perfusion value (52±4.8 PU) of the patients who were intubated for more than 8 hours (P<0.05).

Conclusion: In our study, a significant change occurred in skin tissue perfusion before systemic perfusion parameters in CABG, and low perfusion was associated with prolonged intubation time.

Keywords: Skin perfusion, Laser speckle contrast imaging, Extracorporeal circulation, Coronary artery bypass grafting, Extubation time

1. INTRODUCTION

Detection of predictive factors for perioperative complications is important in terms of protecting organs and stopping possible organ damage in the intraoperative and postoperative period [1]. Organ damage begins with disruption of tissue microcirculation [2]. Invasive methods have been defined to obtain direct or indirect information about tissue microcirculation. These include assessment of blood gases, mixed-venous oxygen saturation (SvO_2 > 70%) [3], lactate level (<2 mmol/L) [4], veno-arterial carbon dioxide difference (<6 mmHg) [5], gastric tonometry (pH>7.35) method [6]. However, these approaches have some limitations such as being invasive and providing site-specific information. Therefore, non-invasive monitoring is needed.

The laser speckle contrast imaging (LSCI) is a system that does not require special expertise and is used to evaluate the perfusion of tissues such as the retina and skin without using any dyes [7]. The technique is based on the principle of processing backscattered light from a tissue surface illuminated by laser light. It can give real-time images without touching the tissue. The light reflected from the erythrocytes moving in the tissue creates ripples on the detector. These fluctuations appear as color spectrum and give numerical values as LSCI perfusion unit [8]. In recent studies on tissue perfusion, it has been shown that skin perfusion is closely related to the microcirculation of internal organs [9-11]. By evaluating the skin microcirculation, the vascular mechanisms of systemic diseases such as hypertension, diabetes and chronic renal failure are investigated [12]. Ijzerman et al., found impaired skin vasodilation in people with high coronary heart disease scores [13]. In this way, it has been shown that a non-invasive evaluation of peripheral tissue can be used in the diagnosis of a systemic vascular disease.

The primary aim of our study is to measure the skin tissue perfusion with LSCI during the surgery in patients who will undergo coronary artery bypass grafting (CABG) and to investigate its relationship with systemic tissue perfusion markers. The secondary aim of our study is to investigate the

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relationship between skin tissue perfusion and intubation times in the intensive care unit.

2. PATIENTS and METHODS

Patients

Fifteen American Society of Anesthesiologists patients aged between 43 and 80 who were going to undergo on-pump CABG operation were included in this prospective study. The Ethics Commitee of the University approved the study (ATADEK: 2015-15/17) and written informed consent was obtained from the patients. Exclusion criteria: Active congestive heart failure, emergency cardiac surgery, need for inotropic support before surgery, elevation of any systemic hypoperfusion parameter before surgery, coagulopathy, renal failure, liver dysfunction, local or systemic infection and inflammation.

Anesthesia and Surgery

Anesthesia and surgical management of the patients were performed by the same anesthesiologist and surgical team. Standard anesthesia and surgical techniques were used. The night before surgery, patients were given Alprazolam (0.5mg [p.o]) (Xanax®). Midazolam (Dormicum®) (125 µg/kg i.m.) was administered 30 minutes before the operation. A standard monitoring regimen including invasive arterial pressure, central venous pressure, peripheral oxygen saturation, 5-lead electrocardiogram and end-tidal CO_2 monitoring was applied.

Anesthesia induction was performed with fentanyl (20-30 µg/kg, i.v.) and propofol (2-3 mg/kg, i.v.), and tracheal intubation was with rocuronium (0.6-1.0 mg/kg, i.v.). Anesthesia was maintained with the minimum alveolar concentration (MAC) :1 sevoflurane in air/oxygen, and rocuronium and fentanyl in maintenance doses. The ventilation of the patients was adjusted to maintain normoxia and normocapnia.

Standard extracorporeal circulation (ECC) techniques were used with pump flow 2.0–2.4 L/min/m^2 body surface area and moderate systemic hypothermia (32°C). Cardiac arrest was achieved with intermittent antegrade cold-blood cardioplegia. Homogeneous cooling and reheating were done.

The erythrocyte suspension was transfused so that hemoglobin levels were ≥ 7 g/dL at the pump and ≥ 8 g/dL after reperfusion. To monitor tissue perfusion during surgery, blood gas analysis was performed during ECC, as well as monitoring of systemic blood pressure, venous-arterial carbon dioxide difference, lactate levels and urine flow rate. Freshly frozen plasma and platelet transfusions were used according to laboratory and clinical findings. An isotonic (0.09% NaCl) solution was used to supplement the volume lost through evaporation and urine. After the operation, the patients were transferred to the cardiac surgery intensive care unit.

Sampling and Laser Speckle Contrast Imaging System

Patients' hemodynamics (mean arterial pressure [MAP], heart rate) and blood gas measurements (PO_2 (mmHg), PCO_2 (mmHg) and lactate (mmol/L)) were measured and recorded at 5 time points: T1-Before induction of anesthesia, T2-After induction of anesthesia, T3-10th minute of extracorporeal circulation (ECC), T4-After cross-clamp (CC) and T5-End of surgery. The skin area on the palmar surface of the left 2nd, 3rd and 4th fingers of the patients was used to monitor perfusion changes with LSCI during surgery. A commercially available system (Moor Instruments, Devon, UK) using a class 1 laser diode was used for LSCI measurements. Laser speckle images were acquired using a charged-coupled device (CCD) camera and converted to speckle contrast images by pseudocoloring where perfusion scaled from blue (low perfusion) to red (high perfusion). Finally, the extubation times of the patients in the intensive care unit were recorded.

Statistical Analysis

The normal distribution of all datasets was presented as mean ± SD after evaluation with the Kolmogorov–Smirnov test. Statistical analysis was performed using GraphPad Prism v5.0 (GraphPad Software, La Jolla, CA, USA). A one-way ANOVA-Bonferroni post hoc test was used for comparisons. $P<0.05$ was considered statistically significant. In the experimental setup where the tissue perfusion change was accepted as 60%, the power analysis with 80% power and 0.5 alpha error resulted in n=15 sample numbers.

3. RESULTS

The hemodynamic and blood gas data sets of the patients are shown in Table I. There was no significant difference in heart rate, MAP, or serum lactate levels during the surgery when compared with pre-induction ($P>0.05$). A significant increase was found in blood pO_2 (mmHg) values after induction, the 10th minute of ECC and after CC when compared with pre-induction ($P<0.05$). The blood pCO_2 (mmHg) value was found to be significantly lower than the post-operative value after CC ($P<0.05$).

Table I. *Hemodynamic and blood gas parameters*

	Before Induction	After Induction	ECC 10. min	After CC	End of Surgery
Heart rate (min^{-1})	71 ± 2	68 ± 5	-	72± 5	84± 5
MAP (mmHg)	80 ± 2	67 ± 4	55± 4	70 ± 3	76± 4
pH	7.41 ± 0.01	7.40 ± 0.02	7.42 ± 0.01	7.45 ± 0.01	7.40 ± 0.02
PO_2 (mmHg)	104 ± 19	178 ± 21*	197 ± 9*	193 ± 13*	145 ± 13
PCO_2 (mmHg)	36 ± 1	37± 1	37± 1	32 ± 1+	37 ± 1
Lactate (mmol/L)	1.2 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.4 ± 0.2	1.5 ± 0.1

**P<0.05; Before induction, +p<0.05; According to the end of surgery time point. MAP: Mean arterial pressure, ECC: Extracorporeal circulation, CC: Cross-clamping, PO2:Partial oxygen pressure, PCO2: Partial carbon dioxide pressure*

Findings obtained from the LSCI are presented in Figure 1, and the related images are presented in Figure 2. The perfusion values (Perfusion Unit,_PU) of the patients were measured as 301±23 PU before anesthesia induction, 207±19 PU after anesthesia

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induction, 102±10 PU at the 10th minute of ECC, 107±11 PU after CC and 103±9.8 PU after surgery. The skin perfusion levels on the palmar surface of the fingers decreased significantly from the pre-induction measurement and remained low until the end of the operation (P<0.05).

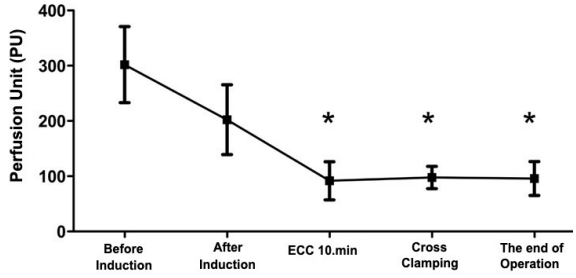

Figure 1. *Change in LSCI perfusion level throughout the surgery*

**P<0,05; Compared to before induction. ECC: Extracorporeal circulation*

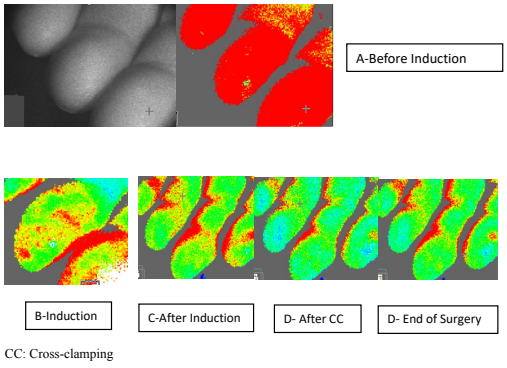

Figure 2. *Laser Speckle images of the change of skin perfusion in the fingers during the surgery*

The relationship between intubation times in the intensive care unit and skin perfusion is shown in Figure 3. It was found that the median perfusion values (98±11 PU) of the patients who were intubated for less than eight hours (388±36 minutes) were higher than the perfusion values (52±4.8 PU) of the patients who were intubated for more than eight hours (634±45 minutes) (P<0.05).

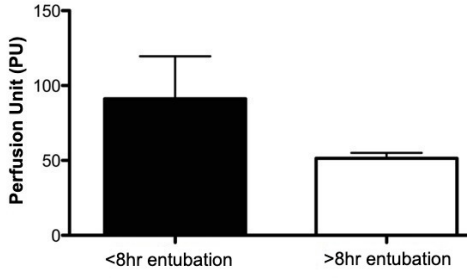

Figure 3. *Postoperative LSCI perfusion levels according to postoperative intubation times*

4. DISCUSSION

The main finding of our study is that hypoperfusion, which cannot be detected by standard hemodynamic monitoring techniques in CABG surgery, can be detected by LSCI. In addition, in our study, it was found that the level of skin perfusion was directly related to the duration of intensive care intubation.

In surgeries where macrocirculation is monitored with standard monitoring techniques, unexpected organ dysfunction may occur in the postoperative period [14]. In adition, improving macrocirculation data does not always improve microcirculation [15]. In cases such as sepsis, it is known that microcirculation is impaired while macrohemodynamic values are not impaired yet. Yin Wu et al., observed liver functions, MAP, heart rate, and liver microcirculation with LSCI in rats with cecal sepsis. They found that perfusion values decreased in LSCI while MAP and heart rate were not impaired in the early (12th hour) period of sepsis [16]. In the same period, they found that the subjects' plasma bilirubin, aspartate transaminase (AST) and alanine transaminase (ALT) levels also increased. However, they also observed signs of sepsis in the histopathological examination of the liver of rats. As a result of the study, they stated that the evaluation of septic hepatic microcirculation with LSCI would provide invaluable information for the early diagnosis and treatment of sepsis.

Depending on the damaged organ, findings such as cognitive dysfunction, urea, creatinine, AST, ALT, troponin-I and pro-brain natriuretic peptide (BNP) elevations, ischemic ECG (electrocardiogram) changes, and hypoxemia due to respiratory failure may occur in the postoperative period. These signs and symptoms may be temporary or have long-term effects. The main cause of organ damage during surgery is the deterioration of the oxygen supply-consumption balance by worsening the microcirculation in the organ. MAP, heart rate, and peripheral tissue oxygen saturation provide limited information on organ microcirculation. De Backer et al. investigated the relationship between macrohemodynamic parameters (MAP, cardiac output) and microcirculatory markers in sepsis. Microcirculation measurements were made in the sublingual region with sidestream darkfield (SDF) technology. As a result of their research, they found a weak correlation between global hemodynamic values and sepsis survival parameters, while they found a high correlation with SDF microcirculation values [17].

Macrohemodynamic monitoring is insufficient for the evaluation of organ perfusion. Therefore, a more reliable method is needed. SvO_2, lactate level monitoring, veno-arterial carbon dioxide differences can be mentioned among the methods showing tissue microcirculation. However, the need for invasive catheterization is its most important limitation [18]. Therefore, there is a need for non-invasive monitoring. It is important in anesthesia management to have information about the microcirculation of organs with noninvasive methods, especially in long-term surgeries. Various techniques have been developed, including fluorescent intravital microscopy,

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SDF imaging, orthogonal polarization spectral imaging (OPS), and laser doppler flowmeter (LDF) [7].

As a new technique, LSCI can monitor continuous and real-time tissue perfusion over a larger area. LSCI has been used successfully to assess microcirculation in the skin, gastrointestinal mucosa, and cerebral cortex [19]. Ambrus et al. investigated the relationship between the blood flow in the intra-abdominal organs and the microcirculation in the same organ with LSCI in their study in pigs. They performed a central block with an epidural catheter on the experimental animals. There were similar changes in both the blood flow of the intra-abdominal organs and perfusion values with LSCI [20]. Eriksson et al. measured the microcirculation of the liver with LSCI intraoperatively in liver surgery. They observed that the perfusion unit was zero on the LSCI when the patients' hepatic artery and hepatic vein were fully occluded [21].

In addition to demonstrating macrohemodynamic changes in organs with laser speckle imaging, studies showing similar vascular changes in the skin are available in the literature [22]. The earliest finding in the pathogenesis of cardiovascular diseases is vascular endothelial disruption. Systemic endothelial disruption causes similar changes in the microvascular structure of cutaneous tissue [23]. Therefore, monitoring the microcirculation of the cutaneous tissue can provide information about systemic events. Galliard et al., compared patients with systemic sclerosis with healthy volunteers. They measured postocclusive hyperemia after 5 minutes of ischemia with LSCI. They found that distal digital perfusion was decreased in patients with systemic sclerosis. In this way, they showed that the diagnosis and treatment follow-up of a systemic vascular disease can be made from skin tissue [24]. Kiss et al., performed fulminant sepsis in pigs with E.coli and followed the microvascular changes in the skin with LSCI. While no change was found in the control group, they detected early microvascular deterioration in the skin in pigs with sepsis. Thus, they showed that early detection of systemic vascular changes is possible with non-invasive evaluation [25]. Barcelos et al. measured skin microcirculation with LSCI in patients with subacute and acute endocarditis. It was stated that noninvasive skin circulation monitoring in patients with endocarditis would be beneficial in the follow-up of the disease and in the early diagnosis of complications [22].

In our study, systemic macrohemodynamic parameters were monitored at the beginning of the operation, at the end of the operation and during the ECC in CABG. At the same time, the cutaneous microcirculation was monitored by LSCI. While no significant changes were observed in MAP, heart rate, or blood lactate levels throughout the operation, LSCI perfusion values started to decrease after induction and remained low until the end of the operation (Figure 1). In addition, we evaluated intubation times as a secondary output in our study. Extubations made in the first 8 hours in the intensive care unit after CABG are considered early extubations (fast track). It is known that these patients have a better outcome. It was found that patients who were intubated for more than 8 hours in the ICU had lower perfusion values (Figure 3).

The pressure-volume relationship is lost in long-lasting major surgeries. Therefore, standard monitoring techniques cannot provide information about organ microcirculation. Advanced hemodynamic monitoring is both an invasive method and has certain limitations [26] In addition, when choosing advanced hemodynamic monitoring methods, their advantages and limitations should be well known. It is best to choose the methods that will give us the most accurate information according to the patient and the surgery [27]. LSCI can monitor the cutaneous microcirculation without contacting the patient and can provide valuable information about the systemic microcirculation.

Among the limitations of our study, we can mention that the microvascular reactivity of the measured skin tissue is affected by the ambient temperature [13]. If the operating room temperature is low, microvascular reactivity is reduced [28]. This should be taken into account during measurements.

Conclusion

A sudden decrease in the perfusion of skin tissue in the extracorporeal circulation and a prolonged intubation time associated with this decrease were detected. In addition, despite the decrease in tissue perfusion, no change was observed in other measured macrohemodynamic parameters. This suggests that clinically, skin perfusion measurement may be a potential microcirculation monitoring parameter. However, further studies are needed to evaluate the relationship between skin perfusion, environmental conditions and internal organs.

Compliance with Ethical Standards

Ethical approval: This study was approved by the Acibadem University Ethics Committee (approval number: ATADEK: 2015-15/17) and conducted following the guidelines of the 1964 Helsinki Declaration. Written informed consent was obtained from the patients.

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Authors Contributions: HU and FT: Idea, FT: Design, HU and MT: Inspection, FT: Sources, MO and HU: Materials, EE, PG, MT and HU: Data collection and/or processing, UA and MT: Analysis and/or comments, UA, and HU: Literature review, HU: Writing the manuscript, HU, FT and UA: Critical review. All authors approved the final version of the manuscript.

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Original Article

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Innovative distal bolt-locking screw tibial nailing method and conventional nailing: A comparison of outcomes

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ABSTRACT

Objectives: Distal bolt-locking screw (DSBLS) tibial nailing offers an innovative method in which the nail is locked upon entering the screw. The current study compares the clinical, radiological, and functional outcomes of DSBLS tibial nails with conventional tibial nails.

Patients and Methods: We retrospectively evaluated 38 tibial fractures of 37 patients treated with intramedullary nailing. In Group 1, 21 fractures were treated with DSBLS nailing, while in Group 2, 17 fractures were treated with conventional nailing. Duration of surgery, time to weight-bearing, time to union, presence of deformity, return to work and sports, complications, American Orthopedic Foot and Ankle Society Score (AOFAS) and Olerud-Molander Ankle Score (OMAS) values were compared between the groups.

Results: Group 1 patients had significantly shorter time to full weight-bearing than patients in Group 2 (P=0.032). There was no significant difference between the groups in functional comparisons according to the AOFAS. In contrast, the outcomes of Group 2 were better than those of Group 1 according to the OMAS (P=0.475 and P=0.037). The outcomes for the other variables were similar.

Conclusion: In this method, patients can bear weight in a shorter time. The results of DSBLS nailing are as good as conventional nails, and it can be safely preferred in treating tibial fractures with intramedullary nailing.

