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CONTENTS

REVIEW

Is Human Vomeronasal Organ A Myth or A Neglected Structure?
Ayşegül Fırat, Özlem Önerci Çelebi, Hatice Mürvet Hayran 213

ORIGINAL ARTICLES

The Effect of Statin Use on In-Hospital Mortality in Covid-19 Patients
Abdurrahman Akyüz, Burhan Aslan, Ferhat Işık, Murat Çap, İlyas Kaya, Özgür Atlı, Ümit İnci, Ercan Taştan, Önder Bilge, Metin Okşul, Mehmet Zülküf Karahan 219

Morphometry in Classification of Hippocampal Sclerosis
Güneş Güner, Figen Söylemezoğlu 227

Bioinformatic Analysis of Expression Pattern and Prognostic Value of Oxidoreductase ERO1L in Pancreatic Cancer
Begüm Kocatürk 233

Are COVID-19 Vaccine Preference and COVID-19 Risk Differ In Individuals Living with HIV from The Normal Population?
Çağlayan Merve Ayaz, Aliye Baştuğ 245

In silico Activity and Target Prediction Analyses of Three Triazolothiadiazine Derivatives
Ceren Sucularlı, Birsen Tozkoparan, Sevim Peri Aytaç 251

Comparison of Visual Rating Scale Based on Brain 18F-FDG-PET and Montreal Cognitive Assessment Test in Probable Alzheimer's Disease
Elifcan Aladağ, Elif Bulut, Pınar Demirayak, Bilge Volkan Salancı, Kader Karlı Oğuz, Belkıs Erbaş, Eser Lay Ergün, Burcu Balam Doğu, Mustafa Cankurtaran, Meltem Gülhan Halil 261

Effects of the COVID-19 Pandemic on Patients with Schizophrenia Spectrum Disorders
Elçin Özçelik Eroğlu, M. İrem Yıldız, A. Elif Anıl Yağcıoğlu, Özge Türkoğlu, Oğuz Kaan Yalçınkaya, Aygün Ertuğrul, Sevilay Karahan, M. Kâzım Yazıcı 267

Event-free Survival in Patients with Chronic Myeloid Leukemia Receiving Front-line Imatinib Mesylate
Nesrin Damla Eyüpoğlu, Olgu Erkin Çınar, Salih Aksu, Yahya Büyükaşık, Nilgün Sayınalp, Haluk Demiroğlu, Hakan Göker, Osman İlhami Özcebe, İbrahim C. Haznedaroğlu 277

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The Impact of Covid-19 Pandemic on the Clinical Course of Pediatric Skin Disorders: A Cross-Sectional Study
Ecem Bostan 284

Does COVID-19 Affect the Course of Trophoblastic Gestational Disease in Partial Hydatidiform Moles; Is It A Viral or A Pandemic?
Müjde Can İbanoğlu, Seval Yılmaz Ergani, İrem Özge Uzunoğlu, Belgin Savran Üçok, Yıldız Akdaş Reis, Kadriye Yakut Yücel, Cantekin İskender, Yaprak Engin Üstün 292

CASE REPORTS

A Case of Crimean-Congo Haemorrhagic Fever (CCHF) Mimicking the COVID-19 Disease
Zahit Taş, Meliha Çağla Sönmezer, Gülçin Telli Dizman, Ahmet Çağkan İnkaya, Ömrüm Uzun, Murat Akova 299

Coexistence of Geographic Tongue and Palmoplantar Pustulosis: A Case Report
Yasuhiro Horiuchi 303

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REVIEW

Is Human Vomeronasal Organ A Myth or A Neglected Structure?

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ABSTRACT

The human vomeronasal organ (VNO) is a structure situated under the anteroinferior side of the nasal septum. It is mainly described in the rodents and found as a part of the accessory olfactory system. It has been shown to consist of specialized olfactory sensory cells, which function in perception of pheromones. With a large number of literature on the human VNO, there is little concensus of its persistence and functionality in human. During a routine dissection of nasal cavity, we recognized a one-sided vomeronasal pit in one of the six fresh-frozen cadavers (17 %) and described the position and structure of this rare structure morphologically.

The present study summarizes the literature about the VNO and describes its structural and functional findings.

Keywords: Human vomeronasal organ, nasal biopsy, vomeronasal pit, nasal septum

INTRODUCTION

The vomeronasal organ (VNO) is a structure situated under the anteroinferior side of the nasal septum. It is part of the accessory olfactory system and has been shown to consist of specialized olfactory sensory cells, which function in perception of pheromones and also they produce gonadotropin-releasing hormone [1]. VNO has been extensively studied in vertebrates, but there is still a lot to be understood on its function and persistence.

If a chemical message triggers specific effects in the receiver, this chemical substance is said to be a pheromone. The pheromones either act rapidly on receiver's behaviour or cause a sustained change on receiver's hormonal physiology. For example, a sexually mature male mouse's pheromone is able to trigger puberty in young females. Our knowledge about pheromones mainly covers rodents that live mainly in darkness and essentially communicates thru chemicals [2]. The chemical structure of the pheromone is mainly not known. In some rodent studies, volatile pheromones are small airborne molecules that contain steroids, peptides and proteins [3-5]. Aphrodisin is an example of proteins that is found in the vaginal secretions of female hamster and it triggers reproductive behavior in young males by activating the vomeronasal organ. Likewise, major urinary proteins emitted in mouse urine are detected by the other's vomeronasal organ and they act as an authentic signature of that individual [2].

The VNO contains two tubular structures on either side of the nasal septum. They open either onto the nasopalatine ducts which connect the oral cavity to the nose, or onto the nasal cavities. The pheromones reach the opening either via the nostrils or by the nasopalatine duct, depending

on the species. It has an internal duct that is closed at the back and communicates outside thru a small aperture depending on the species, Medial side of the lumen is lined by the sensory epithelium containing receptors and lateral side containes vascularized erectile tissue innervated by autonomic nervous system. Stimulation of this tissue induces contraction that results in pumping of the chemicals into the lumen. An accessory olfactory bulb that lies behind the main olfactory bulb transmits information towards the amygdala and the anterior hypothalamus in vertebrates. These regions are involved in gonadotropin secretion and sex hormone activity [2].

VNO is first indicated in human by Ruysch (1703, 1724), a Dutch anatomist, as an organ near the nasal septum of an infant. He did not mention an accurate description or provide a name, but he identified a nasal canal close to palate to represent the VNO [6-8].Then Kölliger (1877) became the first investigator of the VNO histologically in both fetus and adult human [9]. VNO is also known as Jacobson's organ, because Jacobson (1811) intensively studied the VNO across a variety of mammals, but he denied the existence of VNO in humans [10].Potiquet (1891) first discussed the VNO in human and he named the structure as Jacobson's organ [1,6,7,11].

The human VNO develops very early in utero [2,8-13]. The nerve fibers of the VNO extend together with a cluster of migrating gonadotropin releasing hormone (GnRH)-secreting cells from the olfactory placode toward the brain. It contains bipolar neurons and also generates GnRH secreting cells as in other species. Embryologically, these GnRH-secreting cells, developed from the olfactory placode and migrating to the arcuate nucleus of hypothalamus, are responsible from the development of reproductive system at puberty [13]. Subsequently compared to other mammals the VNO of the adult human shows some signs of regression [10-13]. Most of the studies agree on its non-functional status. The cavity of the openings can be visible on some endoscopical examinations but not in all patients. In gross descriptions, there is a depression on the side of nasal mucosa, along the anteroinferior 1/3 of the nasal septum, approximately 3-16 mm inside the nares.14 The opening into the nasal cavity is a depression called VNO pit. In a recent demonstration by Trotier et. al., endoscopically VNO pit can vary in appearance and may be invisible on inspection. In this study, they estimated around 92% of evidence of at least one VNO pit in subjects with no septal surgery [14]. But the position remarkably changes among researches. There are many reports of VNO located in the nasal septum in adult humans describing VNO as a blind ended diverticulum or a tube like sac with a diameter of approximately 0.2-0.6 cm and a depth of approximately 2 mm in the septal mucosa [14-16]. Smith et al. (1998) gave a distance of 7 to 10 mm from the depressions attributed to the VNO pit at the base of the nasal cavity [17]. Trotier et al. (2000) described vomeronasal "cavities" as 6.2 to 10.7 mm above the "crest of the palatine bone" [14]. Other studies have located the VNO openings 1-3 mm above the nasal cavity floor [18,19]. Such varied descriptions may be due to methodological differences, regional variations existing within the human VNO itself, individual variation of VNOs among humans, or due to the description of multiple, non-homologous structures that resemble the human VNO [20]. In addition, some authors have suggested that gross indicators are highly unreliable for locating the VNO [15,21]. In the literature, the frequency of the VNO in human ranges from 25 to 90% [22]. This wide variation may be related to the investigation method and the difficulty of detecting the opening of the VNO [18]. Zbar et al., described three types of openings of the VNO according to its size, while Besli et al. (2004) reported three types based on the shape of the opening: oval, fissural or elliptical [18,23]. Even with a large number of literature on the human VNO, there is little concensus of its persistence and functionality. While its precise function is unknown, it is believed to be associated with pheromone recognition and food flavour perception [15,16].

According to the histological studies, the VNO is covered with simple or pseudo-stratified columnar epithelium with microvilli lining the tubular sac, supported by a lamina that is rich in capillaries [24].Some authors have indicated the presence of cells similar to bipolar sensory neurons, but

did not identify any axons between the epithelial cells [16,25]. On the other hand, Monti-Bloch et al. demonstrated depolarization in the epithelium of the VNO, during local stimulation using substances secreted by the human skin [25]. Trotier et al. showed that most cells in the vomeronasal epithelium expressed keratin, a protein that is not expressed by the olfactory neurons [14]. Vomeronasal epithelial cells of human were not stained by an antibody against the olfactory marker protein, a protein expressed in vomeronasal receptor neurons of other mammals. Moreover, an antibody against protein S100, expressed in Schwann cells, failed to reveal the existence of vomeronasal nerve bundles that would indicate a neural connection with the brain. Positive staining was obtained with the same antibodies on specimens of human olfactory epithelium. The lack of neurons and vomeronasal nerve bundles, together with the results of other studies, suggests that the vomeronasal epithelium, unlike in other mammals, is not a sensory organ in adult human [14]. Notably, it has recently been shown that there are morphological connections of the VNO cells with the underlying capillaries. These, along with the expression of calcium-binding protein in part of these cells, suggest a potential endocrine activity [26]. If so, we would have the first evidence of an alternative function than the usually assumed pheromone sensing one for the VNO.

Some odorant chemicals like steroids have been studied as human pheromone prototypes in adults and some have activated the anterior hypothalamus. The effects observed are more psychological (mood shift, increased attention) than physiological and are context-dependent. Such effects are very far from the pheromonal effects observed in animals (stereotypic behavioral effects, neuroendocrine changes). They can at most be considered to be possible modulators of certain psychological variables [27].

MATERIALS AND METHODS

This study was approved by the Ethical Committee of the Faculty of Medicine. The cadaver dissections are conducted at the Department of Anatomy, Faculty of Medicine. For this study, the anteroinferior part of the septum were sampled bilaterally from six fresh frozen head and neck specimens using standard punch biopsy. For each specimen a careful exploration for possible vomeronasal pit area was conducted. Suspected sites were sampled by three biopsies taken appoximately 5 mm apart. The biopsy was about 5 mm x 2 mm in diameter and provided sufficient depth for examining the epithelium and lamina propria. The specimens were immediately fixed in 2.5% gluteraldehyde (R 1010, Agar Scientific Ltd.) and post-fixed in 1% osmium tetroxide (Catalogue number: 56H1140, Sigma-Aldrich, Germany). After post-fixation tissues were dehydrated in increasing ethanol (159010, Merck KGaA, Germany) concentrations. After washing with propylene oxide (Catalogue number: 8.07027.1000, Merck KGa, Germany) for 30 minutes, samples were embedded in epoxy resin (Araldite CY212 kit, AGR 1030, Scientific Ltd.). Semi-thin sections, approximately 2 µm in thickness were stained with 1% methylene blue solution (methylene blue 1 g, BDH Ltd. Standard stain, borax 1 g, distilled water 100 ml) and examined under the camera lucida of a Nikon Optiphot (Nikon Corporation, Japan) light microscope. Histologic evaluation of the epithelium, glands, vessels, connective tissue and neural elements from nasal mucosa was performed for each sample.

RESULTS

In one of the six cadavers (17 %) we recognized a one-sided vomeronasal pit on the right side. The epithelium of sample taken from the vomeronasal pit had the appearance of a pseudostratified epithelium lining a lumen (Figure 1). It is critical to describe the positional and structural variations in the human VNO where histological verification has been made. To that end, the present study describes the location of the adult human VNO as it relates to grossly identifiable surface marking the vomeronasal pit on the cadaveric nasal septum, 8.2 mm above the nasal floor. This study was not designed for a functional molecular evaluation nor was further immunohistochemistry staining

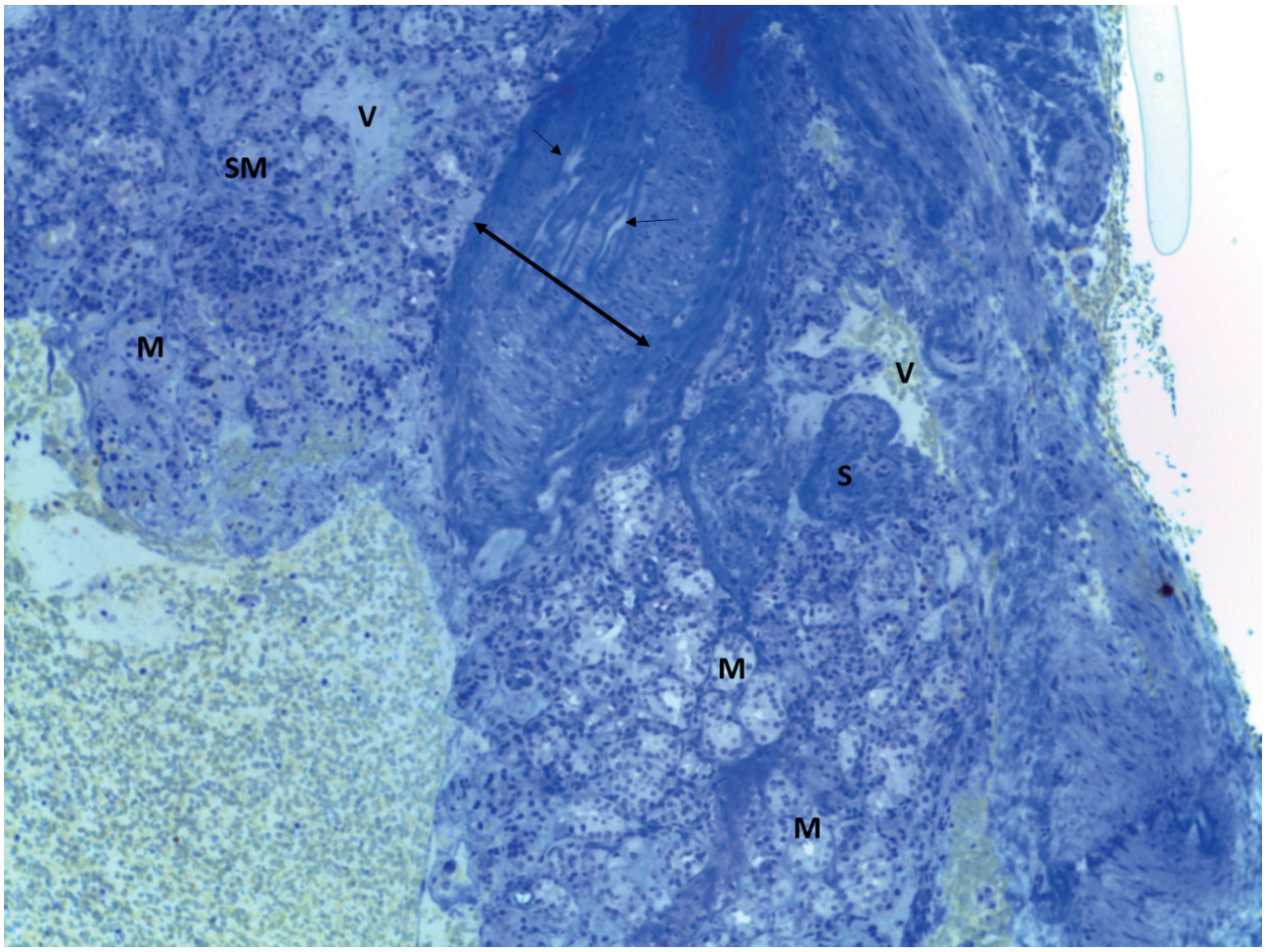

Figure 1. Human vomeronasal organ (double-sided arrow) lined by pseudostratified epithelium.around a lumen (arrow). (M: mucous gland; S: serous gland; SM: seromucous gland; V: vessel). Methylene- blue staining, x200.

planned. The aim was to microscopically examine and recognize the microanatomy of nasal epithelium and related normal nasal cavity tissue components using accessible cadaver samples.

In general, epithelial lining of the anterior-inferior septum was keratinized stratified squamous cilliated epithelium. We observed a thin lamina propria and few connective tissue fibers in all samples. Serous, seromucous and mucous glands were shown in the anteroinferior septum. We have also recognized abundant blood vessels with smaller diameter than other parts of the nasal cavity that we reported in a previous study [28]. In the anteroinferior site of the septum, we haven't differentiated any axons or neuronal extensions.

CONCLUSION

During a routine nasal mucosa dissection we had a chance to describe the histology of human VNO from one of our head and neck specimens. We processed our sample by routine preparation techniques for nasal cavity investigations using transmission electron microscopy. The samples were fixed, processed, embedded, sectioned, and stained for transmission microscopy. However, this preparation technique is not ideal on cadaver tissues and should only be used for live patients. Therefore we could not had a chance to show the ultrastructure of that sample. We thought that the final decision would be made by performing immunohistochemical studies, due to the fact that whether these cells carry the properties of gustatory receptors and contain any axons at their basal portions. These features cannot be determined by standard light microscopy methods.

In this study we reviewed and summarized all perspectives of a neglected and rare structure: the human vomeronasal organ. Questions still remain unanswered about its function, role and persistence in humans. The VNO still continues to incite interest and argument. Recovering the literature, we should finally say that generally in

adult human, the only sensory channel that might possibly allow detection of pheromones and all odorable chemicals in the nasal cavities would be the olfactory system itself.

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Author contribution

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

Ethical approval

This review study does not require ethical approval.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Stoyanov GS, Matev BK, Stoyanov P, et al. The Human Vomeronasal (Jacobson's) Organ: A Short Review of Current Conceptions, With an English Translation of Potiquet's Original Text. Cureus 2018;10(5): e2643.

[2] Trotier D. Vomeronasal organ and human pheromones. Eur Ann Otorhinolaryngol Head Neck Dis 2011;128:184-190.

[3] Nodari F, Hsu FF, Fu X, et al. Sulfated steroids as natural ligands of mouse pheromone sensing neurons. J Neurosci 2008;28:6407-6418.

[4] Leinders-Zufall T, Ishii T, et al. Structural requirements for the activation of vomeronasal sensory neurons by MHC peptides. Nat Neurosci 2009;12:1551-1558.

[5] Touhara K. Sexual communication via peptide and protein pheromones. Curr Opin Pharmacol 2008;8:759-764.

[6] Bhatnagar KP and Smith TD. The human vomeronasal organ. V. An interpretation of its discovery by Ruysch, Jacobson, or Kölliker, with an English translation of Kölliker (1877). Anat Rec B New Anat 2003;270:4-15.

[7] Bhatnagar KP and Reid KH. The human vomeronasal organ - I: Historical perspectives. A study of Ruysch's (1703) and Jacobson's (1811) reports on the vomeronasal organ with comparative comments and English translations. Biomed Res 1996(7);pp.219-229.

[8] D'Aniello B, Semin GR, Scandurra A, et al. The vomeronasal organ: a neglected organ. Frontiers in Neuroanatomy 2017;11:70.

[9] Kölliker A. Über die Jacobson'schen Organe des Menschen. Leipzig: Wilhelm Engelmann. 11 p + 2 plates with explanation of figures. 1877.

[10] Jacobson L. Description anatomique d'un organe observe dans le mammiferes. Ann Mus Hist Natl 1811;18:412–424.

[11] Potiquet M. Le canal de Jacobson. Rev Laryngol 1891;2:737–753.

[12] Smith TD, Siegel MI, Mooney MP, et al. Prenatal growth of the human vomeronasal organ. Anat Rec 1997;248:447–455.

[13] Wray S. From nose to brain: development of gonadotrophinreleasing hormone-1 neurones. J Neuroendocrinol 2010; 22:743–753.

[14] Trotier D, Eloit C, Wassef M, et al. The vomeronasal cavity in adult humans. Chem Senses 2000;25:69–380.

[15] Bhatnagar KP and Smith TD. The human vomeronasal organ. III. Postnatal development from infancy to the ninth decade. J Anat 2001;199:289–302.

[16] Meredith M. Human vomeronasal organ function: a critical review of best and worst cases. Chem Senses 2001;26:433-445.

[17] Smith TD, Siegel MI, Burrows AM, et al. Searching for the vomeronasal organ of adult humans: preliminary findings on location, structure, and size. Microsc Res Techn 1998;41:483-491.

[18] Zbar RI, Zbar LI, Dudley C, et al. A classification schema for the vomeronasal organ in humans. Plast Reconstr Surg 2000;105(4):1284-1288.

[19] Moran DT, Jafek BW, Rowley III JC. The vomeronasal (Jacobson's) organ in man: ultrastructure and frequency of occurrence.J Steroid Biochem Molec Biol 1991;39:1545-1552.

[20] Smith TD, Buttery TA, Bathnagar KP, et al. Anatomical position of the Vomeronasal Organ in postnatal humans. Ann Anat 2001;183:475-479.

[21] Jacob S, McClintock MK. Psychological state and mood effects of steroidal chemosignals in women and men. Horm Behav 2000;37:57-78.

[22] Abolmaali ND, Kühnau D, Knecht M, et al. Imaging of the Human Vomeronasal Duct. Chem Senses 2000;26(1):35-39.

[23] Besli R, Saylam C, Veral A, et al. The existence of the vomeronasal organ in human beings. J Craniofac Surg 2004;15(5):730-735.

[24] Knecht K, Kfihnau D, Hiittenbrink K-B, et al. Frequency and localization of the putative vomeronasal organ in humans in relation to age and gender. Laryngoscope 2001;111:448-452.

[25] Monti-Bloch L, Jennings-White C, Berliner DL. The Human Vomeronasal System. A Review Ann. N.Y. Acad Sci 1998;855:373-389.

[26] Wessels Q, Hoogland PV, Vorster W. Anatomical evidence for an endocrine activity of the vomeronasal organ in humans. Clin Anat 2014;27:856–860.

[27] Savic I, Berglund H. Androstenol–a steroid derived odor activates the hypothalamus in women. PLoS One 2010;5:e8651.

[28] Firat A, Onerci-Celebi O, Tuncel A, et al. Microscopic study of human nasal cavity microanatomy using semi-thin resin embedding and methylene blue staining. J Histotechnol 2019;42(1):13-18.

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ORIGINAL ARTICLE

The Effect of Statin Use on In-Hospital Mortality in Covid-19 Patients

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ABSTRACT

Objective: Our aim in this study was to determine whether statins with anti-inflammatory and antithrombotic properties reduce in-hospital mortality in Covid-19 patients.

Materials and Methods: 1752 patients hospitalized with the diagnosis of Covid-19 between September and December 2020 were retrospectively analyzed. The patients were grouped according to statin use and their characteristics were compared. The parameters associated with mortality were examined.

Results: For the patients, the median age was 64 years (53-74 interquartile range [IQR]), 804 (45.9%) were over the age of 65, 903 (51.5%) were male, 167 (9.5%) were using statins, and mortality developed in 381 (21.7%) of the patients. The multivariate logistic regression model was applied using statistically significant parameters in the univariate analysis of mortality development. The group using statins was included in the regression model because it was statistically borderline significant (p=0.052). According to this model; increased age (odds ratio (OR) =1.051, 95% confidence interval (CI) 1.039-1.063, p<0.001), male gender (OR=1.002, 95%CI 0.993-1.010, p=0.705), decrease in lymphocyte count (OR=0.452, 95%CI 0.337-0.607, p<0.001) increase in potassium level (OR=1.306, 95%CI 1.025-1.664, p=0.031), increase in aspartate transaminase (AST) level (OR=1.004, 95%CI 1.001-1.007, p=0.003), increase in D-dimer level (OR=1.000, 95%CI 1.000-1.000, p=0.011), increase in procalcitonin level (OR=1.027, 95%CI 1.000-1.053, p=0.046), increase in CRP level (OR=1.007, 95%CI 1.005-1.008, p<0.001), and the development of myocardial injury (OR=3.045, 95%CI 1.864-4.976, p<0.001) was found to be associated with increased mortality. Statin use (OR=1.221, 95% CI 0.759-1.966, p=0.410) was not associated with mortality.

Conclusion: According to our study, statin use was not associated with an increase or decrease in-hospital mortality in patients hospitalized with a diagnosis of Covid-19.

Keywords: Covid-19, in-hospital mortality, statin

INTRODUCTION

The World Health Organization (WHO) recognized Covid-19 diseaseas a pandemic in March 2020, and unfortunately, the disease continues to spread globally at an alarming rate [1].

Acute Respiratory Distress Syndrome (ARDS) and thromboembolism are two major consequences that result in a high fatality rate in hospitalized Covid-19 patients [2,3]. According to current research, fatal consequences of SARS-CoV-2 infection originate from cytokine storm caused by an excessive inflammatory response [4]. SARS-CoV-2 invasion and destruction of epithelial cells in alveoli causes excessive inflammatory response consequently ARDS. Thromboembolism complications occur as a result of the release of inflammatory cytokines, intravascular endothelial dysfunction, thrombin production, and clot accumulation [5-7]. Although statins have traditionally been used to lower serum cholesterol, they show pleiotropic effects with anti-inflammatory, immunomodulatory, and antithrombotic properties [8-10]. This condition makes statins an attractive class of drugs in the adjunct therapy of Covid-19. Statins have been demonstrated to improve outcomes in patients with community-acquired pneumonia in studies [11,12]. However, the use of statins in Covid-19 patients is still contentious as result of several researches [13,14].

Our study aims to examine the effect of statin use on in-hospital mortality in Covid-19 patients.

MATERIALS and METHODS

This study is a retrospective study that included all reverse transcription-polymerase chain reaction (RT-PCR) positive Covid-19 patients hospitalized in our hospital between September and December 2020. The study protocol was approved by the ethics committee of our hospital. Patients older than 18 years were included in the study. Exclusion criteria were as follows; missing medical records, patients hospitalized for more than 28 days (considered decompensated or end-stage of chronic organ failure (e.g. decompensated heart failure, decompensated cirrhosis, or decompensated chronic renal failure), patients with active malignancy or acquired immunodeficiency syndrome (AIDS) or pregnancy. Patients who were prescribed statins for more than 1 month were considered the statin user group.

The demographic information, clinical characteristics, radiological images, and laboratory data of the patients were obtained from the patient files and the hospital digital system. Concomitant diseases such as hypertension, coronary heart disease, heart failure, atrial fibrillation, diabetes mellitus, chronic lung diseases (asthma/chronic obstructive pulmonary disease), cerebrovascular diseases, chronic renal failure, and the medications used by the patients were obtained from the medical history. In-hospital medications and interventions were carried out according to the Covid-19 Guidelines, published by the Ministry of Health of the Republic of Turkey [15]. To protect patient privacy, each patient was given a code before data collection. It was carefully double-checked by experienced physicians to verify the accuracy of the data. Definition of ARDS and septic shock were assessed according to the WHO intermediate guideline [16].

Statistical Analysis

The IBM SPSS software suite was used to conduct all statistical analyses (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). The continuous variables were presented given as mean±SD and median interquartile range 25-75% (IQR) in case of non-normal distribution. The categorical variables were expressed as percentages. The distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Depending on the data distribution, the continuous variables were compared using the Student's t-test or the Mann-Whitney U test. The categorical variables were compared using Chi-square or Fisher's exact tests if appropriate. The non-normally distributed numerical and categorical variables were analyzed with the Mann-Whitney U test in the two groups. Univariate and multivariate logistic regression analyses were performed to evaluate the relationship between statin use and the development of mortality. In stepwise multivariable regression analysis (Backward,

Wald); effect size was adjusted with a univariate significance level of <0.05 for all variables. Adjusted odds ratios (OR) along with the confidence interval (CI) 95% were presented. A 2-tailed p-value<0.05 was considered to be statistically significant.

RESULTS

A total of 1752 patients hospitalized in our hospital with the diagnosis of Covid-19 were included in our study. For the patients, the median age was 64 years (53-74 interquartile range [IQR]), 804 (45.9%) were over the age of 65, 903 (51.5%) were male, 167 (9.5%) were using statins, and mortality developed in 381 (21.7%) of the patients. The characteristics of the study population are summarized in Table 1. In the group using statins; age, systolic blood pressure, and diastolic blood pressure levels were higher (p=0.004, p=0.012, and p=0.002, respectively). In addition, comorbidities such as coronary artery disease, heart failure, hypertension, diabetes mellitus, and asthma were more common (p <0.001, p <0.001, p <0.001, p <0.001, and p=0.031, respectively). While creatinine level and potassium level were higher (p <0.001, and p=0.007) in statin users, AST level was lower (p=0.039). Antiaggregant, ACEI, ARB, beta-blocker, calcium channel blocker, diuretic, oral antidiabetic, and insulin use were more common (p <0.001, p <0.001, p <0.001, p <0.001, p <0.001, p <0.001, p <0.001, and p <0.001, respectively) in the statin user group. Also, mechanical ventilator need and ICU hospitalization rates were higher (p=0.041, and p=0.008) in statin users. Although the mortality rate was higher in the group using statins, it was not statistically significant (p=0.056).

In Table 2, the multivariate logistic regression model was applied using statistically significant parameters in the univariate analysis of mortality development. The group using statins was included in the regression model because it was statistically borderline significant (p=0.052). According to this model; increased age (odds ratio (OR) =1.051, 95% confidence interval (CI) 1.039-1.063, p<0.001), male gender (OR=1.002, 95%CI 0.993-1.010, p=0.705), decrease in lymphocyte count (OR=0.452, 95%CI 0.337-0.607, p<0.001) increase in potassium level (OR=1.306, 95%CI 1.025-1.664, p=0.031), increase in aspartate transaminase (AST) level (OR=1.004, 95%CI 1.001-1.007, p=0.003), increase in D-dimer level (OR=1.000, 95%CI 1.000-1.000, p=0.011), increase in procalcitonin level (OR=1.027, 95%CI 1.000-1.053, p=0.046), increase in CRP level (OR=1.007, 95%CI 1.005-1.008, p<0.001), and the development of myocardial injury (OR=3.045, 95%CI 1.864-4.976, p<0.001) was found to be associated with increased mortality. Statin use (OR=1.221, 95% CI 0.759-1.966, p=0.410) was not associated with mortality.

DISCUSSION

In this study, we found that advanced age, male gender, increased levels of potassium, AST, D-dimer, procalcitonin, CRP, low lymphocyte count, and myocardial damage were associated with increased mortality. We determined that using statins does not affect mortality.

Similar to previous studies [17], we found that advanced age, male gender, increased potassium, AST, D-dimer, procalcitonin or CRP level, low lymphocyte count, and myocardial damage were associated with increased mortality. In our study, the rate of statin use in patients with coronary heart disease was 59.9%. This low rate of statin use may be attributed to the side effects of statins, lack of health insurance, or polypharmacy.

Statins have antiplatelet, anticoagulant, anti-inflammatory, and immunomodulatory properties in addition to reducing the cholesterol level. Statins cause a decrease in platelet activity as a result of an increase in nitric oxide, a potent inhibitor of platelet aggregation. Thrombomodulin (TM) acts as a cofactor of thrombin in the process of activation of activated protein C (APC), which proteolytically inactivates factors V and VIII and acts as an anticoagulant. Statins have been shown to increase the expression of TM and APC [18]. Statins have been reported to have pleiotropic effects on respiratory tract infection and acute lung injury [19,20]. By activating intracellular signaling pathways, it has been demonstrated that statins have a variety of helpful anti-inflammatory and immunomodulatory characteristics [21,22]. In addition, many in vitro and in vivo research results have shown antiviral efficacy of statins against influenza virus [23,24].

Table 1. Characteristics of the study population

Parameters	All patients (n=1752)	Statin users (n=167)	Non Statin users (n=1585)	p value
Age, years	64(53-74)	66(58-73)	64(52-74)	0.004
Gender (male), n (%)	903(51.5)	95(56.9)	808(51.0)	0.146
SBP, mmHg	120.0(110.0-122.8)	120.0(110.0-130.0)	120.0(110.0-120.0)	0.012
DBP, mmHg	70.0(60.0-80.0)	70.0(70.0-80.0)	70.0(60.0-80.0)	0.002
Coronary heart disease, n(%)	280(16.0)	100(59.9)	180(11.4)	<0.001
Chronic heart failure, n (%)	72(4.1)	19(11.4)	53(3.3)	<0.001
Hypertension, n(%)	809(46.2)	134(80.2)	675(42.6)	<0.001
Diabetes mellitus, n(%)	474(27.1)	99(59.3)	375(23.7)	<0.001
COPD, n(%)	106(6.1)	14(8.4)	92(5.8)	0.184
Asthma, n(%)	119(6.8)	18(10.8)	101(6.4)	0.031
Cerebrovascular diseases, n(%)	81(4.6)	8(4.8)	73(4.6)	0.914
Chronic renal diseases, n(%)	68(3.9)	10(6.0)	58(3.7)	0.138
Chronic AF, n(%)	59(3.4)	5(3.0)	54(3.4)	0.778
Neutrophil count, 10^3/uL	5.56(3.80-8.20)	5.41(4.11-7.68)	5.45(3.78-8.23)	0.758
Lymphocyte count, 10^3/uL	1.06(0.74-1.43)	1.10(0.75-1.60)	1.06(0.74-1.41)	0.111
Platelet count, 10^3/uL	210(168-264)	203(159-259)	210(169-265)	0.153
Hemoglobin, g/dl	13.4(12.1-14.5)	13.3(12.0-14.5)	13.4(12.1-14.5)	0.314
Creatinine, mg/dl	0.93(0.79-1.21)	1.03(0.83-1.40)	0.92(0.78-1.19)	<0.001
Potassium, mmol/l	4.18(3.84-4.54)	4.31(3.89-4.73)	4.17(3.84-4.52)	0.007
AST, U/L	33(24-47)	30(23-39)	33(24-48)	0.039
ALT, U/L	25(17-39)	24(16-37)	25(17-40)	0.147
Ferritin, ng/ml	468(236-882)	406(219-730)	474(237-915)	0.065
D-dimer increase, ng/ml	272(177-445)	259(173-436)	272(177-445)	0.936
Procalcitonin, ng/ml	0.11(0.06-0.24)	0.12(0.06-0.29)	0.11(0.06-0.24)	0.505
C-reactive protein, mg/l	83.0(40.2-128.0)	73.1(33.9-117.0)	83.4(40.4-129.4)	0.191
Bilateral lesions, n(%)	1682(96.0)	161(96.4)	1521(96.0)	0.912
Antiaggregant users, n(%)	464(26.5)	124(74.3)	340(21.4)	<0.001
OAC users, n(%)	47(2.7)	3(1.8)	44(2.8)	0.456
ACEI users, n(%)	274(15.6)	65(38.9)	209(13.2)	<0.001
ARB users, n(%)	385(22.0)	58(34.7)	327(20.6)	<0.001
Beta-blocker users, n(%)	357(20.4)	84(50.3)	273(17.2)	<0.001
Calcium channel blocker users, n(%)	395(22.5)	58(34.7)	337(21.3)	<0.001
Diuretic users, n(%)	446(25.5)	65(38.9)	381(24.0)	<0.001
Spironolactone users, n(%)	32(1.8)	4(2.4)	28(1.8)	0.564
Alpha-blocker users, n(%)	36(2.1)	5(3.0)	31(2.0)	0.368
Digoxin users, n(%)	16(0.9)	1(0.6)	15(0.9)	0.653
Oral antidiabetic users, n(%)	367(20.9)	81(48.5)	286(18.0)	<0.001
Insulin users, n(%)	136(7.8)	36(21.6)	100(6.3)	<0.001
Mechanical Ventilation, n(%)	384(21.9)	47(28.1)	337(21.3)	0.041
Septic shock, n(%)	190(10.8)	23(13.8)	167(10.5)	0.201
ARDS, n(%)	332(18.9)	41(24.6)	291(18.4)	0.052
Hospital stays, days	8(6-12)	8(7-11)	8(6-12)	0.191
ICU stays, n(%)	525(30.0)	65(38.9)	460(29.0)	0.008
Myocardial injury, n(%)	106(6.1)	15(9.0)	91(5.7)	0.091
Mortality, n(%)	381(21.7)	46(27.5)	335(21.1)	0.056

Data are shown as % for categorical and as median (interquartile range) for continuous variables. Categorical data were compared using chi-square test and continuous data using Mann-Whitney U test

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; AF, atrial fibrillation; ALT, alanine transaminase; ARB, angiotensin receptor blocker; ARDS, acute respiratory distress syndrome; AST, aspartate transaminase; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; ICU, intensive care unit; MV, mechanical ventilation; OAC, oral anticoagulant; SBP, systolic blood pressure

Table 2. Univariate and multivariate logistic regression analysis for in-hospital mortality

Variable	Univariable			Multivariable		
	Unadjusted OR	95% CI	P-value	Adjusted OR	95% CI	P-value
Age, years	1.060	1.050-1.070	<0.001	1.051	1.039-1.063	<0.001
Gender (male), n (%)	1.434	1.140-1.805	0.002	1.469	1.076-2.005	0.015
SBP, mmHg	1.013	1.005-1.021	0.001	1.002	0.993-1.010	0.705
DBP, mmHg	1.002	0.997-1.008	0.448			
Coronary heart disease, n(%)	1.962	1.482-2.600	<0.001	1.090	0.717-1.656	0.687
Chronic heart failure, n (%)	2.110	1.286-3.461	0.003			
Hypertension	1.839	1.462-2.315	<0.001	1.046	0.690-1.585	0.832
Diabetes mellitus, n(%)	1.385	1.083-1.773	0.010	1.127	0.795-1.598	0.502
COPD, n(%)	2.219	1.470-3.352	<0.001	1.284	0.773-2.132	0.335
Asthma, n(%)	1.116	0.719-1.732	0.625			
Cerebrovascular diseases, n(%)	1.860	1.155-2.996	0.011			
Chronic renal diseases, n(%)	1.643	0.970-2.785	0.065			
Chronic AF, n(%)	2.209	1.287-3.793	0.004			
Neutrophil count, 10^3/uL	1.155	1.123-1.187	<0.001			
Lymphocyte count, 10^3/uL	0.292	0.222-0.383	<0.001	0.452	0.337-0.607	<0.001
Platelet count, 10^3/uL	1.000	0.998-1.001	0.461			
Hemoglobin, g/dl	0.864	0.813-0.917	<0.001	0.950	0.877-1.029	0.210
Creatinine, mg/dl	1.218	1.131-1.312	<0.001	1.003	0.901-1.117	0.955
Potassium, mmol/l	1.650	1.360-2.002	<0.001	1.306	1.025-1.664	0.031
AST, U/L	1.004	1.002-1.006	0.001	1.004	1.001-1.007	0.003
ALT, U/L	1.002	1.000-1.004	0.060			
Ferritin, ng/ml	1.001	1.001-1.001	<0.001			
D-dimer, ng/ml	1.000	1.000-1.000	<0.001	1.000	1.000-1.000	0.011
Procalcitonin, ng/ml	1.065	1.032-1.099	<0.001	1.027	1.000-1.053	0.046
C-reactive protein, mg/l	1.008	1.007-1.010	<0.001	1.007	1.005-1.008	<0.001
Antiaggregant users, n(%)	1.779	1.397-2.267	<0.001	0.777	0.532-1.136	0.193
OAC users, n(%)	1.895	1.025-3.502	0.041			
ACEI users, n(%)	1.144	0.843-1.551	0.388			
ARB users, n(%)	1.431	1.102-1.858	0.007	1.036	0.715-1.500	0.853
Beta-blocker users, n(%)	2.017	1.557-2.613	<0.001	1.478	0.988-2.211	0.057
Calcium channel blocker users, n(%)	1.685	1.305-2.174	<0.001	1.338	0.927-1.930	0.120
Diuretic users, n(%)	1.149	0.890-1.484	0.287			
Spironolactone users, n(%)	1.418	0.651-3.090	0.380			
Alpha-blocker users, n(%)	2.961	1.519-5.772	0.001			
Digoxin users, n(%)	3.654	1.362-9.801	0.010			
Oral antidiabetic users, n(%)	1.198	0.914-1.572	0.191			
Insulin users, n(%)	1.748	1.195-2.558	0.004	1.407	0.828-2.392	0.207
Statin users, n(%)	1.419	0.989-2.034	0.052	1.221	0.759-1.966	0.410
Miyocardial injury, n(%)	6.352	4.223-9.555	<0.001	3.045	1.864-4.976	<0.001

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; AF, atrial fibrillation; ALT, alanine transaminase; ARB, angiotensin receptor blocker; AST, aspartate transaminase; CI, confidence interval; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; OAC, oral anticoagulant; OR, odds ratio; SBP, systolic blood pressure

Statins adjust the antiviral response in human epithelial and bronchial cells, which form the first line of defense against invading pathogens such as H1N1. Significantly reduces the production of proinflammatory cytokines such as TNF-alpha and IL-6 [25,26]. Despite these beneficial effects of statins, it was shown in a study that plasma IL-18 levels were higher in patients using statins and that higher levels of IL-18 were associated with higher mortality in patients with sepsis-induced ARDS [27]. Zhang et al., in a retrospective study involving a total of 13,981 patients, 1219 of whom received statin therapy, a potential reduction in all-cause mortality was determined in Covid-19 patients receiving statin therapy. They found that in-hospital statin use was associated with a 42% reduction in 28-day mortality risk [14]. Although our data may be biased by remaining confounding factors, including patient selection, treatment indication, socioeconomic status, and lack of adjustment for prehospital medication, this controversial finding deserves further investigation. On the other hand, in their study Butt et al. analyzed a total of 4842 patients diagnosed with Covid 19, of which 843 were prescribed a statin at least once in the past 6 months. In this study, there were outpatients and hospitalized patients. The main conclusion of the study was that statin use before Covid-19 diagnosis was not associated with an improvement or worsening in the clinical course of Covid-19 infection [28]. A meta-analysis of 9 studies involving 3449 patients by Hariyanto and Kurniawan showed that the use of statins did not improve the severity and mortality of Covid-19 infection [29]. Oh et al. showed that the probability of developing Covid-19 in the statin treatment group was 35% lower than the control group in their study involving 122040 patients with 22633 statin users, but showed that there was no difference in in-hospital mortality between the statin treatment and control groups [30].