Keywords: DSBLS, Distal bolt-locking screw, Tibia, Tibia nailing, Tibia fracture

1. INTRODUCTION

Among long bone fractures, tibia fractures are the most common [1]. The primary goal in treating tibial fractures is to achieve reasonable alignment and return the patient to daily life as soon as possible. Although, different implants such as plates and external fixators can be applied in treating tibial fractures, intramedullary nailing is also an effective option [2]. Intramedullary nails are preferred for tibial fractures due to essential advantages such as allowing stable fixation, causing less damage to soft tissue, allowing earlier weight bearing with weight sharing, and being linked to lower infection rates [3].

Tibial nails can be used in treating metaphyseal transition zone fractures and tibial diaphyseal fractures. As a result of the anatomical structure of the tibia expanding in the distal part, less bone-nail contact occurs in this region, and it is more difficult to obtain the desired stability. Therefore, distal locking screws are essential factors affecting stability [4]. Although, this problem has been tried to be overcome with various screw configurations or additional screws such as poller screws in classical nailing approaches, distal locking screws for the stable fixation of tibial fractures remain an important problem for orthopedic surgeons [5].

The distal bolt-locking screw (DSBLS) tibial nail was developed to address the problems caused by distal locking screws in the nail treatment of tibial fractures [6]. In the DSBLS method, in contrast to conventional nails, the nail is inserted into the bolt screw. This design allows for stability in different axes and axial compression [7]. In the current study, we compare the clinical, radiological, and functional outcomes of the DSBLS as an innovative method with the results of conventional intramedullary distal locking tibial nails. DSBLS is a method used in the current treatment of tibial fractures [6]. However, there needs to be a convincing study comparing the results of DSBLS and conventional nails in the literature. With this study, we aimed to contribute to the literature by comparing and evaluating the outcomes of the two methods.

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2. PATIENTS and METHODS

This study was carried out retrospectively with the approval of the ethics committee (approval number: 2019/0259). Patients who underwent surgery for tibial fractures between 2012 and 2018 and were treated by intramedullary nailing were evaluated. Patients who died in the postoperative period, underwent intramedullary nailing for the treatment of pseudoarthrosis, and did not attend regular follow-up visits were excluded. With these criteria, 38 tibial fractures of 37 patients were evaluated in the study. The patients were divided into two groups according to the type of nail used in treating their tibial fractures. Patients for whom DSBLS tibial nailing was used were considered Group 1, and patients for whom conventional tibial nailing was used were considered as Group 2. In the treatment of the 21 fractures in Group 1, intramedullary tibia nail with distal bolt-locking screw "the TibiA" (TST, Istanbul, Türkiye) was used (Figure 1). The Trigen Meta-Nail (Smith & Nephew, Memphis, TN, USA), a conventional distal locking nail, was used in the treatment of the 17 fractures in Group 2.

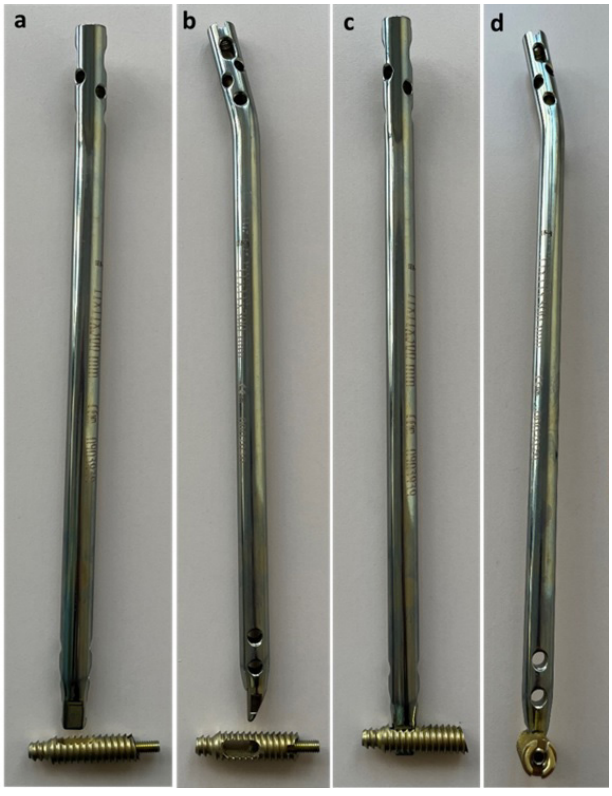

Figure 1. a: *Coronal view of the DSBLS and the nail.* ***b:*** *The tibial nail in the sagittal plane and the entrance hole of the nail in the DSBLS.* ***c:*** *Locking of the tibial nail to the DSBLS in the coronal plane* ***d:*** *Locking of the tibial nail to the DSBLS in the sagittal plane*

The neurovascular status of patients admitted to the emergency department after trauma was evaluated. Patients with open fractures received antibiotic and tetanus prophylaxis. Anteroposterior (AP) and lateral (LAT) tibial radiographs were obtained, and temporary fixation was applied with a splint. The traumas that caused the fractures were analyzed. Fractures were classified by the Arbeitsgemeinschaft für Osteosynthesefragen/ Orthopaedic Trauma Association (AO/OTA) classification, and the Gustilo-Anderson classification was used for open fractures [8,9].

Patients received 1 g of first-generation cephalosporin preoperatively. The operation duration of the patients were recorded. Antibiotic prophylaxis was terminated at the 24th postoperative hour. Low-molecular-weight heparin was given to patients for prophylaxis of venous thromboembolism. On postoperative day 1, two-way (AP-LAT) radiography was performed with the ankle and knee joints visible. Patients with other concomitant fractures and those who could not be ambulated for other reasons were given in-bed exercises. Non-disabled patients walked on their feet.

After discharge, a routine outpatient follow-up program was applied, and the patients were evaluated at weeks 2, 4, 6, 8, 10, 12, and 16 and months 6 and 12 with control radiographs. Information about postoperative time to fracture union, time to weight bearing, return to work and sports were recorded in outpatient clinic visits. All participants were divided into four groups day 1, 1-20 days, 20-45 days, and >45 days according to time to weight bearing. Complications such as anterior knee pain, venous thromboembolism, infection, nonunion, malunion, heterotrophic ossification, reflex sympathetic dystrophy, and screw breakage were followed. The presence of varus-valgus deformity was evaluated on AP radiographs of the tibia, and the presence of antecurvatum-recurvatum deformity was evaluated on LAT radiographs. Angulation of more than 5° in any plane was considered malalignment [10]. Full weight bearing was regarded as the patient stepping on the foot without pain. Evaluation of union was performed radiologically. The Olerud-Molander Ankle Score (OMAS) and the American Orthopedic Foot and Ankle Society Score (AOFAS) were used for clinical evaluation [11, 12].

Surgical Technique

All patients were positioned supine. After sterile covering, the skin and subcutaneous tissues were cut with a 5-cm incision distally from the patella. The tibia was reached by passing through the center of the patellar tendon. The first entry site was determined and entered with fluoroscopic control at the point where the tibial plateau turned to the anterior cortex. The fracture was reduced, the guide wire was placed, and the tibia was reamed appropriately. After the nail was pushed to the location of the distal bolt screw, a Kirschner (K) wire was inserted from the medial of the tibia at the supramalleolar level, parallel to the joint in the coronal plane and to the midline of the tibia in the sagittal plane. After confirming position of the K-wire with the scope, the medial and lateral cortex were drilled sequentially with a 5-mm drill through the wire, and then solely the medial cortex was drilled with an 8.5-mm drill. The bolt screw was inserted into the drilled holes with its wide opening proximally facing the tibia. A nail was inserted into the placed screw, and locking was completed with a set screw. Proximal screws were placed using the guide (Figure 2).

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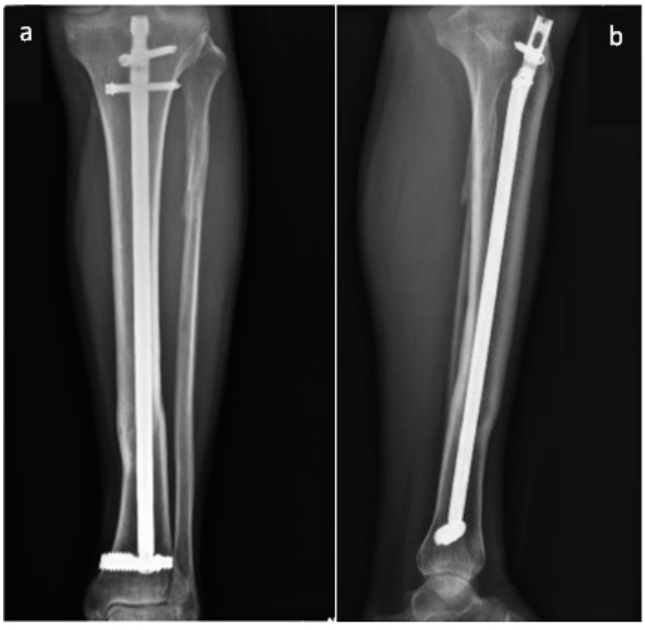

Figure 2. *X-ray images of a patient treated with DSBLS at 43 months postoperatively a: AP view b: LAT view*

In Group 2, the tibia was entered with the same approach used in the first group during intramedullary nailing applications. After reduction, the guide wire was placed, and reaming was performed. An intramedullary nail of appropriate length and thickness was placed. Distal locking screws were locked with a magnetically assisted system (Smith & Nephew, Memphis, TN, USA). Proximal screws were placed using the guide (Figure 3).

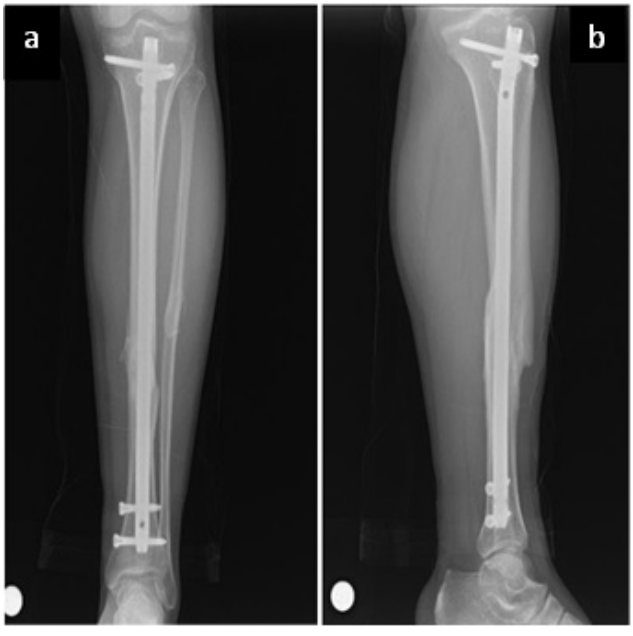

Figure 3. *X-ray images of a patient treated with the conventional nail at 36 months postoperatively a: AP view b: LAT view*

Statistical Analysis

The collected data were evaluated using IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). Descriptive analyses were used for the groups. Data for categorical variables were presented as number (%); data for continuous variables were presented as mean ± standard deviation or mean (standard deviation). Since parametric test conditions were not met in general, differences in value between groups for continuous and discrete variables were evaluated with the Mann-Whitney U test. Chi-square independence test was applied to analyze the significance of differences between groups in terms of qualitative variables. The relationships among quantitative variables were evaluated with Spearman's correlation coefficients. P value <0.05 was accepted as a sign of statistical significance.

3. RESULTS

Twenty-three (62,1%) of the patients were male, and 14 (37,9%) were female. The mean age of Group 1 was 47.48±17.71 years, and that of Group 2 was 42.35±17.12 years. (P=0.332). The mean follow-up period of all participants was 37.6 (12-84) months. The mean follow-up period was 30.19±9.04 months for Group 1 and 46.76±19.94 for Group 2. The follow-up period of Group 1 was statistically significantly shorter than Group 2 (P=0.005).

The tibial fractures of the patients were due to non-vehicular traffic accidents (NVTAs), vehicular traffic accidents (VTAs), falls, gunshot wounds (GSWs), and sports injuries. Tibial fractures were classified according to the AO/OTA classification, and the Gustilo-Anderson classification was used to evaluate open fractures [8,9]. Traumas causing fractures and the classifications of the fractures are given in Table I.

The mean operative duration of the patients in Group 1 was 125±30.28 min.; in Group 2, this duration was 124±32.45 min. Statistically, the difference between the groups was not significant. (P = 0.714). Time to union was 8.11±1.83 weeks in Group 1, and 9.75±3.35 weeks in Group 2, the difference between them was not statistically significant (p=0.289). The time to return to work was 9.09±8.21 months in Group 1 and 8.06±3.31 months in Group 2. The time to return to sports was 9.79±7.91 months in Group 1 and 9.53±4.95 months in Group 2. In terms of time to return to work or time to return to sports there was no statistically significant difference between the groups. (P=0.422 and P=0.470, respectively). the American Orthopedic Foot and Ankle Society Score (AOFAS) and Olerud Molander Ankle Score (OMAS) values and times to weight bearing for the two patient groups are given in Table II.

In the coronal plane, 15 patients (71.4%) in Group 1, 12 patients (70.6%) in Group 2 had neutral alignment. In Group 1, 2 (9.5%) patients had varus alignment; no varus alignment was seen in Group 2. Valgus alignment was seen in 4 (19%) cases in Group 1, 5 (29.4%) in Group 2. Thus, there was no statistically significant difference between the groups for coronal plane alignment (P=0.36). In Group 1, alignment in the sagittal plane was neutral in 16 (76.2%) cases, antecurvatum in 2 (9.5%), and recurvatum in 3 (14.3%). In Group 2, it was neutral in 15 (88.2%) cases

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and antecurvatum in 2 (11.8%). No recurvatum alignment was observed in Group 2. In the sagittal plane, no statistically significant difference was found between the groups (P=0.267). Other complications seen in these cases are given in Table III. All complications were evaluated together and compared in the groups, there was no statistically significant (P=0.75).

***Table I**. Types of trauma causing fractures and fracture classifications according to AO/OTA*

	Group 1 n (%)	Group 2 n (%)	χ^2	p
Trauma				
Fall	8 (38.1)	7 (41.2)		
GSW	1 (4.8)	1 (5.9)		
Sports	0 (0)	1 (5.9)		
VTA	3 (14.3)	1 (5.9)		
NVTA	9 (42.9)	7 (41.2)	2.094	0.853*
Type of fracture				
Closed	16 (76.2)	13 (76.5)		
Type 1 open	1 (4.8)	0 (0)		
Type 3 open	4 (19)	4 (23.5)	0.913	0.529*
Fracture classification				
42A1	4 (19.0)	9 (52.9)		
42A2	0 (0)	2 (11.8)		
42A3	2 (9.5)	2 (11.8)		
42B2	9 (42.9)	3 (17.6)		
42C2	1 (4.8)	0 (0)		
42C3	1 (4.8)	0 (0)		
43A1.1	3 (14.3)	0 (0)		
43B1.3	1 (4.8)	0 (0)		
43C1.3	0 (0)	1 (5.9)	17.168	0.024*

**Fisher's Exact/Fisher-Freeman-Halton Test*

***Table II**. AOFAS /OMAS values and times to weight bearing*

	Group 1 (n=21)		Group2 (n=17)			
	Mean (SD)	median	Mean (SD)	median	U	p*
AOFAS	91.86 (6.67)	90	93.71 (5.83)	95	154.500	0.475
OMAS	83.90 (11.30)	85	91.18 (11.66)	95	108.500	0.037
Postop. full weight bearing (day)	**n (%)**		**n (%)**		χ^2	p**
<1	2 (9.5)		0 (0)			
1-20	4 (19)		2 (11.8)			
20-45	5 (23.8)		0 (0)			
>45	10 (47.6)		15 (88.2)		7.753	0.032

**Mann-Whitney U test **Fisher Exact test/Fisher-Freeman-Halton Test , AOFAS: The American Orthopaedic Foot and Ankle Society Score, OMAS: The Olerud-Molander Ankle Score*

***Tablo III.** Complications in the groups*

Complication	Group 1 (n=21) n (%)	Group 2 (n=17) n (%)	χ^2	p
Anterior knee pain	9 (42.9)	4 (23.5)	1.559	0.212
Irritation	5 (23.8)	7 (41.2)	1.311	0.252
Emboli	2 (9.5)	0 (0)	1.709	0.492*
Infection	1 (4.8)	0 (0)	0.831	0.362*
Nonunion	1 (4.8)	0 (0)	0.831	0.362*
Heterotrophic Ossification	0 (0)	0 (0)	-	-
Sudeck atrophy	0 (0)	1 (5.9)	1.269	0.447*
Screw breakage	0 (0)	1 (5.9)	1.269	0.447*

** Fisher Exact / Fisher-Freeman-Halton Test*

4. DISCUSSION

There are different options for the treatment of tibial fractures, but intramedullary nailing is primarily recommended because of its superiority over other methods [13]. Especially for fractures in the distal third of the tibia, it is challenging to maintain proper alignment, reduction, and stable fixation. Conventional nailing has overcome these difficulties by placing multiple screws on different planes. Mohammed et al. stated that one distal locking screw is insufficient for fractures in the distal region, and if more than one screw cannot be placed, the choice of implant type should be changed [14]. Vallier et al., reported a malalignment rate of 23% after intramedullary nailing in the treatment of distal tibial fractures [10]. Richard et al., similarly reported a malunion rate of 25% in their study [15]. We evaluated the locations of the fractures in our study and found that the number of distal third fractures in Group 1 was statistically significantly higher than in Group 2. Despite this difference between the groups, there was no statistically significant difference between them in terms of malalignment, and the results were similar to the literature.

The most crucial difficulty in intramedullary nailing is the placement of distal locking screws in the nailing [16]. Previously, distal locking screws were placed with fluoroscopy, but later electromagnetic methods started to be used, and operation durations were shortened thanks to that development [17]. Uruc et al., concluded in their study that the duration of surgery with the electromagnetic locking method was significantly shorter, and Langfitt et al., reported that surgical durations were shorter when the electromagnetic locking method was used for distal locking in their study compared to conventional nails [18,19]. In our study, there was no significant difference between the groups with regard to the surgical duration (P=0.714). Although, a magnetically assisted locking system (Smith & Nephew Trigen Sureshot) was used in Group 2, we think that the application technique applied for DSBLS nailing was the source of similar results being obtained between the two groups. With conventional nails, the distal locking screw is placed inside the nail, whereas with DSBLS nailing, the nail is placed inside the screw, providing ease of application for distal locking.

There are different results in the literature regarding the union time of tibial fractures after intramedullary nailing. Robertson et al., reported the mean time to union of tibial fractures as 20.9

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weeks [20]. In the first of two studies in which the results of DSBLS nailing alone were given, Küçükdurmaz et al., reported a mean union time of 9 weeks, and Atiç et al., reported a mean union time of 20.9 weeks [6,21]. In our study, there was no significant difference between the groups with regard to union time, and the results were compatible with those of Küçükdurmaz et al. Although, the same type of nail was used, we think that the difference between the results of Küçükdurmaz et al. and Atiç et al. was related to the time to weight bearing of their patients. Küçükdurmaz et al., allowed their patients to bear weight on the first postoperative day, while Atiç et al., allowed weight bearing to begin in the 9th week. In the literature, it has been shown that early weight bearing accelerates fracture union [22,23]. In our study, we aimed to have our patients bear weight as soon as possible in the postoperative period. In the comparison between the two groups in terms of time to weight bearing, we concluded that DSBLS nailing allowed for a shorter time to weight bearing compared to conventional nails (P=0.032).

The primary goal in the treatment of tibial fractures is to return the patient to daily life with a pain-free and fully functional limb. In their study, Dogra et al., reported an average time to return to work of 5 months, while Arangio et al., reported an average time to return to work of 11 months [24, 25]. In our study, the average time to return to work was 9.09±8.21 months in Group 1 and 8.06±3.31 months in Group 2, and there was no significant difference between the groups. In a systematic review of 16 studies by Robertson et al., the average time to return to sports after tibial fracture was 10.25 months [20]. In our study, the average time to return to sports was 9.79±7.91 months in Group 1 and 9.53±4.95 months in Group 2. There was no significant difference between the two groups, and the results were similar to the literature.

Ankle movements may be affected, especially in cases of distal tibial fractures. Therefore, ankle function should also be considered in the evaluation of clinical results of tibial fractures. Guo et al., reported the mean AOFAS of 89.1 among their patients treated with intramedullary nailing [26]. In their study, Kariya et al., reported that the mean AOFAS was 82.1 [27]. Matthew et al., found the mean OMAS of 91.4 as clinical outcomes of their patients [28]. Ibrahimi et al., reported a mean OMAS of 87.9 in the study [29]. In our study, the mean AOFAS was 91.86±6.67 and 93.71±5.83 with no significant difference between the groups. When OMAS criteria were considered, the mean value of Group 1 was found to be 83.90±11.30, while this value was 91.18±11.66 in Group 2. The results of Group 2 were statistically significantly better in terms of the OMAS. We think the larger number of distally located fractures in Group 1 and the shorter follow-up period compared to Group 2 may have been effective for this situation.

Anterior knee pain is the most common complaint after treatment of tibial fractures with intramedullary nailing [30]. Keating et al., reported that anterior knee pain occurred in 53% of the patients in their study [31]. Court-Brown et al. reported this rate as 56.2% in their study[32]. In a meta-analysis, Katsoulis et al., found the average incidence of anterior knee pain to be 47.4% [33]. In our study, the most common complication that we observed in patients was anterior knee pain, reported by 34.2% of our patients. There was no statistical significance between the groups and our results are consistent with the literature. In our study, five patients in Group 1 and seven patients in Group 2 complained of screw irritation. Although, there were more patient complaints in Group 2, there was no significant difference between the groups. Superficial skin infection was observed in two patients in Group 1 and was treated with oral antibiotics. Reflex sympathetic dystrophy was observed in one patient in Group 2. Venous thromboembolism was detected in two patients in Group 1. The distal locking screw broke in one patient in Group 2. In Group 1, nonunion was observed in the left tibia of a patient with bilateral tibial fractures, and the union was achieved by applying exchange nailing. When all complications were considered together, the results were similar between the groups.

The first limitation or our study is its retrospective structure. The fact that a single surgeon did not perform the operations is another limitation. We think that different results could be obtained in a homogeneous group with a higher number of patients with fractures located in the distal tibia.

Conclusion

In conclusion, DSBLS nailing is a method for solving the problems encountered with distal locking screws. With DSBLS nailing, patients can bear weight earlier. The radiological and functional results and complications of DSBLS nailing are similar to conventional nails. This approach can be safely preferred in the treatment of tibial fractures with intramedullary nails.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Medeniyet University, Goztepe Training and Research Hospital Ethics Committee (approval number: 2019/0259).

Conflict of Interest: The authors declare that they have no conflicts of interest.

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

Authors Contributions: HK:Data collection ,writing – original draft preparation AD:Conceptualization, methodology, EUK:Writing – reviewing and editing, FK:Supervision

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The impact of chiral switch on drug labeling in Turkey: indication, posology, and adverse effects

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ABSTRACT

Objective: Chiral switch, which involves replacing racemic drugs to market them as pure enantiomers, is presumed to improve efficacy and safety. Data on how chiral switch-related changes are represented in summary of product characteristics (SmPC) is scarce. We aimed to compare the indication, posology, and safety expressions in SmPCs of racemates and their pure enantiomers.

Materials and Methods: We examined SmPCs of nine drug pairs (racemate/pure enantiomer) that underwent chiral switching among top 100 utilized active substances throughout Turkey. We evaluated the expressions in "indications", "posology", and "adverse effects" (AE) subheadings. Daily doses were examined based on "Defined Daily Dose" (DDD) metric.

Results: We detected indication differences in four drug pairs, including absence of "peptic ulcer" in dexlansoprazole and "prevention of depression relapses" in escitalopram. DDDs of pure enantiomers decreased in most of the pairs. Recommended daily doses of esomeprazole and dexibuprofen per DDD were lower than their racemates. Cautions about use in renal and/or hepatic insufficiency varied in three pairs. AE expressions differed in seven drug pairs, mainly citalopram/escitalopram.

Conclusion: This study demonstrated few indication differences in SmPCs of the drug pairs frequently used in Turkey and underwent chiral switching. However, dose reductions and distinctions in safety expressions were remarkable.

Keywords: Chirality, Chiral switch, Pure enantiomer, Racemate, Summary of product characteristics, Health policy

1. INTRODUCTION

Chirality, an important geometric property of chemical compounds, is simply defined as the non-overlapping of the molecule with its mirror image. This feature is also frequently encountered in drug molecules [1-4]. It is known that more than half of the conventional medications with small-molecule structure in the current pharmaceutical market contain at least one asymmetric center. The share of pure enantiomers in newly authorized medications has progressively grown over the years [5,6]. Among small-molecule pharmaceuticals available on the market, pure enantiomers were triple the racemates as reported in our recently published work [7].

Although, chiral compounds are common in body components, it is well known that many critical physiological processes are stereoselective and utilize just one potential enantiomer [1]. This geometric characteristic has been shown to cause substantial pharmacokinetic and pharmacodynamic variations for drug molecules [8]. The variations in efficacy and safety which pure enantiomers might exhibit have sparked controversy regarding whether these compounds should be studied as distinct drug candidates [1,9,10]. The replacement of the already approved racemate with the single enantiomer form on the market is referred to as chiral switch. The goals of this process include more accurate pharmacodynamic profile, broader therapeutic index, improved safety profile, reduced risk of undesired drug interactions, rapid onset of effect, and dosage reduction [1,2]. Most authorities, however, do not require that the pure enantiomer be compared to the racemate product during the medication approval procedure. Furthermore, there is a scarcity of data on the efficacy and safety variations attributed to chiral switch in labeling information of the drugs, creating a knowledge gap in this context [1,11,12]. Summaries of product characteristics (SmPCs) are official sources of information about

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the drug product for healthcare professionals [13]. We aimed to compare the SmPCs of the products that have undergone chiral switch and marketed as racemate and pure enantiomer in Turkey in terms of indication, posology, and safety.

2. MATERIALS and METHODS

In this study, we evaluated SmPCs of drugs that have undergone chiral switch process. While determining the active substances, the chirality status of the 100 most used drugs in Turkey were examined. For this purpose, we used outpatient drug sales data of 2021 obtained from the Turkey branch of IQVIA (formerly Quintiles and IMS Health), which is a global provider of advanced analytics to the healthcare industry. IQVIA provides complete and statistically validated data without any projection, measuring the number of packs at the wholesale distribution level [14]. Among top 100 active substances marketed as non-combination preparations, we identified seven racemates (ibuprofen, lansoprazole, salbutamol, cetirizine, rabeprazole, ketoprofen, amlodipine) and three pure enantiomers (dexketoprofen, esomeprazole, escitalopram) that underwent chiral switch to include in the study. We also added the racemate/pure enantiomer counterparts of those identified active substances. As both the racemate and the pure enantiomer of the ketoprofen/dexketoprofen pair were listed in top 100 drugs, we achieved a total of 18 drugs/nine drug pairs to include in the study. We selected the SmPCs of the identified drugs from the database of Turkish Medicines and Medical Devices Agency [15]. The SmPC of a particular drug brand was eligible if it (i) was a non-combination medicinal product; (ii) belonged to an oral formulation (to minimize comparability issues implied by possible expression discrepancies, given the majority of marketed medications are in oral form), (iii) was preferably an original brand. If an original brand was available, the one that contains the strength of drug dose recommended by the World Health Organization's (WHO) defined daily dose (DDD) metric was selected. If an original brand was not available, we selected the brand with the latest approval date among the ones that had the same formulation and DDD in the database (Table I). We examined the similarities and differences of the expressions in the SmPCs of the chiral-switched racemate and pure enantiomer pairs under "therapeutic indications", "posology" and "adverse effects (AE)" headings. We analyzed all indications under the heading of "therapeutic indications" and only those AEs categorized as "very common" and "common". Subsequently, we classified the shared and different parameters for each heading. In posology analyses, we evaluated the similarities and differences by comparing the daily recommended dose, daily maximum dose, and the need for dose adjustments in renal failure and liver failure for drug pairs based on DDD [16]. The study does not contain patient data and does not require ethics committee approval.

3. RESULTS

Indications

Among the three PPI pairs, we found no difference between rabeprazole and dexrabeprazole in terms of indications. Lansoprazole was indicated in duodenal, gastric, and non-steroidal anti-inflammatory drug (NSAID)-associated ulcers, *H. pylori* eradication, and Zollinger-Ellison syndrome, whereas its pure enantiomer, dexlansoprazole, was not. In omeprazole/esomeprazole pair, the latter was additionally indicated for short-term maintenance of hemostasis after parenteral PPI treatment. For the differences in selective serotonin reuptake inhibitor (SSRI) pair, citalopram was additionally indicated in prevention of relapses/recurrences of depression whereas escitalopram was distinctly indicated in social and generalized anxiety disorders. In NSAID pairs, ibuprofen was additionally indicated in symptomatic treatment of certain rheumatoid conditions while therapeutic indication of dexibuprofen specified dental pain distinct from its racemate. We found no difference for the drug pairs of ketoprofen/dexketoprofen, cetirizine/levocetirizine, salbutamol/levosalbutamol, and amlodipine/S-amlodipine (Table II).

Posology

We determined that DDDs of pure enantiomers was lower in four of the six drug pairs for which DDD information was available. Among the remaining, esomeprazole had higher DDD (30 mg) than that of omeprazole (20 mg) while dexlansoprazole/lansoprazole pair had no difference in DDD. In terms of recommended daily doses in the SmPCs, esomeprazole and dexibuprofen had lower DDDs compared to that of their racemates. The maximum daily doses of dexibuprofen, dexketoprofen, levosalbutamol, and S-amlodipine were lower than that of their racemates (Table III).

The expressions about dose adjustments for renal failure overall showed consistency except for omeprazole/esomeprazole and ibuprofen/dexibuprofen pairs. While omeprazole did not require dose adjustment, its pure enantiomer had a warning to use carefully in severe failure. Ibuprofen was indicated to use as lowest possible dose in renal failure where dexibuprofen was not recommended if the condition was severe. The major difference in terms of hepatic dysfunction was observed for the lansoprazole/dexlansoprazole pair where the racemate required halving of the dose in severe liver failure for which the pure enantiomer was recommended against use (Table III).

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Table I. *Distribution of racemate and pure enantiomer drug pairs undergoing chiral switch.*

Racemate		Pure enantiomer	
Active substance	**Drug brand**	**Active substance**	**Drug brand**
Omeprazole	Demeprazol® 20 mg capsules*	Esomeprazole	Nexium® 20 mg enteric-coated pellet tablets
Lansoprazole	Lansoprol® 30 mg enteric-coated micropellet capsules*	Dexlansoprazole	Dexapol® 30 mg enteric-coated micropill-containing capsules*
Rabeprazole	Pariet® 20 mg enteric tablets	Dexrabeprazole	Rabby-D® 10 mg enteric-coated tablets*
Ibuprofen	Brufen® 600 mg film-coated tablets	Dexibuprofen	Tradil® fort 400 mg film tablets
Ketoprofen	Bi-Profenid® 100 mg ER tablets	Dexketoprofen	Arveles® 25 mg film-coated tablets
Citalopram	Cipram® 20 mg film-coated tablets	Escitalopram	Cipralex® 10 mg film-coated tablets
Cetirizine	Zyrtec® 10 mg film-coated tablets	Levocetirizine	Xyzal® 5 mg film-coated tablets
Salbutamol	Ventolin® 100 mcg pressurized inhalation suspension	Levosalbutamol	Inhawell® 100 capsule-containing powder for inhalation*
Amlodipine	Norvasc® 5 mg tablets	S-amlodipine	S-Nor® 2.5 mg tablets*

**Generic brand, ER, extended-release*

Table II. *Comparison of chiral active substance pairs in terms of licensed indications.*

Drugs	Mutual indications	Differences
Omeprazole	– Reflux esophagitis (treatment and prophylaxis) – Symptomatic GERD – Zollinger-Ellison syndrome – H. pylori eradication – NSAID-related peptic ulcer (treatment and prophylaxis) – Peptic ulcer prophylaxis	– Peptic ulcer treatment
Esomeprazole		– Short-term maintenance of hemostasis in peptic ulcers
Lansoprazole	– Reflux esophagitis treatment – Reflux esophagitis prophylaxis – Symptomatic GERD	– Duodenal ulcer – Gastric ulcer – *H. pylori* eradication – NSAID associated ulcer – Zollinger-Ellison syndrome
Dexlansoprazole		
Rabeprazole	– Active duodenal ulcer – Active benign gastric ulcer – GERD treatment – GERD maintenance therapy – Symptomatic GERD – Zollinger-Ellison syndrome – H. pylori eradication in peptic ulcer patients	
Dexrabeprazole		
Ibuprofen	– Osteoarthritis symptoms – Dysmenorrhea – (Acute±) Musculoskeletal pain	– RA symptoms – Ankylosing spondylitis symptoms – Acute gouty arthritis – Postoperative pain
Dexibuprofen		– Dental pain
Ketoprofen	– Osteoarthritis symptoms – RA symptoms – Ankylosing spondylitis symptoms – Acute gouty arthritis – Acute musculoskeletal pain – Postoperative pain – Dysmenorrhea	
Dexketoprofen		
Citalopram	– Depression treatment – Panic disorder – Obsessive compulsive disorder	– Prevention of depression relapses/recurrences
Escitalopram		– Social anxiety disorder – Generalized anxiety disorder
Cetirizine	– Symptoms of allergic rhinitis – Urticaria symptoms	
Levocetirizine		
Salbutamol	– Asthma symptoms – COPD symptoms	
Levosalbutamol		
Amlodipine	– Essential HT – Chronic stable angina – Vasospastic angina	
S-amlodipine		

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Table III. *Comparison of DDD and the posology characteristics of chiral drug pairs declared in SmPCs.*

Drugs	Daily recommended dose	DDD	Maximum daily dose	Dose in renal impairment	Dose in hepatic impairment
Omeprazole	1 x DDD	20 mg (GERD)	N/A	Dose adjustment is not required	10-20 mg daily
Esomeprazole	(2/3) x DDD*	30 mg[a] (GERD)	N/A	Mild to moderate: No dosage recommendation Severe: Should be cautious	Mild to moderate: No dosage recommendation Severe: 20 mg maximum
Lansoprazole	1 x DDD	30 mg (GERD)	N/A	Dose adjustment is not required	Moderate to severe: Half the daily dose
Dexlansoprazole	1 x DDD	30 mg[c] (GERD)	N/A	Dose adjustment is not required	Mild: No dosage adjustment required Moderate: Maximum of 30 mg daily Severe: Use not recommended
Rabeprazole	1 x DDD	20 mg (GERD)	N/A	Dose adjustment is not required	Dose adjustment is not required
Dexrabeprazole	10 mg (GERD)	(N/A)[d]	N/A	Dose adjustment is not required	Dose adjustment is not required
Ibuprofen	1 to (3/2) x DDD	1200 mg (rheumatoid arthritis)	2 x DDD	Minimum possible dose	Minimum possible dose
Dexibuprofen	3/4 x DDD*	800 mg[b] (rheumatoid arthritis)	3/2 x DDD[#]	Mild to moderate: Reduced initial dose (not specifically specified) Severe: Use not recommended	Mild to moderate: Reduced initial dose (not specifically specified) Severe: Use not recommended
Ketoprofen	(2/3) to (4/3) x DDD	150 mg (rheumatoid arthritis)	4/3 x DDD	Reduced initial dose (not specified) Severe: Contraindicated	Minimum effective daily dose Severe: Contraindicated
Dexketoprofen	(2/3) to 1 x DDD	75 mg[b] (rheumatoid arthritis)	1 x DDD[#]	Mild: 50 mg daily Moderate to severe: Use is not recommended	Mild: 50 mg daily Moderate to severe: Use is not recommended
Citalopram	1 x DDD	20 mg (depression)	2 x DDD	Mild to moderate: No dosage adjustment required Severe: Should be cautious	Mild to moderate: Initial 10 mg, maximum 20 mg Severe: Caution, slow titration
Escitalopram	1 x DDD	10 mg[b] (depression)	2 x DDD	Mild to moderate: No dosage adjustment required Severe: Should be cautious	Mild to moderate: Initial 5 mg, maximum 10 mg Severe: Caution, slow titration
Cetirizine	1 x DDD	10 mg	N/A	Mild: No dosage adjustment required Moderate: 5 mg daily Severe: 5 mg every other day ESRD/dialysis: Contraindicated	Dose adjustment is not required
Levocetirizine	1 x DDD	5 mg[b]	N/A	Mild: No dosage adjustment required Moderate: 5 mg every other day Severe: 5 mg every three days ESRD/dialysis: Contraindicated	Dose adjustment is not required
Salbutamol	(1/8) to (1/2) x DDD	0.8 mg inh. aerosol/ powder (asthma)	1 x DDD (800 mcg)	No data	No data
Levosalbutamol	100 mcg every 4-6 hours	(N/A)[d]	100 mcg (1 inh. dose)[#] 6 times a day	Should be cautious	Dose adjustment is not required
Amlodipine	1 x DDD	5 mg (HT)	2 x DDD	Dose adjustment is not required	Minimum initial dose, slow titration
S-amlodipine	2.5 mg (initial dose)	(N/A)[d]	5 mg[#]	Dose adjustment is not required	Minimum initial dose, slow titration