Our results showed that statin use did not result in an increase in-hospital mortality, similar to the results of previous studies. Although statins produce anti-inflammatory and antithrombotic effects, there is no clear evidence that these drugs reduce morbidity and mortality in Covid-19 patients. The use of statins may cause an increase in IL-18 levels and potential detrimental effects may occur [27] and may result in a neutral effect on covid-19, balanced by beneficial effects such as anti-inflammatory and antithrombotic properties.

Limitations

The most important limitation was that our study was a single-center, retrospective study, and the number of patients was small. There was a lack of data from hospital records such as smoking history and body mass index. In addition, since our study was retrospective, an important limitation emerged as there was no data on the doses and drug compliance to prescribed statins.

CONCLUSION

Our study showed that using statins was not associated with an increase or decrease in mortality in Covid-19 patients. Although statins are known to have beneficial effects such as anti-inflammatory and antithrombotic properties, large-scale randomized clinical trials are needed to determine the benefits of statins in Covid-19 patients.

Author contribution

Study conception and design: AA, and BA; data collection: FI, MÇ, İK, ÖA, and Üİ; analysis and interpretation of results: ET, ÖB, and MO; draft manuscript preparation: AA, and MZK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ethics Committee of SBU Diyarbakır Gazi Yaşargil Education and Research Hospital (Protocol no: 778/29.05.2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Mahase E. Covid-19: WHO declares pandemic because of "alarming levels" of spread, severity, and inaction. BMJ. 2020;368:m1036.

[2] Hasan SS, Capstick T, Ahmed R, et al. Mortality in COVID-19 patients with acute respiratory distress syndrome and corticosteroids use: a systematic review and meta-analysis. Expert Rev Respir Med. 2020;14:1149–63. https://doi.org/10.1080/17476348.2020.1804365

[3] Malas MB, Naazie IN, Elsayed N, et al. Thromboembolism risk of COVID-19 is high and associated with a higher risk of mortality: a systematic review and metaanalysis. EClinicalMedicine. 2020;29–30:100639. https://doi.org/10.1016/j.eclinm.2020.100639

[4] Hojyo S, Uchida M, Tanaka K, et al. How COVID-19 induces cytokine storm with high mortality. Infamm Regen. 2020;40:37. https://doi.org/10.1186%2Fs41232-020-00146-3

[5] Joly BS, Siguret V, Veyradier A. Understanding pathophysiology of hemostasis disorders in critically ill patients with COVID-19. Intensive Care Med. 2020;1:1–4. https://doi.org/10.1007/s00134-020-06088-1

[6] Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020;395:1417–8. https://doi.org/10.1016/S0140-6736(20)30937-5

[7] Abou-Ismail MY, Diamond A, Kapoor S, et al. The hypercoagulable state in COVID-19: incidence, pathophysiology, and management. Thromb Res. 2020;194:101–15. https://doi.org/10.1016/j.thromres.2020.06.029

[8] Radenkovic D, Chawla S, Pirro M, et al. Cholesterol in relation to COVID-19: should we care about it? J Clin Med. 2020;9:1909. https://doi.org/10.3390/jcm9061909

[9] Castiglione V, Chiriacò M, Emdin M, et al. Statin therapy in COVID-19 infection. Eur Hear J Cardiovasc Pharmacother. 2020. https://doi.org/10.1093/ehjcvp/pvaa042

[10] Lee KCH, Sewa DW, Phua GC. Potential role of statins in COVID19. Int J Infect Dis. 2020;96:615–7. https://doi.org/10.1016/j.ijid.2020.05.115

[11] Fedson DS. Treating infuenza with statins and other immunomodulatory agents. Antiviral Res. 2013;99:417–35. https://doi.org/10.1016/j.antiviral.2013.06.018

[12] Grudzinska FS, Dosanjh DP, Parekh D, et al. Statin therapy in patients with community-acquired pneumonia. Clin Med (Northfeld Il). 2017;17:403–7. https://doi.org/10.7861/clinmedicine.17-5-403

[13] Cariou B, Goronfot T, Rimbert A, et al. Routine use of statins and increased mortality related to COVID-19 in inpatients with type 2 diabetes: Results from the CORONADO study. Diabetes Metab. 2020. https://doi.org/10.1016/j.diabet.2020.10.001

[14] Zhang XJ, Qin JJ, Cheng X, et al. Inhospital use of statins is associated with a reduced risk of mortality among individuals with COVID-19. Cell Metab. 2020;32(176–187):e4. https://doi.org/10.1016/j.cmet.2020.06.015

[15] Republic of Turkey Ministry of Health. COVID-19 (SARS-CoV-2 INFECTION) GUIDE. https://covid19.saglik.gov.tr/TR-66299/covid-19-tedavi.html

[16] World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected. 2020. https://www.who.int/publications-detail/clinicalmanagement-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected

[17] Izcovich A, Ragusa M, Sanguine V, et al. Prognostic factors for severity and mortality in patients infected with COVID-19: A systematic review. PLoS One. 2020 Nov 17;15(11):e0241955. https://doi.org/10.1371/journal.pone.0241955

[18] Owens AP 3rd, Mackman N. The antithrombotic effects of statins. AnnuRevMed. 2014;65:433–45. https://doi.org/10.1146/annurev-med-051812-145304

[19] Van De Garde EM, Hak E, Souverein PC, et al. Statin treatment and reduced risk of pneumonia in patients with diabetes. Thorax. 2006;61(11):957–61.

[20] Jacobson JR, Barnard JW, Grigoryev DN, et al. Simvastatin attenuates vascular leak and inflammation in murine inflammatory lung injury. Am J Physiol Lung Cell Mol Physiol. 2005; 288(32–36):L1026–32.

[21] Liao JK, Laufs U. Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol. 2005;45:89–118.

[22] Terblanche M, Smith TS, Adhikari NKJ. Statins, bugs and prophylaxis: intriguing possibilities. Crit Care. 2006;10(5):168.

[23] Mehrbod P, Ideris A, Omar AR, et al. Evaluation of antiviral effect of atorvastatin on H1N1 infection in MDCK cells. Afr J Microbiol Res. 2012;6(27):5715–9. https://doi.org/10.5897/AJMR12.1011

[24] Vandermeer ML, Thomas AR, Kamimoto L, et al. Association between use of statins and mortality among patients hospitalized with laboratory-confirmed influenza virus infections: a multistate study. J Infect Dis. 2012;205(1):13–9.

[25] Inoue I, Goto S, Mizotani K, et al. Lipophilic HMG-CoA reductase inhibitor has an antiinflammatory effect: reduction of mRNA levels for interleukin-1, interleukin6, cyclooxygenase- 2 and p22phox by regulation of peroxisome proliferatoractivated receptor (PPAR) in primary endothelial cells. Life Sci. 2000;67(8): 863–76.

[26] Mehrbod P, Zowalaty ME, Omar AR et al. Statins reduce the expression of proinflammatory cytokines in influenza A virus infected CrFK cells. Acta Virol. 2012;56(4):353–5.

[27] Rogers AJ, Guan J, Trtchounian A, et al. Association of Elevated Plasma Interleukin-18 Level With Increased Mortality in a Clinical Trial of Statin Treatment for Acute Respiratory Distress Syndrome. Crit Care Med. 2019;47(8):1089-96. https://doi.org/10.1097/CCM.0000000000003816

[28] Butt JH, Gerds TA, Schou M, et al. Association between statin use and outcomes in patients with coronavirus disease 2019 (COVID-19): a nationwide cohort study. BMJ Open. 2020 Dec 4;10(12):e044421. https://doi.org/10.1136/bmjopen-2020-044421

[29] Hariyanto TI, Kurniawan A. Statin therapy did not improve the in- hospital outcome of coronavirus disease 2019 (COVID-19) infection. Diabetes Metab Syndr 2020;14:1613–5.

[30] Oh TK, Song IA, Jeon YT. Statin Therapy and the Risk of COVID-19: A Cohort Study of the National Health Insurance Service in South Korea. J Pers Med. 2021 Feb 10;11(2):116. https://doi.org/10.3390/jpm11020116

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ORIGINAL ARTICLE

Morphometry in Classification of Hippocampal Sclerosis

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ABSTRACT

Hippocampal sclerosis (HS) is evaluated in 3 categories by the latest (2013) ILAE classification. The distinction between these categories rely on the histopathological assessment of pyramidal neuron loss in 4 CA sectors. In order to evaluate neuron loss assessment done manually by a neuropathologist, cell counts were carried out from representative photomicrographs of each section. NeuN immunohistochemistry was applied on hippocampus sections of 28 samples of epilepsy surgery, photographed at x100 magnification to represent each of the 4 sectors, and neuron density was calculated per photo. This density data was compared to the pathology reports' diagnoses. HS type 1 cases were predominant (n=23) with few type 2 and type 3 cases (3 and 2, respectively). Percentage of neuron loss calculated per photos, ILAE classification guidelines and pathological diagnoses rendered without any calculation were relatively well-correlated; with HS type 2 and 3 displaying slight changes from recommendations. Data also display accurate pathological diagnoses of HS without special equipment or cell density calculation. HS types 2 and 3 in Turkey may display variant cell density properties which may warrant further clarification.

Keywords: Epilepsy, hippocampus, hippocampal sclerosis, gliosis, NeuN, immunohistochemistry, classification

INTRODUCTION

Epilepsy is a prevalent disease characterized by periodic and unanticipated seizure episodes, many precipitating and etiological factors, and variable seizure frequencies and types. Although much progress has been made in epilepsy diagnosis and therapy, there remains about a third of epilepsy patients that do not respond to current therapy modalities; in these cases epilepsy surgery may be preferred. A relatively recent surgical procedure in our country, epilepsy surgery is a new area for our pathologists, requiring experience in neuropathology and knowledge of neuroanatomy [1,2].

Hippocampal sclerosis (HS) is the prototype of surgically treatable epileptic syndromes; surgery consists of selective amygdalahippocampectomy or amygdalahippocampectomy accompanied by temporal lobectomy [3]. HS is characterized by pyramidal neuron loss and gliosis of variable intensity and location, along with dentate gyrus findings [4,5]. These different histological patterns have been classified in several attempts, the most recent and well-accepted one is the classification offered in 2013 by the International League Against Epilepsy (ILAE) [4]. ILAE 2013 classification renders three categories according to histological patterns and location of pyramidal neuron loss and gliosis (HS type 1 with losses in CA1 and CA4, the CA1-predominant HS type 2 and the CA4 predominant HS type 3) [4].

Although the 2013 ILAE classification is admittedly a semiquantitative scheme depending on patterns and intensity of pyramidal neuron loss, its use is still recommended, as different HS patterns are

considered to be associated with variable etiologies, clinical findings and therapy response, possibly reflecting different epileptogenetic mechanisms [4-7].

In the present study, digitalized microscopic images are annotated and their neuron counts quantified with the aid of computerized image analysis software, in an attempt to assess the feasibility of such computer-aided morphometric techniques in HS subtyping.

METHODS

The study was approved by the Hacettepe University Non-interventional Clinical Research Ethics Board (31/05/2021, 2022/09-67). Twenty-eight cases that underwent epilepsy surgery (amygdalectomy, hippocampectomy, temporal lobectomy) in Hacettepe University Hospitals between 2017-2019 whose samples were assessed and reported by a single neuropathologist (FS) and whose paraffin blocks were readily available were included in the study. Twenty three of these were reported as HS type 1, three were HS type 2 and two were HS type 3 according to 2013 ILAE criteria. NeuN immunohistochemistry was implemented to show intact neurons with a Leica Bond Max autostainer. Briefly, 4-micrometer thick formalin fixed paraffin embedded tissue sections were deparaffinized, rehydrated and endogenous peroxidase blockage was carried out, followed by antigen retrieval at 100 °C with ER2 (EDTA) solution. Primary antibody (NeuN, Zymed, A60, 1/50) was applied for 25 minutes at room temperature. After incubation with the appropriate secondary antibody at room temperature for 90 min, the signals were visualized with DAB chromogen, slides were dehydrated, and hematoxylin counterstaining was applied.

Neu-N immunostained slides were reviewed by a pathologist (GG) on an Olympus BX53 microscope connected to an Olympus CellSens Entry 2.3 image capture – camera system. At x100 magnification, one area representing each CA sector of each HS case was picked and photographed (Fig. 1A). Photomicrographs were visualized with ImageJ [8] and its "Cell Counter" mode was used to manually count NeuN positive cells.

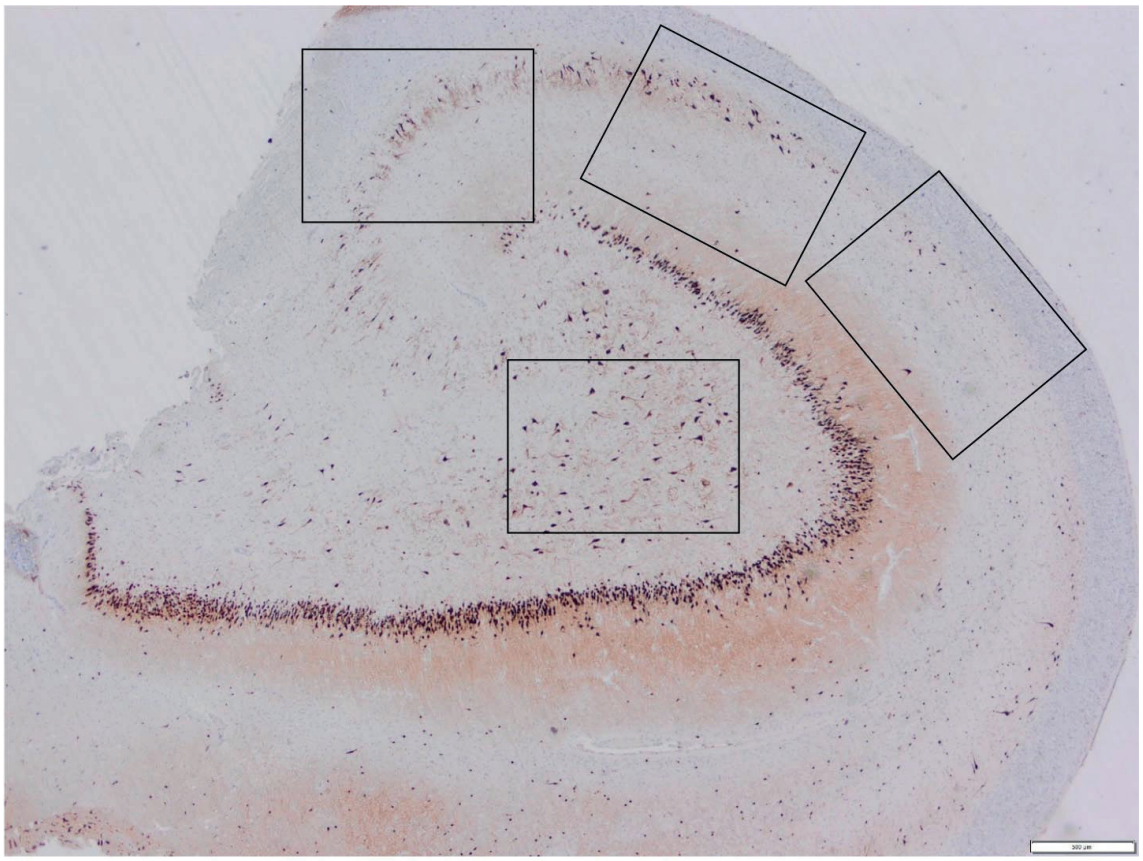

Figure 1A. Representative areas photographed from a Neu-N immunostained hippocampal section (x2.5, HS type 1). ROI for CA4 is shown mid-picture, CA3, CA2 and CA1 are in the upper part of the picture from left ro right, respectively. Scale bar: 500 um.

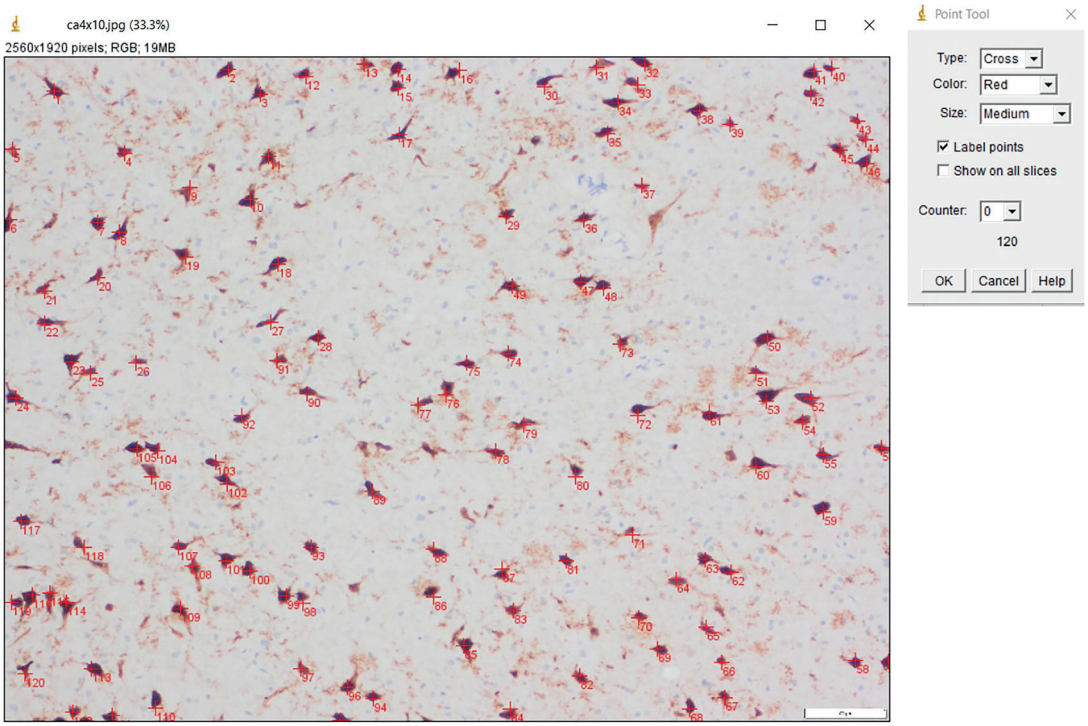

Figure 1B. Manual cell counting of a x10 image with ImageJ. Neu-N immunostaining, x10 magnification. Scale bar: 100 um.

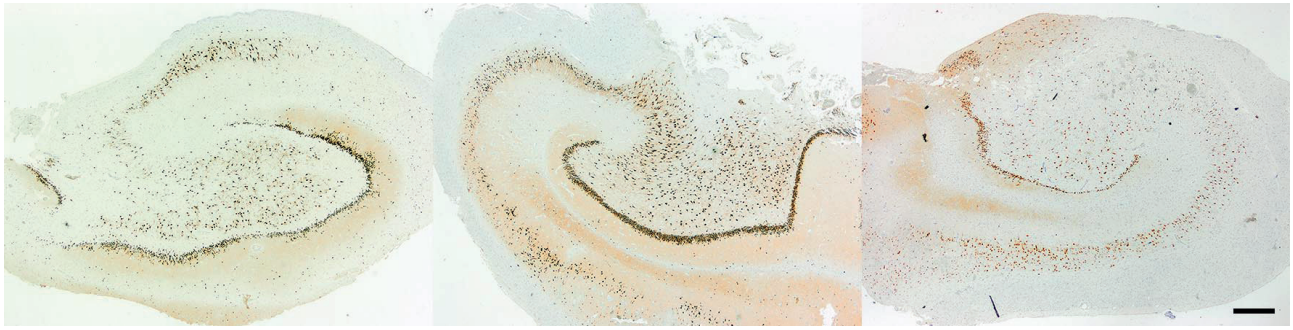

Figure 2. Examples of HS type 1 (left), HS type 2 (mid) and HS type 3 (right). Neu-N immunostaining, x2,5. Scale bar: 500 um.

The number of neurons were divided by the area of the photomicrograph to obtain cell densities (n/mm2) (Fig. 1B). The autopsy cases (n=5) intended to be controls did not react with NeuN antibodies in the immunohistochemistry assay, probably due to prolonged fixation, which prompted the count of neurons on photomicrographs of H-E stained slides. Cell densities were compared via Kruskal-Wallis tests in the R package (Jamovi) [9]. A p value lower than 0,05 was considered statistically significant.

RESULTS

Main clinicopathological features of the cases are shown in Table 1. A low-power (x2,5) view of exemplary HS type 1, HS type 2 and HS type 3 cases are depicted in Fig. 2.

A comparison of neuron loss rates in our cases and the corresponding ILAE criteria [4] is given in Table 2. Sector-based paired comparisons of neuron densities per category are given in Table 3.

Table 1. Major clinicopathological features of the cases with average number of neurons per sector

				Hippocampal neuron counts, mean (min-max)			
Reported diagnosis	n	Sex (M/F)	Average age (min-max)	CA1	CA2	CA3	CA4
HS1	23	13 / 10	20.33 (2-44)	36.2 (1-100)	54.4 (18-121)	37.3 (3-99)	45.9 (4-135)
HS2	3	2 / 1	17.77 (15-22)	72.9 (59-82)	107.04 (99-119)	113.8 (93-132)	204.59 (172-238)
HS3	2	0 / 2	10.5 (10-11)	140.9 (135-147)	96.0 (79-119)	107.7 (69-168)	111.4 (71-175)
Control	5	3 / 2	6.12 (0.17-33)	186.2 (118-253)	135.0 (89-206)	126.0 (75-174)	185.1 (138-268)

Table 2. Average rates of cell loss per sector in cases and the rates of neuron loss in ILAE criteria

DIAGNOSIS	PYRAMIDAL NEURON LOSS (%)			
	CA1	CA2	CA3	CA4
HS1	76.1	56.7	65.8	68.7
ILAE HS1 CRITERIA*	>%80	%30–50	%30–90	%40–90
HS2	61.5	24.3	12.9	-7.9
ILAE HS2 CRITERIA*	APPROX. %80	<%20	<%20	<%25
HS3	26.3	30.2	10.3	35.7
ILAE HS3 CRITERIA*	<%20	<%25	<%30	Approx. %50

* ILAE 2013 [4]

Table 3. P values obtained by sector-by-sector comparisons of HS groups (Dwass-Steel-Critchlow-Fligner)

CA1	HS1	HS2	HS3	Control
HS1	.	0.1	0.097	0.003
HS2	0.1	.	0.307	0.114
HS3	0.097	0.307	.	0.651
Control	0.003	0.114	0.651	.

CA3	HS1	HS2	HS3	Control
HS1	.	0.036	0.226	0.006
HS2	0.036	.	1	0.879
HS3	0.226	1	.	0.98
Control	0.006	0.879	0.98	.

CA2	HS1	HS2	HS3	Control
HS1	.	0.156	0.397	0.012
HS2	0.156	.	0.991	0.724
HS3	0.397	0.991	.	0.651
Control	0.012	0.724	0.651	.

CA4	HS1	HS2	HS3	Control
HS1	.	0.029	0.321	0.003
HS2	0.029	.	0.656	0.879
HS3	0.321	0.656	.	0.866
Control	0.003	0.879	0.866	.

The 23 cases in our HS type 1 category had a rate of 76.1% neuron loss in CA1, 56.7% in CA2, 65.8% in CA3 and 68.7% in CA4. In the HS type 1 category, CA1 and CA4 sectors have considerably less neuron density when compared to controls (p=0.003 in both). Among the three cases of HS type 2 at hand, neuron loss rates were 61.5%, 24.3%, 12.9% in sectors CA1, CA2 and CA3, respectively, while there was no significant neuron loss in CA4. In HS type 2, which is characterized by CA1 neuron loss, there is no statistically significant loss in CA1 sector among our cases (p=0.114). In our HS type 3 group (n=2), CA4 neuron density was not statistically different from the controls (p=0.866). However, the CA4 neuron loss rate of 35.7%, clearly higher than that of other sectors in the HS type 3 category (26.3%, 30.2% and 10.3% losses in sectors CA1, CA2 and CA3 respectively), was noted (Table 2).

DISCUSSION

Histopathology, like in all other acts of pathological assessment, is of paramount importance in HS diagnosis. However, semi-quantitative evaluation, like ILAE criteria, possess the inherent possibility of inter- and intra-observer variability/discrepancy. Most of the time, such variability can be corrected and repeatability enhanced by stepping out of tradition and implementing morphometry-based techniques. Morphometry, in its broadest sense, is the quantitative assessment of size and shape. In pathology, morphometry aims to reach reproducible and accurate data from cell and tissue samples [10]. Technological advances allow for computer-based morphometric analysis of raw data from digitalized slides [11].

With the forthcoming inevitable addition of other modalities such as deep learning and artificial intelligence to this mix, histopathologic subtyping, classification and grading will become experience-independent endeavours [12].

Hippocampus is a part of the archicortex and histologically consists of CA sectors, dentate gyrus, fimbria, subiculum, parasubiculum and entorhinal cortex. In its simplest sense, it is made up of neuronal layers folded into a "C" shape. One of these neuronal layers is the dentate gyrus. The other contains pyramidal neurons and is named the cornu ammonis, due to its resemblance to the horn of the ancient Egyptian god Ammon Ra. Cornu ammonis consists of 4 sectors, numbered consecutively from 1 to 4 [13].

The widely-used ILAE 2013 classification scheme divides HS into three categories depending on pyramidal neuron loss, dentate gyrus neuron loss and gliosis patterns in the 4 CA sectors [4,5]. Despite good interobserver agreement overall, subjectivity is still an issue in semi-quantitative assessment of neuronal loss in different hippocampal sectors [4]. Diagnostic criteria themselves don't reveal stiff cut-off points; for example, for the CA4-dominant HS type 3, criteria suggest neuronal loss rates of <20%, <25%, <30% and "approximately" 50% for sectors CA1, CA2, CA3 and CA4, respectively [4]. For determining the rates of pyramidal neuron loss required by the classification, the reviewer needs to have a visual grasp on the normal neuronal densities of all CA sectors.

The neuron loss rates of the cases reported in our facility are, for the most part, quite close to those recommended by the ILAE 2013 classification [4] (Table 2). In HS type 2 category, our cases displayed a lower percentage of neuron loss than recommended in CA1 sector (61.5% vs 80%), a higher rate of neuron loss in CA2 sector (24.3% vs <20%) and in contrast, a higher percentage of CA4 sector neurons than controls (7.9% higher than controls). In HS type 3 category, a slightly higher rate of neuron loss was detected in sector CA1 (26.3% vs the recommended <20%) and CA2 (30% vs 30.2% vs the recommended <25%); sector CA4 registered a lower rate of neuron loss in our cases than recommended (35.7% vs 50%). It is logical to claim that a higher than control neuron count on CA4 might stem from possible miscount of Neu-N positive dentate gyrus neurons as CA4 neurons; these two are intimately close [13]. Morphological differences of these two types of cells are well delineated elsewhere [13]. Whether other discrepancies are a result of low case numbers in each HS group or true phenotypical variance in the Turkish population remains to be seen, preferably in bigger cohorts.

The present study has obvious limitations. The number of cases in the HS type 2 and 3 categories are low, which render any statistical test relatively indecisive. For example, despite the 61.5% neuron loss in CA1 sector of our HS type 2 cases, there is no statistically significant difference between CA1 sectors of our HS type 2 cases and controls (p=0.114). During the actual reporting process, the experienced neuropathologist reviewed all serial slides of the case, including special stains and considered relevant clinical data; also assessed the dentate gyrus neurons and gliosis in the process. Yet the cell counts used in the present study involves a pathologist with relatively low experience in the field that used a single hippocampus section to pick a single representative photograph per CA sector. Relevant literature emphasizes the importance of increasing the numbers of regions of interest (ROI) assessed and having experienced pathologists determine the ROIs [12]. Dentate gyrus abnormalities were also not specifically addressed.

These results are however promising for the utilization of digital pathology-enhanced morphometry as an ancillary technique in pathology. Even a manual Neu-N count done on photomicrographs taken on x10 or x20 magnification with the aid of a simple image processor can aid a regular pathologist with relatively limited experience in hippocampal pathology diagnose hippocampal sclerosis.

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Author contribution

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

Ethical approval

The study was approved by the Hacettepe University Non-interventional Clinical Research Ethics Board (Protocol no. 2022/09-67/31.05.2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Blumcke I, Aronica E, Miyata H, et al. International recommendation for a comprehensive neuropathologic workup of epilepsy surgery brain tissue: A consensus Task Force report from the ILAE Commission on Diagnostic Methods. Epilepsia. Mar 2016;57(3):348-58. doi:10.1111/epi.13319

[2] Öz BS, F. Pathologic Evaluation of Epilepsy Surgery. Epilepsi. 2012;18(1):53-59. doi:10.5505/epilepsi.2012.88528

[3] Blumcke I, Spreafico R, Haaker G, et al. Histopathological Findings in Brain Tissue Obtained during Epilepsy Surgery. N Engl J Med. Oct 26 2017;377(17):1648-1656. doi:10.1056/NEJMoa1703784

[4] Blumcke I, Thom M, Aronica E, et al. International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a Task Force report from the ILAE Commission on Diagnostic Methods. Epilepsia. Jul 2013;54(7):1315-29. doi:10.1111/epi.12220

[5] Thom M. Review: Hippocampal sclerosis in epilepsy: a neuropathology review. Neuropathol Appl Neurobiol. Aug 2014;40(5):520-43. doi:10.1111/nan.12150

[6] Thom M, Liagkouras I, Elliot KJ, et al. Reliability of patterns of hippocampal sclerosis as predictors of postsurgical outcome. Epilepsia. Sep 2010;51(9):1801-8. doi:10.1111/j.1528-1167.2010.02681.x

[7] Gunbey C, Soylemezoglu F, Bilginer B, et al. International consensus classification of hippocampal sclerosis and etiologic diversity in children with temporal lobectomy. Epilepsy Behav. Nov 2020;112:107380. doi:10.1016/j.yebeh.2020.107380

[8] Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. Jul 2012;9(7):671-5. doi:10.1038/nmeth.2089

[9] The Jamovi Project. Version 1.6. 2021. https://www.jamovi.org

[10] Collan Y. Morphometry in pathology: another look at diagnostic histopathology. Pathol Res Pract. Nov 1984;179(2):189-92. doi:10.1016/S0344-0338(84)80126-0

[11] Hamilton PW, Bankhead P, Wang Y, et al. Digital pathology and image analysis in tissue biomarker research. Methods. Nov 2014;70(1):59-73. doi:10.1016/j.ymeth.2014.06.015

[12] Kubach J, Muhlebner-Fahrngruber A, Soylemezoglu F, et al. Same same but different: A Web-based deep learning application revealed classifying features for the histopathologic distinction of cortical malformations. Epilepsia. Mar 2020;61(3):421-432. doi:10.1111/epi.16447

[13] Duvernoy HM. The human hippocampus : functional anatomy, vascularization, and serial sections with MRI. 3rd ed. Springer; 2005:viii, 232 p.

acta medica

ORIGINAL ARTICLE

Bioinformatic Analysis of Expression Pattern and Prognostic Value of Oxidoreductase ERO1L in Pancreatic Cancer

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ABSTRACT

Objective: As people continue to succumb to the progression of various forms of cancer, the extreme lethal nature of pancreatic cancer in particular suggests that new therapeutic targets and novel regulatory mechanisms need to be explored.

Materials and Methods: We examined ERO1L expression in different cancer types using cBioPortal and Oncomine exploration tools. Next, we analyzed ERO1L levels in pancreatic cancer and healthy tissues via online public databases. The prognostic value of ERO1L and its correlation with clinopathological features were investigated using the UCSC, TNMplot and cBioPortal databases. The correlation analyses were then performed using data obtained from GEPIA, cBioPortal and the Gene Expression Omnibus.

Results: The enzyme ERO1L was found to be highly expressed in pancreatic cancer and elevated in tumor compared to healthy tissue. Its levels correlated with the hypoxia level and ER stress activation status of the pancreatic cancer tissues. ERO1L and VEGFA levels were also found to be correlated exclusively in tumor tissue, thus underlying its pro-oncogenic nature.

Conclusion: Oxidoreductase ERO1L is a potential prognostic marker and its oncogenic effects might be regulated via hypoxia/ER stress/ERO1L/VEGFA axis in pancreatic cancer.

Keywords: ERO1L, hypoxia, pancreatic cancer, ER stress, VEGFA

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INTRODUCTION

Cancer is a worldwide health problem causing the death of millions of people each year [1]. Pancreatic cancer accounts for about 7% of these deaths while its intense lethal character results in a 5 year survival rate of less than 9% [2]. Only a few newly diagnosed patients are eligible for surgical resection [3] and the prevalent detection of distant metastasis and local recurrence renders the use of systemic chemotherapy (i.e gemcitabine, FOLFILINOX), or radiation therapy as the main treatment options. However, the poor response rate indicates that there is an urgent need for understanding the molecular mechanisms of pancreatic cancer progression and finding novel therapy targets.

The endoplasmic reticulum(ER) is an organelle in a cell where newly synthesized proteins are folded to be delivered to their final destination. The formation of both intermolecular and intramolecular disulfide bonds play a key role in this folding process [4]. Protein disulfide isomerase (PDI), by interacting with endoplasmic reticulum Oxidoreductase 1(ERO1), is the enzyme responsible for the formation of these bonds [5]. During this process, PDI is reduced

upon oxidizing proteins and which leads to the formation of disulfide bridges. ERO1 reoxidizes PDI thus preparing it for an upcoming cycle of protein folding. Perturbations in this machinery causes the formation of misfolded proteins which results in disrupted ER homeostasis and organelle stress.

Ero1 exists in two isoforms being Ero1-alpha(ERO1L) and Ero1-beta(ERO1LB). Interestingly, while the transcription of both isoforms can be regulated by ER stress(ERS), only ERO1L shows responsiveness to hypoxia which is a well known pathological hallmark of cancer thus making the ERO1L an interesting candidate for cancer research [6-8]. It has been reported that ERO1L is amply expressed in a variety of tumors and its expression shows association with poor prognosis [8-11]. ERO1L was found to promote angiogenesis by regulating vascular endothelial growth factor A (VEGFA) at the both transcriptional and post-translational level [12]. This regulation also plays a significant role in the metastatic potential of breast cancer[13]. The ERO1L/VEGFA axis also modulates key oncogenic features in hepatocellular carcinoma [11]. The profound presence of two ERO1L promoting insults, being hypoxia and ERS [14], and increased VEGFA levels [15] in pancreatic cancer prompted us to speculate that hypoxia/ERS/ERO1L/VEGFA might be an axis regulating pancreatic cancer progression.

In the present study, we evaluated the role of ERO1L in pancreatic cancer progression using in silico analysis tools. We primarily investigated if ERO1L level is increased in pancreatic cancer compared to other cancers and further upregulated in tumor compared to healthy tissue. We then examined ERO1L's association with prognostic and clinopathological parameters and investigated a potential pathway that might facilitate ERO1L-driven carcinogenesis.

MATERIALS AND METHODS

ERO1L expression analysis among different cancer types

The expression levels of ERO1L among different cancers were analyzed using publicly available exploration tools namely cBioPortal (http://www.cbioportal.org/index.do) [16,17] and Oncomine (https://www.oncomine.org/resource/login.html) [18]. The Tumor Cancer Genome Atlas Pan Cancer data set (TCGA Pan Can) and Bittner Multi-cancer data set, Ramaswamy Multi-cancer data set were used in cBioPortal and Oncomine respectively to analyze ERO1L transcript levels in different tumor types.

Examination of gene transcript levels across Normal and Tumor tissue

Comparison of the transcript level of ERO1L between normal and tumor tissue was undertaken using data obtained from the Oncomine database [18]. Statistical analysis of the data was performed using Students' t-test with threshold search criteria of fold change>2, p-value<0.01 and gene ranking: 1% [19]. The TNMplot online database (https://www.tnmplot.com/) [20] online database contains omics data from various sources. The distribution of ERO1L expression in diversified cancers and corresponding healthy tissues was examined using this bioinformatic tool. To verify differential expression in TNMplot, the Mann-Whitney U test was used and significant differences with $p<0.05$ were marked with the (*) sign. An ERO1L, HIF1A, DDIT3 and HSPA5 expression profile between pancreatic cancer and its complementary normal tissue was also obtained using the Gene Expression Profiling Interactive Analysis (GEPIA2) website (http://gepia2.cancer-pku.cn/) [21]. The GTEx (Genotype-Tissue Expression) and TCGA data were then matched using the ANOVA differential method and the analysis was performed using the default threshold settings. Differentially expressed genes (DEGs) in the pancreatic cancer dataset GSE28735 from GEO (http://www.ncbi.nlm.nih.gov/geo/) were determined using GEO2R and utilizing the following threshold criteria: $|\log FC|\geq 1$, $p<0,05$. It is known that GSE28735 contains 45 samples of pancreatic tumor and their adjacent non-tumor tissue [22].