**Different from the Defined Daily Dose (DDD) posology of the World Health Organization, a: those whose DDD is increased compared to its racemate, b: those whose DDD is reduced compared to its racemate, c: those whose DDD is unchanged compared to its racemate, d: Those who do not have DDD but whose daily dose is reduced compared to their racemate in the SmPC declaration, #: represents those with reduced maximum daily dose compared to its racemate.*

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Table IV. *Comparison of chiral drug pairs in terms of very common and common adverse effects.*

Drugs	Mutual adverse effects	Differences
Omeprazole	-Headache	-
Esomeprazole	-Abdominal pain, constipation, diarrhea, bloating, vomiting, nausea, benign fundic gland polyps	-
Lansoprazole	-Headache -Nausea, diarrhea, abdominal pain, constipation, flatulence	-Dizziness -Vomiting -Dry mouth/throat -Increased liver enzymes -Urticaria, itching, redness -Fatigue
Dexlansoprazole		-Fundic gland polyps
Rabeprazole	-Infection	-Fundic gland polyps
Dexrabeprazole	-Insomnia -Headache, dizziness -Cough, pharyngitis, rhinitis -Diarrhea, vomiting, nausea, abdominal pain, constipation, flatulence -Non-specific pain, back pain -Asthenia, flu-like illness	-
Ibuprofen	-Nausea, vomiting, abdominal pain -Rash	-Dyspepsia, diarrhea, flatulence, constipation, melena, hematemesis, GI bleeding
Dexibuprofen	-Headache, dizziness -Fatigue	**-Dyspepsia, diarrhea** -Somnolence, vertigo
Ketoprofen	-Nausea, vomiting, dyspepsia, abdominal pain	-
Dexketoprofen		-Diarrhea
Citalopram	-Decreased appetite -Anxiety, abnormal dreams, female anorgasmia, decreased libido, agitation -Tremor, paresthesia -Yawning -Nausea -Diarrhea, constipation, vomiting -Increased sweating -Myalgia, arthralgia -Impotence, ejaculation disorder	**-Insomnia, somnolence** **-Headache** **-Dry mouth** -Weight reduction -Dizziness, attention disorder, migraine, amnesia -Tinnitus -Palpitations -Rhinitis -Dyspepsia, abdominal pain, bloating, excessive salivation -Itching -Asthenia -Fatigue
Escitalopram		-Increased appetite, weight gain -Irritability, confusion, apathy -Headache -Insomnia, somnolence, dizziness -Sinusitis -Dry mouth -Weakness, fever
Cetirizine	-Headache -Somnolence -Dry mouth -Weakness	-Nausea -Pharyngitis -Dizziness
Levocetirizine		-
Salbutamol		-Tremor, headache -Tachycardia
Levosalbutamol		-Dizziness -Pain -Asthma, pharyngitis, rhinitis
Amlodipine	**-Somnolence, dizziness, headache** **-Edema** -Visual impairment, diplopia -Palpitations -Facial flushing -Dyspnea -Abdominal pain, nausea, dyspepsia, change in bowel movements -Ankle swelling, muscle cramps -Fatigue, asthenia	-
S-amlodipine		

Very common adverse reactions are denoted by bold font.

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Adverse effects

In PPI pairs, we identified the major AE difference between lansoprazole/dexlansoprazole was the frequent prevalence of "dizziness, vomiting, dry mouth/throat, increased liver enzymes, urticaria/itching/redness, and fatigue" in lansoprazole compared to its enantiomer which distinctly exhibited "fundic gland polyps" as frequent AEs. Among substantial differences in gastrointestinal and central nervous system AEs between the SSRI pair, insomnia/somnolence, headache, and dry mouth was listed as "very frequent" for citalopram and as "frequent" for escitalopram. In addition, these drugs also differed in terms of their frequent weight-related AEs that citalopram was associated with weight loss compared to the association of escitalopram with weight gain (Table IV).

In NSAID pairs, ibuprofen indicated dyspepsia and diarrhea as frequent AEs, which were listed as very frequent for dexibuprofen. On the other hand, this pure enantiomer lacked flatulence, constipation, melena, hematemesis, and gastrointestinal bleeding, AEs which were categorized as frequent for ibuprofen. The antihistamine racemate cetirizine showed nausea, pharyngitis, and dizziness as frequent AEs, which were not listed as frequent or very frequent by its pure enantiomer, levocetirizine. Similarly, the frequent AEs of tremor, headache and tachycardia of the salbutamol was not observed in the frequent/very frequent AE category of its pure enantiomer (Table IV).

4. DISCUSSION

Although, health authorities have defined criteria for the official drug documents, especially SmPCs, it is known that problems can be encountered regarding their standardization and compliance with the current literature [17,18]. Moreover, the reflection of the data on the efficacy and safety differences resulting from the chiral switch to drug labels is limited [1,19]. The up-to-dateness of these documents, approved by health authorities, is important to achieve all global health goals positioned within the framework of "good health and well-being" [19,20]. Our study focused on the prominent differences in the SmPCs of the preparations that have undergone chiral switch and are available in both the racemate and pure enantiomer form in the pharmaceutical market.

Since, the pure enantiomer of three of the four racemates available in Turkey is also available in the market, PPIs are among the drug groups that have undergone chiral switch substantially. As a result of the chiral switch, esomeprazole, dexrabeprazole and dexlansoprazole were included in the global pharmaceutical market [21-23]. The advantages expected to emerge with the chiral switch of PPIs include increased bioavailability, prolonged intragastric pH control, and less inter-individual variability in drug metabolism [22,24]. On the other hand, it is known that the active substances in this group are similar in terms of clinical efficacy and therapeutic areas [25]. Six of the seven indication differences between the PPI pairs examined in our study were related to peptic ulcer treatment. Although, there are studies showing that the pure enantiomer of the lansoprazole/dexlansoprazole pair, in which the majority of these differences was observed, may be effective in the treatment of *H. pylori*-associated peptic ulcer [26,27], it was noteworthy that dexlansoprazole is not indicated for duodenal, gastric and NSAID-related ulcers. Besides, the additional indication for the short-term maintenance of hemostasis in esomeprazole compared to omeprazole may be related to the fact that this PPI pair provides similar intragastric pH control, but the pure enantiomer provides >24 hours of intragastric pH stabilization in more patients compared to the racemate [21]. In a meta-analysis including randomized trials of the lansoprazole and dexlansoprazole where almost all AE differences were observed, it was reported that these drugs were similar in terms of safety endpoints [12,28].

One of the potential advantages of chiral switch is the limitation of drug doses to which the patient is exposed [29]. In the conversion of drugs in which one of the two enantiomers in the structure of the racemate provides the therapeutic effect and the other is inert, the therapeutically effective enantiomer is purified and the dose of the drug to which the patient will be exposed can be reduced accordingly [30]. Except for esomeprazole and dexlansoprazole, the recommended daily dose of pure enantiomers was lower than their racemates in all of the pairs examined in the study, suggesting that this target of chiral switch can often be achieved. While, the recommended daily doses of the racemate and the pure enantiomer were the same in the two PPI pairs in which no dose reduction was observed, it was noteworthy that the DDD value of esomeprazole was higher than that of omeprazole. Of the 17 studies in a meta-analysis comparing esomeprazole to its racemate, nine received higher doses of esomeprazole than omeprazole. Based on the results of 10 of these 17 studies, the safety profile of the two drugs was reported to be generally similar [31]. The fact that studies provide evidence in favor of the safety of high doses of esomeprazole may have paved the way for the widespread use of the drug at these doses. However, the direct relationship of this situation with chirality is doubtful. Another pair of PPIs, lansoprazole and dexlansoprazole, were similar in terms of both recommended daily dose and DDD values. This situation may be related to the fact that dexlansoprazole is presented in a dual-delayed release (DDR) formulation rather than stemming from chiral switch. In the DDR formulation, the drug consists of a mixture of two different types of granules, one release in the proximal duodenum and the other in the distal small intestine. It is stated that the drugs in this formulation require a higher daily dosage administration as they release for a longer period of time than conventional delayed-release PPIs [31]. This may suggest that the posology differences observed in both drug pairs are unlikely to be chirality related.

Ibuprofen and ketoprofen, which are arylpropionic acid derivatives, are among the racemic NSAIDs that undergo chiral switch. The S-enantiomers of these drugs are mainly responsible for the anti-inflammatory effect and have been introduced to the market after chiral switch. This switch is aimed at faster onset of action and less individual variability in drug response [1,32]. On

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the other hand, the fact that the R-enantiomers of these drugs are converted to S-enantiomers as a result of chiral inversion in the body (approximately 60% in ibuprofen, ≤15% in ketoprofen) has led to debates that the biological effect difference between drugs is limited [1,30,32]. Indication and AE differences in NSAIDs were mainly observed in the ibuprofen/dexibuprofen pair. Only ibuprofen was approved for the symptomatic treatment of certain rheumatic disorders, but dexibuprofen was not. However, in a review of eight clinical studies and three observational studies, dexibuprofen was reported to be similarly effective with its racemate in these conditions [33]. In terms of safety, the frequencies of "dyspepsia" and "diarrhea" were stated more frequently in dexibuprofen, but the number of gastrointestinal AE expressions were higher in ibuprofen. In fact, a study conducted with osteoarthritis patients in Austria, reported that gastrointestinal complaints were >2 times higher in ibuprofen compared to the enantiomer [34]. On the other hand, a Korean study conducted in children reported no safety difference between the two drugs [35]. This suggests that the differences reflected in the safety profile may be related to the indication and target patient population. Indication and AE expressions of ketoprofen/dexketoprofen, the other NSAID pair studied, were largely similar. In a systematic review evaluating 24 studies, it was reported that these two drugs have similar efficacy and safety profiles [36].

Unlike the ones in citalopram, the SmPC of escitalopram showed indication statements about anxiety disorders. It has been suggested that the S-enantiomer (escitalopram) of citalopram is the part that is effective in the treatment of anxiety disorders [37]. Escitalopram was reported to be superior to its racemate in terms of efficacy in seven studies comparing citalopram [38]. The fact that the pure enantiomer drug has indications related to anxiety, unlike its racemate, may be associated with this evidence. However, although it is not among the indications of citalopram, it has been reported that this chiral mixture has benefits in anxiety disorders and included in the guidelines, albeit trailing behind escitalopram [39,40]. As in this case, it might be suggested that physicians, while prescribing drugs, do not necessarily adhere to the approved indications and may use some drugs off-label for conditions where their efficacy has been established. We observed substantial AE differences statements for the citalopram/escitalopram pair, especially regarding the central nervous and gastrointestinal system. Theoretically, separation of escitalopram from its R-enantiomer is expected to positively affect the safety profile of the drug, but it is stated that the frequency of AEs and tolerability of drugs are similar in the majority of clinical studies in the literature [1,12,41]. It is unclear if AE declaration differences as those seen in this drug pair are the product of statistical evaluation of clinical trial-based safety data or post-marketing routine pharmacovigilance data. Considering that documentation errors may have contributed to this situation, our findings may imply that this problem, which complicates standardizing and homogenizing the label expressions, merits further examination.

The fact that levocetirizine has up to 30 times greater affinity for H1 receptors than dextrocetirizine and exhibits approximately 10 times more potency is among the reasons for the chiral switch of cetirizine [1,42]. On the other hand, the superiority of levocetirizine to cetirizine in terms of effectiveness is controversial. The efficacy of the drugs was compared in three studies evaluated in a meta-analysis, and only one study reported that levocetirizine was more effective than its racemate [12]. As would be expected, this suggest overlapping of the indication statements in the respective SmPCs. In terms of safety, the expressions "nausea", "pharyngitis" and "dizziness" in the SmPC of cetirizine were not found in levocetirizine. However, the same meta-analysis, reported no superiority of either drug to each other in terms of safety [12]. The discrepancies between the data sourced for SmPCs and the results of the limited studies in the literature suggest that more studies are needed to compare the two drugs in terms of efficacy and safety. Similar to that in cetirizine/levocetirizine, the indication expressions in the labels of the salbutamol/levosalbutamol pair were similar, while "tremor and headache" and "tachycardia" AEs found in salbutamol were not present for levosalbutamol. Increased potency and reduced airway hyperreactivity have been pointed out as potential advantages of the chiral switch seen in this drug pair [1,41]. As a result of the two studies comparing the drugs, it was reported that there was no significant difference between the racemate and the pure enantiomer in terms of AEs such as tremor and headache [43].

In our study, edema and ankle swelling were expressed in both amlodipine and S-amlodipine SmPCs, with similar frequencies for both drugs. A meta-analysis reported that peripheral edema of the lower extremities was less common in S-amlodipine users compared to racemic amlodipine users, but the evidence was weak in quality [44]. However, a more recent randomized controlled trial reported that S-amlodipine used at half the dose of the racemate caused less leg edema [45,46]. Furthermore, it was reported that nearly 90% of patients who had edema while taking racemic amlodipine had their symptoms resolved after switching to the S-enantiomer form [46]. This suggests that there may be problems with updating drug labels or failing to respond soon enough in the light of recently added studies to the literature.

Our study has some limitations. First of all, the chirality characteristics of all drugs available in the market could not be examined. This limitation was tried to be mitigated as much as possible by examining the chirality characteristics of the mostly consumed drugs in Turkey. However, although there are similarities in some groups, it should be kept in mind when evaluating the results of this study that the most consumed drugs in Turkey may not necessarily exhibit parallel trends with the rest of the world. Another limitation of the study can be considered as not examining the sections other than indications, posology, and AEs in the SmPCs of drug pairs. The lack of DDD values for some pure enantiomers can be considered as a limitation, especially for posology-based findings. Given the possibility of variances depending on SmPC documentation of the preparations of each active ingredient, the fact that all the marketed preparations of the drug pairs examined in the study could not be assessed is another limitation. Nevertheless,

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considering that the drug labels should provide standard expressions between the preparations of the same active ingredient, it highlights the need for the parties to re-examine the substantial discrepancies discovered in the SmPCs for all relevant preparations.

Conclusion

We shed light on the differences observed in chiral switch products and the reflections of changes in drug labels. Accordingly, we observed a few differences in indications, several dosage reductions, and substantial differences in AE profile amongst the drug pairs. The limited clinical efficacy and safety benefits of the pure enantiomer after chiral switch suggest that a more selective approach should be followed before its use. Generally, reflections of chiral switch to recommended doses have been observed. On the other hand, although chiral drug pairs are similar to each other in terms of indication and AE according to the literature, it seems that various differences can be encountered in SmPCs due to both documentation problems and lack of up-to-dateness. The potential advantages of chiral switch in terms of efficacy, safety, and suitability are likely to be retained in the future. It is crucial to ensure uniformity while the data on chiral switch are reflected in drug-related official documents. Our study is expected to contribute to the relevant stakeholders in terms of developing SmPCs to provide accurate and up-to-date information. In this context, updated SmPCs will be expected to contribute more to the maintenance of good health and well-being.

Compliance with Ethical Standards

Ethics Committee: The study does not contain patient data and does not require ethics committee approval.

Conflict of Interest: The authors declare that they have no conflicts of interest.

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Authors Contributions: NIKS, CV, AB, VA, and AA: Study conception, design and data collection, NIKS, CV, OG and VA: Analysis and interpretation of results, NIKS, CV, OG, VA and AA: draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

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Cholinergic cognitive enhancer effect of *Salvia triloba* L. essential oil inhalation in rats

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ABSTRACT

Objective: Current treatment of Alzheimer's disease is provided by cholinesterase inhibitors. *Salvia triloba* L. (syn. *Salvia fruticosa* Mill.), a species mostly consumed as refreshing herbal tea in traditional medicine, is rich in 1,8-cineole that is known to have cholinesterase inhibiting effects. In this study, we investigated cognitive enhancer effects of *S. triloba* essential oil inhalation on healthy control rats and rats with scopolamine induced memory impairment.

Materials and Methods: *S. triloba* samples from different geographical locations of Turkey were hydro-distilled and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). The optimum sample with the highest 1,8-cineole and lowest camphor, α-thujone and β-thujone content was selected. In vitro cholinergic and antioxidant potentials of the selected essential oil were calculated. Cognitive enhancer and anti-amnestic effects of the inhaled essential oil on rats were assessed by means of Morris water maze. The bioavailability of 1,8-cineole in blood of rats was measured by GC-MS.

Results: The group that inhaled *S. triloba* significantly outperformed control group, namely faster achieving peak escape latency performance in Morris water maze. However, *S. triloba* inhalation failed to restore scopolamine induced learning impairment.

Conclusion: In this study, we report positive effects of inhaling *S. triloba* essential oil as a complementary treatment for supporting cognitive functions.

Keywords: Cognitive enhancers, Essential oil, Gas chromatography, 1,8-Cineole, Antioxidants, Morris water maze

1. INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disorder, which is characterized by impaired memory and cognitive functions, and behavioral problems leading to significant disturbances in daily activities and the most common cause of dementia [1, 2]. Acetylcholine is one of the neurotransmitters that is associated with the etiopathogenesis of AD. Drastic decrease in acetylcholine levels in the brain has been revealed as the most remarkable biochemical change of AD and major cortical cholinergic innervation by the basal forebrain in the medial septum and nucleus basalis of Meynert is known to be impaired in AD patients [3].

The animal models of AD also proved cholinergic lesions caused learning impairment, and those converging results led to the cholinergic hypothesis of AD related dementia. Accordingly, Morris water maze, is widely adopted to assess behavioral impairments in rodents due to age-related cognitive deficits and in commonly used transgenic mouse models of AD [4]. Scopolamine, a tropane alkaloid with muscarinic antagonist effects, has been the most commonly used pharmacological model to induce amnesia [5].

The cholinergic hypothesis was further supported by improvement in AD related cognitive decline by acetylcholinesterase (AChE) inhibitor therapies. Inhibition of AChE and butyrylcholinesterase (BChE) at the cholinergic synapse, increases acetylcholine levels and boosts cholinergic transmission. Although, cholinesterase inhibitors (ChE-Is) such as donepezil, rivastigmine, galantamine and the N-methyl-D-aspartate (NMDA) antagonist memantine are approved as main pharmacological options for cognitive enhancing treatment in AD, they are unfortunately not capable of reversing the neurodegenerative process [3]. Morris water maze has been commonly recruited to investigate the anti-dementia effects of

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ChE-Is on rodent models of hippocampus-dependent memory losses [6].