Survival analysis

RNA-seq expression values for ERO1L and the clinical data of pancreatic cancer patients were obtained from TCGA-PAAD data set. Analysis of Kaplan-Meier overall survival, disease specific survival, disease free interval undertaken using data recorded in the UCSC Cancer Genomics Browser (https://genome-

cancer.ucsc.edu) [23]. A log-rank test (test statistics and p-value) was then conducted for the statistical analysis of ERO1L high and and low groups. p-value<0.05 indicates statistical significance.

Analysis of ERO1L expression in tumors having different clinopathological features

The expression data of ERO1L in tumors and clinopathological features (recurrence, KRAS status and survival status) of the corresponding patients was obtained from the TCGA-PAAD data set and analyzed using cBioPortal. Results were presented as mean ± SD. Student's t-tests were performed to compare the difference between two groups. A violin plot showing the correlation between ERO1L and histological grade was produced using data sourced via the UCSC Cancer Genomics Browser. One-way ANOVA test was used to determine differential expression of ERO1L among three different histological grades. The expression of ERO1L in normal, cancerous and metastatic tissues were compared using TNMplot and the Kruskal Wallis test was used to assess statistical significance. The differences being considered statistically significant if p<0.05.

Hypoxia Score and ERO1L expression analysis

Winter hypoxia scores, buffa hypoxia scores and an expression heatmap showing ERO1L expression z-scores from the TCGA-PAAD dataset were obtained from cBioPortal. Hypoxia scores were determined for the $ERO1^{high}$ group (expression z-score>1) and $ERO1^{low}$ group (expression z-score<-1). Student's t-test was then used to determine statistical significance between groups.

Correlation analysis

The correlation between ERO1L, HIFA vs. ER stress signature genes (a gene set comprised of 113 genes obtained from GSEA Molecular Signatures Database HALLMARK_UNFOLDED_PROTEIN_RESPONSE) and ERO1L vs. VEGFA was analyzed using the GEPIA2 database. Correlation analysis among aforementioned parameters were performed by Pearson's correlation test. ERO1L expression levels and winter, buffa hypoxia scores of corresponding tumors in the TCGA-PAAD dataset were obtained using cBioPortal. The correlation between ERO1L vs. Winter Hypoxia Score and ERO1L vs. Buffa hypoxia score was then analyzed using Pearson's correlation test. Likewise, the correlation between VEGFA and ERO1L expression in pancreatic non-tumor and tumor tissues in the GSE28735 dataset was identified by Pearson's correlation tests.

RESULTS

The transcript levels of ERO1L is increased in pancreatic tumors

The unique expression profile of cancer tissues is used for several aims including biomarker discovery, cancer subtype identification, survival prediction and novel therapeutic target determination [24]. To establish if ERO1L has a possible role in cancer progression, we first aimed to determine its differential expression pattern in numerous cancers. Analyses using several databases revealed that ERO1L is highly expressed in tumors and pancreatic cancer is among the ten cancer types with highest ERO1L expression (Figure 1A). To further support this notion, different datasets were also examined using in silico analysis tools and ERO1L was found to be expressed significantly higher in pancreatic cancer compared to other cancer types (Figure 1B-C).

Next, we wanted to ascertain if the increasing transcript levels of ERO1L are unique for tumor tissue thus we compared ERO1L levels in tumor and normal tissues using ONCOMINE. Pan-cancer analysis results displayed that ERO1L is upregulated in 43 datasets associated with the bladder, brain and CNS, breast, colorectal, gastric, kidney, lung, lymphoma, ovarian, pancreatic, prostate cancer whereas 16 datasets showed opposite results (Figure 2A). The increased ERO1L expression in tumor tissues including pancreatic cancer was also verified using the TNMplot (Figure 2B) and GEPIA (Figure 2C) databases. Last but not least, differential gene expression analysis of 45 matching pancreatic cancer and healthy tissues from GSE28735 supported lower levels of ERO1L in normal tissue (Figure 2D). Overall, these results suggest that ERO1L might play a specific role in pancreatic cancer.

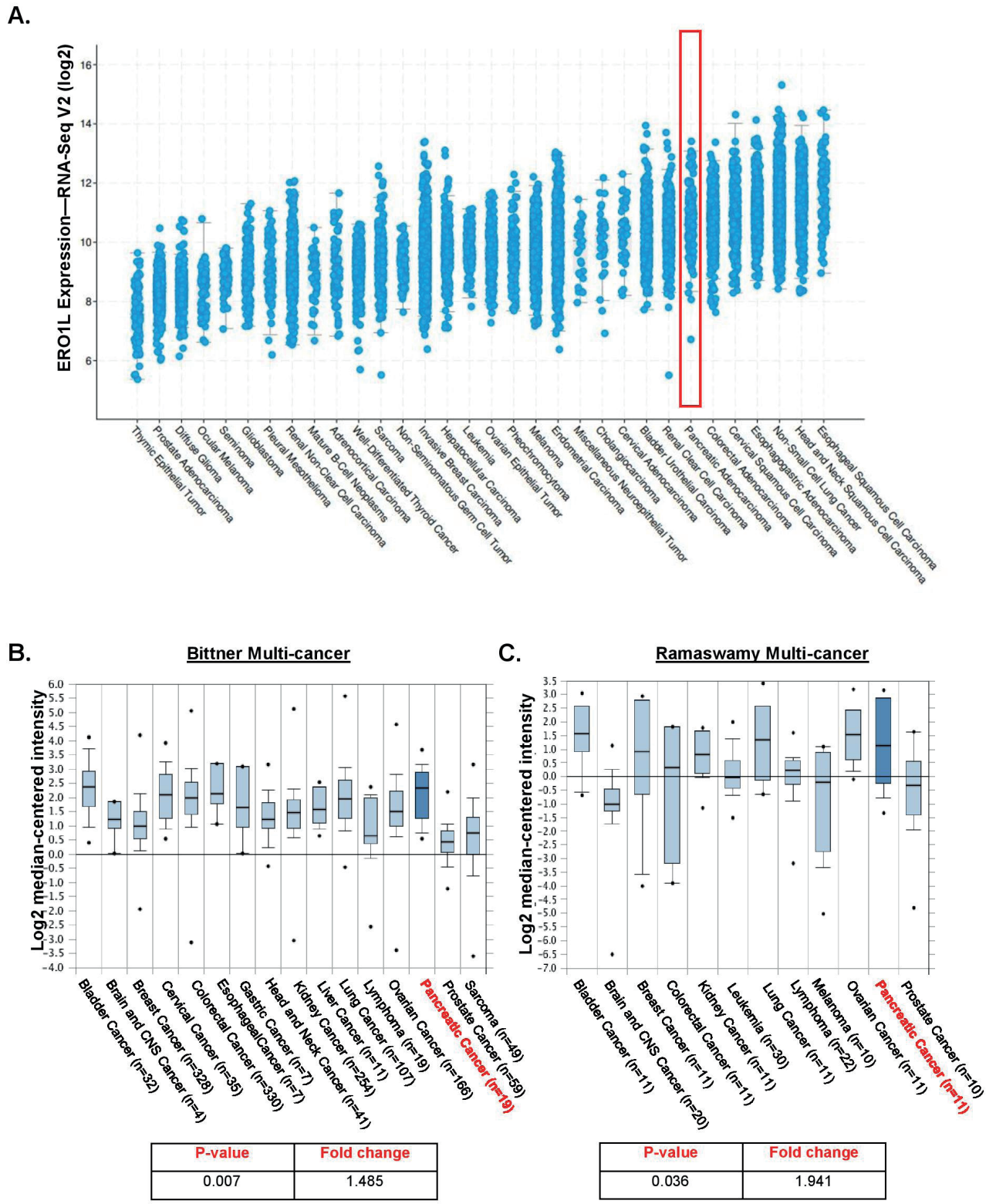


Figure 1. Expression pattern of ERO1L across cancers. A) Abundance of ERO1L was analyzed using the cBioPortal database. Cancer types aligned based on their median ERO1L transcript level. Gene expression profiling of ERO1L in B) Bittner and C) Ramaswamy multi-cancer datasets from Oncomine indicate remarkably high ERO1L expression in pancreatic cancer compared to other types of cancer. p<0,05 shows statistical significance.

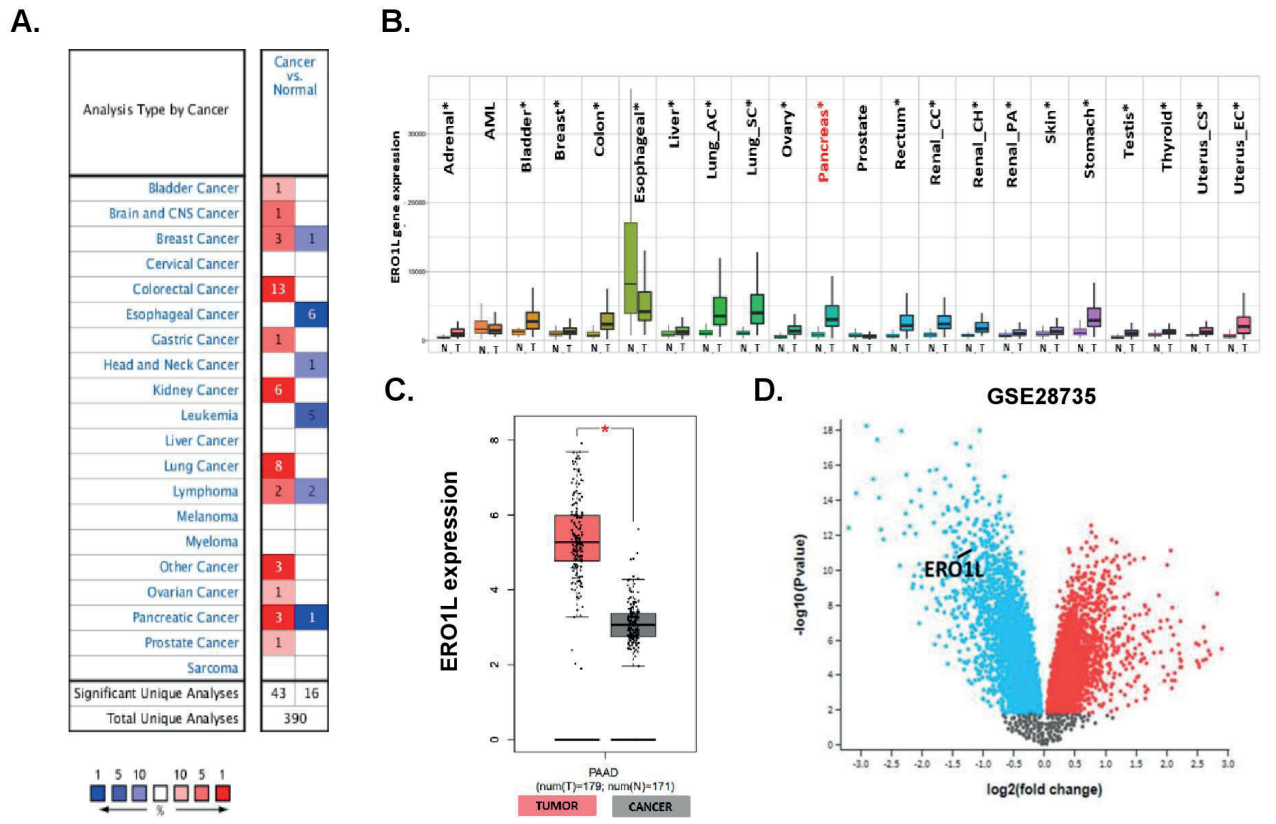


Figure 2. Differential expression of ERO1L in pancreatic normal and tumor tissues. A) The ERO1L expression levels were analyzed between normal and tumor tissues using the Oncomine database. The plot shows the numbers of datasets with higher (red) or lower (blue) ERO1L levels in cancer tissue compared to corresponding normal tissue. B) Relative ERO1L expression was investigated between healthy and cancer tissue using TNMplot. The data illustrates a universal upregulation of ERO1L in tumor tissues. C) The expression pattern of ERO1L between tumor (TCGA) and normal (GTEX) pancreatic tissue was investigated by GEPIA2 using ANOVA differential method. * indicates p-value<0.01. D) ERO1L was found to be among DEGs between pancreatic tumor and non-tumor tissue in GSE28735 and was shown lower expression in normal counterpart (p value: 5,58e-12, log FC: -1,166).

ERO1L gene expression is associated with poor prognosis in pancreatic cancer patients

The predictive value of ERO1L in pancreatic cancer was determined with UCSC. We used TCGA pan-cancer data to analyze survival differences between ERO1L high(red line) and ERO1L low(blue line) groups by using the ERO1L median expression level as the cutoff. Log-rank test showed that in pancreatic cancer patients, the high ERO1L expression group had a shorter overall survival (Figure 3A), disease specific survival (Figure 3B), disease free interval (Figure 3C) and progression free interval (Figure 3D). Collectively, this data indicate that pancreatic cancer patients with high ERO1L expression have a shorter survival time than those with low ERO1L expression.

ERO1L transcript levels shows positive correlation with clinical behaviour of pancreatic cancers

We also examined if ERO1L levels are associated with the clinical features of pancreatic cancers. Analysis using the TCGA dataset in cBioPortal revealed that recurring pancreatic cancer patients showed elevated ERO1L levels compared to disease-free patients (Figure 4A). We also analyzed ERO1L levels in pancreatic cancer patients expressing WT or mutated KRAS. KRAS plays a pivotal role in signaling pathways regulating cancer progression. Mutations causing constitutive activation of KRAS are commonly seen in pancreatic cancer and shown to not only promote the proliferative and migrative capacity of cells

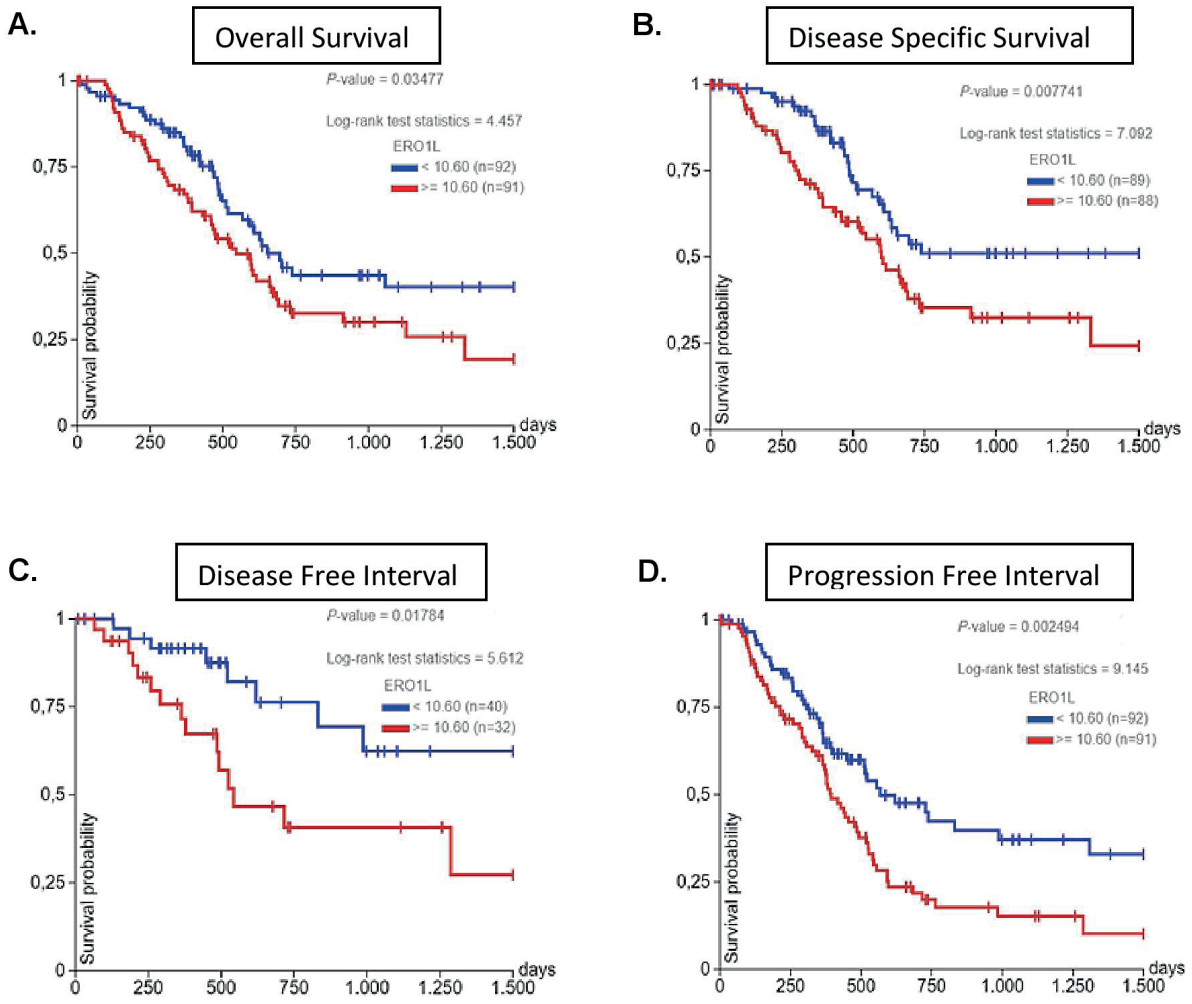
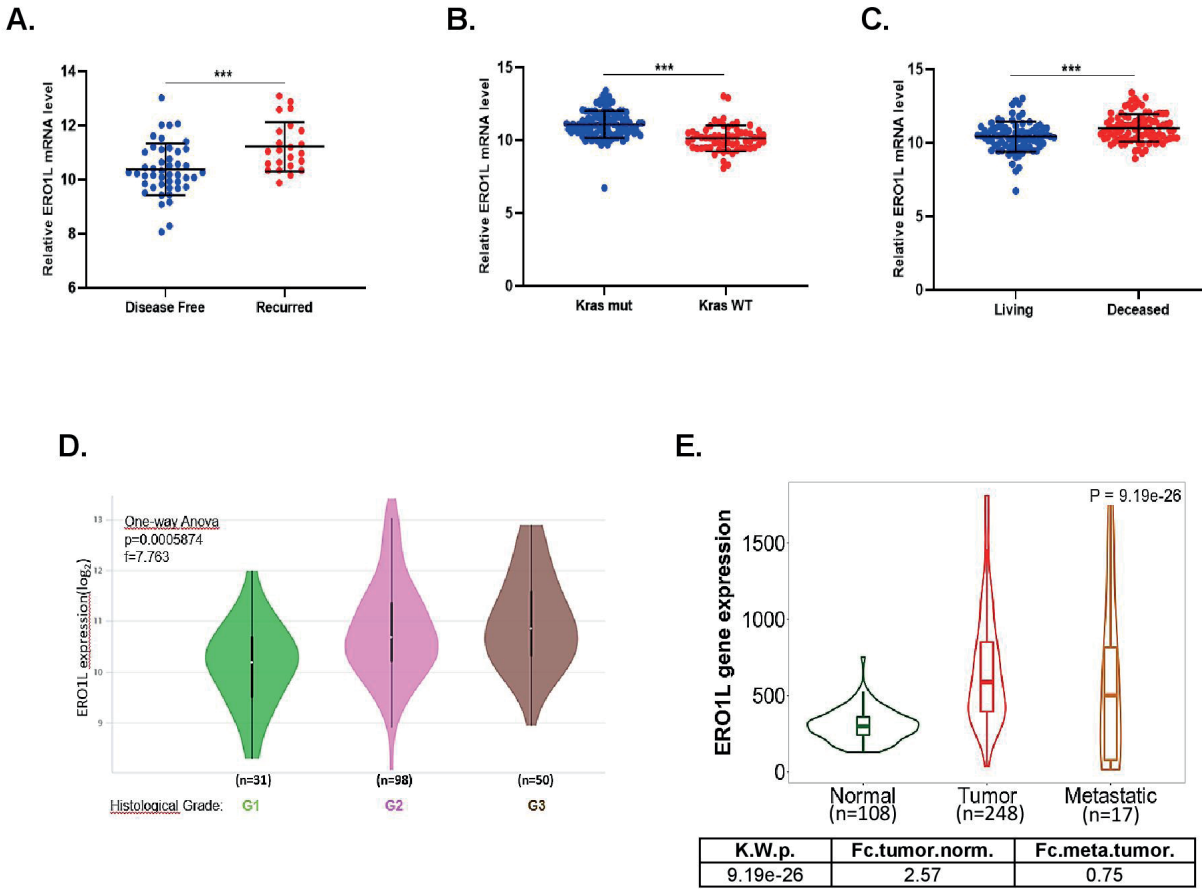


Figure 3. Prognostic value of ERO1L in pancreatic cancer. Kaplan-Meier curves comparing high and low expressions of ERO1L show that higher ERO1L expression (red line) results in shorter A) Overall survival, B) Disease specific survival, C) Disease free interval and D) Progression free interval. p<0,05 indicates statistical significance.

but also confer survival disadvantage[25,26]. Our results revealed that tumors carrying mutant KRAS also have higher ERO1L levels (Figure 4B). In addition, pancreatic tumor tissues of deceased patients showed increased ERO1L expression compared to tissues obtained from living patients (Figure 4C). To further evaluate ERO1L's correlation with clinical parameters, we used the UCSC database to investigate ERO1L's transcript levels in tumors with different histological grades. ERO1L was upregulated gradually along with the increased histological grade (Figure 4D). ERO1L levels were also higher in metastatic pancreatic tumor compared to normal tissues. Altogether, these findings strongly suggest that ERO1L has a pro-oncogenic role and hence can be used as a biomarker and therapeutic target.

Hypoxia is a master regulator of ERO1L expression level in pancreatic tumors

Next, we sought to understand the molecular basis of ERO1L upregulation in pancreatic tumors. It is known that oxygen deprivation occurs in tumors when cancer cells multiply rapidly, a phenomenon known as hypoxia. Hypoxic tumors show resistance to therapy and have a more aggressive phenotype [27]. Tumors with high ERO1L levels also show prevalent hypoxia [28] indicating a possible connection between them. This connection was verified in lung adenocarcinoma. Lung cancers with high ERO1L levels have been shown to carry a prominent hypoxic signature [29]. The severe hypoxic nature of pancreatic cancers also prompted us to investigate if this hallmark of cancer might regulate ERO1L levels in pancreatic tumors [30].



K.W.p.	Fc.tumor.norm.	Fc.meta.tumor.
9.19e-26	2.57	0.75

Figure 4. The relationship between ERO1L expression and clinopathological features. Log2-transformed ERO1L mRNA expression levels in the A) recurring or disease-free B) Kras mutant or Kras WT C) deceased or living PAAD samples was examined using cBioPortal. D) Violin plot showing the association between ERO1L expression and histological grade in patients with pancreatic cancer(UCSC). E) Boxplot comparing ERO1L levels in paired normal, tumor and metastatic tissues from gene chip data at TNMplot. (*** means p<0.001)

We used the TCGA dataset in cBioPortal and explored the correlation between ERO1L levels and hypoxia. Our analyses revealed that tumors with strong ERO1L expression exhibited significantly elevated Winter (Figure 5A-B) and Buffa (Figure 5D-E) hypoxia scores. These findings were also supported by the observed positive correlation of ERO1L expression levels with Winter (Figure 5C), and Buffa (Figure 5F) hypoxia scores. Taken together, these results clearly demonstrate that hypoxia modulates ERO1L expression in the pancreatic tumor microenvironment.

Hypoxia regulates ERO1L levels via ERS activation

The disturbed homeostasis in the tumor environment triggers the formation of ERS activating stimuli including hypoxia [31] thus ERS activation is prevalent in tumor tissues [32]. Previous studies showed that ERS activation regulates ERO1L levels on a trancriptional level [7,8,33]. Based on these findings we hypothesized that ERS might be the intermediary modulator between hypoxia and ERO1L transcription. To verify this link we first investigated if hypoxia and ERS are upregulated in pancreatic cancer tissue. Analysis using the TCGA and GTEx datasets in GEPIA revealed that hypoxia marker HIF1A (Figure 6A) [34] and ERS activation markers DDIT3 (Figure 6B) and HSPA5 (Figure 6C) [35] are upregulated in pancreatic tumor tissues. These results imply a functional hypoxia and ERS axis in the pancreatic tumor microenvironment. We then sought to establish whether ERS is regulated by hypoxia in tumor tissue. The correlation analysis tool in GEPIA revealed that HIF1A shows a strong correlation with ERS activation signature (Figure 6D) indicating the validity of this regulation. Close correlation of ERS signature and ERO1L levels

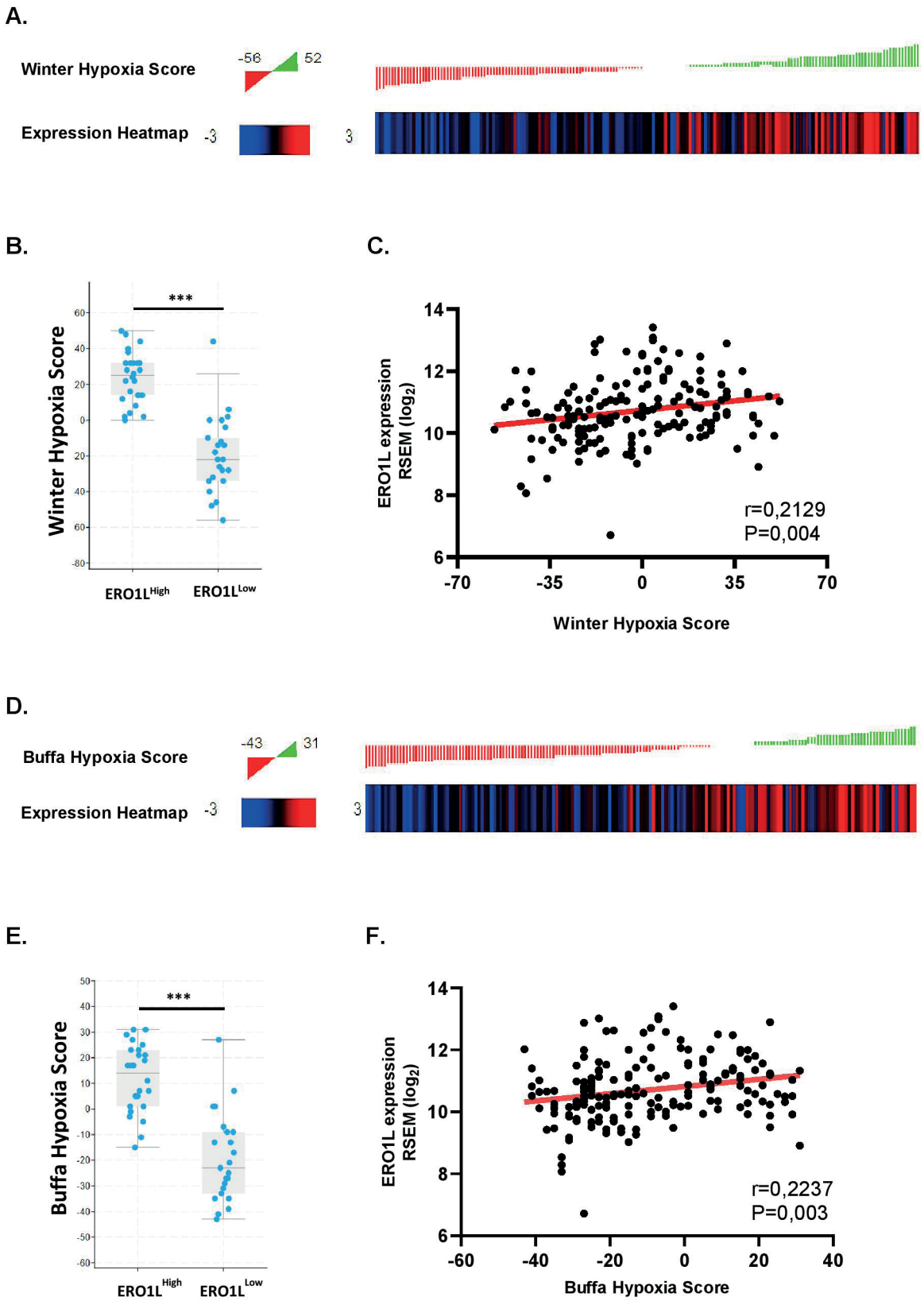


Figure 5. ERO1L is upregulated with hypoxia A) The score bar and heatmap showing winter hypoxia score and ERO1L expression z-scores respectively. B) Winter hypoxia scores in ERO1high and ERO1low tumors in TCGA-PAAD cohort. C)The correlation between ERO1L expression and winter hypoxia score in 177 TCGA pancreatic cancer tissues. D) The score bar and heatmap showing buffa hypoxia score and ERO1L expression z-scores respectively. E) Buffa hypoxia scores in ERO1high and ERO1low tumors in TCGA-PAAD cohort. F) The correlation between ERO1L expression and buffa hypoxia score in 177 TCGA pancreatic cancer tissue. p<0,05 indicates statistical significance. (*** means p<0.001)

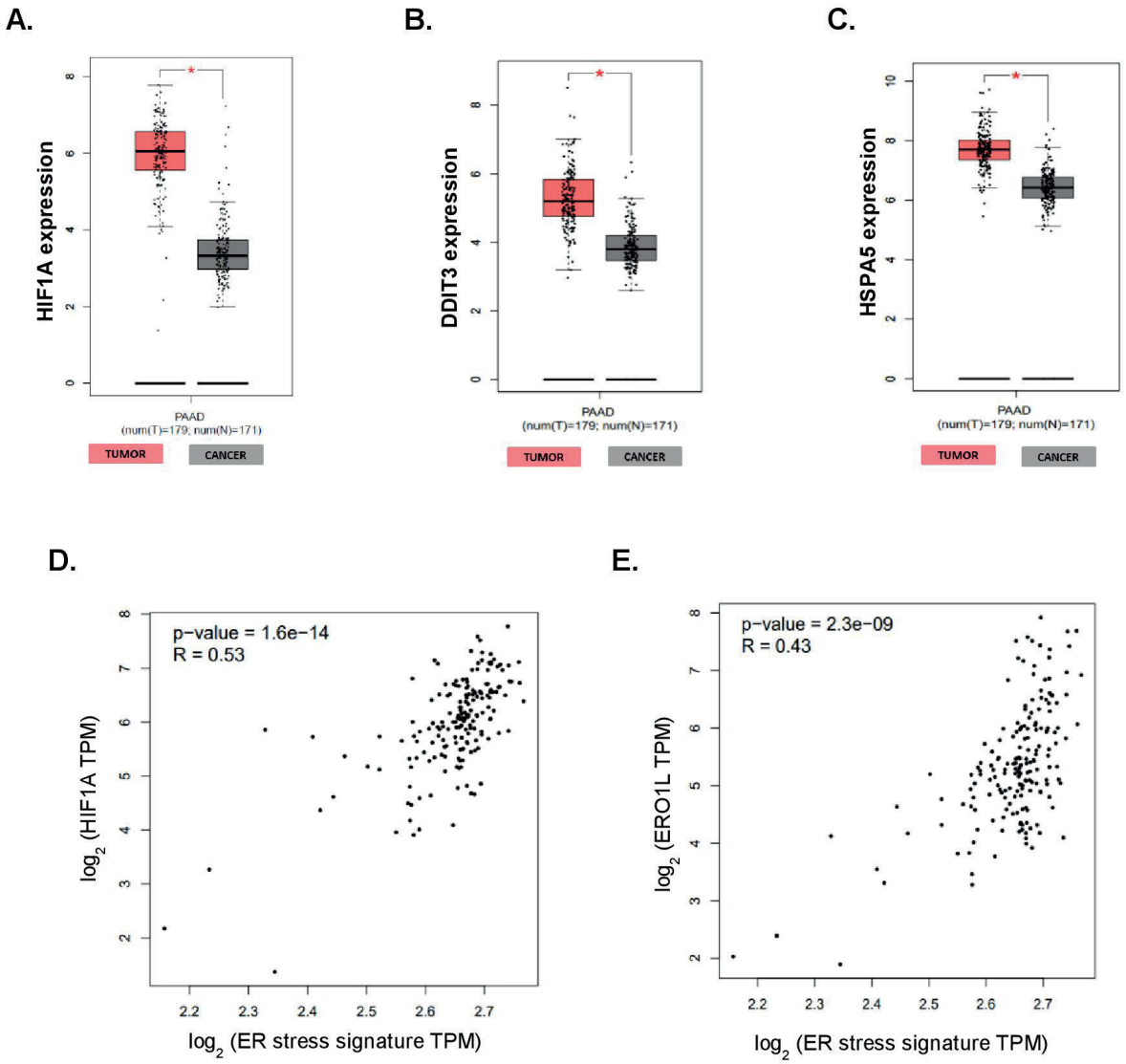


Figure 6. Hypoxia and ER stress markers' correlation with ERO1L expression. Box plots derived from GEPIA comparing the expression of A) hypoxia (HIF1A) and B,C) ER stress (DDIT3 and HSPA5) markers in PAAD(TCGA) and normal tissues(GTEx). The positive correlation of D) hypoxia marker HIF1A and E) ERO1L with ER stress related genes were analyzed by GEPIA (* means p<0.01).

(Figure 6E) strongly suggest a critical role for ERS in ERO1L upregulation. In brief, these results provide strong evidence that hypoxia driven ERS activation modulates ERO1L levels and accounts for the poor prognosis of patients with pancreatic cancer.

ERO1L and VEGFA are exclusively correlated in cancerous tissues of pancreas

Metastasis occurs when cancer cells spread from the primary site to other body parts. The low 5-year overall survival rate of pancreatic cancer patients is further reduced if their tumors are metastatic [36]. ERO1L's higher expression in metastatic pancreatic cancer (Figure 4E) and its significant contribution to poor patient prognosis (Figure 3A) led us to speculate that ERO1L might be involved in the regulation of genes that are involved in metastasis. VEGFA has been shown to stimulate tumor metastasis [37] and its transcript and secretion levels have been regulated by ERO1L especially in a hypoxic environment [38]. To better understand if this also holds true for pancreatic cancer, we analyzed the correlation between ERO1L and VEGFA expression. Our data revealed that ERO1L expression level was not correlated with VEGFA levels in healthy tissue (Figure 7A) whereas there was a strong correlation in tumor tissue

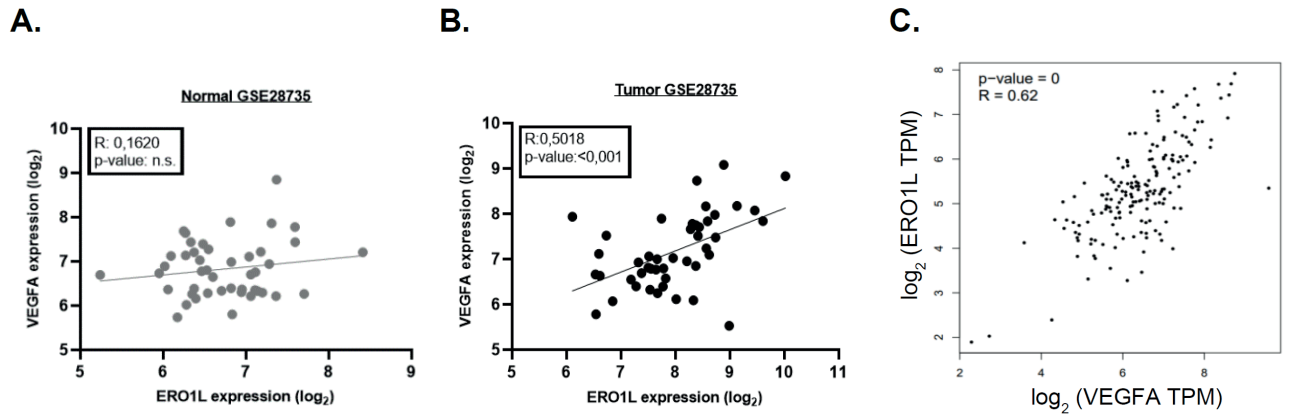


Figure 7. Correlation analysis of VEGFA and ERO1L in tumor and normal tissue A)The lack of correlation was observed between ERO1L and VEGFA in normal pancreatic tissues of PAAD patients based on the GSE28735 data set. The positive correlation of VEGFA and ERO1L was observed in pancreatic cancer tissues based on the B) GSE28735 data set and C) TCGA cohort analyzed by GEPIA. p<0,05 was used as the significance threshold.

(Figure 7B-C). Together, these results provide strong evidence that ERO1L is a principal prognostic marker in pancreatic cancer. Its levels were regulated via hypoxia-ERS axis and its contribution to tumor progression might very well be driven by VEGFA modulation in tumor tissues.

DISCUSSION

The strong proliferative nature of cancer cells is thought to accompany the high protein expression required during the formation of new cells. These proteins need to be properly folded in the ER prior to taking up their final positions in the cell. Disulfide bonds play a crucial role in the folding process and their formation is regulated by PDI/ERO1L axis. Thus, a disturbance in this pathway not only impair organelle homeostasis but also distort the protein repertoire which in turn may modulate oncogenic events in tumors [4,5]. ERO1L levels are known to be regulated by hypoxia and ERS [6,7]. Moreover, ERO1L controls VEGFA levels, a well known angiogenic molecule, transcriptionally and post-transcriptionally [12]. However, the role of the hypoxia/ERS/ERO1L/VEGFA axis in human pan-cancer has not been identified and whether ERO1L can be used as a biomarker or therapeutic target is still obscure.

In our study, we have focused on pancreatic cancer due to prominent presence of ERO1L increasing stimuli, being hypoxia and ERS [14]. Our results also verified the notable hypoxic and ERS active nature of pancreatic cancers by showing the presence of upregulated hypoxia marker HIF1A and ERS markers DDIT3, HSPA5 in tumors compared to healthy tissues. The existence of prevalent ERO1L augmenting stimuli in pancreatic cancer is supported by our data showing ERO1L's significantly higher expression levels in pancreatic cancer compared to other cancer types. Moreover, its expression profile is upregulated in pancreatic tumor compared to normal tissues. Notably, we detected a strong prognostic value for ERO1L in pancreatic cancer as well. Patients with high ERO1L levels had lower overall survival and disease specific survival. This data was also supported by the increased ERO1L levels seen in deceased patients. In addition, high ERO1L also resulted in shortened disease free interval and progression free interval. The elevated ERO1L levels in recurred patients were in line with this finding and suggested that regulating ERO1L levels might be a valuable tool for follow-up treatment. The presence of KRAS mutations in pancreatic cancer is a well known indicative for poor prognosis [39]. KRAS mutation plays a pro-oncogenic role and there are no drugs directly targeting mutant KRAS which in turn making the treatment of pancreatic cancer patients carrying KRAS mutation harder. Our results revealed increased ERO1L levels in tumors carrying mutant KRAS thus showing that ERO1L might be a used as a novel therapy target in these patients. Overall, our results showed that ERO1L was a plausible oncogenic gene with a prognostic value in pancreatic cancer. Additionally, based on the results from the Oncomine and TNMplot, ERO1L

expression is upregulated in many other cancer types including bladder, breast, lung and colorectal cancer thus it might very well be that ERO1L is involved in the progression of other cancer types as well.

Previous studies pinpointed hypoxia and ERS activation as the main regulators of ERO1L expression [6,8]. The increment in ERO1L levels with increasing hypoxia and ER stress signature scores verified those findings. Since hypoxia is a well known inducer of ERS [31], we hypothesized that ERS activation bridges hypoxia and ERO1L expression. The strong correlation between hypoxia marker HIF1A vs. ERS and ERS vs. ERO1L indicated that hypoxia-induced ERS might very well be the axis leading to ERO1L upregulation in the tumor microenvironment.

Subsequent to determination of upstream regulators of ERO1L levels, we aimed to unveil the downstream effectors that might regulate pancreatic tumor progression. Angiogenesis plays a key role in cancer progression. ERO1L was found to be involved in VEGFA, a master regulator of angiogenesis, modulation at transcriptional and post-transcriptional level [12,28]. Analysis using gene expression data series GSE28735 and GEPIA exhibited that ERO1L shows correlation with VEGFA levels exclusively in pancreatic tumor tissues. This may also explain our data showing high ERO1L expression in metastatic tumors based on the fact that angiogenesis has an essential role in metastasis. The development of resistance against the common treatment regimen, chemotherapy has led to a continuing search for novel therapeutic targets in pancreatic cancer. The increased levels of VEGF and its receptors in pancreatic cancer makes anti-angiogenic therapy a strong candidate for its treatment. In this context, bevacizumab, a recombinant humanized monoclonal antibody that binds to VEGFA, seems to be a promising target. However, high VEGFA levels have been shown to be an underlying factor for developing resistance to bevacizumab treatment [40]. Although it is tempting to speculate that decreasing VEGFA levels by blocking ERO1L might improve bevacizumab's efficacy, further studies are warranted. Taken together, our data uncover a mechanistic axis involving hypoxia, ERS, ERO1L, VEGFA and tumor progression in pancreatic cancer.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Sung, H, Ferlay, J, Siegel, RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 71(3): 209-249.

[2] Siegel, RL, Miller, KD, Jemal, A Cancer statistics, 2020. CA Cancer J Clin. 2020; 70(1): 7-30.

[3] Strobel, O, Neoptolemos, J, Jager, D, et al. Optimizing the outcomes of pancreatic cancer surgery. Nat Rev Clin Oncol. 2019; 16(1): 11-26.

[4] Feige, MJ, Hendershot, LM Disulfide bonds in ER protein folding and homeostasis. Curr Opin Cell Biol. 2011; 23(2): 167-175.

[5] Benham, AM, van, LM, Sitia, R, et al. Ero1-PDI interactions, the response to redox flux and the implications for disulfide bond formation in the mammalian endoplasmic reticulum. Philos Trans R Soc Lond B Biol Sci. 2013; 368(1617): 20110403.

[6] Gess, B, Hofbauer, KH, Wenger, RH, et al. The cellular oxygen tension regulates expression of the endoplasmic oxidoreductase ERO1-Lalpha. Eur J Biochem. 2003; 270(10): 2228-2235.

[7] Pagani, M, Fabbri, M, Benedetti, C, et al. Endoplasmic reticulum oxidoreductin 1-lbeta (ERO1-Lbeta), a human gene induced in the course of the unfolded protein response. J Biol Chem. 2000; 275(31): 23685-23692.

[8] Zhang, J, Yang, J, Lin, C, et al. Endoplasmic Reticulum stress-dependent expression of ERO1L promotes aerobic glycolysis in Pancreatic Cancer. Theranostics. 2020; 10(18): 8400-8414.

[9] Kutomi, G, Tamura, Y, Tanaka, T, et al. Human endoplasmic reticulum oxidoreductin 1-alpha is a novel predictor for poor prognosis of breast cancer. Cancer Sci. 2013; 104(8): 1091-1096.

[10] Seol, SY, Kim, C, Lim, JY, et al. Overexpression of Endoplasmic Reticulum Oxidoreductin 1-alpha (ERO1L) Is Associated with Poor Prognosis of Gastric Cancer. Cancer Res Treat. 2016; 48(4): 1196-1209.

[11] Yang, S, Yang, C, Yu, F, et al. Endoplasmic reticulum resident oxidase ERO1-Lalpha promotes hepatocellular carcinoma metastasis and angiogenesis through the S1PR1/STAT3/VEGF-A pathway. Cell Death Dis. 2018; 9(11): 1105

[12] Tanaka, T, Kutomi, G, Kajiwara, T, et al. Cancer-associated oxidoreductase ERO1-alpha drives the production of VEGF via oxidative protein folding and regulating the mRNA level. Br J Cancer. 2016; 114(11): 1227-1234.

[13] Varone, E, Decio, A, Chernorudskiy, A, et al. The ER stress response mediator ERO1 triggers cancer metastasis by favoring the angiogenic switch in hypoxic conditions. Oncogene. 2021; 40(9): 1721-1736.

[14] Hidalgo, M Pancreatic cancer. N Engl J Med. 2010; 362(17): 1605-1617.

[15] Costache, MI, Ioana, M, Iordache, S, et al. VEGF Expression in Pancreatic Cancer and Other Malignancies: A Review of the Literature. Rom J Intern Med. 2015; 53(3): 199-208.

[16] Cerami, E, Gao, J, Dogrusoz, U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012; 2(5): 401-404.

[17] Gao, J, Aksoy, BA, Dogrusoz, U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013; 6(269): l1.

[18] Rhodes, DR, Kalyana-Sundaram, S, Mahavisno, V, et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia. 2007; 9(2): 166-180.

[19] Gou, R, Zhu, L, Zheng, M, et al. Annexin A8 can serve as potential prognostic biomarker and therapeutic target for ovarian cancer: based on the comprehensive analysis of Annexins. J Transl Med. 2019; 17(1): 275.

[20] Bartha, A, Gyorffy, B TNMplot.com: A Web Tool for the Comparison of Gene Expression in Normal, Tumor and Metastatic Tissues. Int J Mol Sci. 2021; 22(5).

[21] Tang, Z, Li, C, Kang, B, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017; 45(W1): W98-W102.

[22] Zhang, G, Schetter, A, He, P, et al. DPEP1 inhibits tumor cell invasiveness, enhances chemosensitivity and predicts clinical outcome in pancreatic ductal adenocarcinoma. PLoS One. 2012; 7(2): e31507.

[23] Goldman, MJ, Craft, B, Hastie, M, et al. Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol. 2020; 38(6): 675-678.

[24] Rapin, N, Bagger, FO, Jendholm, J, et al. Comparing cancer vs normal gene expression profiles identifies new disease entities and common transcriptional programs in AML patients. Blood. 2014; 123(6): 894-904.

[25] di Magliano, MP, Logsdon, CD Roles for KRAS in pancreatic tumor development and progression. Gastroenterology. 2013; 144(6): 1220-1229.

[26] Windon, AL, Loaiza-Bonilla, A, Jensen, CE, et al. A KRAS wild type mutational status confers a survival advantage in pancreatic ductal adenocarcinoma. J Gastrointest Oncol. 2018; 9(1): 1-10.

[27] Brahimi-Horn, MC, Chiche, J, Pouyssegur, J Hypoxia and cancer. J Mol Med (Berl). 2007; 85(12): 1301-1307.

[28] May, D, Itin, A, Gal, O, et al. Ero1-L alpha plays a key role in a HIF-1-mediated pathway to improve disulfide bond formation and VEGF secretion under hypoxia: implication for cancer. Oncogene. 2005; 24(6): 1011-1020.

[29] Liu, L, Wang, C, Li, S, et al. ERO1L Is a Novel and Potential Biomarker in Lung Adenocarcinoma and Shapes the Immune-Suppressive Tumor Microenvironment. Front Immunol. 2021; 12: 677169.

[30] Shah, VM, Sheppard, BC, Sears, RC, et al. Hypoxia: Friend or Foe for drug delivery in Pancreatic Cancer. Cancer Lett. 2020; 492: 63-70.

[31] Chen, X, Cubillos-Ruiz, JR Endoplasmic reticulum stress signals in the tumour and its microenvironment. Nat Rev Cancer. 2021; 21(2): 71-88.

[32] Han, CC, Wan, FS New Insights into the Role of Endoplasmic Reticulum Stress in Breast Cancer Metastasis. J Breast Cancer. 2018; 21(4): 354-362.

[33] Matsusaki, M, Okuda, A, Matsuo, K, et al. Regulation of plant ER oxidoreductin 1 (ERO1) activity for efficient oxidative protein folding. J Biol Chem. 2019; 294(49): 18820-18835.

[34] Bostrom, PJ, Thoms, J, Sykes, J, et al. Hypoxia Marker GLUT-1 (Glucose Transporter 1) is an Independent Prognostic Factor for Survival in Bladder Cancer Patients Treated with Radical Cystectomy. Bladder Cancer. 2016; 2(1): 101-109.

[35] Jia, SZ, Xu, XW, Zhang, ZH, et al. Selenoprotein K deficiency-induced apoptosis: A role for calpain and the ERS pathway. Redox Biol. 2021; 47: 102154.

[36] Chen, X, Liu, F, Xue, Q, et al. Metastatic pancreatic cancer: Mechanisms and detection (Review). Oncol Rep. 2021; 46(5).

[37] Kim, M, Jang, K, Miller, P, et al. VEGFA links self-renewal and metastasis by inducing Sox2 to repress miR-452, driving Slug. Oncogene. 2017; 36(36): 5199-5211.

[38] Lei, Y, Zang, R, Lu, Z, et al. ERO1L promotes IL6/sIL6R signaling and regulates MUC16 expression to promote CA125 secretion and the metastasis of lung cancer cells. Cell Death Dis. 2020; 11(10): 853.

[39] Tao, LY, Zhang, LF, Xiu, DR, et al. Prognostic significance of K-ras mutations in pancreatic cancer: a meta-analysis. World J Surg Oncol. 2016; 14: 146.

[40] Van, CE, Paccard, C, Chiron, M, et al. Impact of Prior Bevacizumab Treatment on VEGF-A and PlGF Levels and Outcome Following Second-Line Aflibercept Treatment: Biomarker Post Hoc Analysis of the VELOUR Trial. Clin Cancer Res. 2020; 26(3): 717-725.

acta medica

ORIGINAL ARTICLE

Are COVID-19 Vaccine Preference and COVID-19 Risk Differ in Individuals Living with HIV from The Normal Population?

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ABSTRACT

Objectives: Coronavirus disease 2019 (COVID-19) may have a severe course in high-risk patients and people living with Human immunodeficiency virus (HIV, PLWH)) are also in this risk group. The aim of the study was to compare the history of COVID-19, vaccination status, vaccine doses, and vaccine preferences of PLWH with the normal population.

Materials and Methods: This study was a retrospective cross-sectional survey study. The PLWH were study group and patients without chronic disease were selected as a control group.

Results: A total of 326 patients, 163 HIV positive and 163 without chronic disease, were included in the study. Of the patients, 142 (88.1%) were male, and the mean age was 46.69 ± 13.72 years. The number of patients who were not vaccinated was 36 (11.1%). When unvaccinated PLWH were evaluated, it was observed that women were less vaccinated than male patients (p=0.01). In PLWH, 145 (89.0%) of patients were vaccinated with single dose, 129 (79.1%) of patients with double dose, and 123 (75.5%) of patients with full dose; in the control group, 145 (89.0%) of patients with single dose, 131 (80.9%) of patients with double dose and 126 (77.3%) of patients with full dose were vaccinated. There was no difference between the groups in the preference of inactivated and mRNA vaccines (p=1.0). Before vaccination, 42 (12.9%) patients were infected. Twenty (12.3%) of these patients were in PLWH group, while 22 (13.5%) patients were in the control group. There were 28 (9.8%) patients who had COVID-19 during or after vaccination, and 10 (6.9%) of them were in PLWH group; 18 (12.4%) of them were in the control group.

Conclusion: In our study, no difference was found in the vaccination status, vaccine preference, vaccination doses and COVID-19 history between two groups.

Keywords: COVID-19, vaccination, HIV

INTRODUCTION

It is unclear whether people living with human immunodeficiency virus (PLWH, HIV) are at higher risk for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and for poorer clinical outcomes after infections [1]. There are many reasons to assume that PLWH are a high-risk group: Antibody responses are impaired in PLWH and there are risk factors associated with SARS-CoV-2 infection that can lead to poor clinical outcomes, including hypertension, diabetes, smoking, cardiovascular and lung disease [2-6]. As an alternative hypothesis, the results of more severe coronavirus disease 2019 (COVID-19) may be due to excessive immune activation, therefore it has been stated that PLWH may actually be at lower risk for poor clinical outcomes following SARS-

CoV-2 infection due to reduced immunological responses [7]. However, there is not yet sufficient data to support or refute any of these hypotheses [6].

With the active use of many vaccines worldwide, which can eliminate all the negative effects of the pandemic period and hope for the end of the pandemic, the return to normal life has begun. It has been observed that the disease is more controllable with vaccine applications [8]. In the first months of the vaccination campaign in Turkey, people classified as high risk group started to be vaccinated and PLWH was also included in this group [9]. Vaccination against COVID-19 is recommended by the World Health Organization (WHO) for PLWH even if there is a decreased immune response to vaccines [6,8].

In this study, it was aimed to compare the history of COVID-19, vaccination status, vaccine doses and preferences of PLWH with the normal population without chronic disease.

MATERIALS AND METHODS

Study design and patients

The study was a retrospective cross-sectional questionnaire study including patients who applied to outpatient clinic between June 1, 2021 and 30 November, 2021. PLWH who were followed up in Infectious Diseases and Clinical Microbiology clinic of Ankara City Hospital, were selected as study group; patients with similar age and gender, who did not have a chronic disease that suppressed the immune system and applied to the clinic within the specified date range for the study, were selected as the control group. The patients were first called by phone, and after consent form was obtained during the face-to-face interview at their subsequent visits from those who accepted the study; age, gender, COVID-19 history before or after vaccination, vaccine preference and number of doses were recorded. The last viewed count of CD4+ T cells before vaccination of the patients in the study group was obtained from the hospital automation system. According to the recommendations of the Republic of Turkey Ministry of Health, patients who received two doses of mRNA vaccine, three doses of inactivated vaccine, one or two doses of mRNA vaccine after the 2nd dose of inactivated vaccine, and patients who did not pass three months after the 2nd dose of inactivated vaccine were considered to be fully vaccinated [9].

Statistical analysis

Nominal variables were presented as number and percentage, whereas continuous variables were presented as mean ± standart deviation (SD) and median with interquartile range (IQR). The continuous variables' distribution was performed using the Kolmogorov-Smirnov test where appropriate. An Independent-sample t-test was applied to analyze normally distributed data, whereas Mann-Whitney was applied to analyze nonnormally distributed data. The Pearson Chi-square and Fisher's exact tests were applied to examine categorical data. A p-value of .05 or less was overall considered statistically significant for all analyses and comparisons. The IBM Statistical Package for the Social Sciences (SPSS) version 24 (Chicago, USA) was used to perform statistical analyses.

Ethics approval

The study was approved by Clinical Research Ethics Committee of Ankara City Hospital (E1- 21-2220).

RESULTS

A total of 326 patients, 163 HIV positive and 163 without chronic disease, were included in our study. Both groups were equal in terms of age and gender distribution, with 142 (88.1%) men and 21 (12.9%) women in each group. The mean age of the patients was 46.69 ± 13.72. The median CD4+ T cell count is 540 (IQR:430) cells/mm^3 in PLWH, and CD4+ T cell count of 11 (6.7%) patients was lower than 200 cells/mm^3.

The number of unvaccinated individuals in the entire study population was 36 (11.1%), and this number was equally distributed in both groups (n=18, 11.0%). No gender difference was found among unvaccinated patients (p=0.06). However, when unvaccinated PLWH were evaluated, it was observed that women (28.6%) had significantly less vaccination compared to male patients (8.5%) (p=0.01), and this difference was not seen in unvaccinated patients in the control group (p=1.0).

It was seen that the mRNA vaccine was preferred by 79.0% (n=113) of patients in both groups. Among patients with CD4+ T cell count below 200 cells/mm3 (n=11, 6.7%), 9 (81.8%) of them preffered mRNA vaccine; two (18.2%) of the remaining patients were vaccinated with inactived SARS-CoV-2 vaccine. All patients with low CD4+ T cell count were fully vaccinated. Demographic characteristics, vaccination rates and vaccine preferences of the patients were shown in Table 1.

The number of patients with SARS-CoV-2 infection history was 69 (21.2%), and this number was 30 (18.4%) patients in PLWH; there were 39 (23.9%) patients in the control group. After full-dose vaccination, SARS-CoV-2 infection was detected in 7 (5.9%) patients in PLWH;11 (8.7%) patients in the control group. None of the patients in the study or control groups, who received a single dose of mRNA vaccine after 2 doses of inactivated vaccine or two doses of mRNA vaccine after 2 doses of inactivated vaccine, did not develop SARS-CoV-2 infection. Two (1.2%) patients with low CD4+ T cell count had a history of SARS-CoV-2 infection, one of them prior vaccination and the other one after 2 doses of inactivated vaccine. The SARS-CoV-2 infection history of patients was shown in Table 2 according to vaccination.

It was determined that one (0.6%) patient in the study group and two (1.2%) patients in the control group died, respectively. One patient in the control group and one patient in the study group died due to COVID-19. The other patient in the control group died due to sepsis associated with urinary infection. There was no history of vaccination against SARS-CoV-2 infection in deceased patients.

DISCUSSION

Since the beginning of the pandemic, many studies have been conducted to evaluate the transmission routes of SARS-CoV-2 infection, the

Table 1. Comparison of demographic characteristics, vaccination rates and vaccine preferences of patients in the groups

Characteristics	PLWH (n=163) (n, %)	Controls (n=163) (n, %)	*p*-value
Age, mean (years, standart deviation)	46.69 ± 13.72	46.69 ± 13.72	1.0[a]
Male gender	142 (87.1)	142 (87.1)	1.0[b]
Vaccination status (unvaccinated)	18 (11.0)	18 (11.0)	1.0[b]
Vaccination history (single dose)	145 (89.0)	145 (89.0)	1.0[b]
Vaccination history (double doses)	129 (79.1)	131 (80.9)	0.89[b]
Full-dose vaccination	123 (75.5)	126 (77.3)	0.79[b]
Vaccine preference (mRNA vaccine)	113 (79.0)	113 (79.0)	1.0[b]

N: number; %: percentages; PLWH: People living with Human immunodefficiency virus.

[a]Independent sample t and [b]Chi-square tests were used.

Table 2. Distribution of SARS-CoV-2 infection before, during and after vaccine types and doses

Characteristics	PLWH		Controls		*p*-value
	N	n (%)	N	n (%)	
History of SARS-CoV-2 infection	163	30 (18.4)	163	39 (23.9)	0.28*
Prior vaccination	145	20 (12.3)	145	22 (13.5)	1.0*
During vaccination	145	10 (6.9)	145	17 (11.7)	0.16*
After full-dose vacciantion	123	7 (5.9)	126	11 (8.7)	0.46*
History of SARS-CoV-2 infection after vaccine types and doses					
First dose of inactivated vaccine	50	-	65	1 (1.5)	-[#]
Second dose of inactivated vaccine	48	4 (8.3)	65	10 (15.4)	0.76[q]
Third dose of inactivated vaccine	16	2 (12.5)	16	2 (12.5)	1.0[q]
First dose of mRNA vaccine	95	1 (1.1)	80	3 (3.7)	0.33[q]
Second dose of mRNA vaccine	86	3 (3.5)	71	2 (2.8)	0.60[q]

N: number; %: percentages; PLWH: People living with Human immunodefficiency virus; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

*Pearson Chi-square and [q]Fisher's exact tests were used. [#]Statistical analysis was not made due to the small number of patients.

risk of transmission, the course of the disease, laboratory results, treatment methods and trends in vulnearable groups. PLWH were also included in the vulnerable group, and the coexistence of COVID-19 and HIV and its results have been investigated [10]. Studies evaluating the prevalence of COVID-19 in PLWH are few and they are showing conflicting results. In the literature, besides the studies reporting that SARS CoV-2 infection is more common in PLWH than in the normal population [11], there are also studies showing that there is no difference [12]. In our study, no difference was found in terms of the incidence of COVID-19 and the history of having COVID-19 before and after vaccination between the normal population and PLWH.

While there are studies showing that SARS-CoV-2 infection can be more severe in PLWH [13-16], there are also studies showing that it does not progress differently from the normal population [17-20]. Studies conducted in the first months of the pandemic period mostly consist of case reports or series, and especially the risk factors that cause the worsening of COVID-19, clinical presentations and disease results were examined [19,21,22]. In the later stages of the pandemic, with the increase in the diversity of the affected population, specific studies on PLWH have begun. In these studies, the effects of compliance with anti-retroviral therapy (ART), HIV viral load, CD4+ T cell count and the presence of comorbidities on the course of the disease were investigated, and these results seem to be inconsistent with each other [15,17,20]. The incompatible results in the studies revealed the necessity of considering the presence of confounding factors and reviewing patient-specific risk factors when evaluating the COVID-19 diagnosis, hospitalization and mortality data in PLWH.

With the initiation of vaccination against COVID-19, PLWH has been among the priority groups our country [9]. mRNA and inactivated vaccines were applied according to the preference of the patients, and vaccination programme is still ongoing. [8,23]. Vaccination against COVID-19 is recommended, although the World Health Organization and Centers for Disease Control and Prevention have stated that there is a limited possibility of efficacy in PLWH. In studies examining the efficacy and safety of two different vaccines administered in our country, it was found that the mRNA vaccine was effective, safe and had mild side effects; [24-26]; on the other hand, some inactivated vaccine studies did not include PLWH [27-29]. In the included studies, it has been shown to be effective and safe, but with less antibody responses [30-32].

In our study, there was no difference in preference between the two vaccines against COVID-19; and there was also no difference in the frequency of SARS-CoV-2 infection after immunization with mRNA or inactivated vaccines between two groups.

In a study examining vaccination anxiety and attitude among PLWH, age, gender, religious belief, marital status, occupation, income and education level were not found to have an effect on this attitude [33]. In another study conducted in black PLWH, it was stated that 97% of the patients had a feeling of insecurity about COVID-19, and more than 50% of this insecurity was related to the COVID-19 vaccine and treatments [34]. In a study from China showed that individuals who had a higher education, engaged in occupations with a higher risk of COVID-19 infection, received influenza or pneumonia vaccine in the past three years, believed in the effectiveness of vaccines and received media information regarding COVID-19 vaccine were more likely to be vaccinated. But concerning about adverse reactions, negative impact on the progression of HIV or ART, comorbidities, being unmarried and older age were negatively associated with vaccination [35]. In our study, the number of unvaccinated patients was 18 (11.0%) in each group and no difference was found. Women living with HIV were vaccinated less than men. This could be explained by the low number of female patients included in the study.

It was known that at the beginning of the pandemic period, patients' applications to health centers were delayed due to closure [2,6]. In this period, the drug reports about the chronic diseases of the patients in our country were extended by the Ministry of Health without the need to go to the hospital. This application, which was done with the benefit of the patient in mind, might have caused negative results such as not being able to answer questions about the vaccines and not being able to resolve their hesitations, since PLWH can take their medications

without coming to the hospitals. Considering that PLWH are applying to the hospitals more frequently for examinations, important duties fall on the health care providers who follow these individuals for the continuation of the normalization process. Explaining the importance, necessity and effectiveness of vaccination at each visit and creating social awareness for the continuity of normalization will only be possible with the effort of health caare professionals.

In conclusion, despite the many negative effects of the COVID-19 pandemic, it has contributed to remind health-care professionals of the importance of close follow-up of the high-risk patient population and taking the necessary urgent measures to protect them from the disease.

PLWH is included in this high-risk group. Vaccination of these patients is of great importance for the continuity of their well-being and for the provision of community immunity. In our study, vaccination status, vaccine preference, vaccination doses, history of SARS-CoV-2 infection before and after vaccination between PLWH and the normal population were evaluated and no difference was found.

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Author contribution

Study conception and design: ÇMA and AB; data collection: ÇMA and AB; analysis and interpretation of results: ÇMA and AB; draft manuscript preparation: ÇMA and AB. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Clinical Research Ethics Committee of Ankara City Hospital (Protocol no. E1-21-2220).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Brown LB, Spinelli MA, Gandhi M. The interplay between HIV and COVID-19: summary of the data and responses to date. Curr Opin HIV AIDS. 2021;16(1):63-73.

[2] Lesko CR, Bengtson AM. HIV and COVID-19: Intersecting Epidemics With Many Unknowns. Am J Epidemiol. 2021;190(1):10-6.

[3] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62.

[4] Zheng Z, Peng F, Xu B, Zhao J, Liu H, Peng J, et al. Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. J Infect. 2020;81(2):e16-e25.

[5] Virata MD, Shenoi SV, Ladines-Lim J, Villanueva MS, Barakat LA. Cumulative burden of non-communicable diseases predicts COVID hospitalization among people with HIV: A one-year retrospective cohort study. PLoS One. 2021;16(12):e0260251.

[6] Yang Y, Iwasaki A. Impact of Chronic HIV Infection on SARS-CoV-2 Infection, COVID-19 Disease and Vaccines. Curr HIV/AIDS Rep. 2021:1-12.

[7] Mascolo S, Romanelli A, Carleo MA, Esposito V. Could HIV infection alter the clinical course of SARS-CoV-2 infection? When less is better. J Med Virol. 2020;92(10):1777-8.

[8] World Health Organization. Accessed date: 05 Deecember 2021. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines/advice.

[9] Republic of Turkey Ministry of Health. Accessed date: 05 December 2021. Available from: https://covid19asi.saglik.gov.tr.

[10] Centers for Disease Control and Prevention. Accessed date: 12 December 2021. Available from: https://clinicalinfo.hiv.gov/en/guidelines/covid-19-and-persons-hiv-interim-guidance/interim-guidance-covid-19-and-persons-hiv.

[11] D'Souza G, Tong W, Gustafson D, Alcaide ML, Lahiri CD, Sharma A, et al. SARS-CoV-2 Infection Among People Living With HIV Compared With People Without HIV: Survey Results From the MACS-WIHS Combined Cohort Study. J Acquir Immune Defic Syndr. 2022;89(1):1-8.

[12] Castel AD, Wilbourn B, Magnus M, Greenberg AE. SARS-CoV-2 and HIV: Epidemiology, Treatment, and Lessons Learned from HIV. AIDS Rev. 2020;22(3):133-42.

[13] Geretti AM, Stockdale AJ, Kelly SH, Cevik M, Collins S, Waters L, et al. Outcomes of Coronavirus Disease 2019 (COVID-19) Related Hospitalization Among People With Human Immunodeficiency Virus (HIV) in the ISARIC World Health Organization (WHO) Clinical Characterization Protocol (UK): A Prospective Observational Study. Clin Infect Dis. 2021;73(7):e2095-e106.

[14] Bhaskaran K, Rentsch CT, MacKenna B, Schultze A, Mehrkar A, Bates CJ, et al. HIV infection and COVID-19 death: a population-based cohort analysis of UK primary care data and linked national death registrations within the OpenSAFELY platform. Lancet HIV. 2021;8(1):e24-e32.

[15] Western Cape Department of Health in collaboration with the National Institute for Communicable Diseases. Risk Factors for Coronavirus Disease 2019 (COVID-19) Death in a Population Cohort Study from the Western Cape Province, South Africa. Clin Infect Dis. 2021;73(7):e2005-e15.

[16] Tesoriero JM, Swain CE, Pierce JL, Zamboni L, Wu M, Holtgrave DR, et al. COVID-19 Outcomes Among Persons Living With or Without Diagnosed HIV Infection in New York State. JAMA Netw Open. 2021;4(2):e2037069.

[17] Shalev N, Scherer M, LaSota ED, Antoniou P, Yin MT, Zucker J, et al. Clinical Characteristics and Outcomes in People Living With Human Immunodeficiency Virus Hospitalized for Coronavirus Disease 2019. Clin Infect Dis. 2020;71(16):2294-7.

[18] Karmen-Tuohy S, Carlucci PM, Zervou FN, Zacharioudakis IM, Rebick G, Klein E, et al. Outcomes Among HIV-Positive Patients Hospitalized With COVID-19. J Acquir Immune Defic Syndr. 2020;85(1):6-10.

[19] Durstenfeld MS, Sun K, Ma Y, Rodriguez F, Secemsky EA, Parikh RV, et al. Impact of HIV Infection on COVID-19 Outcomes Among Hospitalized Adults in the U.S. medRxiv. 2021.

[20] Sigel K, Swartz T, Golden E, Paranjpe I, Somani S, Richter F, et al. Coronavirus 2019 and People Living With Human Immunodeficiency Virus: Outcomes for Hospitalized Patients in New York City. Clin Infect Dis. 2020;71(11):2933-8.

[21] Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med. 2020;180(7):934-43.

[22] Blanco JL, Ambrosioni J, Garcia F, Martínez E, Soriano A, Mallolas J, et al. COVID-19 in patients with HIV: clinical case series. Lancet HIV. 2020;7(5):e314-e6.

[23] Centers for Disease Control and Prevention. Accessed date: 12 December 2021. Available from: https://www.cdc.gov/hiv/basics/covid-19.html.

[24] Bergman P, Blennow O, Hansson L, Mielke S, Nowak P, Chen P, et al. Safety and efficacy of the mRNA BNT162b2 vaccine against SARS-CoV-2 in five groups of immunocompromised patients and healthy controls in a prospective open-label clinical trial. EBioMedicine. 2021;74:103705.

[25] Bajema KL, Dahl RM, Evener SL, Prill MM, Rodriguez-Barradas MC, Marconi VC, et al. Comparative Effectiveness and Antibody Responses to Moderna and Pfizer-BioNTech COVID-19 Vaccines among Hospitalized Veterans - Five Veterans Affairs Medical Centers, United States, February 1-September 30, 2021. MMWR Morb Mortal Wkly Rep. 2021;70(49):1700-5.

[26] Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020;383(27):2603-15.

[27] Akova M, Unal S. A randomized, double-blind, placebo-controlled phase III clinical trial to evaluate the efficacy and safety of SARS-CoV-2 vaccine (inactivated, Vero cell): a structured summary of a study protocol for a randomised controlled trial. Trials. 2021;22(1):276.

[28] Tanriover MD, Doğanay HL, Akova M, Güner HR, Azap A, Akhan S, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet. 2021;398(10296):213-22.

[29] Fadlyana E, Rusmil K, Tarigan R, Rahmadi AR, Prodjosoewojo S, Sofiatin Y, et al. A phase III, observer-blind, randomized, placebo-controlled study of the efficacy, safety, and immunogenicity of SARS-CoV-2 inactivated vaccine in healthy adults aged 18-59 years: An interim analysis in Indonesia. Vaccine. 2021;39(44):6520-8.

[30] Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. N Engl J Med. 2021;385(10):875-84.

[31] Feng Y, Zhang Y, He Z, Huang H, Tian X, Wang G, et al. Immunogenicity of an inactivated SARS-CoV-2 vaccine in people living with HIV-1: a non-randomized cohort study. EClinicalMedicine. 2022;43:101226.

[32] Lv Z, Li Q, Feng Z, Zheng X, NaYin, Yang H, et al. Inactivated SARS-CoV-2 vaccines elicit immunogenicity and T-cell responses in people living with HIV. Int Immunopharmacol. 2022;102:108383.

[33] Qi L, Yang L, Ge J, Yu L, Li X. COVID-19 Vaccination Behavior of People Living with HIV: The Mediating Role of Perceived Risk and Vaccination Intention. Vaccines (Basel). 2021;9(11).

[34] Bogart LM, Ojikutu BO, Tyagi K, Klein DJ, Mutchler MG, Dong L, et al. COVID-19 Related Medical Mistrust, Health Impacts, and Potential Vaccine Hesitancy Among Black Americans Living With HIV. J Acquir Immune Defic Syndr. 2021;86(2):200-7.

[35] Zhao H, Wang H, Li H, Zheng W, Yuan T, Feng A, et al. Uptake and adverse reactions of COVID-19 vaccination among people living with HIV in China: a case-control study. Hum Vaccin Immunother. 2021:1-7.

acta medica

ORIGINAL ARTICLE

In silico Activity and Target Prediction Analyses of Three Triazolothiadiazine Derivatives

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This study has been presented previously in 5th International BAU Drug Design Congress.

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ABSTRACT

Objective: Polypharmacology, interaction of one drug with multiple targets, emerged as an effective approach in drug discovery and development. Bioinformatics and cheminformatics methods are essential tools for determination of polypharmacological profiles of newly synthesized or known compounds and drugs. Previously, three novel triazolothiadiazine derivatives; **1h**, **3c** and **3h**, have been shown to induce apoptosis and cause cell cycle arrest on liver cancer cells. The aim of this study is to find possible action mechanisms and potential targets for these three triazolothiadiazine derivatives, and to investigate their potential as new therapeutic agents by using computational methods.

Materials and Methods: PASS software was used to identify biological activities and Swiss Target Prediction and BindingDB databases to predict potential targets for **1h**, **3c** and **3h**. PDE4A, ALR and DUSP1 proteins were selected for molecular docking analysis following the protein modeling of the three proteins.

Results: Activity prediction results show that **1h**, **3c** and **3h** might have phosphatase and signal transduction pathway inhibitor, hepatocyte growth factor antagonist, anti-inflammatory and antifungal activities. These derivatives are predicted as inhibitors of several phosphodiesterases by activity and target prediction tools.

Conclusion: Based on prediction and molecular docking results, it is proposed that these compounds may have therapeutic properties through new predicted targets.

Keywords: Molecular docking, pharmacology, similarity searching, triazolothiadiazines

INTRODUCTION

Polypharmacology is considered as an emerging approach in discovery and development of new drugs. The idea behind polypharmacology comes from the notion; a drug can act on different targets of a disease pathway or several disease pathways [1]. Bioinformatics approaches provide tools for drug development processes, such as target discovery and prediction of drug-target interactions [2,3]. Using computational methods for such studies ease the drug discovery and development process, and reduce the expanses on drug discovery [4,5]. Therefore, *in silico* activity and target prediction methods are valuable tools to estimate probable activities and targets for newly synthesized compounds.

For newly synthesized compounds, *in silico* target fishing is used to predict potential targets by mining chemical databases. Similarity searching is one of the approaches for searching potential targets for

a compound. The main idea for similarity searching is chemically similar compounds may interact with similar protein targets [6]. Therefore, by comparing the structure of newly synthesized compound with the structure of the known compounds with known targets, the potential targets for novel compound can be predicted.

Molecular docking is performed to assess the potential interaction or binding geometries according to the structure of the novel compound and target protein. Potential targets are virtually docked to novel compounds to identify potential interactions [7,8].

Previously, some novel triazolothiadiazine derivatives (**1a–3j**) have been synthesized, characterized and searched for their anti-proliferative effects on liver cancer cells [9]. Among 30 compounds, 5 of them, **1e**, **1g**, **1h**, **3c** and **3h** have been shown to cause apoptosis and cell cycle arrest at SubG1 phase of cell cycle. Two of the derivatives (**1g** and **1h**) have been studied in detail and proposed as promising anti-proliferative compounds acting by activating ASK-1 and inactivating Akt [9]. In this study, three of these derivatives **1h**, **3c** and **3h**, which may be potential therapeutic agents for cancers were selected. Detailed action mechanisms of these derivatives have not been shown in previous studies, since experimentally testing all possible interactions is not feasible. Biological activities and potential targets of **1h**, **3c** and **3h** were searched by using prediction tools. In order to show the interaction of the derivatives to predicted targets, molecular docking between three of the compounds were performed.

MATERIALS AND METHODS

Simplified Molecular Input Line Entry Specification (SMILES) generation for the compounds 1h, 3c and 3h

A SMILE is an ASCII string, which is used to represent the chemical structure of the compound. SMILES strings of three compounds were generated by using Swiss Target Prediction according to the structure of each molecule ([10], http://www.swisstargetprediction.ch/).

Druglikeness of the compounds 1h, 3c and 3h

Druglikeness of **1h**, **3c** and **3h** according to Lipinski's rule of five for evaluated with SwissADME ([11], http://www.swissadme.ch/index.php).

Computational determination of biological activities of the compounds 1h, 3c and 3h

Biological activity prediction for triazolothiadiazine derivatives was performed by using PASS online version 2.0 ([12], http://www.way2drug.com/passonline/). In order to estimate the probable activities of **1h**, **3c** and **3h**, SMILES strings for each compound loaded to PASS online. The prediction results were provided as predicted activities and corresponding probabilities as Pa (to be active) and Pi (to be inactive), ranging from 0 to 1. In the PASS prediction approach, biological activity is predicted based on the structural formula of a compound for more than 4000 kinds of biological activity with an average accuracy above 95%. Structure-activity relationships are considered for prediction in the training set involving more than 300.000 organic compounds [12]. When the Pa>Pi, the activity is probable for the compound. Pa>0.7 means in an experiment the chance of finding the predicted activity is high. When Pa<0.5, the chance of finding the predicted activity in an experiment is low, however, it also means that there is a chance of finding a structurally new compound. Between those values, which are 0.5<Pa<0.7, the probability of finding the activity in an experiment is less, since the compound is not similar to known pharmaceuticals [13, 14]. Therefore, the higher Pa values increase the chance to find the activity experimentally; also indicate the compound is similar to known pharmaceutical agents.

Target prediction for the compounds 1h, 3c and 3h

Swiss Target Prediction [10] (based on ChEMBL16) and BindingDB ([15], https://www.bindingdb.org/) were used to predict potential targets for each of the three compounds. The SMILES strings of the compounds were inputted to the databases separately and prediction results were reported.

Swiss Target Prediction computes 2D and 3D similarity values for the query compounds against the known ligands. In 2D similarity, FP2

fingerprints are used to define the molecules and Tanimoto coefficient (the number of shared fingerprint patterns/total number of fingerprint patterns) is used to quantify the similarity between molecules. In 3D similarity, different conformations of molecules are produced and Manhattan distances are calculated for all the conformations of each molecule. The target scores are calculated according to the logistic regressions with the 2D and 3D similarity scores of most similar ligands. Target scores rank the possible targets (between 0 to 1, larger when the query compound is the known ligand of the target) and are used to obtain the probability, which assesses the likelihood of the correct prediction [10].

Find My Compound's Targets (FMCT) tool of the BindingDB database was used to find targets for the compounds **1h**, **3c** and **3h**, according to the notion that similar compounds might bind the same proteins. FMCT provided the proposed protein targets with the similarity scores (max similarity) of query compounds to compounds in the database which bind to proposed protein targets, based on Similarity Ensemble Approach (SEA) [15].

Molecular Docking

Phyre2, Protein Homology/analogY Recognition Engine V 2.0, ([16], http://www.sbg.bio.ic.ac.uk/phyre2)) was used to prepare the models of human PDE4A, ALR and DUSP1, since the crystal structures of these proteins in PDB database were partial and/or in complex with other molecules or carried mutated residues. Docking calculations of the predicted target proteins to **1h**, **3c** and **3h** were performed using SwissDock ([8,17], http://www.swissdock.ch/). Following parameters were used for dockings; docking type "Accurate" and flexibility for side chains within 0Å. SwissDock reported FullFitness and Gibbs free energy (ΔG) to evaluate favorable bindings for each molecular docking. For each docking, the most energetically favorable binding, which has a greater negative FullFitness score, of the triazolothiadiazine derivative to the target protein was selected and visualized. UCSF Chimera [18], developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco was used to visualize the results generated by SwissDock and to prepare MOL2 files of the compounds.