Several cognitive enhancer effects of cholinesterase inhibitors have also been reported in healthy population, such as improving retention of aviation skills [7], encoding items in their spatial context [8], episodic memory in young adults [9] and improved verbal memory in healthy elderlies [10]. A systematic review on the cognitive enhancing effects of ChE-Is concluded yield greater effects on low performing participants [11]. Coherent with this fact, donepezil has been reported to ameliorate attention and memory deficits due to sleep deprivation [12], and galantamine treatment has improved sustained attention in abstinent cigarette smokers [13].

Several natural metabolites are capable of selectively inhibiting both AChE and BChE enzymes. *Salvia* (sage) species are frequently used in traditional folk medicine for their memory enhancing effects [14]. The genus *Salvia* (Lamiaceae) has been distributed widely in several regions of the world, especially in the Mediterranean region and Asia, with more than 1000 species [15]. There are 100 species of *Salvia* in Turkey, 57 of which are endemic [16]. Various members of *Salvia* have significant pharmacological activities such as analgesic, anticancer, anti-inflammatory, antimicrobial, antimutagenic, antioxidant, cardioprotective and cytotoxic [17-23]. Several cholinergic enhancing activity of *Salvia* members has also been revealed via numerous *in vitro, in vivo* and *in silico* techniques [17, 18]. Especially, essential oil and extracts of numerous *Salvia* spp. have shown to have AChE and BChE inhibiting activity and antioxidant profile [24-26]. The essential oil of some *Salvia* species and the essential oils of various herbs (*Juniperus communis, Pimpinella peregrina, Lavandula angustifolia,* and *Rosmarinus officinalis*) have been reported to have positive effects on the mood and cognitive functions in the clinic when administered by inhalation [27]. 1,8-cineole in the essential oils is assumed to be the active metabolite that is associated with effects on brain functions [28]. Amongst the mentioned medicinal plants, *S. triloba* L. (syn. *S. fruticose* Mill.) that is rich in 1,8-cineole and camphor, is widely grown in Turkey and mostly consumed as refreshing herbal tea [29].

Excess reactive oxygen species (ROS) can give harm to cellular lipids, proteins or DNA by inhibiting their normal functions and this biological damage is known as "oxidative stress". Oxidative stress has been involved in many diseases including diabetes, cancer and cardiovascular disorders, as well as the aging process [30]. Furthermore, there are literature demonstrating the role of oxidative stress in either pathogenesis or prognosis of several neurodegenerative diseases including AD [31-33]. Although, limited randomized clinic intervention trials of AD have revealed mixed results on benefits from antioxidants, well established pathophysiology of oxidative stress, several *in vitro* studies, animal studies and observational studies depict antioxidants as favorable supplements [31, 34]. Besides, several ChE-Is, including derivates of donepezil and galantamine have been shown to have antioxidant activity in terms of determining their efficacy with multi-targeting [35].

Given the abundant 1,8-cineol content of *S. triloba*, its essential oil was extracted from plant material, analyzed for composition, and confirmed for its cholinesterase inhibitory activity *in vitro*. Based on this exploratory findings, we hypothesized to observe cholinergic enhancer effects of inhaling *S. triloba* essential oil on learning and memory performance of control rats and scopolamine treated amnesic rats. *In vitro* antioxidant profile of the *S. triloba* essential oil was also determined in order to examine its supplementary health promoting effects.

2. MATERIAL and METHODS

Chemicals and Drugs

All chemicals, enzymes and references used in the experiments were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The quality of all chemicals was of analytical grade. Standards used in optimization of GC–MS parameters were also purchased from Sigma.

Plant materials

Aerial parts of *S. triloba* and a commercial essential oil (STC) sample were purchased from different organic agriculture institutes from different regions of Turkey [*S. triloba* – Antalya (STA), Izmir (STI), Yalova (STY) , Balıkesir (STB)] in June 2019. Botanical identification of plant samples were verified by HB. The shade-dried upper ground parts of plant samples were kept at room temperature in air-tight containers until further use. According to the 1,8-cineole, camphor α-thujone and β-thujone amounts calculated by GC-MS analysis, the optimum *S. triloba* sample was selected.

Distillation of essential oils

Aerial parts (100 g) of the plant samples were subjected to hydro-distillation with 1L H_2O for 3h, using a clevenger-type apparatus to produce the essential oils. Condenser of the clevenger was attached to a chiller which was set to 4°C. Aerial parts of *S. triloba* samples from Antalya (STA), Izmir (STI), Yalova (STY) and Balıkesir (STB) afforded an oil with 0.015%; 0.016%; 0.018%; and 0.02% (v/w) yields, respectively. All essential oil samples were stored in amber vials under – 20°C until the day they were analyzed.

GC–MS conditions for essential oil analysis

Qualitative and quantitative analyses were performed by GC-MS. Agilent technologies 7890 A GC system was used with HP-5MS column (30 m x 0.25 mm x 0.25 µm). Oven temperature was started with 60 °C and then steadily increased to 246°C with 3 °C raise per a minute. Helium was used as mobile phase with 0.9 ml/min flow rate. Split mode was used with 50:1 ratio with 1µl sample volume. Relative Retention Indices (RRI) was calculated via comparison with (C4-C40) standards. Identification of the essential oil components were completed by comparison of their relative retention index (RRI) calculated against *n*-alkanes and relative retention times (RT) with those of authentic samples and mass spectra obtained from Wiley 7N library as well as MS literature data was used for the identification [36,

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37]. STA sample was selected for further studies due to its high 1,8-cineole content (40.48%) and significantly low α, β-thujone and camphor content (0.55, 0.80 and 5.15%, respectively).

In vitro tests

Determination of AChE and BChE inhibitory activities

AChE and BChE inhibitory activities of the fractions were studied by a previously elucidated method [38]. AChE (Type-VI-S; EC 3.1.1.7, Sigma, St. Louis, MO, USA) and Horse serum BChE (EC 3.1.1.8, Sigma, St. Louis, MO, USA) were used as enzymes. Acetylthiocholine iodide (AChI) and Butyrylthiocholine chloride (BChC) were used as substrate of enzymes. Adequate amounts of phosphate buffer, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) solution, enzyme and test substance were mixed in 96 well-plate and kept in 25 °C for 15 minutes for incubation. Then, substrates were added to mixture and results were immediately measured at 412 nm via well-plate reader. 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL concentrations of galanthamine was used as positive control to obtain a standard curve for both assays.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity assay was practiced according to method given earlier by Bardakci et al. [39]. Essential oil was diluted with dimethyl sulfoxide (DMSO):H_2O mixture (10% v/v) to adjust the concentration to 1 mg/ml and mixed with 100 µM methanolic DPPH solution. The mixture was preserved at room temperature, away from light, and the loss in absorption was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as reference compound. Moreover, vehicle control test was performed for DMSO:H_2O mixture.

Cupric reducing antioxidant capacity (CUPRAC)

The CUPRAC activity of the DMSO:H_2O (10% v/v) diluted essential oil was measured by using the modified method [39]. Equal volumes of neocuproine, $CuSO_4$ and ammonium acetate buffer were mixed. Then, the essential oil was added to mixture and then kept at room temperature for 1 h, and the absorbance was taken at 450 nm. The results were expressed as mg ascorbic acid equivalent per g DE.

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power activity was assessed spectrophotometrically according to the method described by Bardakci et al. [39]. Correspondingly, essential oil was diluted to 1 mg/ml concentration and 10 µl of it was mixed with 250 µl FRAP solution. After an incubation period of 30 min., the absorbance was retrieved at 593 nm. BHT was used as reference. The results were expressed as mM $FeSO_4$ per g DE.

Determination of total antioxidant capacity by phosphomolybdenum method

Total antioxidant capacity was calculated by phosphomolybdenum method previously published by Bardakci et al. [39]. Diluted essential oil was mixed with the reaction mixture composed of sulfuric acid, ammonium molybdate and sodium phosphate monobasic. After incubation at 95 °C for 90 min., the absorbance was read at 695 nm. Total antioxidant capacity was expressed as mg ascorbic acid equivalent per g DE.

In vivo tests

Animals

Adult male Wistar albino rats, weighing 250-300 g were obtained from Acibadem Mehmet Ali Aydinlar University Experimental Animal Application and Research Center (ACU-DEHAM). All protocols involving animals were approved by Animal Experiments Local Ethics Committee (decision number 2020/13). Food and water were available *ad libitum*. The colony room was kept at 20–24 °C and 60-70% humidity in a 12-hour light-dark cycle. All animals were handled daily and habituated to the experiment room during 3 days preceding the experiments. *In vivo* study consisted of two sections as: behavioral study (n=24) and bioavailability study (n=6). Behavioral study animals were divided into 4 groups depending on drug injection and/or inhalation they received. The behavioral study groups were as follows: Scopolamine injection + *S. triloba* inhalation (Scopolamine + *S. triloba* group), Saline injection + *S. triloba* inhalation (*S. triloba* group), Scopolamine injection + distilled water vapor inhalation (Scopolamine group), Saline injection + distilled water vapor inhalation (control group) (n=6 per group).

Scopolamine hydrobromide was dissolved in an isotonic saline solution (0.9% NaCl). Injections were administered intraperitoneally (i.p.) daily, for 7 days 30 min before water maze test sessions. Scopolamine injections (1 mg/kg) were administered to Scopolamine group and Scopolamine + *S. triloba* group. *S. triloba* group and the control group received isotonic saline injections as sham treatment. Scopolamine dose for inducing amnesia was determined according to an extensive review that investigated effective dose in Morris water maze [5].

All animal groups were individually placed in regular laboratory rodent housing cages of 36x20x14 cm. During the inhalation session, the cage was placed in a 50x40x28 cm transparent plexiglass box (inhalation box) with the vaporizer set up. The vaporizer was designed as follows: 200 µL of the liquid substance to be delivered by inhalation (*S. triloba* essential oil or distilled water) was dropped on a beaker, and the beaker was then placed over a battery powered heater plate (55 °C). The inhalation box was hermetically sealed and the animal was left in the box for 30 minutes. During this period, bioavailability study group, *S.triloba* group and Scopolamine + *S. triloba* groups inhaled *S. triloba* essential oil, while Scopolamine group and the control group inhaled water vapor as sham treatment. Essential oil volume and duration of the implementation were determined based on the study by Bagci et al. with slight modifications [17].

Bioavailability test with GC-MS headspace

Immediately afterwards the inhalation of *S. triloba* essential oil (as described above), 1 mL blood samples were collected under isoflurane anesthesia from each rat and put into 20 mL GC-MS headspace vial and instantly sealed with an aluminum cap. Agilent 7694 E headspace sampler was used with 70 °C extraction temperature, 1 mL sample volume, 110 °C transfer

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line temperature and 16 psi vial pressure for sample injection. Analysis was performed with exactly the same chromatographic method which was used for essential oil analysis. 1,8-cineole standard was used for precise determination.

Morris water maze

All groups received i.p. injection and 30 minutes of inhalation preceding the water maze sessions. The water maze consisted of a black circular tank filled with water to a depth of 30 cm at 25 ± 1 ° C. The pool was divided to four equal quadrants and according to these quadrants 4 start locations (east, north, west, and south) were determined on the perimeter of the tank. A platform (10 × 10 cm) was placed 1 cm below the water and placed at 1/3 length of tank diameter away from tank wall. Geometric shapes and banners were placed on the walls to be used as visual cues by the animals for orienting in water tank. Learning phase was of daily learning blocks for 6 days, and each learning block included 4 consecutive learning trials. In the learning trials, the animal was gently released to water pool facing towards the pool wall from one of the 4 starting locations. It was allowed to swim and climb on the platform for 90 seconds. If an animal failed to find the platform in 90 secs, it was gently picked and left over the platform. By the end of the trials, animals were allowed to rest on the platform for 30 secs, whether they could find the platform by themselves or left there by the experimenter. Starting locations were shifted on every learning trial but the location of the platform and the visual cues on the walls remained fixed through the learning phase. The time to find and climb on the platform by the animal was termed as escape latency. The escape latency for the trials with failure to find the platform is registered as 90 secs (maximum allowed search time). The mean of escape latency for 4 learning trials on a day is the daily escape latency. After completion of the 4th trial, the rat was gently dried and left under a heat lamp before returning to its home cage. The learning performance was mainly observed as the gradual decrease in escape latency on 6 consecutive days (learning phase). On the 7th day, the platform was removed and the animals were left from the second starting point (north) to swim freely for 90 seconds, termed as the probe trial. In the probe trial, the time spent to arrive at the zone where the platform was formerly located (platform latency) and the number of crossings over the platform zone (former place of the platform) are registered. In trials with failure to reach platform zone, platform latency is registered as 90 secs (maximum search time). Shorter platform latency and higher number of platform crossing were interpreted as better memory retention performance. All sessions were recorded and analyzed with Ethovision XT video tracking system (Noldus, The Netherlands).

Statistical Analysis

Each of the *in vitro* tests and analyses were done in triplicate. Escape latencies from 6 days were tested by a repeated measures ANOVA design with experimental animal study group factor with 4 levels (4 animal groups) and day factor with 6 levels (1st day, 2nd day, 3rd day, 4th day, 5th day, 6th day). Huynh-Feldt correction was applied for repeated-measures factors with more than 2 levels as correction for sphericity violation. Tukey test was used for pair-wise group comparisons (post-hoc test). In addition to the repeated measures ANOVA including whole learning phase (6 days), contrast analyses were conducted to elaborate the analyses on the progress of learning. In these contrast analyses, escape latencies from the first day was compared with each of the following days (i.e. 1st day vs. 2nd day, 1st day vs. 3rd day, 1st day vs. 4th day, 1st day vs. 5th day, 1st day vs. 6th day). Bonferroni correction was applied to control familywise error rate in these contrast analyses which involved multiple comparisons, i.e. P values are multiplied with 5 (the number of additional tests).

The platform latency (seconds) and number of platform crossing from the probe trial (7th day) were evaluated with univariate analyses. Normal distribution of platform latency was confirmed in each group by Shapiro-Wilk tests ($p>0.05$) and one-way ANOVA test was conducted to assess group differences. Tukey test was used for post-hoc tests (pair-wise group comparisons). Kruskal-Wallis test was conducted on number of platform crosses and Dunn-Bonferroni correction was applied on post-hoc comparisons. SPSS v. 23 software is used for all statistical analyses.

3. RESULTS

GC-MS analysis of different S. triloba samples and bioavailability

Phytochemical profiles of *S. triloba* essential oils from different geographical regions were evaluated via GC-MS analysis and the results were given in the Table I and Figure 1. In total 32 compounds representing the 86.76-98.09% of the total oils were identified. Results showed that 1,8-cineole amount of the samples varies between 13.22% to 40.48% which was measured 45.31% in commercial essential oil product. Contents of camphor, α and β-thujone are also very noteworthy for *S. triloba* essential oil. Results of the analysis also specified that proportion of these substances fluctuate considerably. α and β-thujone differ from 0.55 to 17.94% and 0.53 to 9.21% respectively. Likewise, camphor content varies between 4.02 to 19.40% in the studied samples.

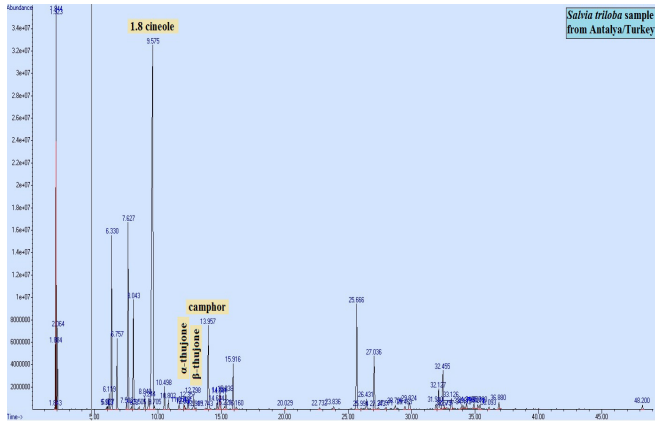

Figure 1. *GC/MS chromatogram of the S. triloba essential oil.*

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Table I. *Essential oil composition of aerial parts of various S. triloba samples*

RT (min)	RRI	Compounds	STA (%)	STY (%)	STI (%)	STB (%)	STC (%)
6.119	927	α-Thujene	0.58	6.60	0.55	0.38	0.75
6.330	933	α-pinene	7.40	8.15	5.39	5.40	6.03
6.757	948	Camphene	3.01	0.11	5.94	4.60	2.03
7.144	976	1-Octen-3-one	-	7.14	0.42	-	0.63
7.627	977	β-pinene	9.54	1.21	-	2.95	9.51
8.043	991	β-myrcene	5.05	0.38	2.18	1.87	2.83
8.940	1017	2-carene	0.66	0.16	0.56	0.52	0.65
9.244	1025	p-cymene	0.83	-	1.15	0.40	0.82
9.398	1029	D-Limonene	-	-	-	1.78	-
9.575	**1034**	**1,8-cineole**	**40.48**	**33.41**	**32.54**	**13.22**	**45.31**
10.498	1058	γ-Terpinene	1.10	0.67	0.80	0.93	1.24
10.802	1066	Sabinenehydrate	0.47	-	-	0.16	0.31
11.654	1088	α-Terpinolene	0.31	-	0.47	0.57	0.35
12.357	**1106**	**α-Thujone**	**0.55**	**0.82**	**3.84**	**17.94**	**2.49**
12.798	**1117**	**β-Thujone**	**0.80**	**0.53**	**3.98**	**9.21**	**4.50**
13.957	**1144**	**Camphor**	**5.15**	**19.40**	**18.53**	**14.55**	**4.02**
14.886	1166	Borneol	1.52	-	2.53	2.47	0.93
15.338	1177	Terpinene-4 ol	-	-	-	-	-
15.916	1191	α-Terpineol	2.73	3.89	-	0.39	1.24
20.016	1288	Isobornyl acetate	-	0.35	2.49	-	0.59
22.743	1348	D-Elemene	-	-	-	-	0.90
23.848	1375	α-Copaene	-	-	-	-	0.35
25.666	1420	Caryophlenne	7.32	4.79	2.33	4.14	6.89
26.431	1439	Aromadendrene	0.66	-	0.11	-	0.73
27.036	1454	α-Humulene	3.48	0.93	1.00	2.31	2.02
29.824	1524	D-Cadinene	0.59	0.81	0.17	-	0.50
31.910	1577	Spathulenol	0.35	-	0.98	-	-
32.127	1583	Caryophylleneoxide	1.56	-	-	0.30	-
32.455	1591	Viridiflorol	2.47	1.00	0.80	5.23	0.29
33.126	1609	Humulene epoxide II	0.76	-	-	-	-
34.316	1641	Cadinol	0.37	-	-	-	-
34.907	1657	Manool	0.35	-	-	-	-
		Total (%)	**98.09**	**90.35**	**86.76**	**89.32**	**95.91**

RT: Retention time, RRI: Relative Retention Index, STA: S. triloba samples from Antalya, STY: S.triloba samples from Yalova, STI: S.triloba samples from Izmir, STB: S.triloba samples from Balıkesir, STC: S. triloba commercial essential oil sample.