RESULTS

Druglikeness of 1h, 3c and 3h

Lipinski's rule of five, which has been used evaluate the druglikeness of a chemical compound, predicts that if a compound has more than 5 H-bond donors, 10 H-bond acceptors, a molecular weight higher than 500 and calculated Log P greater than 5, it may show poorer absorption or permeation [19]. According to predictions, all three compounds met the criteria by having molecular weight between 406-449 (<500), H-bond donors 0 (<5) and H-bond acceptors between 4-5 (<10), although they did not have Log P less than 5, except 1h (Table 1).

Table 1. Physicochemical Properties of 1h, 3c and 3h

Descriptor	**Value 1h**	**Value 3c**	**Value 3h**
Molecular Weight (g/mol)	406.54	448.94	444.52
Consensus Log P	4.85	5.87	5.25
Rotatable Bonds	6	4	5
H-bond Acceptors	4	4	5
H-bond Donors	0	0	0

Biological activity prediction for the compounds 1h, 3c and 3h

Previously, the apoptotic and anti-proliferative activities of the compounds **1h**, **3c** and **3h** were shown experimentally [9]. In order to estimate new possible activities for these derivatives, PASS prediction tool was used. According to PASS predictions, compounds **1h**, **3c** and **3h** might have several biological activities, such as phosphatase and signal transduction pathway inhibitors, had anti-inflammatory effects, and were hepatocyte growth factor and Neuropeptide Y2 antagonists (Pa>0.5, Table 2). All three derivatives predicted as inhibitors of several phosphodiesterases (PDEs), Cyclin-dependent kinase 5 (CDK5), Protein-tyrosine phosphatase 2C (PTP2C, also known as SHP2 and PTPN11) and Dual specificity phosphatase 1 (DUSP1), which regulate removal of phosphate groups from tyrosine residues (Table 2).

Table 2. The predicted activities for 1h, 3c and 3h

	1h		3c		3h	
Activity	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**
Phosphatase inhibitor	0.721	0.009	0.735	0.007	0.705	0.012
Hepatocyte growth factor antagonist	0.635	0.002	0.697	0.002	0.665	0.002
Neuropeptide Y2 antagonist	0.54	0.01	0.57	0.008	0.52	0.013
Signal transduction pathways inhibitor	0.526	0.026	0.779	0.008	0.718	0.011
Antiinflammatory	0.51	0.054	0.562	0.04	0.553	0.043
Calpain inhibitor	0.415	0.012	0.304	0.042	0.296	0.045
Anesthetic general	0.422	0.034	0.189	0.145	0.186	0.149
Growth factor antagonist	0.349	0.013	0.457	0.008	0.459	0.007
Respiratory distress syndrome treatment	0.345	0.016	0.301	0.028	0.303	0.028
Antifungal	0.369	0.057	0.29	0.085	0.263	0.099
Amyloid beta precursor protein antagonist	0.31	0.02	0.511	0.005	0.496	0.005
Alkaline phosphatase inhibitor	0.265	0.021	0.387	0.006	0.36	0.008
Anesthetic	0.256	0.05	-	-	-	-
5 Hydroxytryptamine release inhibitor	0.325	0.12	-	-	-	-
Phosphodiesterase 4A inhibitor	0.207	0.006	0.162	0.009	0.192	0.007
Phosphodiesterase 10A inhibitor	0.194	0.004	0.223	0.004	0.247	0.003
Phosphodiesterase X inhibitor	0.194	0.004	0.223	0.004	0.247	0.003
Cyclin-dependent kinase 5 inhibitor	0.182	0.005	0.214	0.004	0.19	0.004
Protein-tyrosine phosphatase 2C inhibitor	0.175	0.004	0.192	0.004	0.185	0.004
Sphingosine 1-phosphate receptor antagonist	0.206	0.04	0.189	0.054	0.138	0.108
Protein phosphatase inhibitor	0.176	0.039	0.142	0.053	0.16	0.045
Macrophage elastase inhibitor	0.138	0.01	0.099	0.018	0.099	0.018
Neuropeptide Y1 antagonist	0.149	0.03	0.148	0.031	0.148	0.032
Cyclin-dependent kinase inhibitor	0.14	0.023	0.192	0.015	0.159	0.019
Phosphodiesterase IV inhibitor	0.132	0.016	-	-	0.12	0.018
ATPase stimulant	0.22	0.106	-	-	-	-
Protein-tyrosine phosphatase inhibitor	0.152	0.039	0.134	0.047	0.149	0.04
Mucolytic	0.19	0.078	-	-	-	-
Phosphodiesterase 3A inhibitor	0.124	0.016	0.119	0.018	0.124	0.016
Sphingosine 1-phosphate receptor 1 antagonist	0.174	0.072	0.159	0.093	-	-
Mcl-1 antagonist	0.165	0.063	0.155	0.069	0.159	0.066
Dual specificity phosphatase 1 inhibitor	0.179	0.08	0.197	0.056	0.192	0.062
CC chemokine receptor 2B antagonist	0.167	0.069	-	-	-	-
Phosphodiesterase 4D inhibitor	0.108	0.017	0.065	0.037	0.103	0.019
Expectorant	0.181	0.093	-	-	-	-
Phosphodiesterase 4B inhibitor	0.101	0.018	0.066	0.038	0.104	0.017
HIF1A expression inhibitor	0.277	0.204	-	-	-	-
Phosphodiesterase inhibitor	0.114	0.043	-	-	0.098	0.053
Sphingosine 1-phosphate receptor 4 antagonist	0.142	0.075	0.12	0.11	-	-
Cyclooxygenase 3 inhibitor	0.087	0.021	-	-	-	-
Serum-glucocorticoid regulated kinase 1 inhibitor	0.23	0.165	-	-	-	-
Fibrosis treatment	0.116	0.068	0.11	0.077	0.126	0.054
Linoleate diol synthase inhibitor	0.235	0.187	-	-	-	-
Cardiotonic	0.208	0.16	-	-	-	-
Phosphodiesterase III inhibitor	0.08	0.032	-	-	0.067	0.042
Preneoplastic conditions treatment	0.259	0.212	-	-	-	-
Angiotensin II receptor agonist	0.138	0.1	-	-	-	-
Phosphodiesterase 4C inhibitor	0.066	0.029	-	-	0.061	0.033
Complement C5a chemotactic receptor antagonist	0.059	0.025	0.04	0.038	-	-
Antiviral (Rhinovirus)	0.295	0.263	-	-	-	-

Pa, probability to be active; Pi, probability to be inactive

Table 2. Continued

	1h		3c		3h	
Activity	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**
Calcium channel N-type blocker	0.123	0.092	0.144	0.068	0.153	0.061
Biotinidase inhibitor	0.236	0.207	-	-	-	-
Platelet aggregation inhibitor	0.181	0.154	-	-	-	-
PRL phosphatase inhibitor	0.081	0.058	-	-	0.084	0.048
Benzoin aldolase inhibitor	0.054	0.033	-	-	-	-
Sphingosine 1-phosphate receptor 5 antagonist	0.041	0.026	-	-	-	-
Matrix metalloproteinase 1 (membrane-type) inhibitor	0.035	0.024	-	-	-	-
Vesicle monoamine transporter inhibitor	0.032	0.022	-	-	-	-
T-cell protein-tyrosine phosphatase inhibitor	0.032	0.022	-	-	0.03	0.027
Sphingosine 1-phosphate receptor 3 antagonist	0.061	0.056	-	-	-	-
Calcium channel (voltage-sensitive) activator	0.302	0.301	-	-	-	-
Plastoquinol-plastocyanin reductase inhibitor	0.180	0.180	-	-	-	-
Glycogen synthase stimulant	-	-	0.295	0.081	0.27	0.111
Diabetic neuropathy treatment	-	-	0.338	0.134	-	-
Janus tyrosine kinase 2 inhibitor	-	-	0.229	0.052	0.21	0.066
GABA receptor agonist	-	-	0.225	0.056	0.148	0.138
Histamine H1 receptor agonist	-	-	0.218	0.05	-	-
5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor	-	-	0.328	0.202	-	-
Fibromyalgia syndrome treatment	-	-	0.192	0.066	-	-
Chloride channel activator	-	-	0.218	0.1	-	-
Muscle relaxant	-	-	0.217	0.101	-	-
Dihydroorotase inhibitor	-	-	0.168	0.084	-	-
Rheumatoid arthritis treatment	-	-	0.202	0.127	0.169	0.163
Anticonvulsant	-	-	0.237	0.161	-	-
Sporulation kinase A inhibitor	-	-	0.101	0.034	-	-
Autophagy inducer	-	-	0.099	0.047	-	-
Lysyl oxidase inhibitor	-	-	0.227	0.203	-	-
Antianemic	-	-	0.123	0.099	-	-
Cognition disorders treatment	-	-	0.183	0.166	-	-
Premenstrual syndrome treatment	-	-	0.092	0.076	0.091	0.08
Skeletal muscle relaxant	-	-	0.179	0.163	-	-
Lanosterol 14 alpha demethylase inhibitor	-	-	0.111	0.097	-	-
Neuropeptide Y antagonist	-	-	0.085	0.078	-	-
Transglutaminase 2 inhibitor	-	-	0.09	0.085	0.096	0.068
Alpha 2d adrenoreceptor agonist	-	-	0.023	0.019	-	-
Scytalone dehydratase inhibitor	-	-	0.069	0.066	-	-
Potassium channel small-conductance Ca-activated activator	-	-	0.055	0.053	-	-
Glutamate release inhibitor	-	-	0.102	0.1	-	-
Antinociceptive	-	-	-	-	0.334	0.157
CYP2D15 substrate	-	-	-	-	0.264	0.255
Interleukin 1 antagonist	-	-	-	-	0.109	0.097
Antineoplastic	-	-	-	-	0.266	0.175
Calcium channel activator	-	-	-	-	0.203	0.138
Aspulvinone dimethylallyltransferase inhibitor	-	-	-	-	0.304	0.267

Pa, probability to be active; Pi, probability to be inactive

Target prediction for the compounds 1h, 3c and 3h

In order to predict the potential molecular targets of the compounds, Swiss Target Prediction [10] and BindingDB [15] were used. Swiss Target Prediction predicted muscleblind-like proteins (encoded by *MBNLs*), FAD-linked sulfhydryl oxidase ALR (ALR, which is encoded by *GFER*), several phosphodiesterases (encoded by *PDEs*) and microtubule-associated protein tau (encoded by *MAPT*) as potential targets for compounds **1h**, **3c** and **3h** (Table 3).

BindingDB couldn't predict any target for compound **3c**, while Cholinesterases were predicted as a target for both compounds **1h** and **3h**. cAMP-specific 3',5'-cyclic phosphodiesterase 4A (PDE4A), Carbonic anhydrases and Steroidogenic factor-1 (SF-1, encoded by *NR5A1*) were predicted as targets for only **1h** by BindingDB (Table 4).

Molecular Docking for Selected Targets with the compounds 1h, 3c and 3h

Molecular dockings were performed with the selected targets, PDE4A, ALR and DUSP1, which were predicted as targets for all three triazolothiadiazine derivatives by activity and/or target predictions. The most favorable scores between target proteins and compounds were selected and visualized. According to docking results compounds **3c** and **3h** might interact to the same region on DUSP1 protein (Figure 1). All derivatives predicted to interact at the same interaction site on ALR (Figure 2), while they predicted to interact at the different interaction region on PDE4A protein (Figure 3).

Table 4. Target prediction of 1h and 3h, by BindingDB (similarity 0.7)

Predicted targets	Max Similarity	
	1h	3h
Cholinesterases	0.70	**0.70**
cAMP-specific 3',5'-cyclic phosphodiesterase 4A	0.70	-
Carbonic anhydrase	0.70	-
Carbonic anhydrase 2	0.70	-
Carbonic anhydrases; II & IX	0.70	-
Steroidogenic Factor 1	0.70	-

Max Similarity, maximum similarity of the query compounds to BindingDB compounds tested against Targets

Table 3. The predicted targets of 1h, 3c and 3h by Swiss Target Prediction (based on ChEMBL16)

Gene	Target	Prediction probability		
		1h	3c	3h
MBNL1	Muscleblind-like protein 1	0.77	0.55	0.75
MBNL2	Muscleblind-like protein 2	0.77	0.55	0.75
MBNL3	Muscleblind-like protein 3	0.77	0.55	0.75
GFER	FAD-linked sulfhydryl oxidase ALR	0.64	0.53	0.63
PDE4A	cAMP-specific 3', 5'-cyclic phosphodiesterase 4A	0.51	0.34	0.63
PDE4B	cAMP-specific 3', 5'-cyclic phosphodiesterase 4B	0.51	0.34	0.63
PDE4C	cAMP-specific 3', 5'-cyclic phosphodiesterase 4C	0.51	0.34	0.63
PDE4D	cAMP-specific 3', 5'-cyclic phosphodiesterase 4D	0.51	0.34	0.63
PDE10A	cAMP and cAMP-inhibited cGMP 3', 5'-cyclic phosphodiesterase 10A	0.49	0.28	0.46
MAPT	Microtubule-associated protein tau	0.49	0.53	0.53
ALPL	Alkaline phosphatase tissue-nonspecific isozyme	0.4	0.1	0.37
ALPP	Alkaline phosphatase placental type	0.4	0.1	0.37
ALPI	Intestinal-type alkaline phosphatase	0.4	-	-
ALPPL2	Alkaline phosphatase placental-like	0.4	-	-
DYRK1A	Dual specificity tyrosine-phosphorylation-regulated kinase 1A	0.37	0.32	0.41
MCL1	Induced myeloid leukemia cell differentiation protein Mcl-1	-	0.16	-
PGR	Progesterone receptor	-	0.11	-
PDE3B	cGMP-inhibited 3', 5'-cyclic phosphodiesterase B	-	-	0.41
PDE3A	cGMP-inhibited 3', 5'-cyclic phosphodiesterase A	-	-	0.39

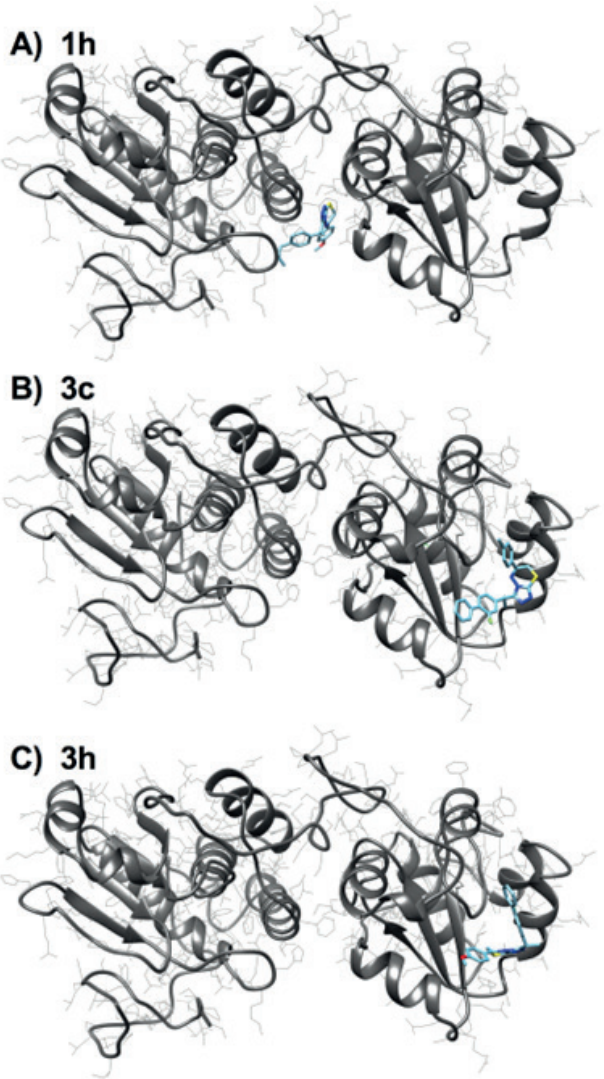

Figure 1. The most favorable molecular docking of DUSP1 and **1h** (A), **3c** (B) and **3h** (C). The modeling of DUSP1 was generated with Phyre2 [16]. SwissDock [8, 17] was used for dockings and the molecular docking results were visualized by UCSF Chimera package [18]. The FullFitness and deltaG Kcal/mol for 1h, -1705.54 and -8.20, for 3c -1680.96 and -8.16 and for 3h -1677.17 and -7.98.

DISCUSSION

Although, conventionally drugs or compounds have been designed to object a single biological target with high selectivity to avoid interaction of a drug with other biological molecules, the complexity of many diseases highlighted the importance of multi target drugs as effective therapeutic candidates [1,20,21]. Computational studies to predict novel activity and targets for known compounds are valuable instruments in using these compounds as therapeutics for diseases.

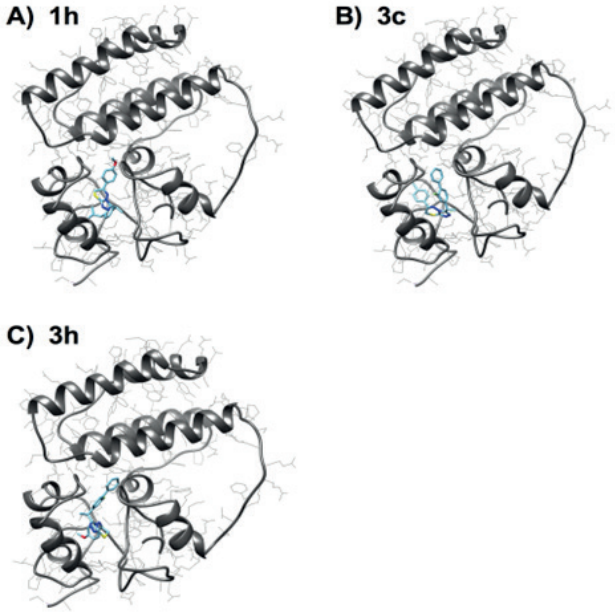

Figure 2. The most favorable molecular docking of ALR and **1h** (A), **3c** (B) and **3h** (C). The modeling of ALR was generated with Phyre2 [16]. SwissDock [8,17] was used for dockings and the molecular docking results were visualized by UCSF Chimera package [18]. The FullFitness and deltaG Kcal/mol for 1h, -1786.17 and -8.39, for 3c -1756.90 and -9.10 and for 3h -1759.01 and -9.17.

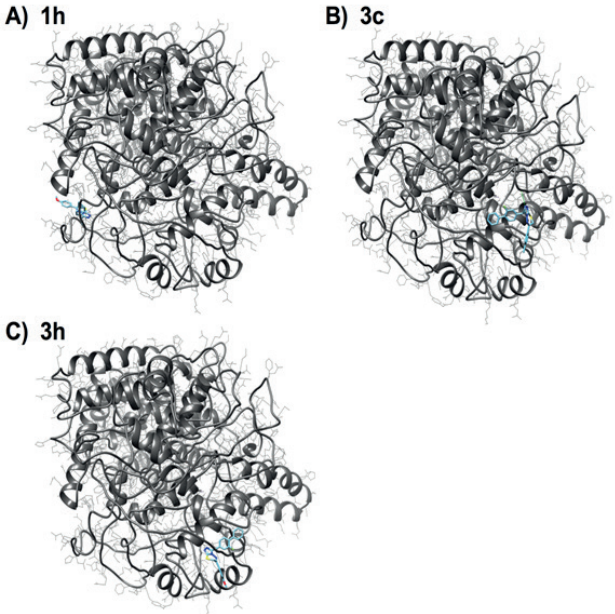

Figure 3. The most favorable molecular docking of PDE4A and **1h** (A), **3c** (B) and **3h** (C). The modeling of PDE4A was generated with Phyre2 [16]. SwissDock [8,17] was used for dockings and the molecular docking results were visualized by UCSF Chimera package [18]. The FullFitness and deltaG Kcal/mol for 1h, -5792.27 and -8.39, for 3c -5764.08 and -8.36 and for 3h -5762.75 and -8.62.

In this study, three triazolothiadiazine derivatives, **1h**, **3c** and **3h**, which have been demonstrated to cause cell cycle arrest at SubG1, indication of apoptosis, in liver cancer cells, was selected [9]. Possible action mechanisms and protein targets of these derivatives, were investigated by using computational methods. According to activity and target prediction results, potential targets and activities of **1h**, **3c** and **3h** were reported. PDE proteins, especially PDE4A, emerged from both biologic activity and target prediction results. Molecular docking analysis showed the potential interaction regions for three derivatives with PDE4A. In addition to PDE4A, possible interactions of three derivatives with ALR and DUSP1, which function in liver regeneration and cell signaling, respectively, were shown by molecular docking analysis. Based on the results presented here, these compounds may have therapeutic properties through new predicted targets.

Results for pharmacokinetic property predictions of 1h, 3c and 3h showed that these compounds might be considered as drug candidates, since they mostly met the Lipinski's rule of five (Table 1).

According to the activity prediction results, all three triazolothiadiazine derivatives had phosphatase inhibitor, alkaline phosphatase inhibitor, hepatocyte growth factor antagonist, Neuropeptide Y2 antagonist, signal transduction pathways inhibitor and anti-inflammatory activities (Table 2). In addition to activity prediction, target prediction results also proposed alkaline phosphatases as targets of **1h**, **3c** and **3h** (Table 3). Phosphatase, especially alkaline phosphatase inhibitor activity of three compounds might be relevant for therapeutic potential of these derivatives in cancers, since the coumarin–triazolothiadiazine hybrid compounds, which have alkaline phosphatase inhibitor activity, were reported as potential anticancer agents [22].

These three triazolothiadiazine derivatives, **1h**, **3c** and **3h**, have been shown to cause cell cycle arrest at SubG1 stage in liver carcinoma cells experimentally [9]. According to results presented in this study, these three triazolothiadiazine derivatives may have potential involvement in liver cell proliferation or regeneration with both activity and target prediction analyses (Table 2 and Table 3). ALR is a critical protein for hepatocyte survival and depletion of ALR caused apoptosis and necrosis in rat hepatocytes [23]. ALR depletion not only affects liver cells, in human derived glioma cells decreased ALR expression caused an increase in apoptosis [24]. Overexpression of ALR induced cell proliferation and inhibited cell death induced by H2O2 in normal human hepatic cell line [25]. In addition to intracellular functions, ALR has extracellular effects; ALR is released from damaged hepatocytes and has been proposed as a hepatic stress/injury marker [26]. In this study, ALR was predicted as target for all three compounds (Table 3). Also in molecular docking analyses, all three derivatives predicted to interact at the same region on ALR protein (Figure 2). These results suggest that in addition to reported factors [9], compounds 1h, 3c and 3h might effect the cell cycle progression in liver cancer cells via their interaction with ALR.

Previously, the action mechanism of one of the compounds, **1h**, was shown to be through the activation ASK-1 and inactivation of Akt proteins [9]. Activity prediction results estimated that all three compounds were protein-tyrosine phosphatase 2C (SHP2) and DUSP1 inhibitors (Table 2). SHP2, a mediator of Erk and PI3K/Akt signaling activation, has been related to cancer by its role in increasing cell proliferation and preventing apoptosis [27]. Therefore, several inhibitors have been developed to target SHP2 [28-30]. Since SHP2 has been shown to activate Akt signaling and prevent apoptosis [27], PASS prediction results on the inhibition of SHP2 by **1h**, **3c** and **3h** might be related with the previous report indicating **1h** inactivated Akt and increased apoptosis [9]. Compound **1h** has also been shown to decrease phosphorylation of ASK-1 and leads to ASK-1 activation. Activated ASK-1 activates JNK protein and causes apoptosis in liver cancer cells [9]. It has been reported that activated PI3-K/Akt signaling pathway cause a decrease in ASK-1 induced apoptosis through ASK-1 phosphorylation by Akt [31]. Therefore, by inhibiting SHP2 activity, compounds **1h**, **3c** and **3h** may inactivate Akt, which increase apoptosis in cells. However, it should be noted that, in addition to Akt, SHP2 is one of the regulators of ASK-1. SHP2 activates ASK1-JNK signaling pathway, by dephosphorylating ASK-1 [32]. PASS prediction tool also proposed compounds **1h**, **3c** and **3h** as DUSP-1 inhibitors (Table 2). In addition, according to molecular docking results, compounds **3h** and **3c** predicted to interact with the same region on DUSP1 protein, while **1h** interacted with a different region (Figure 1). DUSP1 also regulates

JNK mediated apoptosis; DUSP1 inactivates JNK by dephosphorylation and protects cancer cells from apoptosis [33]. Therefore, prediction results propose another action mechanism for these novel compounds on Akt and JNK signaling pathways.

The PDE4 family members that appeared in both activity and target prediction results (Table 2 and Table 3) coded by four genes; *PDE4A*, *PDE4B*, *PDE4C* and *PDE4D* [34]. PDE4A, which has been predicted to be a common target of all three derivatives by activity and target predictions, belongs to a protein family functioning in the cell signaling by hydrolyzing cyclic AMP (cAMP) and cyclic GMP (cGMP) [35]. PDE4A has been proposed as a potential therapeutic target for the anxiety and central nervous system disorders with its role in regulation of anxiety and emotional memory [36]. In addition, the therapeutic effects of inhibition of PDE family members have been shown in several health problems [37]. Phosphodiesterase inhibitors have been used as therapeutics for autoimmune diseases [38] and cancers [39]. Previously, triazolothiadiazines were shown to bind and inhibit PDE4 [40]. According to target and activity prediction and molecular docking results (Figure 3, Table 2 and Table 3), compounds 1h, 3c and 3h might interact with PDE4A. Therefore, **1h**, **3c** and **3h** might be PDE4A inhibitors and their potential therapeutic effect on PDE4A related diseases is worth to evaluate with further experimental studies.

In conclusion, activity and target prediction results proposed new possible activities and targets for the compounds 1h, 3c and 3h. Due to the relevance of predicted activities and targets with cellular mechanisms, all three derivatives might have different therapeutic activities, which need to be tested with experimental studies.

Author contribution

Study conception and design: CS and BT; data collection: CS and SPA; analysis and interpretation of results: CS; draft manuscript preparation: CS. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Reddy AS, Zhang S. Polypharmacology: drug discovery for the future. Expert Rev Clin Pharmacol. Jan 2013;6(1):41-7. doi:10.1586/ecp.12.74

[2] Ferrero E, Dunham I, Sanseau P. In silico prediction of novel therapeutic targets using gene-disease association data. Journal of Translational Medicine. Aug 29 2017;15doi:ARTN 182. 10.1186/s12967-017-1285-6

[3] Yamanishi Y, Kotera M, Kanehisa M, Goto S. Drug-target interaction prediction from chemical, genomic and pharmacological data in an integrated framework. Bioinformatics. Jun 15 2010;26(12):i246-54. doi:10.1093/bioinformatics/btq176

[4] Morrow JK, Tian L, Zhang S. Molecular networks in drug discovery. Crit Rev Biomed Eng. 2010;38(2):143-56.

[5] Knight-Schrijver VR, Chelliah V, Cucurull-Sanchez L, Le Novere N. The promises of quantitative systems pharmacology modelling for drug development. Comput Struct Biotechnol J. 2016;14:363-370. doi:10.1016/j.csbj.2016.09.002

[6] Liu X, Xu Y, Li S, et al. In Silico target fishing: addressing a "Big Data" problem by ligand-based similarity rankings with data fusion. J Cheminform. 2014;6:33. doi:10.1186/1758-2946-6-33

[7] Chen YZ, Zhi DG. Ligand-protein inverse docking and its potential use in the computer search of protein targets of a small molecule. Proteins. May 1 2001;43(2):217-26.

[8] Grosdidier A, Zoete V, Michielin O. SwissDock, a protein-small molecule docking web service based on EADock DSS. Nucleic Acids Res. Jul 2011;39(Web Server issue):W270-7. doi:10.1093/nar/gkr366

[9] Aytac PS, Durmaz I, Houston DR, Cetin-Atalay R, Tozkoparan B. Novel triazolothiadiazines act as potent anticancer agents in liver cancer cells through Akt and ASK-1 proteins. Bioorg Med Chem. Feb 15 2016;24(4):858-72. doi:10.1016/j.bmc.2016.01.013

[10] Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. Nucleic Acids Res. Jul 2014;42(Web Server issue):W32-8. doi:10.1093/nar/gku293

[11] Daina A, Michielin, O., Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports. 2017;(7:42717)

[12] Filimonov DA, Lagunin AA, Gloriozova TA, et al. Prediction of the Biological Activity Spectra of Organic Compounds Using the Pass Online Web Resource. Chemistry of Heterocyclic Compounds. Jun 2014;50(3):444-457. doi:10.1007/s10593-014-1496-1

[13] Goel RK, Singh D, Lagunin A, Poroikov V. PASS-assisted exploration of new therapeutic potential of natural products. Medicinal Chemistry Research. Dec 2011;20(9):1509-1514. doi:10.1007/s00044-010-9398-y

[14] Marwaha A, Goel RK, Mahajan MP. PASS-predicted design, synthesis and biological evaluation of cyclic nitrones as nootropics. Bioorganic & Medicinal Chemistry Letters. Sep 15 2007;17(18):5251-5255. doi:10.1016/j.bmcl.2007.06.071

[15] Gilson MK, Liu T, Baitaluk M, Nicola G, Hwang L, Chong J. BindingDB in 2015: A public database for medicinal chemistry, computational chemistry and systems pharmacology. Nucleic Acids Res. Jan 4 2016;44(D1):D1045-53. doi:10.1093/nar/gkv1072

[16] Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc. Jun 2015;10(6):845-58. doi:10.1038/nprot.2015.053

[17] Grosdidier A, Zoete V, Michielin O. Fast Docking Using the CHARMM Force Field with EADock DSS. J Comput Chem. Jul 30 2011;32(10):2149-2159. doi:10.1002/jcc.21797

[18] Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. Oct 2004;25(13):1605-12. doi:10.1002/jcc.20084

[19] Lipinski CA, Lombardo, F.,Dominy, B.W. , Feeney, P. J. . Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews. 2001;(46):3-26.

[20] Maeda K, Sugino H, Akazawa H, et al. Brexpiprazole I: in vitro and in vivo characterization of a novel serotonin-dopamine activity modulator. J Pharmacol Exp Ther. Sep 2014;350(3):589-604. doi:10.1124/jpet.114.213793

[21] Seeger TF, Seymour PA, Schmidt AW, et al. Ziprasidone (CP-88,059): a new antipsychotic with combined dopamine and serotonin receptor antagonist activity. J Pharmacol Exp Ther. Oct 1995;275(1):101-13.

[22] Ibrar A, Zaib S, Jabeen F, Iqbal J, Saeed A. Unraveling the Alkaline Phosphatase Inhibition, Anticancer, and Antileishmanial Potential of Coumarin-Triazolothiadiazine Hybrids: Design, Synthesis, and Molecular Docking Analysis. Arch Pharm (Weinheim). Jul 2016;349(7):553-65. doi:10.1002/ardp.201500392

[23] Thirunavukkarasu C, Wang LF, Harvey SA, et al. Augmenter of liver regeneration: an important intracellular survival factor for hepatocytes. J Hepatol. Apr 2008;48(4):578-88. doi:10.1016/j.jhep.2007.12.010

[24] Polimeno L, Pesetti B, De Santis F, et al. Decreased expression of the augmenter of liver regeneration results in increased apoptosis and oxidative damage in human-derived glioma cells. Cell Death Dis. Apr 5 2012;3:e289. doi:10.1038/cddis.2012.25

[25] Xia N, Yan R, Liu Q, Sun H, Guo H, Zhang L. [Over-expression of augmenter of liver regeneration promotes proliferation and suppresses hydrogen peroxide-induced apoptosis in LO2 cells]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. Aug 2015;31(8):1017-21.

[26] Vodovotz Y, Prelich J, Lagoa C, et al. Augmenter of Liver Regeneration (ALR) Is a Novel Biomarker of Hepatocellular Stress/Inflammation: In Vitro, In Vivo and In Silico Studies. Mol Med. Nov 2012;18(11):1421-1429. doi:10.2119/molmed.2012.00183

[27] Zhang J, Zhang F, Niu R. Functions of Shp2 in cancer. J Cell Mol Med. Sep 2015;19(9):2075-83. doi:10.1111/jcmm.12618

[28] Liu W, Yu B, Xu G, et al. Identification of cryptotanshinone as an inhibitor of oncogenic protein tyrosine phosphatase SHP2 (PTPN11). J Med Chem. Sep 26 2013;56(18):7212-21. doi:10.1021/jm400474r

[29] Duan YQ, Ma Y, Wang XJ, et al. Design potential selective inhibitors for treating cancer by targeting the Src homology 2 (SH2) domain-containing phosphatase 2 (Shp2) with core hopping approach. Protein Pept Lett. Jun 2014;21(6):556-63.

[30] Yu B, Liu W, Yu WM, et al. Targeting protein tyrosine phosphatase SHP2 for the treatment of PTPN11-associated malignancies. Mol Cancer Ther. Sep 2013;12(9):1738-48. doi:10.1158/1535-7163.MCT-13-0049-T

[31] Kim AH, Khursigara G, Sun X, Franke TF, Chao MV. Akt phosphorylates and negatively regulates apoptosis signal-regulating kinase 1. Mol Cell Biol. Feb 2001;21(3):893-901. doi:10.1128/MCB.21.3.893-901.2001

[32] Yu L, Min W, He Y, et al. JAK2 and SHP2 reciprocally regulate tyrosine phosphorylation and stability of proapoptotic protein ASK1. J Biol Chem. May 15 2009;284(20):13481-8. doi:10.1074/jbc.M809740200

[33] Shen J, Zhang Y, Yu H, et al. Role of DUSP1/MKP1 in tumorigenesis, tumor progression and therapy. Cancer Med. Aug 2016;5(8):2061-8. doi:10.1002/cam4.772

[34] Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. Pharmacol Rev. Sep 2006;58(3):488-520. doi:10.1124/pr.58.3.5

[35] Omori K, Kotera J. Overview of PDEs and their regulation. Circulation Research. Feb 16 2007;100(3):309-327. doi:10.1161/01.RES.0000256354.95791.f1

[36] Hansen RT, 3rd, Conti M, Zhang HT. Mice deficient in phosphodiesterase-4A display anxiogenic-like behavior. Psychopharmacology (Berl). Aug 2014;231(15):2941-54. doi:10.1007/s00213-014-3480-y

[37] Zhang KYJ, Ibrahim PN, Gillette S, Bollag G. Phosphodiesterase-4 as a potential drug target. Expert Opinion on Therapeutic Targets. Dec 2005;9(6):1283-1305. doi:10.1517/14728222.9.6.1283

[38] Kumar N, Goldminz AM, Kim N, Gottlieb AB. Phosphodiesterase 4-targeted treatments for autoimmune diseases. BMC Med. Apr 4 2013;11:96. doi:10.1186/1741-7015-11-96

[39] Savai R, Pullamsetti SS, Banat GA, et al. Targeting cancer with phosphodiesterase inhibitors. Expert Opin Investig Drugs. Jan 2010;19(1):117-31. doi:10.1517/13543780903485642

[40] Skoumbourdis AP, Leclair CA, Stefan E, et al. Exploration and optimization of substituted triazolothiadiazines and triazolopyridazines as PDE4 inhibitors. Bioorg Med Chem Lett. Jul 1 2009;19(13):3686-92. doi:10.1016/j.bmcl.2009.01.057

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ORIGINAL ARTICLE

Comparison of Visual Rating Scale Based on Brain 18F-FDG-PET and Montreal Cognitive Assessment Test in Probable Alzheimer's Disease

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ABSTRACT

Objecives: Recently, imaging biomarkers like flouro-deoxi-glucose positron emission tomography (FDG-PET) become even more important for evaluation probable AD. The aim of this study was to evaluate the possible correlation between Montreal Cognitive Assessment Test (MoCA) and metabolic function of brain regions determined by FDG-PET in patients with probable AD.

Materials and methods: Thirty seven (37) patients who had diagnosis of probable AD were included. MoCA test and metabolic measurements of brain regions by FDG-PET were performed in all patients. A visual scoring was performed to obtain the rates of hypometabolism in brain regions.

Results: Median age of the patients was 77 (minimum 65-maximum 83) years. On the right hemisphere, MOCA test score decreased according to visual FDG-PET score of parietal lobe (15±5.1, 11.8±8.4 and 8.5±5.9; p=0.032). MOCA test score was 16±5.8, 13.1±7.6 and 9.1±6.1 in patients with left temporal lobe and 15.2±5.2, 11.8±7.8 and 8.5±5.9 in patients with left parietal lobe according to visual FDG-PET scores respectively (p=0.035; p=0.02). The comparison of the other right and left hemisphere regions and MOCA test scores were not significant.

Conclusion: The present study is emphasized that the MoCA test which is easily applied in outpatient clinics can be demonstrated the hypometabolism of bilateral parietal and left temporal brain regions related with pathophysiology of AD.

Keywords: Flouro-deoxi-glucose positron emission tomography, hypometabolism, montreal cognitive assessment test, Alzheimer disease

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common cause for dementia [1]. Cognitive impairment related to AD is a progressive condition and the diagnosis is based on clinical evaluation along with brain imaging. Magnetic resonance imaging (MRI) is the modality of choice to exclude non-alzheimer diseases. Additionally, the pattern and extent of brain atrophy on structural MRI scans can be used to support the diagnosis of AD [2]. The degree of brain atrophy can also be demonstrated by MR volumetric measurement but not routinly used because of its detailed and inconvenient nature [3]. Brain flor-18 fluoro-deoxy-glucose (FDG)-positron emission tomography (PET), which has long been frequently used in the diagnosis of neurodegenerative diseases, is deemed as the viable method for in-vivo examination of regional human brain metabolism in healthy human or disease state [4,5].

The Montreal Cognitive Assessment (MoCA) is one of the most common screening instruments developed in 2005. Using a cut-off score of 26 or above provides sensitivity and specificity for excluding normal conditions of 90% and 87%, respectively [6]. Although the MOCA cognitive screening tool has widely used in clinical practice, there are limited data on metabolic function in brain regions associated with MoCA score. The aim of this study was to analyze the relationship between MoCA tests and brain FDG-PET imaging of the patients, who are clinically considered to have AD, the most frequent etiologic cause of dementia.

MATERIALS AND METHODS

Study Population and Neuropsychiatric Assessment

This cross-sectional study was performed with 37 probable AD patients aged over 65 years admitted to the geriatric outpatient clinic of our center. All patients underwent MoCA test and FDG-PET imaging. Brain MRI was performed for all patients to exclude other causes of dementia. MoCA tests were administered to all patients routinely according to the standard instructions. [6-8]. The test consists of 13 tasks organized into eight cognitive and thinking domains including Visuospatial/Executive Function, Animal naming, Clock-drawing test, Attention, Language, Abstraction, Short-term memory, and Orientation. A total score ranges from 0 to 30. The MoCA score was considered abnormal if less than 26 [9].

Written informed consent was obtained from all participants. The study was ethically approved by the Hacettepe University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (16969557-No. 12 and Decision No. GO 15/763-13).

Brain FDG-PET imaging

8 mCi 18F-FDG was administered intravenously when fasting blood glucose levels were within normal values following 12 hours of fasting. Sixty minutes after injection, images in three-dimensional mode were taken from the vertex till upper mediastinum. Axial, coronal and sagittal slices were obtained after making attenuation correction with CT on the images obtained. The slices obtained were assessed visually and using SPM analysis.

The PET images of patients which were present in the PACS system were reevaluated by four specialists working in the Nuclear Medicine Department. A visual scoring was performed between 0 and 2 in order to obtain the rates of hypometabolism in brain regions. (0: normal metabolism, 1: mild hypometabolism, 2: moderate-severe hypometabolism).

Statistical Analysis

Statistical evaluation was performed using Statistical Package for Social Sciences 20 (SPSS) for Windows (IBM SPSS Inc., Chicago, IL) program. The variables were investigating using visual (histograms, probability plots) and analytical methods (Kolmogorov-Simirnov / Shapiro-Wilk's test) to determine whether or not they are normally distributed. Variables with normal distribution were shown as mean ± standard deviation while those without normal distribution were shown as median

with minimum and maximum range. Categorical variables were shown as number and percentage. As the MoCA scores were normally distributed the one-way ANOVA tests were conducted to compare this parameter and visual FDG-PET scores. Levene test was used to assess the homogeneity of the variances. When an overall significance was observed, pairwise post-hoc tests were performed using Tukey's test. $p \leq 0.05$ values were accepted as significant in the intergroup comparison results.

RESULTS

Baseline Demographic Characteristics

Of 37 patients, 20 were (54.1%) male and 17 (45.9%) were female. The median age was 77 years (minimum 65 – maximum 83). In whole population, the rates of comorbidities were as follows; diabetes mellitus (DM) 21.6%, hypertension (HT) 40.5%, coronary artery disease (CAD) 40.5%, thyroid dysfunction 27.0%, osteoporosis 27.0%, asthma/ chronic obstructive pulmonary disease (CIPD) 5.4% and urinary incontinence 29.7%. Median MoCA test score was 12 (minimum 1 – maximum 24). Median education years of the patients were 8 years (minimum 5- maximum 21)

Metabolic Assessment Results

The results of metabolic assessment via FDG-PET in different brain regions are presented in Table 1. The left temporal lobe was found to be the most common site of moderate to severe hypometabolism observed in 16 of 37 patients (43.2%) and followed by the right temporal lobe with 37.8% and left and right parietal lobes with 32.4%. On the other hand, the most common region of normal metabolic activity was noted in the right frontal region with 75.7%. The least common sites of normal metabolic activity were the left and right temporal lobes (40.5% and 43.2%, respectively).

Table 1. Metabolic assessment of different brain regions with FDG PET

	Visual Score of Hypometabolism		
	Score 0 n (%)	Score 1 n (%)	Score 2 n (%)
Right frontal	28 (75,7)	0 (0)	9 (24,3)
Left frontal	25 (67,6)	3 (8,1)	9 (24,3)
Right temporal	16 (43,2)	7 (18,9)	14 (37,8)
Left temporal	15 (40,5)	6 (16,2)	16 (43,2)
Right parietal	17 (45,9)	8 (21,6)	12 (32,4)
Left parietal	16 (43,2)	9 (24,3)	12 (32,4)
Right precuneus	24 (64,9)	7 (18,9)	6 (16,2)
Left precuneus	23 (62,2)	6 (16,2)	8 (21,6)

MoCA and visual FDG-PET scores

The comparison of visual FDG-PET scores and MoCA test results are presented in Table 2. Hypometabolism of right hemisphere regions were not associated the MoCA score except right parietal lob. Mean MoCA test score was 15±5.1, 11.8±8.4, and 8.5±5.9 according to visual FDG-PET score of 0, 1, and 2 in the right parietal region. The difference was statistically significant (p=0.032).

Table 2. Comparison of visual FDG-PET scores and MoCA test results

	Right Hemisphere		Left Hemisphere	
	MoCA Mean (± SD)	p	MoCA Mean (± SD)	p
Parietal		**0.032**		**0.02**
Score 0	15.0 (±5.1)		15.2 (±5.2)	
Score 1	11.8 (±8.4)		11.8 (±7.8)	
Score 2	8.5 (±5.9)		8.5 (±5.9)	
Temporal		0.09		**0.035**
Score 0	13.7 (±5.8)		16 (±5.8)	
Score 1	14.8 (±7.9)		13.1 (±7.6)	
Score 2	9.2 (±6.3)		9.1 (±6.1)	
Frontal		0.13		0.19
Score 0	13.2 (±7)		12.8 (±7.1)	
Score 1	-		16.6 (±6.4)	
Score 2	9.3 (±4.3)		9.3 (±4.3)	
Prekuneal		0.26		0.40
Score 0	13.3 (±6.2)		13.4 (±6.3)	
Score 1	11.2 (±9.1)		10.5 (±7.5)	
Score 2	9 (±4.8)		10.2 (±7.1)	

The mean MoCA score of the patients with moderate to severe visual score of left parietal and left temporal lobe hypometabolism was lower than the other patients. MoCA test score was 16±5.8, 13.1±7.6, and 9.1±6.1 in patients with left temporal lobe visual score 0,1, and 2, respectively (p=0.035).

In patients with left parietal lob visual FDG-PET score 0, 1, and 2, mean MoCA score was 15.2 ±5.2, 11.8±7.8, and 8.5±5.9 (p=0.02) (Figure 1). The comparison of the other left hemisphere regions and MoCA test scores were not significant.

DISCUSSION

In this study, we have systematically investigated the relationship between cognitive performance status and brain glucose metabolism in probable AD patients. Regional glucose hypometabolism has been able to demonstrate highest percentage of variance in the MoCA test scores. This is the first study using visual FDG-PET score to demonstrate the impairment in MoCA performance in patients with probable AD.

There are many methods to evaluate the cognitive impairment of patients in geriatric outpatient clinics. MoCA test is a common screening tool used in cognitive function assessment. The test assesses additionally attention, concentration, working memory, and language [8]. Recently, brain FDG-PET imaging methods have been introduced in clinical and research practice to diagnose of Alzheimer disease [4,5]. A meta-analysis including 27 different studies performed on patients with AD has shown that the sensitivity and specificity of FDG-PET were 91% (confidence interval 86 – 94%) and 86% (confidence interval 79 – 91%), respectively [10]. A pattern of hypometabolism is typically observed in the parietal, temporal and the posterior cingulate cortex regions in the AD [5,11]. Many researchers have shown that the diagnostic sensitivity of hypometabolism in the temporoparietal cortical areas varies; it is approximately 90% for patients with AD [12]. In our study, incidence of hypometabolism was ranked as temporal, parietal, precuneal and frontal similar with literature. When the disease progressed, the frontal lobe might also be affected. Our patients did not have frontal hypometabolism that might be related early phase of Alzheimer.

A few studies have been published searching correlation between geriatric assessment tests and brain FDG metabolism. In a study, decreased 18F – FDG uptake in the left and right precuneus, left fusiform gyrus, and left middle orbitofrontal gyrus have shown trend toward to with decreased MoCA score but significant correlation was shown only left posterior cingulate gyrus, an AD signature area (p=0.007) [13]. In another recently published study performed on 27 AD patients, the range of reduced FDG metabolism was negatively correlated with the total scores of MoCA. In the detailed brain region analyses, significant correlation was shown between reduced FDG metabolism of left parietotemporal and MoCA score (p=0.04) [14]. Similarly in our study MoCA test score did not differ according to visual score of frontal and precuneal hypometabolism. Median MoCA score significant decreased in patient with high left parietotemporal hypometabolism score. Unlike these studies, we have used a visual score including 3 categories to obtain the rates of hypometabolism.

Some study was performed to explain the relationship between domains of MoCA test and functional brain imaging. Clock drawing test (CDT) is a pair of MoCA test requires intact visuoconstructive skills which are mainly represented in the

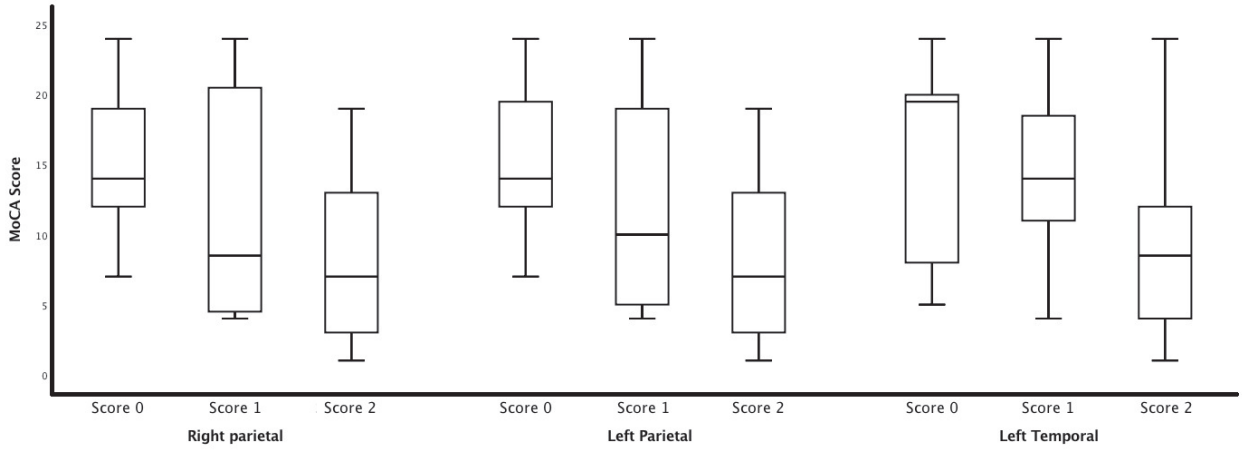

Figure 1. Box plots showing the median Montreal Cognitive Assessment (MoCA) score by visual FDG-PET score of brain regions where were found statistically significant.

parietal lobe. In a study included 71 patients, positive correlation between CDT scores and parietal glucose metabolism in the AD patients (z score=3.68; p<0.001) [15]. Another domain of MoCA test is calculation which is required the linguistic representation and visuospatial imaginary was evaluated on 91 patients. Arithmetic test score of the patients and FDG-PET glucose metabolism showed significant correlation with in the left inferior parietal lobe (r=0.405, p<0.0001) and in the left inferior temporal gyrus (r=0.381, p=0.0002). Abstraction domain of MoCA test requires semantic knowledge and conceptual thinking. On a PET imaging study showed that the metabolic reduction in the left temporal lobe correlates with impairment of abstraction function [16]. In our study, although the subdomains of the MoCA test were not examined individually, a correlation between MoCA scores and hypometabolism of parietal and temporal lobes was demonstrated in parallel with the literature.

This study is one of the study showing that a complex and inaccessible biomarker such as PET-CT mediated metabolic imaging can yield correlated results with MoCA that are easily applied and reproducible in the outpatient clinic.

CONCLUSION

Along with the ever-increasing elder population, the fact that dementia and AD will be encountered more frequently, leads to the renovation and elaboration of the tests to be used in the diagnostic process. Our results emphasize the relationships between brain glucose hypometabolism in patients with AD and their impact on cognitive functioning or vice versa. However, there is a need for prospective studies with larger number of patients and in which the measurements are repeated over time and compared with the baseline values.

Author contribution

Study conception and design: EA and MH; data collection: EA, PD, and EB; analysis and interpretation of results: KKO, BVS, BE, and ELE; draft manuscript preparation: BBY, MC, and MH. All authors reviewed the results and approved the final version of the manuscript.

Informed consent

Written informed consent was obtained from all participants.

Ethical approval

The study was approved by the Hacettepe University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (16969557-No. 12 and Decision No. GO 15/763-13, 06/01/2016).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Scheltens P, De Strooper B, Kivipelto M, et al. Alzheimer's disease. Lancet 2021; 397(10284):1577-1590.

[2] Dubois B, Villain N, Frisoni GB, et al. Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. Lancet Neurol 2021; 20(6):484-496.

[3] Chandra A, Dervenoulas G, Politis M, et al. Magnetic resonance imaging in Alzheimer's disease and mild cognitive impairment. J Neurol 2019; 266(6):1293-1302.

[4] Chetelat G, Arbizu J, Barthel H, et al. Amyloid-PET and (18) F-FDG-PET in the diagnostic investigation of Alzheimer's disease and other dementias. Lancet Neurol 2020; 19(11):951-962.

[5] Marcus C, Mena E, Subramaniam RM. Brain PET in the diagnosis of Alzheimer's disease. Clin Nucl Med 2014; 39(10):e413-422; quiz e423-416.

[6] Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005; 53(4):695-699.

[7] Nousia A, Siokas V, Aretouli E, et al. Beneficial Effect of Multidomain Cognitive Training on the Neuropsychological Performance of Patients with Early-Stage Alzheimer's Disease. Neural Plast 2018; 2018:2845176.

[8] Woodford HJ, George J. Cognitive assessment in the elderly: a review of clinical methods. QJM 2007; 100(8):469-484.

[9] Carson N, Leach L, Murphy KJ. A re-examination of Montreal Cognitive Assessment (MoCA) cutoff scores. Int J Geriatr Psychiatry 2018; 33(2):379-388.

[10] Bloudek LM, Spackman DE, Blankenburg M, et al. Review and meta-analysis of biomarkers and diagnostic imaging in Alzheimer's disease. J Alzheimers Dis 2011; 26(4):627-645.

[11] Shivamurthy VK, Tahari AK, Marcus C, et al. Brain FDG PET and the diagnosis of dementia. AJR Am J Roentgenol 2015; 204(1):W76-85.

[12] Bohnen NI, Djang DS, Herholz K, et al. Effectiveness and safety of 18F-FDG PET in the evaluation of dementia: a review of the recent literature. J Nucl Med 2012; 53(1):59-71.

[13] Zukotynski K, Gaudet V, Kuo PH, et al. The Use of Random Forests to Identify Brain Regions on Amyloid and FDG PET Associated With MoCA Score. Clin Nucl Med 2020; 45(6):427-433.

[14] Jing J, Zhang F, Zhao L, et al. Correlation Between Brain 18F-AV45 and 18F-FDG PET Distribution Characteristics and Cognitive Function in Patients with Mild and Moderate Alzheimer's Disease. J Alzheimers Dis 2021; 79(3):1317-1325.

[15] Lee DY, Seo EH, Choo IH, et al. Neural correlates of the Clock Drawing Test performance in Alzheimer's disease: a FDG-PET study. Dement Geriatr Cogn Disord 2008; 26(4):306-313.

[16] Woo BK, Harwood DG, Melrose RJ, et al. Executive deficits and regional brain metabolism in Alzheimer's disease. Int J Geriatr Psychiatry 2010; 25(11):1150-1158.

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ORIGINAL ARTICLE

Effects of the COVID-19 Pandemic on Patients with Schizophrenia Spectrum Disorders

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ABSTRACT

Objective: To evaluate the mental health of patients with schizophrenia spectrum disorders with the prolongation of the pandemic.

Materials and Methods: This descriptive cross-sectional study was conducted between August-October 2020. Fifty-two patients with schizophrenia spectrum disorders who were hospitalized prior to the onset of the pandemic between March 2019-March 2020 at the inpatient clinic were reassessed during the pandemic. The Positive and Negative Syndrome Scale (PANSS), the Clinical Global Impression-Severity (CGI-S) Scale, Hamilton Depression Rating Scale (HAM-D), Hamilton Anxiety Rating Scale (HAM-A) and Fear of COVID-19 Scale were used to evaluate psychopathology during the pandemic. The PANSS and the CGI severity scores at discharge from the inpatient clinic for each patient were obtained from the medical records review for comparison along with sociodemographic variables.

Results: A total of 34 patients, 33 with schizophrenia (97.1%) and 1 with schizoaffective disorder (2.9%) were included. There was no significant difference between the pre- and during the pandemic assessments in the PANSS total and the CGI severity scores. The PANSS total, the CGI, HAM-D, HAM-A and the Fear of COVID-19 scale scores, medical comorbidity and utilization of psychiatric health care services were significantly higher in patients who reported subjectively increased psychiatric symptoms during the pandemic. There was no significant difference in the change of PANSS total and CGI scores between the two groups. Fear of COVID-19 Scale and HAM-A scores were correlated positively.

Conclusion: During pandemic increase in psychiatric symptoms may be related not only to psychosis but also depression, anxiety. According to the results of patients who reported subjectively increased psychiatric symptoms during the pandemic, while the psychotic symptoms did not worsen during the pandemic, patients with higher anxiety or depression scores reported an increase in their symptoms and were more likely to seek help.

Keywords: COVID-19, Pandemic, Psychosis, Schizophrenia Spectrum Disorder, Tele-medicine

INTRODUCTION

The outbreak of the Corona Virus Disease 2019 (COVID-19) started in Wuhan /China, spread rapidly all around the world and the World Health Organization declared pandemic in March 2020. The elderly and patients with chronic disease or cancer are at higher risk in terms of negative consequences of COVID-19 [1]. From the earliest days of the pandemic, it has been thought that patients with mental illness would be among the risk groups due to their mental symptoms, side effects of psychotropic drug, medical comorbidity and smoking [2]. In a recent population-based study, the hypothesis that people with mental illness are among the risk groups for COVID-19 has been confirmed [3,4]. Cognitive dysfunction, lack of insight, sociodemographic characteristics such as living in congregate housing, homelessness and difficulty in adapting to protective measures are risk factors for patients with schizophrenia/ schizophrenia spectrum disorder to contact COVID-19. It has been shown that protective measures were less effective in these patients and negative consequences related to COVID-19 were seen in higher rates [3,4].

After the declaration of the pandemic, various protective measures such as social distancing, isolation, all of which are psychologically challenging, have been taken to prevent the spread of the disease, and a negative impact of the pandemic on mental health was expected based on previous pandemic knowledge [5]. Prevalence of depression, anxiety, sleep disorders were found to be increased in the general population [6]. The study of Wang and colleagues [7] conducted between January-February 2020 reported that the rate of moderate to severe depression and anxiety symptoms were 16.5% and 28.8%, respectively. In the study conducted between March-May 2020, the rate of depression, anxiety and/or stress at clinical levels was 65.6% [8]. These studies might indicate that the rates of psychiatric symptoms such as depression and anxiety increase with the prolongation of the pandemic [7,8]. From the beginning of the COVID-19 pandemic it has been thought that patients with severe mental illness (SMI) such as schizophrenia, bipolar disorder would be more severely affected by the physical consequences of COVID-19, as well as the psychosocial consequences associated with different aspects of the pandemic [9,10]. On the other hand, the results of a study conducted between April and June 2020 indicated that there was no increase in the psychotic symptoms of patients with schizophrenia who had been evaluated before and during the pandemic [11]. Another study evaluating the impact of the pandemic on the patients with schizophrenia between April-May 2020 showed that these patients were affected less severely than expected [12]. Similarly, an online/telephone survey conducted in a psychiatry clinic in Turkey revealed that majority of patients with schizophrenia did not report any psychotic exacerbation during the first two months of pandemic [13].

Schizophrenia, which is characterized by chronic psychotic symptoms and lead to poor social functioning, is one of the 10 diseases that cause the greatest burden of disease in the world [14], therefore it became an important public health concern how they would be affected from the pandemic. The pandemic has also affected the follow-up and treatment of patients with schizophrenia negatively, due to the interruption of health care. Abrupt changes in mental health care induce relapse by causing decrease in treatment compliance and withdrawal from health care. In order to maintain the continuity of treatment in times of such crises, it's recommended to utilize tele-medicine methods despite some limitations [4,15].

In the majority of studies regarding the effects of the COVID-19 pandemic on mental health, the effects of uncertainty, isolation and quarantine on the general population and health-care workers have been examined. Fewer studies in the literature evaluate the effects of the pandemic on patients with existing mental illness and mental health care [16]. In the few studies which evaluate the effects of the pandemic on mental status of patients with schizophrenia and other psychotic disorders, the early effects of the pandemic on these patients

have been examined [11,12]. Considering the results of studies showing that psychological effects in the general population increase in time with the prolongation of the pandemic, it's necessary to examine how patients with schizophrenia/schizophrenia spectrum disorders are affected by the pandemic in the long term. To our knowledge, in the literature there are no studies which evaluate the long-term effects of pandemic on the mental health of schizophrenia/schizophrenia spectrum disorder patients and their utilization of mental health care. In this study, we aimed to evaluate the effects of the pandemic on the mental health status of patients with schizophrenia/schizophrenia spectrum disorders at the end of the half year past the onset of the pandemic by comparing the psychopathological state of patients who had been hospitalized in the previous year before the onset of the pandemic, to their state at the end of 6-8 months into the pandemic. Hospitalized patients within the past year before the onset of the pandemic were particularly chosen as this would be a group with thorough assessments regarding severity of psychopathology and would be in close follow up after discharge. Our hypothesis was that psychotic symptoms would have increased during the course of the pandemic.

MATERIALS AND METHODS

Subjects

This descriptive cross-sectional study was conducted between August-October 2020 at Hacettepe University Faculty of Medicine, department of psychiatry. Inclusion criteria for the sample was defined as patients with complete medical records and patients who were hospitalized prior to the onset of the pandemic between March 2019-March 2020 at the inpatient clinic with a diagnosis of schizophrenia spectrum disorders. There were no exclusion criteria for this study.

A total of 52 patients diagnosed with schizophrenia/schizophrenia spectrum disorders were identified to be hospitalized between March 2019 - March 2020 at the inpatient clinic. Among these, 6 patients refused to participate in the study, 9 patients refused to come to the hospital or to interview via telepsychiatry methods, 3 patients could not be reached by any means. The remaining 34 patients who provided informed consent were included in the study.

Approval from the Hacettepe University Faculty of Medicine Ethics Committee was obtained for this study (Number: 2020/12-78).

Method

Patients were either interviewed face to face or via telepsychiatry methods to complete study assessments consisting of a questionnaire including sociodemographic such as age, sex and clinical characteristics. The other clinical characteristics such as diagnosis, duration of illness, pharmacological treatment, duration of treatment, length of stay in hospital, treatment compliance before the pandemic, medical and psychiatric comorbidity, Positive and Negative Syndrome Scale (PANSS) and Clinical Global Impression-Severity (CGI) scores at discharge from the inpatient clinic were obtained from the medical records for each patient. We also evaluated subjective increase in psychiatric symptoms by asking "Do you feel an increase in your psychiatric symptoms during the pandemic?". According to the subjective evaluation of the psychiatrist, patients' information level about the pandemic was graded as a high, medium and low. The PANSS [17,18], CGI [19], Hamilton Depression Rating Scale (HAM-D) [20,21], Hamilton Anxiety Rating Scale (HAM-A) [22-24] and Fear of COVID-19 Scale were used to evaluate the psychopathology. The Fear of COVID-19 Scale is a unidimensional 7 item 5-point likert type scale which was developed by Ahorsu and colleagues [25], and is used to assess the severity of fear caused by the pandemic and a higher score indicates more severe fear of COVID-19. The Turkish validity and reliability study has been recently conducted by Satıcı and colleagues [26].

Statistical Analysis

Statistical analysis was conducted using the SPSS 23.0 package software for Windows. Descriptive statistics were expressed as mean ± standard deviation for continuous variables and as number

and frequencies (percentages) for categorical variables. Participants have been categorized into two groups: Those who reported subjective increase in the severity of psychiatric symptoms during the pandemic and those who did not. The differences in categorical variables between two groups were analyzed using Chi-square test of Fisher's Exact Test and the differences in numerical variables between two independent groups were analyzed using the Mann Whitney U test for independent samples when variables were not normally distributed. Wilcoxon-signed rank test was used to compare the scores of PANSS and CGI pre- and during the pandemic. Spearman's correlation analysis was used to evaluate the relationship between fear of COVID-19 scale and other variables. The p value of <0.05 was accepted as a statistically significant.

RESULTS

Sociodemographic and clinical characteristics

A total of 34 patients, 33 with schizophrenia (97.1%) and 1 with schizoaffective disorder (2.9%) were included. Twelve (35.3%) of the patients were female, 22 (64,7%) were male. Twenty-two patients were evaluated in the outpatient clinic face to face and 12 were evaluated via tele-psychiatry methods. The mean duration from discharge of patients until the declaration of the pandemic was 122 (±103,3) days and the mean duration from the declaration of the pandemic to evaluation for the study was 179 (±30.49) days. Only 1 of the patients included in the study had confirmed COVID-19 infection and had survived without any problems. Eighteen patients (52.9%) were not working and 1 of these patients was dismissed from his former job due to the pandemic, 1 had left his job voluntarily. Five patients (14.7%) were living alone. While 22 patients were being treated with clozapine, 12 patients were using other antipsychotics as monotherapy or in combination with another antipsychotic. Thirty-one patients stated that they had information about the pandemic; according to the subjective evaluation of the psychiatrist, the level of the information was high in 15 (44.1%); medium in 13 (38.2%) and low in 3 (8.8%8). The patients indicated newspaper, television, internet, social media and official institutions as sources of information. Number of patients who reported increased severity of psychiatric symptoms were 14 (41.2%). Major sociodemographic and clinical characteristics during the pandemic are shown in Table 1.

Before discharge from hospital, the mean (±SD) PANSS total score of the patients was 65.9±13.2 and CGI score was 3.6±0.7. The assessments during the pandemic revealed that the mean (±SD) PANSS total score was 62.3±16.5, CGI score 3.5±1.6, HAM-D score 7.9±8.9, HAM-A score 6,9±9,8 and the Fear of COVID-19 Scale score 17.5±7.5. There was no significant difference between the pre- and during the pandemic assessments in the PANSS Total and CGI scores (z=-1.515, p=0,130; z=-0.677, p=0.498, respectively)

When patients who reported subjectively increased psychiatric symptoms during the pandemic (Group 1, N: 14) were compared to those who did not (Group 2, N:20), age, sex, education, age of onset, duration of illness, duration of treatment, treatment compliance were not significantly different between the groups. There was also no significant change in the difference between the PANSS total and CGI scores between two groups. On the other hand, the PANSS total, CGI, HAM-D, HAM-A and the Fear of COVID-19 scale scores and utilization of psychiatric health care services were significantly higher in Group 1 (Table 2).

There were 12 patients (35.3%) with a HAM-D score of ≥8 which indicates the presence of depression. When compared to patients with HAM-D scores <8, the mean PANSS total score, CGI-S were significantly higher in patients with depression (p scores <0.001). Patients with depression reported a higher rate of subjective increase in their psychiatric symptoms (p=0.001).

There were 8 patients (23.5%) with HAM-A scores ≥8 which means anxiety. Patients with anxiety had significantly higher scores in PANSS, CGI, the Fear of COVID-19 scales compared to patients with HAM-A <8 (p scores 0.001, 0.002, <0.001, respectively). Patients with anxiety reported a higher rate of subjective increase in their psychiatric symptoms (p<0.001).

There was a positive correlation between the Fear of COVID-19 Scale and HAM-A scores (r=0.377, p=0.028).

Table 1. Sociodemographic and clinical characteristics of the patients

Variables	Mean±SD	Median (25-75 percentiles)
Age	39,8±14,7	39 (26-48)
Duration of education (years)	10.8±4,7	11 (8-15)
Age of onset	24,1±10,9	22 (15-30)
Duration of illness (month)	175,5±114,5	168 (96-276)
Duration of treatment (month)	130,9±100	120 (31-204)
Length of stay in hospital (day)	49,8 ±31.2	47 (29-57)
PANSS at discharge	65.9±13.2	62 (56-74)
during pandemic	62.3±16.5	63 (47-77)
CGI at discharge	3.6±0.7	3 (3-4)
during pandemic	3.5±1.6	3 (3-5)
HAM-D	7.9±8.9	5 (1-13)
HAM-A	6,9±9.8	3 (0-7)
Fear of COVID19 scale	17.5±7.5	17 (13-22)
	n (%)	
Marital Status		
Married	6 (17.6)	
Single	28 (82,4)	
	n (%)	
Diagnosis of psychiatric disorders		
Schizophrenia	33 (97,1)	
Schizoaffective	1 (2.99)	
Psychiatric comorbidity		
Depression	8 (23.5)	
Anxiety Disorder	3 (8.8)	
Other	5 (14.7)	
Comorbid Medical Disease		
Diabetes mellitus	5 (14.7)	
Hypertension	2 (5.9)	
Coronary artery disease	1 (2.9)	
Asthma	1 (2.9)	
Other	5 (14.7)	
Treatment during assesment		
Clozapine	22 (64.7)	
Other antipsychotics	12 (35.3)	
Smoking		
Yes	18 (52,9)	
No	16 (47,1)	
Alcohol		
Yes	7 (20,6)	
No	27 (79,4)	
Treatment compliance before pandemic		
High	24 (70,6)	
Medium-Low	10 (29,4)	
Utilization of mental health services during pandemic		
Yes	15 (44,1)	
No	19 (55,9)	

SD: standard deviation, PANSS: Positive and Negative Syndrome Scale, CGI: Clinical Global Impairment, HAM-D: Hamilton Depression Rating Scale, HAM-A: Hamilton Anxiety Rating Scale

Table 2. Comparison of patients reporting subjective increase in psychiatric symptoms (Group 1) and those who did not (Group 2)

Variables	Group 1 (N=14)	Group 2 (N=20)	Statistical Analysis
	Mean±SD	Mean±SD	
Age	39.0±15.0	40.3±14.9	p=0.823*
Duration of education (year)	9.4±5.1	11.8±4.2	p=0.217*
Age of onset	22.3±10.8	25.4±11.1	p=0.377*
Duration of illness (month)	185.4±121.6	168.6±112.0	p=0.641*
Duration of treatment (month)	134.3±100.4	128.4±102.2	p=0.823*
PANSS Total	75.1±11.8	53.4±13.2	p<0.001*
CGI	4.4±0.9	2.9±0.9	p<0.001*
HAM-D	11.3±9.8	3.4±4.3	p<0.001*
HAM-A	14.3±11.6	1.7±2.3	p<0.001*
Fear of COVID-19 Scale	22.9±7.2	14.0±5.2	p=0.001*
	n (%)	n (%)	
Sex			
Female	4 (11.8)	8 (23.5)	p=0.717†
Male	10 (29.4)	12 (35.3)	
Clozapine use			
Yes	9 (64.3)	13 (65.0)	p=1.000†
No	5 (35.7)	7 (35.0)	
Medical comorbidity			
Yes	10 (29.4)	6 (17.6)	p=0.042†
No	4 (11.8)	14 (41.2)	
Distrupted treatment compliance during pandemic			
Yes	4 (28.6)	4 (20)	p=0.689†
No	10(71.4)	16 (80)	
Utilization of mental health services during pandemic			
Yes	10 (71.4)	5 (21)	p=0.020†
No	4 (28.6)	15 (79)	

*: Mann Whitney U Test, †: Chi-square test of Fisher's Exact Test

SD: standard deviation, PANSS:Positive and Negative Syndrome Scale, CGI: Clinical Global Impairment, HAM-D: Hamilton Depression Rating Scale, HAM-A: Hamilton Anxiety Rating Scale

DISCUSSION

The aim of this study was to reevaluate patients with schizophrenia spectrum disorders who had been hospitalized during the year before the outbreak of COVID-19 in the midst of the pandemic, and to examine the effect of this ongoing crisis on their psychopathology. The most interesting finding of the study was that there was no difference regarding the severity of psychopathology between the two time points, as indicated by the PANSS and CGI scores at discharge and in the reevaluation during the pandemic. In addition, although the patients in the group who subjectively stated that their severity of psychiatric symptoms increased during the pandemic had higher scores on all scales, there was no significant difference in the objective change of PANSS-Total and CGI scores between the two groups.

Several risk factors such as advanced age, chronic disease, smoking and immunosuppression have been determined for COVID-19, and patients with mental illness were also included in these risk groups [2]. It was found that patients with a new psychiatric diagnosis in the previous year before the pandemic have more COVID-19 than those without mental illness and that mortality is higher in these patients [7]. It has also been argued that there are different risk factors for each mental disorder [7]. When it has been stated that the high rates for COVID-19 in patients with schizophrenia

may have resulted from difficulties in applying protective measures such as isolation, quarantine due to delusions, cognitive dysfunctions, poor insight or/and the role of similar mechanisms such as inflammation in both diseases [4,7]. Smoking habits, medical comorbidities which are common in patients with schizophrenia also lead to high mortality rates [2,7]. In addition, since the beginning of the pandemic, it has been mentioned that both the disease itself and its consequences along with mandatory protective measures all have negative effects on mental health in the general population, and that people with preexisting mental illness will be affected even more negatively [27]. Nevertheless, in the current literature, studies showing the relationship of these risk factors with psychotic exacerbations include only case reports [28,29]. On the contrary, in one study, it has been shown that the prevalence of psychosis among university students did not increase during the pandemic compared to pre-pandemic period [30]. Similarly, majority of patients with schizophrenia didn't report any psychotic exacerbation in a study conducted in Turkey [13]. In our study, no significant difference was found between the pre- and during the pandemic psychopathology severity scores consisting of PANSS and CGI evaluations, this finding is consistent with the evidence showing that the COVID-19 pandemic does not cause a significant increase in psychotic symptoms. These results have previously been interpreted as those with SMI are more resilient to the psychological effects of a pandemic [11,31].

In the literature several variables that protect patients with schizophrenia from being negatively affected by disasters in acute and subacute period are defined [32]. Psychopathology -related (i.e., negative symptoms, lack of insight, or drug treatment) and treatment related variables (qualified and competent follow-up process during and after the disaster, as in the present case of pandemic) may play a compensating role in the negative ef¬fects of the event. Parallel to this opinion, Katz and colleagues [33] have previously reported that patients with schizophrenia may remain clinically stable after disasters. They offered the vulnerability stress coping model as the mechanism of sustaining clinical stability. This coping model suggests that the subjective perception of distressing events in clinically stable schizophrenia patients does not form an immediate negative interpretation. In addition various studies show that individuals using the avoidance strategy to cope with the negative effects of distressing events generally have more severe psychopathology compared to patients who mainly use other coping mechanisms [34,35]. These mechanisms, which have been suggested to lead to protective effects, in the acute and subacute periods of disasters, may have also contributed to the resilience of schizophrenia patients in terms of psychotic symptoms in our study. To our knowledge, there are no studies evaluating long term effects of disasters or other mass events like a pandemic on the psychotic symptom severity of patients with schizophrenia.

Stigmatization in schizophrenia is an important problem in patients' access to treatment, and it is divided into public stigma and self-stigma [36]. It is argued that self-stigmatization in schizophrenia patients may decrease with the increase in perceived similarities with the general population [36]. In this context, the applying of restrictions to prevent the spread of the disease to the world reduces the internal stigmatization of patients with schizophrenia, and therefore may have been protective against increase in psychotic symptoms. However, in order to support this interpretation, studies investigating how self-stigma has been affected by the pandemic in patients with schizophrenia are needed.

In studies conducted in the general population at different time points, it has been shown that negative effects on mental health increase with the prolongation of the pandemic [6,8]. Studies suggesting schizophrenia patients are more resilient to the mental effects caused by the pandemic have been conducted in the earlier period of the COVID-19 [11,12]. When compared to other studies, in our study patients were evaluated at a later period of the pandemic and it was found that there was no increase in psychotic symptoms parallel to the results of the studies conducted in the early periods of pandemic. This result suggests that resilience may not be limited to the early period of the pandemic for patients with schizophrenia.

Another finding of this study is that the patients who reported an increase in their psychiatric symptoms during the pandemic had higher scores in all psychopathology severity rating scales in the pandemic period. Although these patients also had

higher PANSS total scores before discharge from the hospital compared to those who did not report an increase in their symptoms during the pandemic, no difference between two groups in terms of change in the PANSS total scores were found. This finding may suggest that the symptoms which patients claim to be exacerbated may not be the psychotic symptoms, but other mental symptoms that may occur during the pandemic period. Patients who had more anxiety and depression symptoms at follow up, as indicated by higher HAM-D and HAM-A scores, reported a subjective increase in their psychiatric symptoms and their total PANSS scores were found to be higher. This finding is most likely due to the fact that PANSS evaluation includes multiple domains of psychopathology including depression and anxiety, and not only psychotic symptoms. We suggest that this subjective increase could be related with higher depression and anxiety symptoms assessed during the pandemic. In our clinic, baseline HAM-A and HAM-D are not used in the routine of patients with a diagnosis of schizophrenia, and therefore we could not compare the pre-pandemic and pandemic depression and anxiety scores. The lack of HAM-D and HAM-A baseline evaluations to further support the view that the subjective increase of symptoms during the pandemic could be related with increasing depressive and anxiety symptoms, is an important limitation.

Previously, higher levels of avoidance predicted higher residual stress symptoms at 5 weeks after an earthquake in schizophrenia patients [32]. Subacute effects of these particular coping mechanism may also have contributed to the observed anxiety symptoms in our study group. One study showed that patients with SMI had more anxiety associated with the COVID-19 pandemic [38] and the higher anxiety scores of patients who reported an increase in their symptoms supports this finding. Since anxiety may have unfavorable outcomes in patient with SMI, screening anxiety in patients with psychiatric disorders is important in terms of early interventions [38]. In our study, we determined that although the pandemic did not affect the positive psychotic symptoms negatively, patients having higher anxiety or depression scores reported an increase in their symptoms and were more likely to seek mental health services than others. This finding supports the importance of screening for anxiety and depression in patients with schizophrenia spectrum disorders in periods of crisis such as a pandemic.

With the pandemic, measures such as postponing appointments and reducing patient quota in healthcare services have been taken which cause disruptions in health care [39]. On the other hand, some patients have avoided referral to hospitals because of COVID-19 even if they have complaints [40]. In this study, the rate of referral to psychiatry was significantly higher in patients who reported an increase in their psychiatric symptoms during the pandemic, this finding is important in terms of showing that these patients had the opportunity of utilizing a health care service during the pandemic. Since the beginning of the pandemic, the use of telemedicine methods has been suggested in order to prevent disruptions in health care, in our clinic a telephone line and a triage system has been established with the declaration of the pandemic. Our results supported that tele-psychiatry interventions are important to facilitate the utilization of mental health care in patients with increased psychiatric symptoms [41].

Previous studies conducted during the pandemic evaluated psychopathology by self-report scales and one of the strengths of this study is that evaluation and rating of psychopathology of the patients was mainly based on psychiatric interview and structured scales. The relatively small sample size and the subjective evaluation of whether there is an increase in the symptoms of the psychiatric disorder and whether treatment compliance is kept and access to psychiatric services is present during the pandemic, are among the limitations of the study.

In conclusion, although studies with larger samples are needed to explore the impact of the pandemic on patients with schizophrenia spectrum disorders, it appears that this group of patients are more resilient to the effect of stress related to the pandemic and this resilience continues into the 6-8 months into the pandemic. However, it should be kept in mind that the prolongation of the pandemic could still cause negative consequences upon daily life and mental health care. Although positive psychotic symptoms do not appear to be exacerbated, patients report discomfort and morbidity due to an increase in depressive and anxiety symptoms. The overall effect of the pandemic on the psychopathology of the schizophrenia spectrum patients could lead to

more substantial change in the months to follow and therefore should be reexamined. In addition, the importance of tele-medicine applications for accessing to health care during the pandemic is evident, despite limitations they should be utilized in the follow up of seriously mentally ill patients in different clinical settings.

Author contribution

Study conception and design: EÖE, MİY, AEAY, AE, and MKY; data collection: OKY and ÖT; analysis and interpretation of results: SK, EÖE, MİY, AEAY, AE, and MKY. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Hacettepe University Faculty of Medicine Ethics Committee (Protocol no. 2020/12-78/23.06.2020).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Jordan RE, Adab P, Cheng KK. Covid-19: risk factors for severe disease and death. BMJ. 2020;368: m1198.

[2] Padala SP, Dennis RA, Caceda R. Why COVID-19 Is Especially Difficult for Those With Schizophrenia: Reasons and Solutions. Prim Care Companion CNS Disord 2020; 2.

[3] Wang Q, Xu R, Volkow ND. Increased risk of COVID-19 infection and mortality in people with mental disorders: analysis from electronic health records in the United States. World Psychiatry 2021;20:124-130.