GC-MS-Head Space analyses were also conducted on the blood of essential oil inhaled rats for evaluating bioavailability of major principle of *S. triloba* oil, 1,8-cineole. Results of the analysis revealed that significant amount of 1,8-cineole was present in the blood stream of the rats and possible bioactivity of the essential oil inhalation may be attributed to bioavailable 1,8-cineole content (Figures 2 and 3).

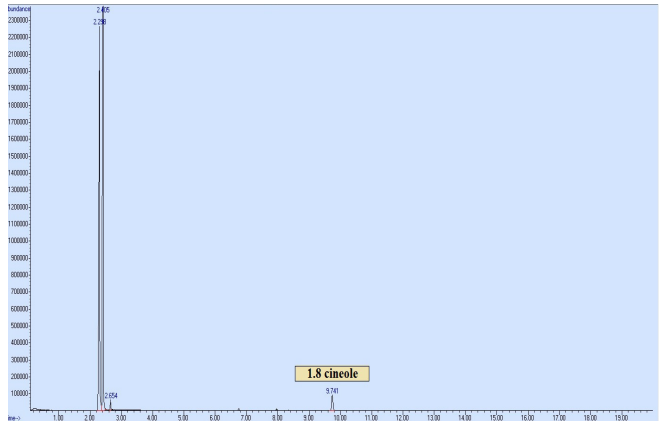

Figure 2. *GC/MS chromatogram of the S. triloba essential oil inhaled rat blood sample.*

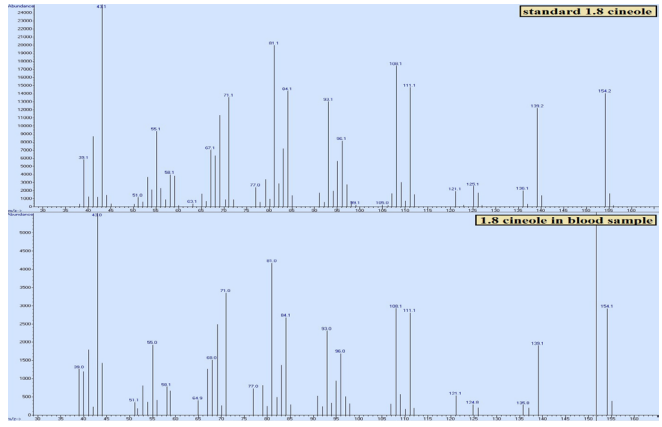

Figure 3. *Mass spectra of standard 1,8 cineole (upper pane) and 1,8 cineole in S. triloba essential oil inhaled rat blood sample (lower pane).*

In vitro evaluation of AChE and BChE inhibitory and antioxidant activities

AChE and BChE inhibitory activities of the STA essential oil are summarized in Table II. *S. triloba* essential oil possessed significant AChE and BChE inhibitory activities with respect to a strong anti-cholinesterase inhibitor, galanthamine. Results revealed that STA showed 841.47841.47 ± 15.57 mg GALAE/g AChE and 914.16 ± 9.58 mg GALAE/g BChE inhibition activity.

Table II. *In vitro AChE and BuChE inhibition of S. triloba essential oil (n=3)*

	AChE[#A] inhibition (mg GALAE /g)	BuChE[§A] inhibition (mg GALAE /g)
***S. triloba* essential oil (STA)**	841.47 ± 15.57	914.16 ± 9.58

[#] Acetylcholinesterase, [§] Butyrylcholinesterase, [A] Results were stated as the mean of triplicates and standard deviation (S.D.) and as mg galantamine equivalents (GALAE) in 1 g sample

In vitro antioxidant evaluation of STA was implemented with four different methods; DPPH radical scavenging activity (DPPH), Ferric reducing antioxidant power (FRAP), Cupric reducing antioxidant capacity (CUPRAC) and Total Antioxidant Capacity (TOAC). Result of the assays were given in Table III. It was determined that STA exhibite

d significant *in vitro* antioxidant activity.

Table III. *In vitro antioxidant activity of S. triloba essential oil (n=3)*

	DPPH[#A] (mg -BHTE/g)	FRAP[##A] (mg BHTE/g)	CUPRAC[§B] (mg AAE/g)	TOAC[§§B] (mg AAE/g)
***S. triloba* essential oil (STA)**	211.64 ± 9.88	71.31 ± 0.36	341.25 ± 12.39	578.94 ± 9.31

[A] *Results were stated as the mean of triplicates § standard deviation (S.D.) and as mg butylated hydroxytoluene equivalents (BHTE) in 1 g sample.* [B] *Results were stated as the mean of triplicates § standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g sample.* [#] *2,2-diphenyl-1-picrylhydrazyl.* [##] *Ferric reducing antioxidant power.* [§]*Cupric reducing antioxidant capacity.* [§§] *Total Antioxidant Capacity*

Morris water maze

Animals in the *S. triloba* inhalation groups did not display any peripheral cholinergic side effects such as hypersalivation, vomiting or frequent micturition and/or defecation. The gradual decrease in daily escape latencies was indicative of learning the hidden platform in the water maze, shown in Figure 4. Escape latencies were significantly different between the groups [F (3,20) = 21.553; P<0.001].

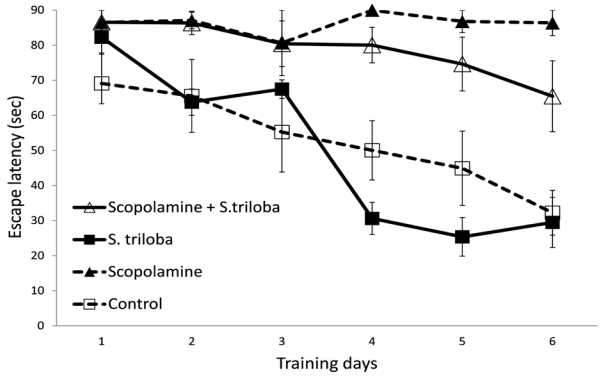

Figure 4. *Morris water maze acquisition (learning) performance, escape latencies during 6 training days.*

Post-hoc tests revealed that the escape latencies of Scopolamine group were significantly longer than *S. triloba* group and control group (P<0.001 for both comparisons). Scopolamine + *S. triloba* group also displayed significantly longer escape latencies compared with *S.triloba* group and control group (*S. triloba*) (P<0.001, P=0.001, respectively). In reference to the control group, learning was not observed in the two groups with scopolamine induced learning impairment. Pair-wise group comparisons also revealed no significant difference between Scopolamine group and Scopolamine + *S. triloba* group. Escape latencies were also comparable between control group and *S. triloba* group by the end of 6 day of learning phase.

In addition to the GROUP effects in the overall (6 days) escape latencies, GROUP x DAY interaction was also significant [F(15,100) = 3.948; P<0.001]. Contrast analyses comparing escape latencies from 1st day against the following days revealed significant group differences on 4th, 5th and 6th days (P<0.0001, P=0.001, P=0.006, respectively). In the *S. triloba* group and control group (i.e. group that displayed learning), GROUP x DAY interactions in the escape latencies were significant [F(5,50) = 2.861; P=0.031]. Contrast analyses comparing escape latencies against the 1st day revealed that *S. triloba* group displayed significantly shorter escape latency on 4st day compared with the control group (P=0.01, Bonferroni corrected, Figure 5).

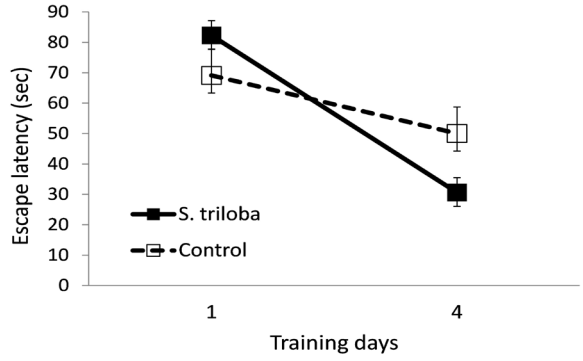

Figure 5. *Morris water maze test escape latencies of 1st and 4th days of learning comparing S. triloba vs. control group.*

On the probe day, the number of platform crossing was significantly different between 4 groups [$X^2(3)$ = 13.772, P= 0.003]. The control group and *S. triloba* group performed significantly more crosses than scopolamine group (P=0.02, P=0.005, respectively). Scopolamine + *S. triloba* group displayed few crosses on average and it was lower than that of *S. triloba* (P= 0.052). There was no significant difference between control group and *S. triloba* or Scopolamine + *S. triloba* groups. Results of post-hoc tests are listed in Table IV (also see Figure 6).

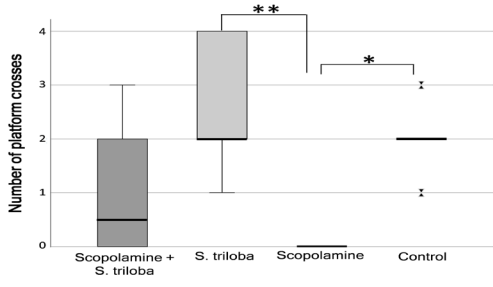

Figure 6. *Morris water maze test memory retention performance, boxplot chart with medians of number of platform crosses (*P< 0.05; **P< 0.01).*

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Platform latency was significantly different between 4 study groups [F (3,20) = 4.05; P<0.05]. Post-hoc tests revealed that, platform latency was significantly shorter in control and *S.triloba* groups compared with the Scopolamine group (P<0.05 for both comparisons). Results of post-hoc tests are listed in Table IV (also see Figure 7).

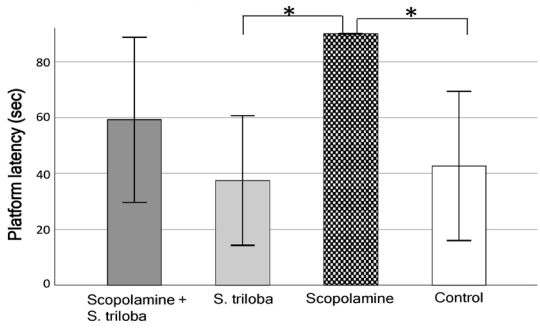

Figure 7. *Morris water maze test memory retention performance, bar graph of platform latency (*P < 0.05).*

Table IV. *Morris water maze pair-wise group comparison, p values for number of platform crosses and platform latency on probe trial*

Pair-wise group comparisons	Number of platform crosses	Platform latency
Control vs. *S. triloba*	0.9	0.989
Control vs. Scopolamine	0.02*	0.045*
Control vs. Scopolamine + *S. triloba*	0.837	0.755
Scopolamine vs. *S. triloba*	0.005**	0.024*
Scopolamine vs. Scopolamine + *S. triloba*	0.872	0.571
S. triloba vs. Scopolamine + *S. triloba*	0.375	0.278

**P < 0.05, **P< 0.01*

4. DISCUSSION

Phytochemical profiles of *S. triloba* essential oils from different geographical regions were evaluated via GC-MS analysis and the results were given in the Table 1 and Figure 1. Previous studies revealed that phytochemical profile of essential oil composition of *Salvia* species even *S. trioba* essential oils might be highly variable [26, 39]. In a recent study, phytochemical composition of *S. triloba* samples from two different localities showed variations [40]. 1,8-cineole content of the samples was measured up to 47.1% however; there are significant variation for the profile depending on the geographic differences and essential oil obtaining methods. In this study, phytochemical analysis was performed on the essential oils which were obtained from aerial parts of *S. triloba* from different locations of Turkey and essential oil purchased from market. Results revealed that phytochemical profiles of *S. triloba* essential oils are highly variable depending upon the geographical differences. 1,8-cineole is known as the major component of *S. triloba* essential oil and the responsible molecule for its various bioactivities especially for its memory-enhancing activity [28]. Nonetheless, previous studies demonstrated that *S. triloba* essential oil samples on the market might contain high amount of camphor, α-thujone and β-thujone ketones which are infamous for their toxic properties [41, 42]. Camphor, α-thujone and β-thujone contents were highly variable likewise 1,8-cineole content. For further *in vitro* and *in vivo* investigations on cognitive properties of *S. triloba* essential oil, appropriate sample selection is crucial. Thujone content is lowest in the essential oils from Yalova (STY) and Antalya (STA) however camphor content is overwhelmingly higher in STY when compared to STA (19.40% and 5.15%, respectively). In addition, 1,8-cineole content is highest in STA (40.48%) which is very close to commercial essential oil (STC) (45.31%). In the light of these data, although, STC contains the highest amount of 1,8-cineole content, STA was selected as the most suitable candidate for further bioactivity studies due to its high amount of 1,8-cineole content and lower amounts of camphor, α-thujone and β-thujone contents.

In the light of prior research pertaining to the significant anti-cholinesterase activities of various extracts and essential oil of *S. triloba*, this study is planned as an investigation on its enzyme inhibitory activity, phenolic profile and antioxidant potentials. Senol et al., revealed that essential oil of *S. triloba* samples have stronger anti-cholinesterase inhibition rather than dichloromethane, ethyl acetate, and ethanol extracts of the same plant [26]. Similar to this study the tested essential oil was dominated with 1,8-cineole (36.25%). The data obtained in this study were in accordance with the results of Savelev et al. [25]. In a recent study, AChE inhibitory activity of *S. triloba* essential oils from Turkey were evaluated [40]. Results demonstrated that both essential oil samples from diverse geographic locations contains high amount of 1,8-cineole, exhibited significant AChE inhibitory activity which are coherent with our findings. Our results and previous data are indubitably indicating that, *S. triloba* essential oil has significant cholinesterase inhibition potential which may lead to potential clinical effects.

It is well known that oxidation and ROS production is highly correlated with the progress of neurodegenerative diseases. Therefore, reducing oxidation and scavenging ROS might be beneficial for preventing such diseases and enhancing cognitive performance [32, 33, 43]. Thus, antioxidative properties of STA essential oil were determined via *in vitro* assays for revealing possible positive effects in our study. DPPH radical scavenging activity of *S. triloba* essential oil from Turkey was previously evaluated. Former results demonstrated that essential oil inhibited DPPH radical by 5.12±0.42% in 1 mg/mL concentration while gallic acid reference showed 92.57 ± 0.1% inhibition at the same concentration [26]. In addition, same study revealed that ferric reducing power of the sample showed 0.302 ± 0.01 absorbance at 1 mg/mL concentration while chlorogenic acid showed 3.618 ± 0.01 at the same concentration. In the present study, DPPH

radical scavenging activity of STA essential oil was measured as 211.64 ± 9.88 mg BHTE/g and FRAP activity was measured 71.31 ± 0.36 mg BHTE/g. Relatively higher antioxidant activity of present sample may be originated by its higher amount of 1,8-cineole and significantly lower amount of camphor when compared to aforementioned study. In a recent study, CUPRAC assay was also employed for evaluation of metal reducing capacity of *S. triloba* essential oils collected from different locations of Turkey [40]. Results showed that both essential oils had significant metal reducing activity, which are consistent with our findings. In our study, *S. triloba* essential oil showed 341.25 ± 12.39 mg AAE/g cupric reducing activity. In addition, TOAC assay was conducted for determining molybdenum reducing potential of *S. triloba* which is the first study of this subject in the literature to our knowledge. Results revealed that essential oil showed 578.94 ± 9.31 mg AAE/g total antioxidant capacity which is coherent with former results measuring metal reducing activity. Especially low molecular weight compounds in the essential oils of *Salvi*a species readily cross blood and blood-brain barrier due to their small molecular size and polarity. In order to verify the absorption of volatile components in *S. triloba* essential oil via inhalation, existence in the blood of rats were examined by comparison of GC-MS spectra of standard 1,8-cineole and blood. The presence of 1,8-cineole in whole *in vivo* test groups were verified for the reliability of the current study.

Control and *S. triloba* groups displayed learning effect in Morris water maze, i.e. gradual decrease in mean escape latency from 70-80 seconds to 30 secs by the end of the learning phase (6^{th} day). Besides, *S. triloba* group reached peak performance 2 days earlier than the control group, this effect was confirmed with contrast analyses. Scopolamine group and Scopolamine + *S. triloba* group displayed little or no learning. Escape latencies of Scopolamine + *S. triloba* group had a tendency to decrease in of 4^{th},5^{th} and 6^{th} days of learning phase. Nevertheless, this slight difference was non-significant, and restorative effect of *S. triloba* inhalation on scopolamine induced learning impairment was invalidated.

On the probe trial, Scopolamine group failed to cross platform zone, as it was expected due to lack of learning. Although, the statistical tests rejected a solid reversing effect of *S. triloba* inhalation, *S. triloba* treated amnesia group performed somewhere in between a drastic memory failure and normal memory performance, (i.e. in the probe trials they performed statistically indifferent from both control group and Scopolamine group due to high variance).

Our results are coherent with several previously reported findings obtained by *S. miltiorrhiza* [44], *S. officinalis* and *S. lavandulaefolia* extracts [45]. These results are also consistent with cognitive enhancer effects of ingesting *S. lavandulaefolia* essential oil capsules on healthy young volunteers [46]. In the present study and those previous studies, the behavioral results of *Salvia* species are mostly attributed to AChE and BChE inhibiting effects of 1,8-cineole. We assume these positive effects of *S. triloba* essential oil on rats' Morris water maze performance are due to cholinergic boosting that was applied preceding the maze sessions. The improved spatial learning due to cholinergic enhancement is mostly explained by facilitation cholinergic projections from septum to hippocampus, retrosplenial and posterior parietal cortices that are essential for spatial navigation and learning [47]. We used GC to confirm high amount of 1,8-cineole in the essential oil and its bioavailability by inhalation. *S. triloba* essential oil also has antioxidant activity that was also a favorable feature by cholinesterase inhibitors [24, 48].