[4] Kozloff N, Mulsant BH, Stergiopoulos V, et al. The COVID-19 Global Pandemic: Implications for People With Schizophrenia and Related Disordersi Schizophr Bull 2020;46:752-757.

[5] Brooks SK, Webster RK, Smith LE, et al. The psychological impact of quarantine and how to reduce it: rapid review of the evidence Lancet 2020;395:912-920.

[6] Huang Y, Zhao N. Generalized anxiety disorder, depressive symptoms and sleep quality during COVID-19 epidemic in China: a web-based cross-sectional survey. Psychiatry Res 2020;288:112954.

[7] Wang C, Pan R, Wan X, et al.. Immediate psychological responses and associated factors during the initial stage of the 2019 coronavirus disease (COVID-19) epidemic among the general population in China. Int J Environ Res Public Health 2020;17:1729.

[8] Tso IF, Park S. Alarming levels of psychiatric symptoms and the role of loneliness during the COVID-19 epidemic: A case study of Hong Kong. Psychiatry Res 2020;293:113423.

[9] Zhand N, Joober R. 2021. Implications of the COVID-19 pandemic for patients with schizophrenia spectrum disorders: narrative review. BJPsych Open 2021;7:e35.

[10] Stefana A, Youngstrom EA, Chen J, et al. The COVID-19 pandemic is a crisis and opportunity for bipolar disorder. Bipolar Disord 2020;22:641-643.

[11] Pinkham AE, Ackerman RA, Depp CA, et al. A Longitudinal Investigation of the Effects of the COVID-19 Pandemic on the Mental Health of Individuals with Pre-existing Severe Mental Illnesses. Psychiatry Res 2020;294:113493.

[12] Koreki A, Nakane J, Kitada S, et al. Impact of COVID-19 on psychiatric day care services. Asian J Psychiatr 2020;54:102442.

[13] Hoşgelen EI, Alptekin K.COVID-19 Salgınının Şizofreni Hastalarındaki Etkisi. Turk J Psychiatry 2021. doi: 10.5080/u26175.

[14] Barbato A. Schizophrenia and public health. WHO 1998.

[15] Sole B, Verdolini N, Amoretti S, et al. Effects of the COVID-19 pandemic and lockdown in Spain: comparison between community controls and patients with a psychiatric disorder. Preliminary results from the BRIS-MHC STUDY. J Affect Disord 2021;281:13-23.

[16] Lakhan R, Agrawal A, Sharma M. Prevalence of Depression, Anxiety, and Stress during COVID-19 Pandemic, J Neurosci Rural Pract 2020;11:519-525.

[17] Kay SR, Fiszbein, A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;13:261-276.

[18] Kostakoğlu AE, Batur S, Tiryaki A, et al. Pozitif ve negatif sendrom ölçeğinin (PANSS) Türkçe uyarlamasının geçerlilik ve güvenilirliği. Türk Psikoloji Dergisi 1999; 14: 23-32.

[19] Guy W. ECDEU Assessment Manual for Psychopharmacology', US Department of Health and Human Services Publication 1976;218-222.

[20] Hamilton M. Development of a rating scale for primary depressive illness. Br J Soc Clin Psychology 1967;6:278-296.

[21] Aydemir A, Örsel S, Dağ I, et al. Hamilton Depresyon Derecelendirme Ölçeği (HDDÖ)'nin geçerliliği, güvenirliliği ve klinikte kullanımı. Psikiyatri Psikoloji Psikofarmakoloji Dergisi. 1996;4:251-259.

[22] Hamilton M. The assessment of anxiety states by rating, Br J Med Psychol 1959;32: 50-55.

[23] Yazıcı MK, Demir B, Tanrıverdi N, et al. Hamilton Anksiyete Değerlendirme Ölçeği, Değerlendiriciler Arası Güvenilirlik ve Geçerlilik Çalışması. Turk J Psychiatry1998;9:114-117.

[24] Matza LS, Morlock R, Sexton C, et al. Identifying HAM-A cutoffs for mild, moderate, and severe generalized anxiety disorder Int J Methods Psychiatr Res 2010; 19: 223-232

[25] Ahorsu DK, Lin CY, Imani V, et al. The Fear of COVID-19 Scale: Development and Initial Validation. Int J Ment Health Addict 2020;1-9.

[26] Satici B, Gocet-Tekin E, Deniz ME, et al. Adaptation of the Fear of COVID-19 Scale: Its Association with Psychological Distress and Life Satisfaction in Turkey. Int J Ment Health Addict 2020;6:1980-1988.

[27] Pfefferbaum B, North CS. Mental Health and the Covid-19 Pandemic. N Engl J Med 2020;383: 510-512.

[28] Lynch A, Bastiampillai T, Dhillon R. Can COVID-19-related anxiety trigger a relapse of schizophrenia? Aust N Z J Psychiatry 2020: 4867420954564.

[29] Brown ER, Gray S, Lo Monaco B, et al. The potential impact of COVID-19 on psychosis: A rapid review of contemporary epidemic and pandemic research. Schizophr Res 2020;222:79-87.

[30] Hajduk M, Dancik D, Januska J, et al. Psychotic experiences in student population during the COVID-19 pandemic. Schizophr Res 2020; 222:520-521.

[31] Pan KY, Kok AAL, Eikelenboom, et al. The mental health impact of the COVID-19 pandemic on people with and without depressive, anxiety, or obsessive-compulsive disorders: a longitudinal study of three Dutch case-control cohorts. Lancet Psychiatry 2021;8:121-129.

[32] Horan WP, Ventura J, Mintz J, et al. Stress and coping responses to a natural disaster in people with schizophrenia. Psychiatry Res 2007;151:77-86.

[33] Katz CL, Pellegrino L, Pandya A, et al. Research on psychiatric outcomes and interventions subsequent to disasters: a review of the literature. Psychiatry Res 2020; 110:201-217.

[34] Morris MC, Evans LD, Rao U, et al. Executive function moderates the relation between coping and depressive symptoms. Anxiety Stress Coping 2015;28:31-49.

[35] Norris FH, Friedman MJ, Watson PJ, et al. 60,000 disaster victims speak: Part I. An empirical review of the empirical literature, 1981-2001. Psychiatry 2002;65:207-239.

[36] Violeau L, Valery KM, Fournier T, et al. How continuum beliefs can reduce stigma of schizophrenia: The role of perceived similarities. Schizophr Res 2020;220:46-53.

[37] Sayeed A, Kundu S, Al Banna MH, et al. Mental Health Outcomes of Adults with Comorbidity and Chronic Diseases during the COVID-19 Pandemic: A Matched Case-Control Study. Psychiatr Danub 2020;32:491-498.

[38] Gonzalez-Blanco L, Dal Santo F, Garcia-Alvarez L, et al. COVID-19 lockdown in people with severe mental disorders in Spain: Do they have a specific psychological reaction compared with other mental disorders and healthy controls? Schizophr Res 2020;223:192-198.

[39] Moreno C, Wykes T, Galderisi S, et al.. How mental health care should change as a consequence of the COVID-19 pandemic. Lancet Psychiatry 2020;7: 813-824.

[40] Goncalves-Pinho M, Mota P, Ribeiro Jet al The Impact of COVID-19 Pandemic on Psychiatric Emergency Department Visits - A Descriptive Study', Psychiatr Q 2020;25:1-11.

[41] Šago D, Martiü V, Šmida D, et al. Telepsychiatry in the time of the covid-19 and earthquake in zagreb as odysseus between scylla and charybdis. Psychiatr Danub 2020;32: 478-481.

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ORIGINAL ARTICLE

Event-free Survival in Patients with Chronic Myeloid Leukemia Receiving Front-line Imatinib Mesylate

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ABSTRACT

Objective: Chronic myeloid leukemia (CML) prognostication at the time of diagnosis is critical to determine the intensity of initial treatment. Event-free survival (EFS) has become a prominent concept of prognosis in the patients with chronic phase CML (CML-CP). The aim of this study is to assess the prognostic impact of bone marrow (BM) and peripheral blood (PB) cellular components, in correlation with the clinical parameters.

Materials and Methods: One hundred forty-three patients with CML-CP on the front-line imatinib mesylate therapy were recruited into this study. Clinical and laboratory characteristics, therapeutic responses were recorded. Sokal, Euro/Hasford, The EUropean Treatment Outcome Study (EUTOS) and The EUTOS long-term survival (ELTS) scores were calculated for the studied patients.

Results: Median follow-up time was 84 (IQR: 54-125) and median front-line therapeutic duration was 56 (IQR:23-89) months. Five-year EFS rate was 62.3% (95% CI: 53.9-70.7). The blast percentage in the BM, EUTOS scores, and basophil percentage in PB were related with the poor therapeutic outcomes in frontline therapy (p=0.002, p=0.002 and p=0.042, respectively). Although Sokal risk classification showed that the intermediate class had a higher event risk compared to the low-risk class (p=0.001), the predictive association disappeared in high-risk classes.

Conclusion: EUTOS score system has better predictive capability for front-line imatinib therapy comparing with other indices. Higher blast percentage in BM and increased basophil percentage in PB are independent risk factors, adversely related with EFS in patients with CML.

Keywords: CML, event-free survival, EFS, first-line, imatinib

INTRODUCTION

Tyrosine kinase inhibitors (TKIs) have game changer effects on the clinical course of chronic myeloid leukemia (CML). Although imatinib mesylate constitutes the major option in the front-line treatment, resistance or intolerance may occur in 50% of patients, which leads to escalation in therapeutic scheme [1,2]. Therefore, CML prognostication at the time of diagnosis is critical to determine the intensity of initial TKI treatment.

Various indices derived from baseline clinical and laboratory features have been used to determine prognosis in CML [3]. Sokal and Euro/Hasford scoring systems which were developed before the TKI era, have been widely used for risk assessment [4,5]. However, it was reported that these scores were less effective than European Treatment and Outcome Study (EUTOS) in event-free survival estimate [6]. Furthermore, a novel predicting system, EUTOS long-term survival (ELTS) score was developed through re-weighing of Sokal score components [7]. Due to improved response rates with TKI treatment, event-free survival (EFS) has become a prominent concept in patients with chronic phase CML (CML-CP). Nevertheless, present scoring systems still need to be improved for perfect EFS estimation.

The aim of this study is to assess the prognostic impact of bone marrow (BM) and peripheral blood (PB) cellular components, correlated with clinical parameters. Our hypothesis was that certain laboratory parameters such as bone marrow blast percentage in addition to current prognostic indices could be effective tools to predict EFS in patients with CML. Elucidation of the exact prognostication in CML could facilitate decision-making in therapeutic management of the patients.

MATERIALS AND METHODS

Ethical approval

During this study, all the ethical considerations was followed in accordance with the 1964 Helsinki Declaration.

Study Population

In our study, one hundred forty-three patients with CML-CP, applied to our clinic between January 2005 and July 2018 were recruited. Exclusion criteria were being under 18 years of age, having a follow-up of less than 24 months, receiving front-line therapy other than imatinib mesylate, and initiating TKI treatment more than 6 months after diagnosis.

Clinical characteristics and laboratory results were collected through electronic record system and patient files, retrospectively. Demographic features, comorbidities, palpable spleen size, complete blood count, BM characteristics, PBS distribution and therapeutic responses were recorded. Sokal, Euro/Hasford, EUTOS and ELTS scores were calculated according to their respective equations [4,5,7,8].

Hematologic, molecular, and cytogenetic responses, primary and secondary resistance were defined through 2013 European LeukemiaNet (ELN) criteria [9]. EFS describes the time between initiation of TKI treatment, and determination of primary or secondary resistance, progression to accelerated phase (AP) or blastic crisis (BC), or moderate to severe adverse event occurrence. Overall survival (OS) defines duration from CML diagnosis to death, by any cause.

Statistical Analyses

The normality of the variable distributions was examined by Kolmogorov-Smirnov test. For categorical variables, proportions and for continuous variables, mean and standard deviation (SD) or median and interquartile range (IQR) were reported based on normality. To evaluate differences in continuous variables, student's t test or Mann-Whitney U test were used based on normality. For the categorical variables, Chi- square or Fisher's exact tests were used. Survival analyses were performed through Kaplan-Meier test, and factors related with EFS were examined through Cox Proportional Hazards Regression Analysis for univariate analysis and backward multivariate adjustments. Statistical analyses were performed using IBM SPSS Statistics (version 25; SPSS, Armonk, NY), probability values were 2-sided and considered statistically significant when $p<0.05$.

RESULTS

General characteristics

One hundred forty-three patients (70 women, 73 men) were enrolled in our study (Figure 1). Median follow-up time was 84 (IQR: 54-125) months and median front-line therapeutic duration was 56 (IQR:23-89) months. At the time of diagnosis, median age was 48 (IQR: 35-59) years. General characteristics of the study population were summarized in Table-1.

During front-line TKİ therapy, 95.3% of the patients achieved complete hematologic response and 83.6% reached major molecular response.

Prognostic scores

All four prognostic scores were calculated for each patient and summarized in Table 1. Sokal scores were positively correlated with Euro/Hasford, EUTOS and ELTS scores (r=0.77, r=0.45, r=0.64 respectively, $p < 0.001$ for all).

Survival analyses

In front-line therapy, the treatment of 66 (46.2%) patients had to be switched to another TKI. Moderate and severe therapeutic adverse events were described in 16 (11.2%) patients. While primary TKI resistance was observed in 19 (13.3%) patients, secondary TKI resistance occurred in 22 (15.4%) patients.

During follow-up at our center, death of any kind occurred in 6 (4.2%) patients. While the 10-year OS rate was 95.2% (95% CI: 90.6-99.8), 5-year EFS rate was determined as 62.3% (95% CI: 53.9-70.7).

Predictive factors for EFS

The results of the univariate regression analyses to determine the factors predicting EFS rate were summarized in Table 2. Although Sokal scores showed that the intermediate class had a higher event risk compared to the low-risk class (HR: 3.117 [95% CI:1.584-6.135], p=0.001), the predictive association disappeared at higher scores (p=0.061). Therefore, numerical scores rather than classifications were used to determine prognosis to avoid lower statistical power due to the limited number of CP-CML patients with high-risk scores. EUTOS score showed a prognostic relationship with EFS, which remained the same after multivariate analyses (Table 2).

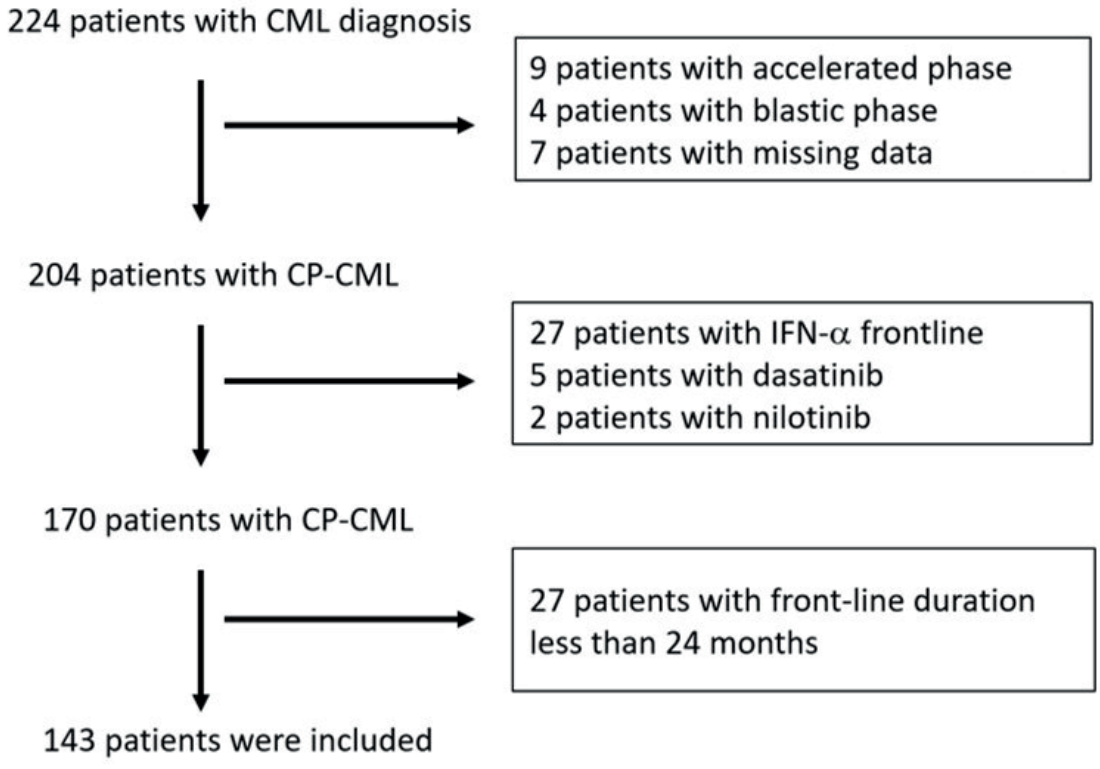

Figure 1. Inclusion diagram

Table 1. Comparison of baseline clinical and laboratory characteristics in different event states

	All patients (N=143)	Event (N= 66)	Censored (N=77)	p value
Age, median (IQR), y*	48 (35-59)	46 (34-54)	49 (38-61)	0.09
Male, N (%)	73 (51)	40 (54.8)	33 (45.2)	0.044
Palpable spleen size, median (IQR), cm	0 (0-2)	2 (0-5)	0	<0.001
Hb†, mean (SD), g/dl	12.4 (1.7)	12.2 (1.7)	12.5 (1.7)	0.39
WBC‡, mean (SD), $x10^3/mm^3$	105.9 (95.8)	122 (105.7)	93.2 (85.9)	0.10
Basophil (%) of PBS§, median (IQR)	0.5 (0.1-2.3)	0.8 (0.1-3.3)	0.4 (0.1-1.2)	0.35
Eosinophil (%) of PBS, median (IQR)	1.2 (0.5-2.1)	1.2 (0.5-2.4)	1.2 (0.5-2.0)	0.83
Platelet, mean (SD), $x10^3/mm^3$	499 (385)	535 (450)	470 (327)	0.38
Myeloblast (%) of PBS, median (IQR)	0 (0-2)	0 (0-3)	0 (0-1)	0.001
Blasts percentage in BM‖, median (IQR)	3 (2-4)	4 (3-4)	3 (2-4)	0.01
Sokal classification				
Low, N (%)	55 (47.4%)	16 (32%)	39 (59.1%)	0.019
Intermediate, N (%)	36 (31.0%)	21 (42%)	15 (22.7%)	
High, N (%)	25 (21.6%)	13 (26%)	12 (18.2%)	
Euro/Hasford classification				
Low, N (%)	76 (65.5%)	28 (56%)	48 (72.7%)	0.06
Intermediate, N (%)	32 (27.6%)	17 (34%)	15 (22.7%)	
High, N (%)	8 (6.9%)	5 (10%)	3 (4.5%)	
EUTOS¶ classification				
Low, N (%)	110 (94.8%)	46 (92%)	64 (97%)	0.40
High, N (%)	6 (5.2%)	4 (8%)	2 (3%)	
ELTS** classification				
Low, N (%)	85 (71.6%)	31 (62%)	52 (78.8%)	0.10
Intermediate, N (%)	25 (21.6%)	15 (30%)	10 (15.2%)	
High, N (%)	8 (6.9%)	4 (8%)	4 (6.1%)	

*: year, †: Hemoglobin, ‡: white blood cells, §: peripheral blood smear, ‖: bone marrow, ¶: The EUropean Treatment Outcome Study, **: The EUTOS long-term survival

Table 2. Univariate and multivariate analyses of EFS predictors

	Univariate regression		Multivariate Regression Model 1		Multivariate Regression Model 2	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Age (year)	0.99 (0.97-1.0)	0.15				
Gender (male vs. female)	0.535 (0.320-0.893)	0.017	0.67 (0.31-1.46)	0.32	0.54 (0.27-1.09)	0.09
Palpable spleen size (cm)	1.055 (1.008-1.105)	0.022	1.09 (0.98-1.21)	0.10		
Hb* (g/dl)	0.93 (0.79-1.1)	0.39				
WBC† ($x10^9$/ml)	1.0	0.15				
Basophil (%) of PBS‡	1.114 (1.005-1.234)	0.04	1.17 (1.006-1.361)	0.042		
Eosinophil (%) of PBS	1.05 (0.89-1.25)	0.55				
Platelet ($x10^9$/ml)	1.0 (0.99-1.0)	0.62				
Myeloblast (%) of PBS	1.08 (0.99-1.19)	0.09				
Blasts% in BM§	1.32 (1.123-1.548)	0.001	1.353 (1.101-1.662)	0.004	1.352 (1.113-1.644)	0.002
Sokal score	1.25 (0.82-1.92)	0.31				
Euro/Hasford score	1.0 (1.0-1.001)	0.08				
EUTOS‖ score	1.012 (1.004-1.021)	0.005			1.017 (1.006-1.027)	0.002
ELTS¶ score	1.49 (0.91-2.43)	0.11				

*: Hemoglobin, †: white blood cells, ‡: peripheral blood smear, §: bone marrow, ‖: The EUropean Treatment Outcome Study, ¶: The EUTOS long-term survival

Similarly, bone marrow blast percentage, spleen size and basophil rates in peripheral blood smear were showed significant predictive relationship. No association was observed between EFS and age, eosinophil percentage or platelet count. In the univariate regression, male patients had a higher risk of events, and their spleen size were significantly higher than women (data not shown). However, the higher risk attributed to gender disappeared in various multivariate regression models.

DISCUSSION

In this study, increased bone marrow blast percentage, peripheral basophil rates and EUTOS scores significantly related to clinical course prediction of real-life patients with CML-CP on front-line imatinib mesylate therapy. Although spleen size, gender and Sokal risk classification appeared to be associated with therapeutic outcomes, multivariable adjustments had indicated their predictive relationship for event-free survival could be limited. Furthermore, in different Euro/Hasford and ELTS risk groups EFS rates were observed as similar. There was no correlation between event-free survival and age of patients.

The estimation of therapeutic responses by prognostic scores is particularly contentious issue. While some authors described that risk stratification was compatible with EFS, the others identified similar EFS duration in different Sokal or Euro/Hasford scores [6,10-14]. In the current study, we observed that ability to anticipate EFS in Sokal risk classes was limited in patients with imatinib mesylate in the frontline. Although there was a prognostic difference between low and intermediate risk groups, EFS rates of high-risk group was similar with low-risk patients, this result could be linked with limited number of patients with high-risk score in CP-CML group. In addition to Sokal risk classification, we also found a linear relationship between EUTOS risk score and EFS. In addition, various studies reported better prediction capacity in EUTOS scoring system consistent with our results, others indicated validation handicaps in the score [6,10,13,15-17]. As opposed to other studies, EFS results were similar among ELTS subgroups in our study [7,11].

According to ELN 2013, blast rate in bone marrow below 15% is a CP criterion [9]. However, many authors reported that a BM blast rate higher than 10% was associated with unfavorable disease course [18-22]. Some authors have even suggested that an excess of blasts in CP could be an early sign of an accelerated phase [18]. In our study, a linear hazard ratio of blast percentage in bone marrow was described regardless of a specific cut-off point. Despite new technological capabilities, our results suggest that histomorphological assessment in CML is still a valuable art.

It was shown that basophilia is an independent prognostic feature correlated with disease progression and TKI resistance in patients with CML [23-25]. Therefore, basophil rates in PB are frequently used laboratory parameters for prognostic indices [5,8]. We also described the relationship between basophil percentage and event rates. Age is also a common variable in overall survival prediction. However, there was no association between EFS estimation and age in our study. This could be associated with our cohort, which was younger than the typical CML median age.

The current study is subject to some limitations. Firstly, due to the study design, calculation of prognostic scores could not be obtained for all patients. However, the retrospective computation of the scores made it possible to evaluate relatively new prognostic systems, such as ELTS score. Secondly, patients' adherence to imatinib therapy and dosage could not be assessed during follow-up. Nevertheless, the study results might have important implications because of providing real-life data. On the other hand, our study also has some strengths. To minimize confounding factors, our study enrolled only patients who received first-line treatment with imatinib and no interferon therapy. In addition, a minimum follow-up period of at least 24 months was set for enrollment in our study to avoid insufficient observation time and to describe a specific patient cohort that is more common in clinical practice.

However, it is important to note that while patient characteristics may predict clinical course, they are not the only determinant of disease prognosis. In addition to patient characteristics, there are other factors that are critical to treatment

management. For example, there have been numerous studies comparing the efficacy of imatinib and new generation TKI therapies [26-28]. Individualization of therapeutic options is an effective tool that improves our position in disease control. Consequently, harmonization of patient characteristics with pharmaceutical data and available facilities would better guide treatment decisions [29].

In conclusion, our results suggest that the EUTOS score system has improved predictive capability for chronic phase CML patients receiving front-line imatinib mesylate therapy. Moreover, higher blast percentage in bone marrow and increased basophil percentage in peripheral blood smear are independent risk factors, adversely related with event-free survival in patients with CML. Large-scale prospective studies are still required to confirm the results of our study.

Author contribution

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

Ethical approval

The study protocol was approved by the ethical committee of Hacettepe University (Protocol No. GO 17/540/ July 2017).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Zackova D, Klamova H, Belohlavkova P, Stejskal L, Necasova T, Semerad L, et al. Dasatinib treatment long-term results among imatinib-resistant/intolerant patients with chronic phase chronic myeloid leukemia are favorable in daily clinical practice. Leuk Lymphoma. 2021;62(1):194-202.

[2] Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, et al. Long-Term Outcomes of Imatinib Treatment for Chronic Myeloid Leukemia. N Engl J Med. 2017;376(10):917-27.

[3] Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966-84.

[4] Sokal JE, Cox EB, Baccarani M, Tura S, Gomez GA, Robertson JE, et al. Prognostic discrimination in" good-risk" chronic granulocytic leukemia. 1984.

[5] Hasford J, Pfirrmann M, Hehlmann R, Allan NC, Baccarani M, Kluin-Nelemans JC, et al. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa Writing Committee for the Collaborative CML Prognostic Factors Project Group. JNCI: Journal of the National Cancer Institute. 1998;90(11):850-9.

[6] Uz B, Buyukasik Y, Atay H, Kelkitli E, Turgut M, Bektas O, et al. EUTOS CML prognostic scoring system predicts ELN-based 'event-free survival' better than Euro/Hasford and Sokal systems in CML patients receiving front-line imatinib mesylate. Hematology. 2013;18(5):247-52.

[7] Pfirrmann M, Baccarani M, Saussele S, Guilhot J, Cervantes F, Ossenkoppele G, et al. Prognosis of long-term survival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia. 2016;30(1):48-56.

[8] Hasford J, Baccarani M, Hoffmann V, Guilhot J, Saussele S, Rosti G, et al. Predicting complete cytogenetic response and subsequent progression-free survival in 2060 patients with CML on imatinib treatment: the EUTOS score. Blood, The Journal of the American Society of Hematology. 2011;118(3):686-92.

[9] Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872-84.

[10] Huang J, Zhao X. [Efficacy of three prognostic scoring systems on evaluating the prognosis for patients with chronic myeloid leukemia]. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2016;41(8):809-14.

[11] Sato E, Iriyama N, Tokuhira M, Takaku T, Ishikawa M, Nakazato T, et al. The EUTOS long-term survival score predicts disease-specific mortality and molecular responses among patients with chronic myeloid leukemia in a practice-based cohort. Cancer Medicine. 2020;9(23):8931-9.

[12] Castagnetti F, Gugliotta G, Breccia M, Stagno F, Iurlo A, Albano F, et al. Long-term outcome of chronic myeloid leukemia patients treated frontline with imatinib. Leukemia. 2015;29(9):1823.

[13] Yahng S-A, Jang E-J, Choi S-Y, Lee S-E, Kim S-H, Kim D-W. Prognostic discrimination for early chronic phase chronic myeloid leukemia in imatinib era: comparison of Sokal, Euro, and EUTOS scores in Korean population. International journal of hematology. 2014;100(2):132-40.

[14] Elbedewy TA, Elashtokhy HEA. The Utility and Applicability of Chronic Myeloid Leukemia Scoring Systems for Predicting the Prognosis of Egyptian Patients on Imatinib: Retrospective Study. Journal of Leukemia. 2016:1-9.

[15] Iriyama N, Hatta Y, Kobayashi S, Uchino Y, Miura K, Kurita D, et al. The European Treatment and Outcome Study score is associated with clinical outcomes and treatment response following European LeukemiaNet 2013 recommendations in chronic-phase chronic myeloid leukemia. International journal of hematology. 2014;100(4):379-85.

[16] Jabbour E, Cortes J, Nazha A, O'Brien S, Quintas-Cardama A, Pierce S, et al. EUTOS score is not predictive for survival and outcome in patients with early chronic phase chronic myeloid leukemia treated with tyrosine kinase inhibitors: a single institution experience. Blood, The Journal of the American Society of Hematology. 2012;119(19):4524-6.

[17] Marin D, Ibrahim AR, Goldman JM. European Treatment and Outcome Study (EUTOS) score for chronic myeloid leukemia still requires more confirmation. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2011;29(29):3944-5.

[18] Braga GW, Chauffaille ML, Moncau JE, Souto EX, Silva MR, Kerbauy J. Chronic myeloid leukemia (CML): prognostic factors and survival analysis. Sao Paulo Med J. 1996;114(1):1083-90.

[19] Kantarjian HM, Smith TL, McCredie KB, Keating MJ, Walters RS, Talpaz M, et al. Chronic myelogenous leukemia: a multivariate analysis of the associations of patient characteristics and therapy with survival. 1985.

[20] Cervantes F, Rozman C. A multivariate analysis of prognostic factors in chronic myeloid leukemia. Blood. 1982;60(6):1298-304.

[21] Jootar S, Ungkanont A, Chuncharunee S, Atichartakarn V. Multivariate analysis of prognostic factors in Philadelphia chromosome positive chronic myeloid leukemia: an update of the first series in Thailand. Asian Pac J Allergy Immunol. 1996;14(1):25-30.

[22] Chikkodi SV, Malhotra P, Naseem S, Khadwal A, Prakash G, Sahu KK, et al. Factors Affecting Early Molecular Response in Chronic Myeloid Leukemia. Clin Lymphoma Myeloma Leuk. 2015;15 Suppl:S114-9.

[23] Valent P, Horny HP, Arock M. The underestimated role of basophils in Ph(+) chronic myeloid leukaemia. Eur J Clin Invest. 2018;48(10):e13000.

[24] Jabbour E, le Coutre PD, Cortes J, Giles F, Bhalla KN, Pinilla-Ibarz J, et al. Prediction of outcomes in patients with Ph+ chronic myeloid leukemia in chronic phase treated with nilotinib after imatinib resistance/intolerance. Leukemia. 2013;27(4):907-13.

[25] Kantarjian HM, Talpaz M, O'Brien S, Smith TL, Giles FJ, Faderl S, et al. Imatinib mesylate for Philadelphia chromosome-positive, chronic-phase myeloid leukemia after failure of interferon-alpha: follow-up results. Clin Cancer Res. 2002;8(7):2177-87.

[26] Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362(24):2251-9.

[27] Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362(24):2260-70.

[28] Kantarjian HM, Hochhaus A, Saglio G, De Souza C, Flinn IW, Stenke L, et al. Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the phase 3 randomised ENESTnd trial. Lancet Oncol. 2011;12(9):841-51.

[29] Haznedaroglu IC, Kuzu I, Ilhan O. WHO 2016 Definition of Chronic Myeloid Leukemia and Tyrosine Kinase Inhibitors. Turk J Haematol. 2020;37(1):42-7.

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ORIGINAL ARTICLE

The Impact of Covid-19 Pandemic on the Clinical Course of Pediatric Skin Disorders: A Cross-Sectional Study

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ABSTRACT

Objectives: As Covid-19 infection continues to affect both the pediatric and adult populations, new-onset and pre-existing skin diseases in addition to the skin diseases with exacerbation, are frequently being reported as the cutaneous manifestations of the pandemic. In the present study, the aim was to investigate the impact of Covid-19 pandemic on the clinical course of the skin diseases observed in the pediatric population.

Materials and Methods: A web-based survey related to the cutaneous disorders seen in the pediatric population (0-18 years) prior to the pandemic and during the pandemic, was formed. The survey was spread using snowball sampling method. The questionnaire was asked to be filled by the parents. Demographical data, Covid-19 related questions, the presence of the new-onset skin disorders and the clinical course of the pre-existing cutaneous diseases during the pandemic were questioned.

Results: Two hundred ninety one children aged between 0-18 years, were included in the study. The mean age was 11.3 ± 4.6 years. One hundred sixty four respondents were female; whereas 127 were male. Ninety seven cases were tested for SARS-CoV-2, 41 had positive RT-PCR result. During the pandemic, 65 children had at least one new-onset cutaneous disease: the most common ones were pruritus (n=23, 35.4%), xerosis (n=17,26.2%), acne vulgaris (n=11, 16.9%) and seborrheic dermatitis (n=11, 16.9%). There was no statistically significant relationship between the presence of any new-onset skin disease during the pandemic and being diagnosed with Covid-19 (p=0.73). However, there was a statistically significant relationship between the number of new-onset acne vulgaris cases and median duration of mask wearing during the pandemic (p=0.025).

Conclusion: The present study shows that the era of the Coronavirus disease, has led to the emergence of new-onset skin problems in the pediatric population due to the use of personal protective equipment. Psychosocial burden of the pandemic also seems to have an impact on the pediatric skin disorders.

Keywords: Covid-19, pediatrics, skin disease

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INTRODUCTION

From the start of Covid-19 pandemic in 31 December 2019, a wide range of systemic manifestations such as fever, dry cough, muscle pain, gustatory and olfactory dysfunction have been associated with the infection. As the outbreak of SARS-CoV-2 infection has continued to spread worldwide, cutaneous manifestations of the disease such as urticaria, morbilliform rash, livedo reticularis, papular or vesicular exanthem, have been associated with the infection [1]. It has also been shown that children tend to exhibit milder symptoms of Covid-19 compared to the adults and deaths are extremely rare in pediatric patients [2]. Just like the clinical course, cutaneous manifestations of the infection also differ between adults and children [3]. Erythema multiforme, Kawasaki disease-like inflammatory multisystemic syndrome, chilblain-like lesions, purpuric/livedoid rash and pityriasis-rosea like eruption are among the most frequently reported cutaneous manifestations of Covid-19 in the pediatric population [3-5]. Thrombosis, coagulopathy, immune complex vasculitis and SARS-CoV-2-induced type 1 interferonopathy have all been implicated in the pathogenesis of these cutaneous manifestations [3-5].

Besides the cutaneous illnesses observed in the individuals with Covid-19, skin problems arising from protective personal equipment (PPE) use and frequent hand washing including irritant/allergic contact dermatitis and acne vulgaris, have also increased significantly among the young adults [6,7]. Social isolation, the extension of the pandemic period, the loss of the beloved ones due to Covid-19 and being separated from the school might have overall had a negative psychosocial influence upon the children which have been associated with the development of new-onset trichotillomania, telogen effluvium (TE) and alopecia areata (AA) cases.

In the present study, the aim was to investigate the influence of Covid-19 pandemic on the clinical course of the skin diseases observed in the pediatric population.

METHODS

The present study was approved by the local ethics committee (the date and decision number: November 19 2021, 2021/029). Additionally, the approval of the Ministry of Health Ethics committee was also obtained (date: October 22 2021; application number: 2021-10-18T21_23_48.) Informed consent was taken from the participants. A web-based questionnaire which composed of 22 questions (Supplementary file 1) was formed using Google forms. The survey was divided into four sections: (I) demographical data of the child (age, number of the siblings/household members, jobs of the parents, the chronic systemic diseases, current medications); (II) questions related to Covid-19 infection [Covid-19 real time polymerase chain reaction (RT-PCR) result, the presence of any Covid-19 associated symptoms, history of close contact to a person with a diagnosis of Covid-19, treatment taken for Covid-19, requirement of hospitalization, use of face masks, duration during which face masks are used in a day); (III) skin problems observed prior to the beginning of Covid-19 pandemic (type of the skin disease, presence of consultation with a physician, treatment used for the disease); (IV) skin illnesses observed during Covid-19 pandemic (types of both new-onset and pre-existing/ongoing skin diseases, presence of referral to a specialist, given treatment, the clinical course of the pre-existing skin diseases during pandemic, the role of stress in the emergence or aggravation of the diseases). The online survey was sent to the individuals who had at least one child between the ages of 0 to 18 years, via e-mail or instant messaging in Turkey. The virtual snowball sampling method was used. The survey was asked to be filled by the parents and children (if the child was able to comprehend and answer the questions). The online survey was filled again for each child.

IBM SPSS for Windows Version 20.0 was used for the statistical analysis. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolomogorov-Smirnov/ Shapiro-Wilk test) to determine whether or not they

are normally distributed. Descriptive analyses were presented using means and standard deviations for normally distributed and medians and interquartile range (IQR) for the non-normally distributed and ordinal variables. Median scores of all groups were compared with the non parametric Mann-Whitney U test. Fisher's exact test or Chi-Square test were utilized for the statistical analysis of categorical variables. P-values below 0.05 were accepted as statistically significant.

RESULTS

Two hundred ninety one children aged between 0-18 years, were included in the study. The mean age was 11.3 ± 4.6 years (range: 1-18). One hundred sixty four (56.4%) respondents were female whereas 127 (43.6%) were male. Sixty four (22%) children had one or more systemic accompanying illness.The mean number of household members living in the same house with the children, were 4.5± 1.5 (range:2-15). Thirty three (11.3%) children was living with one or more household member employed in the healthcare sector. Ninety seven (33.3%) cases were tested for Covid-19, 41 (42.3%) had positive RT-PCR result whereas 56 (57.7%) tested negative. Additionally, 129 (44.3%) cases had close contact to someone with a confirmed diagnosis of Covid-19 whereas 117 (40.2%) children exhibited Covid-19 related symptoms. Hospitalization was not required for any child. In general, non-steroidal anti-inflammatory drugs, vitamin supplements, oral hydration and resting were the most commonly suggested treatment modalities for pediatric patients with Covid-19.

In the online questionnaire, participants were asked if they had any other contributing factors during Covid-19 pandemic which might be associated with the progression of the skin disease. Only five (1.7%) children had lost >5% of his/her own weight in a period of 6 months, 95 (32.6 %) felt stressed or overwhelmed which had a major impact on her/his daily functioning whereas 7 (2.4%) were on an extreme diet during Covid-19 pandemic. Prior to the pandemic, 159 (54.6%) respondents had at least one skin disorder. The most commonly reported cutaneous diseases were acne vulgaris (n=57, 35.8%), xerosis (n=43, 27%) and seborrheic dermatitis (SD) (n=43, 27%). The distribution of different skin diseases is shown in Table 1. Prior to the pandemic, 50 (31.4) out of 159 cases with a skin disease, were diagnosed by a physician and only 83 (52.2%) had used at least one treatment modality for their skin diseases. Forty three (51.8%) used cosmetics/personal care products, 38 (45.8%) used medical treatments prescribed by a physician, 15 (18%) used vitamin supplements and/or herbal medications.