In conclusion, samples of *S. triloba* which is a well-known Anatolian sage, were obtained from different geographical regions of Turkey. The hydro-distilled essential oil compositions of whole samples were identified by using GC-MS. The desired essential oil was selected according to its 1,8-cineole, camphor α-thujone and β-thujone amounts. Afterwards, the essential oil of *S. triloba* was examined for its inhibitory activity on cholinesterases and antioxidant potential. *In vitro* tests exhibited significant AChE and BChE inhibitory activities as well as antioxidant activities. Additionally, cognitive boosting effect was evaluated via Water maze test. Our results refuted a hypothetical reversing effect of *S. triloba* inhalation on the devastating scopolamine induced learning impairment. This negative behavioral result could have several grounds including insufficient *S.triloba* dose or a mild reversing effect could have failed to occur due to insufficient acquisition period. Depending on maze pool size, number of visual cues, or several animal features like age and strain, reaching stable minimum escape latency may take up to 10 days [49]. Nevertheless, *S. triloba* inhalation led to a significant improvement in learning performance compared with control group and an indistinct effect on memory retention in scopolamine induced impairment group. These preliminary results suggest that it is worth studying with variant doses. Also, future *S. triloba* inhalation studies on transgenic AD models can provide more sensitive evaluation of its supplemental effects on cognition and learning.

Compliance with the Ethical Standards

Ethics Approval: All protocols involving animals were approved by Animal Experiments Local Ethics Committee of Acibadem Mehmet Ali Aydinlar University (decision number 2020/13).

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Conflict of interest statement: All authors declare no conflict of interest.

Authors contributions: GSE: contributed to all processes of the study; ME: study design, statistics and interpretation of the behavioral study; THB: Gas Chromatography, *In vitro* studies, analysis and evaluation; HB: supervision and active involvement in plan gathering and essential oil extraction; GS: design, interpretation and supervision for behavioral study.

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Huge thrombosed popliteal artery aneurysm

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ABSTRACT
Popliteal artery aneursyms (PAA) are the most common true peripheral aneurysm. We report a case of 66-year-old patient who has pulsatile mass posterior of the right knee diagnosed with massive PAA.
Keywords: Popliteal artery, Aneurysm, Pulsatile masstime

1. INTRODUCTION

The popliteal artery aneurysms (PAA) are defined as a 50% increase in diameter compared with the normal arterial diameter. PAAs are rare but complicative [1]. Although, mostly asymptomatic, thrombosis, acute and chronic limb ischemia, major amputation may occur [2,3]. Open and endovascular surgery can be performed for treatment. There are various studies that compare the treatment methods but there is no clear consensus regarding management [4-6].

2. CASE REPORT

A 66-year-old male patient was referred to our clinic with the diagnosis of PAA. He had hypertension and coronary vasculary disease (Percutanous coronary intervention-Circumflex artery) for seven years in his medical history. On admission to our center he had a pulsatile mass posterior of the right knee for two months. Computed tomography (CT) angiography revealed a huge aneurysm (67x55 mm) originating from the popliteal artery (Figure 1). Since, PAA is often associated with other large vessel aneurysms, preoperative evaluation is important. We used Doppler ultrasonography to examine abdominal aorta and bilateral carotid arteries. No patology was detected. He underwent open surgery because of intraluminal thrombus suspicion rather than endovascular treatment. During surgery, we detected a massive aneurysm sac filled with thrombus (Figure 2). We explored the aneurysm sac completely, both proximal and distal size was normal. Following systemic heparinization we applied cross-clamp to the proximal and the distal popliteal artery. We replaced the artery with saphenous vein interposition. Distal pulses were palpable at the end of surgery. The pathology result of the aneurysm material were arterial aneurysm formation, intraluminal thrombus and intimal lenfosit aggregates. After a-two-year follow-up the patient was symptom free.

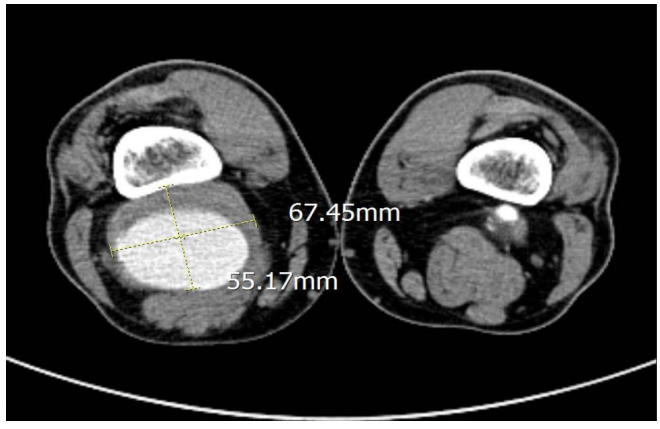

Figure 1. *CT image of the mass*

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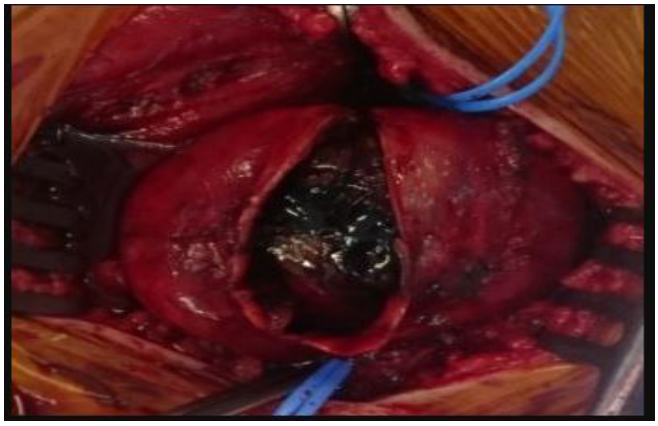

Figure 2. *A massive aneurysm sac filled with thrombus*

3. DISCUSSION

Popliteal artery aneurysm is a rare disease, with the incidence ranging from 0.% to 2.8% but also the most common type of peripheral artery aneurysms. Most cases present bilaterally [7-9]. Most of the patients are asymptomatic. Symptoms can be variable from asymptomatic pulsatile mass to acute limb ischemia due to acute thrombosis or distal embolization. Our patient presented with a pulsatile mass in the right knee. As the aneurysm expends, symptoms and complications increase as well. PAAs with >2 cm are higher rates of tromboembolic events. In our patient there was no thromboembolic symptom despite his PAA being 6 cm. Even though, it is asymptomatic, complications may occur in patients with untreated asymptomatic PAA. The results of asymptomatic patients who underwent surgery were good [5,10]. Therefore, elective surgery is recommended by most of the authors. PAA can be treated with open surgery, endovascular methods or hybrid methods with open surgery. In our patient we preferred open surgery because of intraluminal thrombus suspicion rather than endovascular treatment. Zamboni et al., reported improved results with hybrid method in patients who were presented with thromboembolic symptoms [11]. However, the analysis of POPART registery shows endovascular repair group long-term patency rates are lower when compared with open surgery rates [9]. In the era of percutanous interventions, open surgery is still higly recommended [5,6,9].

Compliance with Ethical Standards

This work was conducted ethically by following per under Helsinki World Medical Association Declaration.

Patient Consent: The patient gave his consent for images and other clinical information relating to his case to be reported in a medical publication.

Conflict of Interest Statement: The authors have no conflicts of interest to declare. **Funding Sources**: This research did not receive any specific grant from funding agencies in the public, commercial, or notfor-profit sectors.

Authors Contributions: ED and KA: Both authors contributed equally.

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A case of granulomatosis of polyangiitis presenting with COVID-19 infection: False-positivity or co-existence?

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ABSTRACT

Coronavirus disease 2019 (COVID-19) was declared a global pandemic and a public health emergency worldwide in March 2020. COVID-19 presents with non-specific symptoms of the upper airway and pulmonary system, which can overlap with other diseases involving the respiratory system as granulomatosis with polyangiitis (GPA). Both diseases have high morbidity and mortality rates and it is important to promptly differentiate and treat them. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) is currently the recommended method for diagnosing COVID-19. Antibody-based tests are used to diagnose both pat and current COVID-19 infections.

We present a previously healthy thirteen-year-old girl who was admitted with upper airway symptoms and pulmonary involvement, and progressed to acute kidney failure. Laboratory findings showed leukocytosis, anemia, elevated kidney function tests and 2+ proteinuria. Computed tomography (CT) of the lungs showed multiple nodules, cavities, and ground-glass opacities (GGOs). We performed RT-PCR tests for COVID-19 for three times. Results were all negative, but the COVID-19 immunoglobulin (Ig)M test sent simultaneously was positive. Based on the cytoplasmic antineutrophilic cytoplasmic antibody (c-ANCA) positivity, upper airway, pulmonary, and renal involvement, she was diagnosed as GPA.

This report highlights that COVID-19 antibody tests can be false-positive in patients with autoimmune diseases including GPA.

Keywords: COVID-19, Granulomatosis with polyangiitis, False-positive antibody test

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a pandemic and a public health emergency by the World Health Organization (WHO) on March 11, 2020 [1]. COVID-19 presents with nonspecific symptoms, including sore throat, rhinorrhea, low-to-high fever, non-productive cough, myalgia, dyspnea, and fatigue. Differential diagnosis with other life-threatening diseases involving respiratory system symptoms can be challenging. Granulomatosis with polyangiitis (GPA) (formerly known as Wegener granulomatosis) is a necrotizing, pauci-immune small-vessel vasculitis that primarily affects the upper and lower respiratory tract, lungs, and kidneys. Although, it is rare in childhood, most pediatric patients with GPA are adolescent girls and they experience a progressive clinical course. Collecting an upper respiratory nasopharyngeal (or oropharyngeal) swab and conducting an evaluation through real-time reverse transcriptase polymerase chain reaction (RT-PCR) is currently recommended for initial COVID-19 testing [2, 3]. Additionally, antibody-based tests may aid in diagnosing both previous and current SARS-CoV-2 infections. In this paper, we aim to present a case of false-positive antibody test results for COVID-19 in a patient with GPA.

2. CASE REPORT

A previously healthy thirteen-year-old girl was admitted to the emergency service with complaints of recurrent otitis media, headache, and rhinorrhea for one month. It was discovered that she had been treated with antibiotics several times during this period. There was no family history of chronic disease. Upon admission, she appeared pale, and her respiratory sounds were decreased. Her body temperature was 37.1°C, blood

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pressure was 115/65 mmHg, and peripheral oxygen saturation was 93%. Cardiac and abdominal examinations were normal. Laboratory findings showed leukocytosis (white blood cell count (WBC): 21.600/mm^3), anemia (hemoglobin (Hb): 4.7 g/dL), hypoalbuminemia (albumin: 2.7 g/dL), elevated kidney function tests (urea: 137 mg/dL, creatinine (Cr): 3.3 mg/dL), hyperuricemia (uric acid: 7.04 mg/dL), and 2+ proteinuria in urinalysis. Microscopic examination of the urine revealed 30-35 erythrocytes, most of which were dysmorphic. There was no evidence of hemolysis in her peripheral smear, and the haptoglobin level was within the normal range. Renal ultrasonography (US) revealed normally sized kidneys with increased parenchymal echogenicity. Computed tomography (CT) of the lungs showed multiple nodules, cavities, and ground-glass opacity (GGO) (Figure 1). The purified protein derivative (PPD) test was negative, and there was no growth of acid-fast bacilli in sputum smears. We collected nasopharyngeal swabs from the upper respiratory tract on two alternate days, and RT-PCR tests for SARS-CoV-2 were negative. Her kidney function tests deteriorated, and she experienced massive hemoptysis on the third day of hospitalization. She developed respiratory failure, which prevented the possibility of a kidney biopsy. Due to the rapid progression of kidney failure, respiratory symptoms, and radiological findings, we conducted an immunologic profile, which revealed a strong cytoplasmic antineutrophil cytoplasmic antibody (c-ANCA) positivity (3+). She was diagnosed with GPA, and methylprednisolone pulse therapy was initiated. Due to the progression of respiratory symptoms and a subfebrile status, we conducted two Ig tests for SARS-CoV-2, both of which showed positive IgM levels (1+), while the IgG level was initially negative and later returned as weakly positive in the second test. However, the RT-PCR test was repeated at the same day with antibody tests for the third time and the result was negative once again. Consequently, favipiravir treatment was initiated. She also received treatment with intravenous cyclophosphamide, intravenous immunoglobulin (IVIG), and plasmapheresis. As her clinical condition was critical, extracorporeal membrane oxygenation and continuous renal replacement therapy were initiated. Unfortunately, despite significant improvement in her respiratory symptoms, she succumbed to fungal septicemia.

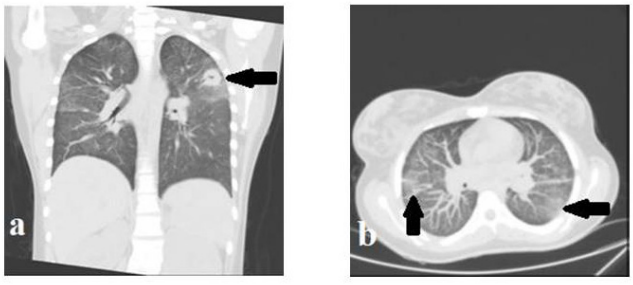

Figure 1. *Thorax tomography images of the patient.*
a. 24x19 mm cavitation in the upper lobe of the left lung
b. Ground-glass opacities in the middle and lower lobe of the right lung and in the upper zone of the left lung

3. DISCUSSION

COVID-19 was declared a pandemic by the WHO on March 11 2020. As of February 3, a total of 103362039 confirmed cases of COVID-19, including 2244713 deaths, have been reported to the WHO. COVID-19 presents with a range of clinical symptoms, including dyspnea, cough, and constitutional symptoms. Lung involvement is the primary cause of intensive care unit admissions and deaths [3, 4].

Granulomatosis with polyangiitis is an uncommon ANCA-associated vasculitis (AAV) that presents with at least three of the following six criteria: granulomatous inflammation, upper airway involvement, pulmonary involvement, kidney involvement, laryngo-tracheo-bronchial obstruction, and positive c-ANCA [5]. Our case met four of the six criteria (upper airway involvement, pulmonary involvement, renal system involvement, and positive c-ANCA) and was diagnosed with GPA. Pulmonary system involvement is the major prognostic factor, and respiratory complications are the most common cause of death in childhood GPA. Therefore, early diagnosis and treatment are crucial. Common radiologic manifestations in GPA include poorly or well-defined nodules of varying sizes, GGOs, consolidations, masses, and cavities, as observed in our patient [6, 7].

The diagnosis of COVID-19 is established based on clinical features, exposure history, RT-PCR testing, and CT findings. The predominant radiologic findings are multifocal bilateral GGOs with a peripheral and posterior distribution, and later, superimposition of consolidations. As reported by Eslambolchi et al., rheumatologic diseases with pulmonary involvement can sometimes obscure, mask, or mimic the features of COVID-19 [7]. The diagnosis of COVID-19 is established based on clinical features, exposure history, RT-PCR testing, and CT findings. The predominant radiologic findings are multifocal bilateral GGOs with a peripheral and posterior distribution, and later, superimposition of consolidations. The diagnosis of COVID-19 also includes antibody-based tests. The sensitivity of antibody tests depends on the time interval between the onset of symptoms and the date the test is performed. Deeks et al., reported that studies have shown the ability of antibody tests to detect SARS-CoV-2 infection is very low in the first week (average sensitivity 30.1%, 95% Confidence Interval (CI) 21.4 to 40.7) and only moderate in the second week post-symptom onset (average sensitivity 72.2%, 95% CI 63.5 to 79.5) [8]. The average sensitivity for IgG/IgM tests across all included studies was estimated to be 91.4% (95% CI 87.0 to 94.4) between 15 and 21 days and 96.0% (95% CI 90.6 to 98.3) between 22 and 35 days [8]. The rate of false-positivity was determined to be 2% in individuals without COVID-19. According to the literature, conditions such as nasopharyngeal carcinoma, colon cancer, duodenal carcinoma, diabetes, diffuse bronchitis, viral infections, and rheumatologic diseases have been reported as potential causes of false-positive test results [9-12]. In our patient, it was challenging to distinguish whether the observed result was due to cross-reactivity or a true infection, as the IgG status changed from negative to weakly positive. In 2004, Wang et al., reported the presence of SARS-CoV antibodies in

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individuals without SARS, which included 114 healthy controls and 104 patients with autoimmune diseases [13]. Among the 114 healthy controls, 4 (3.6%) tested positive for SARS-CoV-IgG antibodies, while all 114 (100%) tested negative for SARS-CoV-IgM antibodies. In contrast, out of the 104 patients with autoimmune diseases, 26 (25%) tested positive for IgG, 5 (4.8%) tested positive for IgM, and 13 (12.5%) tested positive for both IgM and IgG. However, among all samples with positive SARS-CoV-IgG and – IgM antibodies in both autoimmune disease patients and healthy controls, SARS-CoV RNA, and, as in our case, antibodies were all negative by RT-PCR. They suggested that the high levels of autoantibodies to cell antigens in their serum led to a response to antigens in the Vero E6 cell lysates, resulting in false-positivity for SARS-CoV antibodies [13]. Recently, Tzouvelekis et al., also reported a case of GPA with a false-positive COVID-19 antibody test [14].

Based on upper airway, pulmonary, and renal system involvement, radiological findings, positive c-ANCA, and negative RT-PCR results for COVID-19, we diagnosed our patient with GPA and believe that IgM and IgG showed false-positivity for COVID-19.

Patients with chronic diseases are at a higher risk of severe illness and admission to the intensive care unit in the presence of a COVID-19 infection [15, 16]. The co-occurrence of chronic diseases and COVID-19 has been reported previously. Hussein et al. and Uppal et al., reported cases of c-ANCA-associated vasculitis in COVID-19 patients [17, 18]. The first case presented with pulmonary hemorrhage, and the second case presented with pneumonia and acute kidney injury. COVID-19 infection can develop in patients with chronic diseases, and sometimes chronic diseases can be misdiagnosed as COVID-19 infection. It is crucial to promptly differentiate between COVID-19 infection and chronic diseases, especially in cases presenting with respiratory symptoms, as delayed treatment can be life-threatening, particularly in autoimmune diseases like GPA. Our patient was admitted to our hospital with persistent upper airway and pulmonary symptoms that had been ongoing for approximately one month. There are several reasons for the delay in diagnosing these patients. The similarity of the initial symptoms of AAVs and COVID-19, as well as patients' concerns about hospital admission due to the COVID-19 pandemic, can be listed as the most significant reasons. Additionally, false-positive antibody tests contribute to the delay, as they can lead to the misdiagnosis of these patients as having COVID-19. Clinicians must be aware of false-positive antibody tests in rheumatological diseases, especially in atypical cases of COVID-19.

Compliance with Ethical Standards

This work was conducted ethically by following Helsinki World Medical Association Declaration.

Patient Consent: The parents gave their consent for images and other clinical information related to this patient to be reported in a medical publication.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Funding Sources: This research did not receive any specific grant from funding agencies in the public, commercial, or notfor-profit sectors.

Authors Contributions: MK: Collected the data, conceived, designed and wrote the paper, IG:Contributed data, conceived and designed the paper, SG and SP: Contributed data, HA: Supervised the paper. All authors read and approved the final version of this manuscript.

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Review Article

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The therapy for urogenital tuberculosis

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ABSTRACT

Tuberculosis (TB) is a communicable disease that is a major cause of ill health. Urogenital TB was a frequent urological disease in the pre-antibiotic era: about 20% of patients in urological hospitals had renal TB, mostly in the form of pyonephrosis.

We composed a narrative review of the literature with keywords "urogenital tuberculosis', "prostate tuberculosis" "kidney tuberculosis", "treatment of tuberculosis".

Urogenital TB (UGTB) includes TB of the kidney and the urinary tract and male and female genital TB. Each clinical presentation requires tailored antibiotic therapy depending on stage and general management. Anti-TB therapy should be multicomponent, continuous, long-lasting and controlled with a follow-up for 2-3 years. Otherwise, the risks of development of drug-resistance and relapse increase.

Index of suspicion on UGTB is generally low, causing a delay in diagnosis; consequently, complicated forms of UGTB respond poorly to anti-TB therapy, while timely diagnosed "minor" forms are curable medically without surgery. Even with timely diagnosed UGTB, non-optimal therapy may result in over-fibrosis, scarring and strictures of the urinary tract, making surgical repair inevitable. Nevertheless, we have a wide enough spectrum of anti-TB drugs to cure urogenital TB.

Keywords: Urogenital tuberculosis, Prostate tuberculosis, Kidney tuberculosis, Prostate tuberculosis, Treatment

1. INTRODUCTION

Tuberculosis (TB) is a contagious disease with high level of mortality. Even nowadays, with effective antibiotics available, TB is one of the top 10 causes of death throughout the world and the leading cause of death from a single infectious agent [1]. This statement was valid before the coronavirus disease 19 (COVID-19) pandemic which has started in March 2020. The World Health Organization (WHO) emphasized that COVID-19 pandemic reversed recent progress in reducing the global burden of TB disease [2]. The limited possibility of access to medical care resulted in delayed diagnosis and a large global drop in the number of people newly diagnosed with TB in 2020, compared with 2019 [3].

At a glance, decreasing incidence of TB is a positive tendency. However, in fact, the disease was diagnosed too late, when complications had already developed and resulted in an increased mortality rate. The consequence of the large drop in the number of newly diagnosed cases of TB in 2020 is an increase in the number of deaths from TB in this year. Globally, in 2020, there were an estimated 1.3 million deaths among human immunodeficiency virus (HIV)-negative people, and an additional 0.214 million deaths among HIV-positive people [3]. So, if mortality rate of TB was about 15% in 2019, it can be estimated that this rate was doubled in 2020.

Some believe that TB is a disease of poverty, while TB can affect anyone anywhere. Mostly (about 90%) TB affects adults. High incidence rate among children is a mirror of adverse epidemiological situation. Eighty seven percent of newly diagnosed TB patients lived in one of 30 high TB burden countries [1].

Tuberculosis is a disease caused by Mycobacterium complex: Mycobacterium tuberculosis (Mtb) or M. bovis. Lungs are exposed to TB (pulmonary TB, respiratory TB) in the majority

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of patients, but the disease can also affect any other organ (so-called extrapulmonary TB). Extrapulmonary tuberculosis (EPTB) includes any site other than lung parenchyma and can occur both in the presence and absence of active pulmonary TB or history of TB disease.

The spectrum of EPTB fluctuates from region to region, depending on many factors. Urogenital TB is the second-third most common site among all EPTB forms in some regions while it is rare and does not exceed 2-3% and in other regions. Which figure is real? In fact, many patients with urogenital TB remain undiagnosed, so they do not receive anti-TB therapy, that leads to the progress and dissemination of the disease. According to WHO reports only 38% of the patients with TB, caused by multi-resistant pathogen received appropriate therapy in 2019 [4].

Urogenital TB (UGTB) includes kidney and urinary tract TB and male and female genital TB. Co-morbidity with pulmonary TB may be found in 40 – 65% of cases – while isolated UGTB, solo lesion of urinary or genital system, is possible, too. Isolated UGTB is especially difficult to diagnose as it has no specific clinical features and hides under masks of other diseases, mostly urogenital tract infections, cancer and stone disease. In a series, 22% men had acute debut of male genital tuberculosis (MGTB) with no report of contact with TB infection or TB history [5]. Delayed diagnosis may result in surgery, while timely diagnosed disease (on early stages) may be cured by anti-TB drugs. Every second MGTB patient has also pulmonary or renal TB. As a rule, TB epididymitis (especially bilateral) and prostate TB are diagnosed together [5,6]. If there is no other evidence of Mtb, UGTB may be diagnosed based on positive tuberculin skin test, Interferon-Gamma Release Assay (IGRA)-test, QuantiFERON-test, histological picture of biopsy, by X-ray examination, when destructive cavities are revealed. Sterile pyuria is not evident now, as co-morbid UGTB and non-specific urogenital tract infections are diagnosed in 75% [5,7].

Georges Marion, (1869 – 1960), famous French surgeon and urologist had written: "Renal tuberculosis is extremely common. In the urology department every fifth bed patient suffers from renal tuberculosis and more than a third of all renal suppurations are of tuberculous origin. In 80% of cases of renal tuberculosis, the bladder is also affected. The disease progresses slowly, death is almost inevitable" [8].

2. METHODS

This article is structured as a narrative review of the literature with keywords "urogenital tuberculosis', "prostate tuberculosis" "kidney tuberculosis", "treatment of tuberculosis", "anti-TB drugs", "anti-TB therapy". Data were obtained from articles published in Russian and in English from journals indexed in PubMed, Google scholar, e-library. Articles mainly dedicated to anti-TB therapy for UGTB were selected and analyzed.

3. CLASSIFICATION

Urogenital TB is a combined term and covers renal TB, urinary tract TB, male and female genital tract TB [5,9]. Kidney TB is sub-classified into four stages depending on the level of destructive lesion [5,9]: TB of kidney parenchyma, TB papillitis with destruction of one or more calices, cavernous kidney TB with big destructive cavity of parenchyma and final incurable by the therapy stage is polycavernous kidney TB, terminal form with widespread destruction of parenchyma.

Chronic renal failure, flank fistula, arterial hypertension are complications of kidney TB as well as urinary tract TB.

Urinary tract TB is almost always or generally secondary to kidney TB; first appears as an edema and the next stages are infiltration, ulceration and fibrosis with development of a stricture of ureter (mostly in the lower third) or shrinked bladder, when surgery is indicated [8].

Tuberculosis of the urethra is a rare complication. It is usually diagnosed in the stage of a stricture.

Male genital tuberculosis is sub-divided into five categories [5, 9, 10]:

- Category 1: TB of the epididymitis (uni – or bilateral). TB epididymitis is a primary isolated form of UGTB in 21.5% of patients.
- Category 2: TB of the testis. This form of UGTB is always secondary to TB epididymitis. Every third patient has bilateral lesions, every tenth patient has a scrotal fistula. Sixty-two percent of patients with epididymo-orchitis has renal TB as well [10].
- Category 3: TB of the prostate. In 77% of men with any form of TB, prostate TB was found on autopsy, and mostly the disease was not diagnosed while these individuals were alive.
- Category 4: TB of the seminal vesicles. This form of UGTB is always secondary to prostate TB and leads to infertility. Usual outcome of TB of seminal vesicles is calcification [11].
- Category 5: TB of the penis. This form of UGTB is rare. TB of the penis is considered as a sexually transmitted disease [12-14]. TB of the penis may occur as a complication of Bacillus Calmette–Guérin (BCG)-therapy for bladder cancer [15,16].

Complications of MGTB are strictures, fistula, infertility and sexual dysfunction.

There is no unique therapeutic approach to all patients with any form of urogenital TB. Each localization of TB inflammation may require tailored antibacterial therapy as well as additional treatment; so detailed classification is useful for choosing the sort of the therapy.

As only male UGTB is within the scope of urological treatment, evaluation of female genital TB is not covered in this article.

4. THERAPY

Standard antibiotic regimens

Anti-TB therapy should be multi-component, continuous, long-lasting and controlled; after the end of the treatment the patient needs a follow-up for 2-3 years [17]. Otherwise, the risks of development of drug-resistance of the pathogen and relapse increase. WHO considers current anti-TB drugs highly effective and emphasizes that about 85% TB patients can be successfully treated with a 6-month drug regimen –provided that treatment is started timely, before severe complications occur [1]. If Mtb is suspected, standard oral four-component regimen of isoniazid, rifampicin, pyrazinamide and ethambutol may be effective in >90% for pulmonary TB patients [17].

Drug-resistant TB is nowadays a serious threat to global health security. Mtb can be resistant to one or more anti-TB drugs, but if it is resistant to even 3-5 drugs, while being sensitive to isoniazid and rifampicin, the pathogen is considered as mono – or multi-resistant. Strains with multidrug-resistance (MDR), that are defined as resistance at least to isoniazid and rifampicin, and extensively drug-resistance (XDR) that means Mtb are resistant to fluoroquinolones and second-line injectable drugs are very dangerous. WHO estimates that about half a million cases MDR – TB) are estimated to occur each year [2]. WHO recommended regimens of anti-TB therapy can only cure half of MDR-TB patients and 30% of patients with XDR-TB [18].

In general, the standard treatment regimen of UGTB is the same as for pulmonary TB, but there are some points, which should be taken into account. Streptomycin and kanamycin are not recommended for UGTB because of their nephrotoxic side-effect. Fluoroquinolones are widely used as anti-TB drug; mostly moxifloxacin, sparfloxacin, and levofloxacin. All these three fluoroquinolones demonstrate high efficiency in the treatment of patients with pulmonary TB. Nevertheless, levofloxacin should be preferred for the therapy of UGTB. Para-Aminosalicylic acid is indicated for the therapy of UGTB with involvement of pelvic organs and prostate TB. Amoxicillin/clavulanate is a popular antibiotic for urinary tract infections. Recent studies have shown that they are active on M. tuberculosis, too, especially if they are used with meropenem or imipenem together. Cycloserin affects both E. coli and M. tuberculosis, so this drug is indicated for comorbid UGTB and urinary tract infections. Patients with HIV co-infection who are treated by anti-retrovirus therapy should not receive rifampicin. Instead of this antibiotic rifabutin should be used for such patients. Rifampicin, as well as streptomycin is also contraindicated for patients after organ transplantation [19].

The most recent classification of the main anti-TB drugs is displayed in Table I.

Anti-TB drugs may be administered per os and parenterally, the dose is adjusted according to the weight of the patient (Table II).

Without treatment, two-thirds of TB patients will die, and the remaining one-third is likely to suffer from chronic sequelae, which significantly reduce the quality of life. We need new oral TB drugs that are more active and less toxic as well as new regimens with good efficiency and good tolerance both for drug-sensitive and drug-resistant TB [17, 20-26]. In particular, we need drugs that can reduce the duration of the treatment. Shorter regimens will improve patient adherence, reduce cumulative drug toxicities, and reduce clinics' workloads [18].

Table I. *The group classification of anti-TB drugs [20]*

Group	Characteristic	Drugs (abbreviations)
1	First-line anti-tuberculosis drugs used perorally	Isoniazid (INH), Rifamycin (RIF), Ethambutol (EMB), Pyrazinamide (PZA)
2	Injectable anti-tuberculosis drugs	Streptomycin (S), Kanamycin (Km), Amikacin (Am), Capreomycin(Cm), Viomycin (Vi)
3	Fluoroquinolones	Ciprofloxacin (Cfx), Ofloxacin (Ofx), Levofloxacin (Lfx), Moxifloxacin (Mfx), Gatifloxacin (Gfx)
4	Peroral bacteriostatic second-line anti-tuberculosis drugs	Ethionamide (Eto), Protionamide (Pro), Cycloserine (Cs), Terizidone (Trd), *Para*-aminosalicylic acid (PAS), Thioacetazone (Th)
5	Drugs with unknown mechanisms of action (not recommended by WHO for ordinary use to treat MDR TB)	Clofazimine (Cfz), Amoxycillin/clavulanate (Amx/Clv), Clarithromycin (Ctr), Linezolid (Lzd)

Table II. *Recommended daily dosage of anti-TB drugs for adults (mg) [21]*

Drug	Patient's weight		
	33-50 kg	51-70 kg	More than 70 kg (max.)
Isoniazid	300	300-600	600
Rifampicin	450	450-600	600
Pyrazinamide	1000-1500	1500-2000	2500
Amikacin	500-750	1000	1000
Ethambutol	800-1200	1200-1600	1600-2000
Levofloxacin	500	500-750	750-1000
Protionamid/ etionamid	500	750	750-1000
Capreomycin	500-750	750-1000	1000
Para-aminosalicylic acid	3000-5000	5000-8000	8000-12000
Cycloserin	500	500-750	750-100
Delamanid	100 mg twice daily taken with food, for 6 months		
Bedaquiline	400 mg once daily for the first 2 weeks and is then reduced to 200 mg three times weekly for the remaining 22 weeks		
Linezolid	600 mg once daily orally or intravenously		

New drug treatment options

Below we provide a brief characteristic of new and newly introduced old drugs for the treatment of UGTB.

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1. Nitroimidazoles. This group consists of novel anti-TB agents and may be used for TB caused resistant pathogen – both MDR and XDR. Nitroimidazoles act through inhibition of cell wall synthesis and oxidation, and include pretomanid and delamanid. Pretomanid is active against both replicating Mtb and persistors [27], but it is currently not licensed. Delamanid is however, approved by The European Medicines Agency for the treatment of MDR-TB in combination with other anti-TB drugs – but only if standard regimen cannot be used because of total drug resistance or intolerability.

2. Diarylquinolines. Bedaquiline is effective against M. tuberculosis both during active replication and in persistence. Bedaquiline has high activity against resistant pathogen – both MDR and XDR [28]. It has been the first new drug to be approved by the Food and Drug Administration (FDA) for the anti-TB therapy for more than 40 years [17]. Bedaquiline should not be used for monotherapy nowise. The polycomponent anti-TB therapy should include alongside with this new drug pyrazinamide and four second-line anti-TB drugs. However, if MDR and resistance to fluoroquinolones are found together, such a regimen is not appropriate [29]. Co-administration of bedaquiline and delamanid is not recommended due to the potential drug–drug interactions and possible cardiotoxicity [30, 31].

3. Oxazolidinones. Key point of the mechanism of action of linezolid is inhibition of protein synthesis. Linezolid is a first-generation oxazolidinone and has excellent oral bioavailability. Initially, oxazolidinones were approved for the therapy infections caused by of drug-resistant Gram-positive microbes. Severe neurological and haematological toxicity have limited widespread using of this class antibacterials [32,33].

For additional treatment tocopferol, canephron, and trospium chloride and selzink may be used [19]. Evaluation of the outcome of drug treatment of UGTB is difficult for several reasons. Identification of Mtb is not always possible before treatment and healing of kidney and prostate caverns is not possible at all. A special scale has therefore been suggested for the evaluation of the outcome of drug treatment [34].

BCG-induced UGTB

Bacillus Calmette–Guérin are live attenuated M. bovis. Normally, BCG is anti-TB vaccine, and from 1970 BCG is widely used for treatment of non-muscle invasive bladder cancer. BCG-therapy can be associated with complications as alive pathogen has long-time contact with defective urothelium. One of the most severe complication is BCG-induced UGTB, – mainly localized in the bladder or prostate. BCG-induced TB can develop in other systems, too, and even BCG sepsis has been reported in rare cases [35-38]. Diagnosis of BCG – induced bladder TB can be made according to clinical features such as significant dysuria and decreased bladder volume, but not by microbiology. Histological confirmation is crucial, but in about half of patients specific granulomas could not be found, only fibrotic and inflammatory changes occur.

For the treatment of BCG-induced UGTB a short course (2 months) of rifampicin, levofloxacin and isoniazid is recommended. Anti-TB therapy may be terminated in two months if the patient becomes symptom-free relief and urinalysis is normal. If dysuria and / or pyuria persists after 8 weeks, anti-TB treatment should be continued for another two months. The small contracted bladder is an indication for cystectomy or enterocystoplasty. The patient should receive anti-TB therapy for at least 2 months after surgery.

Patients with UGTB should be followed up every 6 months for 1-3 years for early diagnosis of relapse. Control intervals will depend on the type and stage of the disease. Specific prophylaxis against urogenital TB is currently not available.

Side effects of anti-TB drugs

Data on adverse events have not been evaluated globally owing to dissimilar reporting. The assessment of adverse events suggested a risk of excess hepatotoxicity with rifampicin + isoniazid combination. Drug-induced hepatotoxicity is not uncommon with anti-TB drugs. Linezolid is associated with anemia and thrombocytopenia, and care should be taken in patients with anemia. Concomitant use of drugs that prolong QTc should be avoided if possible – such drugs require extra vigilance and monitoring with electrocardiography if prescribed with bedaquiline and fluoroquinolones. CYP3A4 inhibitors and CYP3A4 inducers can interact with bedaquiline: CYP3A4 inhibitors include the azole antifungals (ketoconazole, voriconazole and itraconazole), and macrolide antibiotics other than azithromycin; the azole antifungals in general can safely be used for less than 2 weeks whereas fluconazole could potentially be used for more than 2 weeks.

Monthly examination should be performed for the control of the results of the therapy and estimation of possible indication for surgery.

Conclusions

Urogenital TB is difficult to diagnose as early disease is often asymptomatic. The index of suspicion on UGTB is low, so the correct diagnosis is delayed. Complicated forms of UGTB show with delayed diagnosis may be difficult to treat with standard anti-TB therapy, while in-time diagnosed “minor” forms may be cured by medicines without surgery. Even if we have a case of early diagnosed UGTB, non-optimal therapy may result in over-fibrosis, scarring and strictures of urinary tract, which, again, are an indication for surgery. Nevertheless, we have enough anti-TB drugs to cure UGTB.

Key points

1. Forms of UGTB have different clinical characteristics. Each form requires tailored antibiotic therapy and management.
2. If untreated, two-thirds of newly revealed TB patients will die, and the remaining one-third is likely to suffer from serious sequelae.

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3. Inappropriate therapy may lead to development of overfibrosis, that result in the development of ureteral stricture.
4. Anti-TB therapy should be multi-component, continuous, long-time and controlled.
5. Treatment of UGTB differs from the therapy of pulmonary TB.

Compliance with Ethical Standards

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