During the era of Covid-19, 213 (73.2%) had one or more skin disease. The most common skin diseases were acne vulgaris (n=75, 35.2%), xerosis (n=62, 29.1%) and SD (n=57, 28.8%). The distribution of the skin diseases observed in the era of Covid-19

Table 1. The distribution of the skin diseases prior to the pandemic and during the pandemic

Disease Type	Prior to the Pandemic n (%)	During the Pandemic n (%)
Acne vulgaris	57 (35.8)	75 (35.2)
Xerosis	43 (27)	62 (29.1)
Seborrheic dermatitis	43 (27)	57 (28.8)
Pruritus	34 (21.4)	66 (31)
Telogen effluvium	24 (15.1)	39 (18.3)
Herpes simplex	18 (11.3)	23 (10.8)
Atopic dermatitis	14 (8.8)	16 (7.5)
Verruca vulgaris	10 (6.3)	16 (7.5)
Psoriasis	10 (6.3)	10 (4.7)
Insect bite	9 (5.7)	4 (1.9)
Viral maculopapular rash	4 (2.5)	2 (0.9)
Trichotillomania	3 (1.9)	3 (1.4)
Acral and oral vesicular eruption	3 (1.9)	14 (6.6)
Alopecia areata	2 (1.3)	5 (2.3)
Acral peeling and onychomadesis	2 (1.3)	2 (0.9)
Early hair greying	2 (1.3)	-
Vitiligo	1 (0.6)	2 (0.9)
Rosacea	1 (0.6)	3 (1.4)
Scabies	1 (0.6)	8 (3.8)
Irritant/allergic contact dermatitis	-	24 (11.3)
Impetigo	-	2 (0.9)

are shown in Table 1 and clinical pictures belonging to different diseases are shown in Figure 1 and 2. One hundred ninety one (89.7%) out of 213 children with a skin disease during Covid-19 pandemic, were diagnosed by a physician and 198 (93%) had used at least one treatment for their skin disease. Fifty seven (28.8%) used cosmetics/personal care products, 187 (94.4%) used medical treatments prescribed by a physician or underwent medical procedures (cryotherapy, platelet-rich plasma etc.) performed by a physician, 9 (4.5%) used vitamin supplements and/or herbal medications. On the other hand, 65 (22.3%) children developed new-onset skin disorders during Covid-19 pandemic (Table 2). The most common skin diseases are pruritus (n=23, 35.4%), xerosis (n=17,26.2%), acne vulgaris (n=11, 16.9%), SD (n=11, 16.9%) and irritant/allergic contact dermatitis (n=9, 13.8%). The average time between the emergence of any new-onset skin disease and Covid-19 diagnosis was 4.7± 3.9 months (range: 1-12). Prior to the pandemic, out of 159 children with at least one preceding cutaneous disease, 92 (57.9%) showed increase in the symptoms of the disease (Table 2). Out of 41 patients with a confirmed diagnosis of Covid-19, 26 (63.4%) had at least one skin disease during Covid-19 pandemic. The most prevalent diseases were TE (n=8, 30.8%), acne vulgaris (n=8, 30.8%) followed by pruritus (n=7, 26.9%) and SD (n=6, 22%).

Table 2. The distribution of new-onset skin disorders and pre-existing skin disorders with exacerbation

Disease Type	New-onset skin disorders n (%)	Pre-existing skin disorders with exacerbation n (%)
Telogen effluvium	6 (9.2)	13 (14.1)
Vitiligo	1 (1.5)	1 (1.1)
Alopecia areata	3 (4.6)	1 (1.1)
Psoriasis	1 (1.5)	4 (4.4)
Seborrheic dermatitis	11 (16.9)	32 (34.8)
Pruritus	23 (35.4)	18 (19.6)
Atopic dermatitis	5 (7.7)	5 (5.4)
Scabies	5 (7.7)	1 (1.1)
Verruca vulgaris	6 (9.2)	9 (9.8)
Acne vulgaris	11 (16.9)	41 (44.6)
Irritant/allergic contact dermatitis	9 (13.8)	-
Insect bite	1 (1.5)	2 (2.2)
Xerosis	17 (26.2)	26 (28.3)
Impetigo	2 (3.1)	-
Acral peeling with onychomadesis	1 (1.5)	-
Rosacea	2 (3.1)	-
Herpes simplex	6 (9.2)	7 (7.6)
Acral vesicular eruption	9 (13.8)	1 (1.1)
Maculopapular rash	-	2 (2.2)
Trichotillomania	-	2 (2.2)

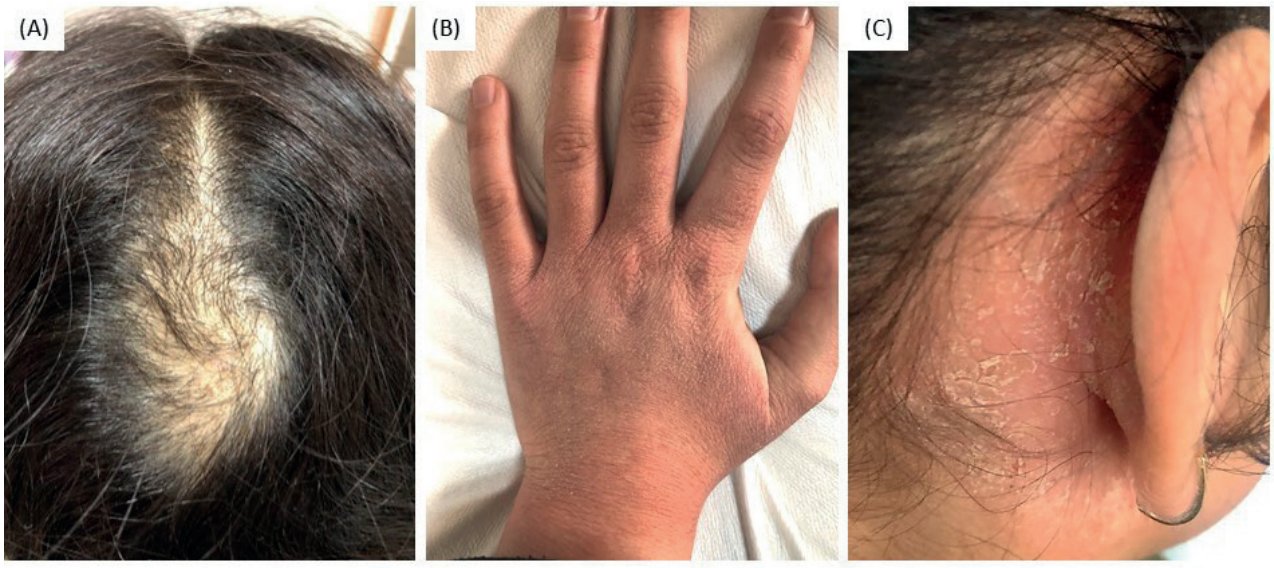

Figure 1. Some clinical pictures of the skin diseases observed in pediatric patients during Covid-19 pandemic: a case of trichotillomania (A), irritant contact dermatitis due to frequent hand washing (B), retroauricular dermatitis due to face mask use (C).

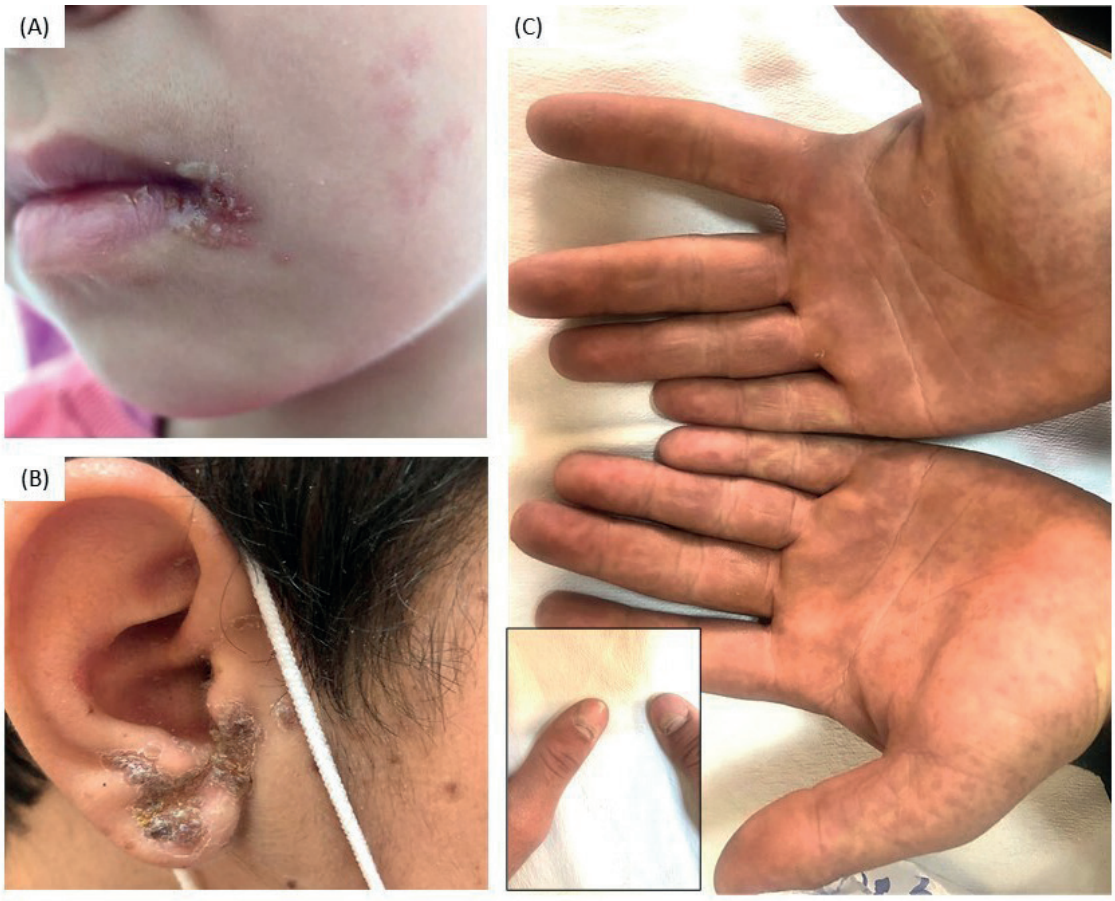

Figure 2. Some clinical pictures of the skin diseases observed in pediatric patients during Covid-19 pandemic: labial herpes simplex (A), vesicular/crusted acral eruption on the ear lobes (B), palmar dusky plaques (C) and onychomadesis (inset) belonging to the same patient.

The parents and children were also asked to compare the clinical course of the pre-existing skin diseases between the pre-pandemic and pandemic eras. In 56 (35.2%) individuals, the clinical course of the cutaneous disease was not reported to have any change, in 92 (57.9%) children the severity of the skin disease increased during Covid-19 pandemic whereas 11 (6.9%) showed decrease in the severity of the symptoms during Covid-19 pandemic. Of 157 children who developed either a new-onset skin disorder or showed increase in the severity of a pre-existing skin disease, 84 (53.5%) thought that stress played a significant role in the development of the new-onset skin disease or exacerbation of the pre-existing skin disease.

There was no statistically significant relationship between the presence of any new-onset skin disease during Covid-19 pandemic and being diagnosed with Covid-19 (p=0.73) (Table 3). Again, we were not able to find any significant difference between the presence of Covid-19 related symptoms and presence of any new-onset skin disorder (p=0.054) (Table 4). No statistically significant difference was found between Covid-19 RT-PCR positivity and the clinical course of the pre-existing skin disease (p=0.058) (Table 5). Lastly, 256 (88 %) out of 291 children were using masks regularly in daytime. The mean duration of mask-wearing in a day, was found to be 6.3±2.4 hours (range: 0.5-12). Statistically significant relationship was found between the presence acne vulgaris (both new-onset and pre-existing cases) among children

Table 3. There was no statistically significant relationship between the presence of any new-onset skin disease during Covid-19 pandemic and being diagnosed with Covid-19 (p=0.73)

	Presence of any new-onset skin disease during Covid-19 pandemic		
Covid-19 RT-PCR Results	Not present n (%)	Present	Total n (%)
Negative	16 (57.1)	10 (62.5)	26 (59.1)
Positive	12 (42.9)	6 (37.5)	18 (40.9)
Total	28 (100)	16 (100)	44 (100)

Table 4. There was not any significant difference between the presence of Covid-19 related symptoms and presence of any new-onset skin disorder (p=0.054)

	Presence of any new-onset skin disorder		
Presence of any Covid-19 related symptoms	Not present n (%)	Present	Total n (%)
Negative	47 (70.1)	35 (53.8)	82 (62.1)
Positive	20 (29.9)	30 (46.2)	50 (37.9)
Total	67 (100)	65 (100)	132 (100)

Table 5. No statistically significant difference were found between Covid-19 RT-PCR positivity and the clinical course of the pre-existing skin disease (p=0.058)

	The Clinical Course of the Pre-existing Skin Disorder			
Covid-19 RT-PCR results	No change n (%)	Symptoms increased n (%)	Symptoms decreased n (%)	Total n (%)
Negative	10 (50.0)	20 (66.7)	0 (0)	30 (62.1)
Positive	10 (50.0)	10 (33.3)	3 (100)	23 (37.9)
Total	20 (100)	30 (100)	3(100)	53 (100)

and the median duration of mask wearing during Covid-19 pandemic (p=0.001). Again, we found a statistically significant relationship between the number of new-onset acne vulgaris cases and the median duration of mask wearing (p=0.025).

DISCUSSION

SARS-CoV-2 infection, since its first emergence keeps on having a substantial influence upon mankind's physical and mental health. In various studies, it has been shown that Covid-19's systemic manifestations differ significantly between adults and children [8]. Pediatric patients with Covid-19 tend to exhibit milder symptoms of the disease compared to the adults; the most common signs are fever and cough [8]. Furthermore, the characteristic laboratory finding of the adult patients with Covid-19, lymphopenia, is not frequently observed in the children and atypical clinical manifestations such as diarrhea is found to appear more commonly in pediatric patients [9]. Just like symptoms and laboratory findings, cutaneous manifestations of SARS-CoV-2 infection are also distinctive in the pediatric population [4].

In a review article by Khalili et al [10], it was reported that pseudochilblain lesions, dactylitis, acral erythema, erythema multiforme, acute urticaria, livedo-like lesions, morbilliform eruption, acro-ischemia, chicken-pox-like rash, petechia and purpura were among the skin manifestations of Covid-19. Most patients were asymptomatic or showed only a few mild symptoms, the duration between the emergence of the systemic symptoms and cutaneous findings ranged between 1 day and weeks [10]. In another review by Shah et al [11], acral erythematous and violaceous maculopapular lesions were found to be the most prevalent skin findings in children followed by erythema multiforme, Kawasaki-like disease and varicella-like exanthem. The induction of type 1 interferon release by the virus, activation of the JAK-STAT signalling pathway along with coagulation pathway, thrombotic vasculopathy and immune-complex deposition are all implicated in the etiopathogeneses of the various cutaneous manifestatons of Covid-19 infection [4,12,13].

In addition to these skin diseases observed in the individuals diagnosed with Covid-19, skin problems due to frequent hand washing and hand sanitiser use such us irritant contact dermatitis, have also risen among the children [6]. In an observational study by Borch et al [6], it was shown that school children had a higher relative risk of developing irritant contact dermatitis compared to preschool ones. Frequent hand washing was found to be a significant risk factor for the emergence of irritant contact dermatitis [6]. Furthermore, continuous use of hand sanitisers was blamed in the pathogenesis of contact dermatitis with atypical clinical presentations [14]. A case of contact urticaria mimicking allergic contact dematitis due to the use of disposable polypropylene surgical mask, in a 7-year old girl was also reported by Corazza et al [15]. In a retrospective study by Altun [7], the most common causes for admission to an outpatient dermatology clinic in Turkey between 30 March and 30 April 2020, were investigated. The most prevalent, new-onset diseases in the pediatric group, were found to be acne, scabies, diaper dermatitis, atopic dermatitis and other eczematous eruptions [7]. In both children and adult age groups, the most frequent reason for admission was acne vulgaris [7]. In the present study in which new-onset pediatric skin diseases were evaluated

from the start of the outbreak until February 2022, the most common new-onset cutaneous illnesses were pruritus (n=23, 35.4%), xerosis (n=17,26.2%), acne vulgaris (n=11, 16.9%), SD (n=11, 16.9%) and irritant/allergic contact dermatitis (n=9, 13.8%). Our results were partially similar with the results of Altun's study, in that face mask-induced acne and SD cases constitued a substantial part of the newly emerged skin problems. Distinctively, pruritus, xerosis and contact dermatitis were found to be the other most frequent new-onset skin problems in the present study. Both the methods of the two studies (single-center retrospective study, physician-diagnosed illnesses vs online survey, physician and/or parent-assessed illnesses) and different time intervals in which the studies were carried out, might be responsible for the different outcomes of the two studies. Additionally, in the present study, no statistically significant relationship was determined between the presence of any new-onset skin disorder during Covid-19 pandemic and being diagnosed with Covid-19 (p=0.73). We believe that small proportion of cases (n=41, 18.7%) with a confirmed diagnosis of Covid-19, might be accountable for this result. Low nasopharyngeal swab RT-PCR positivity in children [4,16], higher rate of asymptomatic pediatric patients [17] and the lower rate of hospital admission among the pediatric patients due to mild-to-moderate symptoms might have caused the real positive cases to be missed out.

In another case letter from Turkey, two different hair loss patterns (AA and TE) were described in two pediatric patients with Covid-19-associated multisystemic inflammatory syndrome [18]. Furthermore, Oner [19] reported three pediatric cases of new-onset trichotillomania which started during Covid-19 pandemic. It was proposed that stress, anxiety, social isolation and negative psychosocial impact of the pandemic upon children, might have all contributed to the development of these hair diseases [18,19]. Supporting this assumption, in the present study, stress and anxiety were thought to have a role in the development of the new-onset skin disease(s) or exacerbation of the pre-existing skin problems in 84 (53.5%) cases. The most common cutaneous disorders which showed deterioration, were acne vulgaris (n=41, 44.6%), SD (n=32, 34.8%) and xerosis (n=26, 28.3%). Higher temperature of the face resulting from uninterrupted mask use, causes an enhanced sebum excretion rate, proliferation of abnormal microbiota and increased permeability of the skin barrier which all contribute to the development or clinical aggravation of acne and SD [20,21]. In line with this observation, in the present research, a statistically significant relationship was determined between the number of new-onset acne vulgaris cases and the median duration of mask wearing in a day (p=0.025). In another study investigating the effects of Covid-19 pandemic upon dermatology-specific health-related quality of life (HRQoL) in pediatric patients aged between 0-4 years, it was revealed that patients with SD and allergic contact dermatitis had a comparable but lower influence of cutaneous diseases on their HRQoL when compared with children with atopic dermatitis [22]. Even though, HRQoL is not evaluated in our study, the exacerbation of the skin disease was observed in 32 patients with SD and 5 patients with atopic dermatitis.

In conclusion, we want to highlight once again that Covid-19 pandemic, has led to the development of new-onset skin problems in the pediatric population due to the infection's direct viral effects, pyschosocial burden of the outbreak and continual use of personal protective equipment.

The present study has some limitations since the results were dependent on the answers of the parents and children obtained through an online questionnaire. The severity of the skin disease(s) was not directly evaluated by a specialist using a score or scale. Prospective, randomized-controlled studies with larger sample size are needed to support our findings.

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Author contribution

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

Ethical approval

The study was approved by the Ministry of Health Ethics Committee (Protocol no. 2021/029/19.11.2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Genovese G, Moltrasio C, Berti E, Marzano AV. Skin Manifestations Associated with COVID-19: Current Knowledge and Future Perspectives. Dermatology 2021;237:1-12.

[2] Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatr 2020;109:1088-95.

[3] Andina D, Belloni-Fortina A, Bodemer C, et al. Skin manifestations of COVID-19 in children: Part 2. Clin Exp Dermatol 2021;46:451-61.

[4] Andina D, Belloni-Fortina A, Bodemer C, et al. Skin manifestations of COVID-19 in children: Part 1. Clin Exp Dermatol 2021;46:444-50.

[5] Andina D, Belloni-Fortina A, Bodemer C, et al. Skin manifestations of COVID-19 in children: Part 3. Clin Exp Dermatol 2021;46:462-72.

[6] Borch L, Thorsteinsson K, Warner TC, et al. COVID-19 reopening causes high risk of irritant contact dermatitis in children. Dan Med J 2020;67:A05200357.

[7] Altun E. The most common pediatric and adult dermatology patient complaints in a month of the COVID-19 pandemic in Turkey. Dermatol Ther 2020;33:e13972.

[8] Cui X, Zhao Z, Zhang T, et al. A systematic review and meta-analysis of children with coronavirus disease 2019 (COVID-19). J Med Virol 2021;93:1057-69.

[9] Cui X, Zhang T, Zheng J, et al. Children with coronavirus disease 2019: A review of demographic, clinical, laboratory, and imaging features in pediatric patients. J Med Virol 2020;92:1501-10.

[10] Khalili M, Iranmanesh B, Mohammadi S, Aflatoonian M. Cutaneous and histopathological features of coronavirus disease 2019 in pediatrics: A review article. Dermatol Ther 2021;34:e14554.

[11] Shah S, Akhade K, Ganguly S, Nanda R, Mohapatra E, Goel AK. Cutaneous manifestations associated with COVID-19 in children: A systematic review. J Family Med Prim Care 2021;10:93-101.

[12] Magro C, Mulvey JJ, Berlin D, et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases. Transl Res 2020;220:1-13.

[13] Castelo-Soccio L, Lara-Corrales I, Paller AS, et al. Acral Changes in pediatric patients during COVID 19 pandemic: Registry report from the COVID 19 response task force of the society of pediatric dermatology (SPD) and pediatric dermatology research alliance (PeDRA). Pediatr Dermatol 2021;38:364-70.

[14] Panda PK, Sharawat IK. Fluctuating Palmar Erythema in a Toddler during COVID-19 Pandemic: Do You Know the Offender? J Trop Pediatr 2021;67:fmab011.

[15] Corazza M, Bencivelli D, Zedde P, Monti A, Zampino MR, Borghi A. Severe contact urticaria, mimicking allergic contact dermatitis, due to a surgical mask worn during the COVID-19 pandemic. Contact Dermatitis 2021;84:466-7.

[16] Tagarro A, Epalza C, Santos M, et al. Screening and Severity of Coronavirus Disease 2019 (COVID-19) in Children in Madrid, Spain. JAMA Pediatr. 2020:e201346. Erratum in: JAMA Pediatr. 2020;174:1009.

[17] Tezer H, Bedir Demirdag T. Novel coronavirus disease (COVID-19) in children. Turk J Med Sci 2020;50:592-603.

[18] Hayran Y, Yorulmaz A, Gur G, Aktas A. Different hair loss patterns in two pediatric patients with COVID-19-associated multisystem inflammatory syndrome in children. Dermatol Ther 2021;34:e14820.

[19] Oner U. Children with trichotillomania in COVID-19 outbreak. J Cosmet Dermatol 2021;20:1967-8.

[20] Veraldi S, Angileri L, Barbareschi M. Seborrheic dermatitis and anti-COVID-19 masks. J Cosmet Dermatol 2020;19:2464-5.

[21] Han C, Shi J, Chen Y, Zhang Z. Increased flare of acne caused by long-time mask wearing during COVID-19 pandemic among general population. Dermatol Ther 2020;33:e13704.

[22] Chernyshov PV, Vozianova SV, Chubar OV. Quality of Life of Infants, Toddlers and Preschoolers with Seborrhoeic, Allergic Contact and Atopic Dermatitis Before and During COVID-19 Pandemic. Dermatol Ther (Heidelb) 2021;11:2017-26.

acta medica

ORIGINAL ARTICLE

Does COVID-19 Affect the Course of Trophoblastic Gestational Disease in Partial Hydatidiform Moles; Is It A Viral or A Pandemic?

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ABSTRACT

Objective: Hydatiform mole (HM) is a non-malignant form of gestational trophoblastic disease (GTD) characterized by failure of normal fetal development and overgrowth of trophoblasts. With this retrospective cohort study, we planned to determine the incidence of PHM, etiology and progression rates to Gestational trophoblastic neoplasia (GTN) during the COVID-19 epidemic.

Materials and Method: This retrospective cohort study was conducted in Ankara Etlik Zübeyde Hanım Women's Health Training and Research Hospital Early Pregnancy Assessment Unit between March 2016 and February 2022. Patients who underwent therapeutic curettage (T/C) with the diagnosis of missed abortion, intrauterine exitus (IUEX), molar pregnancy or incomplete abortion after single spontaneous pregnancy were included in the study. The study group consisted of 138 patients who were diagnosed with partial hydatiform mole as a result of pathological examination in this process. There were 135 patients in the control group.

Results: The number of patients who applied during the pandemic period and were diagnosed with PHM was 62 (44.92%). The mean age of the patients in the study group was 31.97±8.26 years. The mean body mass index of these patients was 26.38±5.13 m^2/kg. The mean paternal age in the study group was 34.95±8.32 years, and it was higher than the paternal age of the patients in the control group (p=0.028). While the incidence of PHM was 1.22/1000 births in our hospital in 2019, this figure was calculated as 3.79/1000 births in 2020. The incidence of progression to GTN at 4 years before the pandemic was 0.02/1000 births; In 2020-2021, this rate was calculated as 0.25/1000 births.

Conclusion: During the pandemic period, along with the increase in the incidence of PHM compared to the pre-pandemic period, an increase in the incidence of progression to GTN disease was detected.

Keywords: COVID-19, pandemic, partial hydatiform mole, gestational trophoblastic neoplasia

INTRODUCTION

Hydatiform mole (HM) is a nonmalignant form of gestational trophoblastic disease (GTD) characterized by disruption of normal fetal development and trophoblast overgrowth [1]. However, it is considered a premalignant disease because it can transform into cancer with local invasion and distant metastasis [2]. Numerous risk factors have been identified for GTD: failed pregnancies in the past, such as miscarriages, folic acid deficiency, excessive maternal age (more than 35 years or less than 20 years), previous molar pregnancy history, use of oral contraceptives, advanced paternal age, use of beta-carotene or animal fats, and smoking [2].

Partial hydatiform mole (PHM); The presence of embryonic or fetal tissue, focal edema, and focal trophoblastic hyperplasia with or without atypia is the hydatiform type in which most 69XXY chromosomes are detectable. Complications are usually less than with complete hydatiform moles and are unlikely to persist (2-4%) [3]. The patient usually reports to the clinic with a missed abortion or incomplete abortion. The incidence of intrauterine growth retardation (IUGR) and fetal malformations is increased in partial mole [4]. PHD is most commonly diagnosed by pathological examination after evacuation of an unsuccessful pregnancy. Previous studies have shown that decidual immune cell infiltrates, particularly FoxP3+ regulatory T cells and CD3+ T cells, are significantly higher in molar pregnancy than in healthy pregnancy [5, 6]. Proinflammatory serum levels are also significantly increased [7]. Similar changes observed in viral infections have already attracted the attention of other researchers. In particular, a study was published suggesting an association between human papillomavirus (HPV) infection and complete molar pregnancy, indicating that the increase in proinflammatory markers was due to a viral etiology [8].

Coronaviruses (CoV) belong to the Coronaviridae family within the Nidovirales [9]. Human CoV infections mainly belong to the α- and β-subgroups and cause the following clinical conditions: severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) [10]. At COVID-19, both T cells and NK cells are reduced. In addition, increased activity of molecules such as cytotoxic perforin and granulizin was found in CD8+ T cells [11]. During pregnancy and the puerperium, infection with COVID-19 can occur through aerosols or direct transmission from mother to fetus [12]. The effects of COVID-19 on the course and outcome of pregnancy are particularly important to pay more attention to this issue. SARS-Cov2 infection during pregnancy has been shown to be associated with spontaneous abortions, preterm births, and intrauterine growth retardation [13].

In the present study, we hypothesize that there is a possible association between the COVID-19 pandemic and the increased incidence of hydatiform mole. With this retrospective cohort study, we aimed to determine the incidence, etiology, and progression rates to GTN of PHM during the epidemic COVID-19 and compare the data with those from 4 years before the pandemic.

MATERIALS and METHOD

The study protocol was approved by the Ethics Committee (28/01/2022, #2022/02). The principles of the Declaration of Helsinki were followed.

This study was a retrospective cohort study. Patients with a singleton or spontaneous pregnancy who underwent dilatation curettage (D/C) with diagnoses of miscarriage, intrauterine exitus (IUEX), molar pregnancy, or incomplete abortion between March 2016 and February 2022 at a tertiary hospital early pregnancy assessment unit were included in the study. The study group consisted of patients diagnosed with partial hydatiform mole based on pathologic examination. The control group consisted of patients who had undergone therapeutic curettage after a single spontaneous pregnancy during the same period and whose pathological findings showed a normal villous structure. During this study period, the first 3 patients with D/C were randomly assigned to the control group on the first day of each month (Figure 1). COVID 19 Polymerase chain reaction tests were not routinely performed on all patients during the study period. Exclusion criteria for

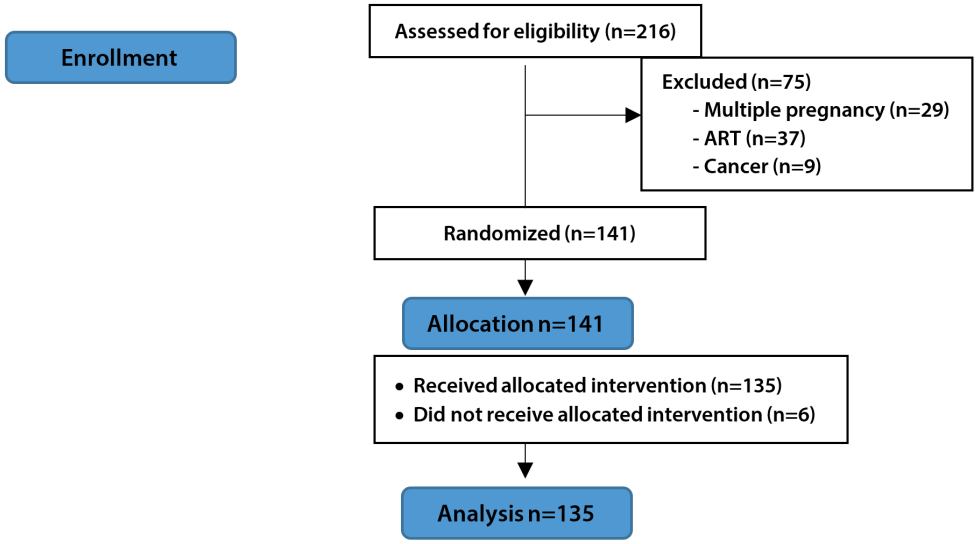

Figure 1. Flow Chart of the Control Groups

the study were maternal history of malignancy, pregnancy by assisted reproductive techniques, patients whose medical records were not available, and multiple pregnancy. Patients' medical records (admission diagnoses, admission status during COVID-19), maternal age, number of births, pre-abortion symptoms, and last menstrual period were reviewed. The period between the pathology reporting date after curettage and the patient's entry in the progress chart was calculated in days. Birth dates in our hospital were broken down by year. Follow-up of patients diagnosed with PHM was examined to determine whether or not GTN status developed.

Statistical analysis

Data analysis was performed using SPSS (statistical package for social sciences, Chicago, IL, USA) 22.0 software. Analytical results were reported as mean ± standard deviation and median (minimum-maximum) for quantitative data and frequency (percent) for categorical variables The distribution of parameters was assessed by Shapiro-Wilk normality tests. The difference between repeated measurements in independent groups was evaluated by the Wilcoxon test. For the normally distributed data, the independent-samples T test was used, and for the nonnormally distributed variables, the Mann Whitney U test was used. The Yates correction, Pearson Chi-square test, and Fisher's exact test were used to examine the relationship between categorical variables by group. A type I error level of 5% overall was used to derive statistical significance.

RESULTS

Between 2016-2022, 138 patients with partial hydatiform moles who consulted the early pregnancy ward of our hospital were diagnosed by pathological examination. There were 135 female patients in the control group. The number of female patients who presented during the pandemic period and were diagnosed with PHM was 62 (44.92%). The mean age of patients diagnosed with PHM was 31.97±8.26 years. The median number of living children in these patients was 1 (0-5). The mean body mass index of these patients was 26.38±5.13 kg/m2. When these data were compared with the control group, there was no statistical difference between the two groups (p=0.194/ p=0.294/ p=0.583). The mean paternal age in the study group was 34.95±8.32 years and was higher than the paternal age of the patients in the control group (p=0.028) (Table 1).

Table 1. Baseline characteristics of the groups

	Control Group (n=135)	Study Group (n=138)	p
Age (year)	30,55 ± 6,58	31,97 ± 8,26	0,194
Number of living birth	1 (0 - 5)	1 (0 - 5)	0,294
Body Mass Index (m^2/kg)	26,75 ± 5,30	26,38 ± 5,13	0,583
Paternal age (year)	32,56 ± 7,20	34,95 ± 8,32	0,028
Time to get results (day)	32,45 ± 46,64	18,75 ± 19,68	<0,001

*Mann Whitney U test

p < 0.05 indicates significant difference. Data are expressed as mean ± standard deviation and median (maximum-minimum).

The mean time to undetectable beta-human chorionic gonadotropin level in the follow-up of patients diagnosed with PHM was 56.39±44.70 days. In patients whose diagnosis was confirmed PHM, the time to return to the hospital to learn the result of the biopsy was shorter (18.75±19.68/ 32.65±46.64) ($p < 0.001$).

The change in the number of patients diagnosed with PHM by year is shown in Figure 2. While the incidence of PHM in our hospital was 1.22/1000 births in 2019, this number was calculated to be 3.79/1000 births in 2020. In the PHM group, the number of patients whose previous pregnancies ended in cesarean section (CS) was 32 (23.2%), the number of patients with smoking habits was 51 (37%), and the number of patients who used oral contraceptives (OC) was 20 (14.5%). Active COVID 19 disease symptoms did not occur in any of the patients who presented during the pandemic period. In this group, the most common reason for hospitalization was a routine examination (n= 70 (50.7%)). The most common diagnosis on admission to the hospital was molar pregnancy (n=52(37.7%)) (Table 2). In the PHM group, during the pandemic period, the most common reason for referral was routine examination (n=32 (51.6%)) and the most common diagnosis on admission to hospital (n=25 (40.4%)) was molar pregnancy. Missed abortion (n=18 (30.8%)); IUEX diagnosis (n=10 (15.4%)); incomplete abortion (n=9 (13.5%)) were the diagnoses at hospital admission. The difference between the two periods is shown in Figure 3. When the diagnoses and reasons for admission were examined, no statistically significant difference was found between the two time periods.

Progression to GTN disease was noted in 6 patients diagnosed with PHM, and 5 of them were patients diagnosed and followed up during the pandemic period. Before the pandemic, the rate of conversion of PHM disease to GTN was 17%. With the declaration of the pandemic, this rate increased to 83%. The incidence of progression to GTN was 0.02/1000 births 4 years before the pandemic; for 2020-2021, this rate was calculated to be 0.25/1000 births.

Table 2. Risk factors for PHM

		n	%
Delivery Method	CS	32	23.2
	Normal Delivery	48	34.8
	Primigravida	58	42.0
Smoking	Yes	51	37.0
	No	87	63.0
Oral Contraceptive using	Yes	20	14.5
	No	118	86.5
Reason for admission to hospital	Control	70	50.7
	Bleeding	40	29.0
	Others	28	20.3
Preliminary diagnosis for hospitalization	IUEX	17	12.3
	Missed abortion	49	35.5
	Incomplete Abortion	20	14.5
	Molar Pregnancy	52	37.7

Chi-square test (χ^2) was used for categorical variables.

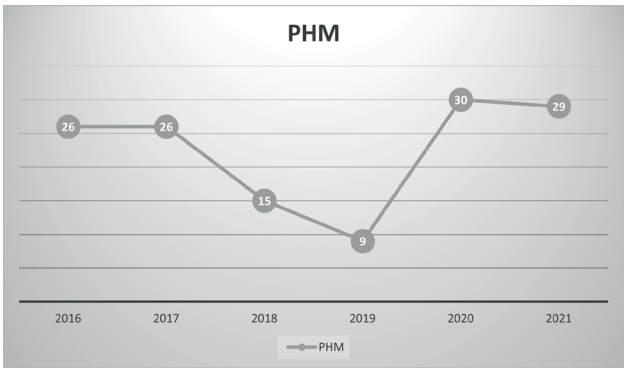

Figure 2. Distribution of the number of PHMs by years

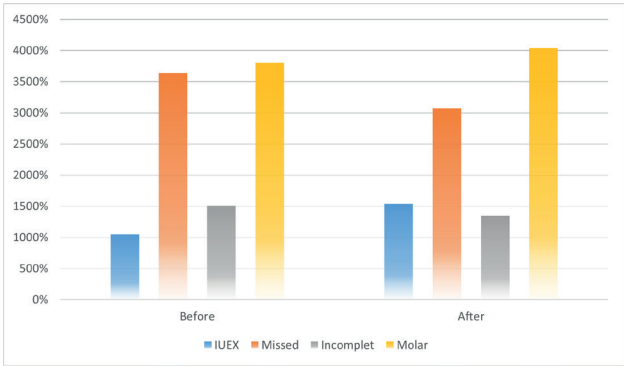

Figure 3. Diagnosis rates of the study group by pandemic

DISCUSSION

In this study, we found that there was an increase in patients diagnosed with partial hydatiform moles in our hospital because of the COVID-19 pandemic. Remarkably, we noted a significant increase in the rate of progression to GTN, particularly in this group of patients.

The pandemic COVID-19 put enormous pressure on health care systems around the world. Long quarantines made access to the health care system difficult, and the impact of the virus led to overcrowding in intensive care units. On the other hand, we did not have sufficient data on the impact of the virus on the fetus or mother during pregnancy from the beginning. Our hospital, which is a tertiary hospital, did not admit any patients with COVID-19 infection during this process and continued its normal service as a clean hospital. For this reason, unlike physicians working in other departments, we had the opportunity to independently assess the impact of the COVID-19 virus on pregnant women, its socioeconomic impact on society, and especially its impact on reproductive health. During this period, an increase in IUEX cases and pregnancy complications (such as preeclampsia, IUGR, threatened preterm labor) was observed in our hospital, whether the mother had COVID-19 infection or not [14, 15]. It is likely that the incidence of this patient group has increased due to a relative increase in density at our hospital as other hospitals in our region treat positive patients. While our total number of births in 2019 prior to the pandemic was 7334, this number increased to 7901 in 2020. The number of patients with suspected molar pregnancy during routine outpatient visits is higher than the number of patients coming to the emergency department with bleeding. A comparison of these hospitalization rates with the pre-pandemic period showed no difference between the two periods. In summary, therapeutic curettage was performed because an unhealthy pregnancy without active symptoms was detected during a routine examination, and the pathologic finding was eventually reported as PHM.

Studies conducted to determine the incidence of molar pregnancy worldwide have reported varying frequencies. Hayashi et al. reported that the incidence of HM in Southeast Asian countries is 2.5-3 times higher than in Europe and America [16]. While the incidence of HM for Turkey was 12.9/1000 births in 1997 [17], it was reported to be 6.60/1000 births in 2004 [18]. In another study by Gul et al, the incidence of gestational trophoblastic disease was reported to be 24.50/1000 births [19]. In these studies, both complete and partial molar pregnancies were reported, and there is no study on the change in incidence during the pandemic period. Abbas et al. published a study in which they stated that the increase in the incidence of HM with the pandemic in Egypt should be noted [20]. Another study conducted in Israel concluded that the incidence of molar pregnancies increased significantly during the pandemic COVID-19, possibly due to late visits to the physician [21]. Between 2016 and 2019, the number of births in our hospital and the number of registrations of pregnant women in the outpatient clinic decreased over the years. No change was observed in the incidence of PHM during the same period. However, during the announcement of the pandemic, the number of births and registrations in the outpatient clinic increased. However, an increase in PHM incidence was also noted.

In a partial hydatiform mole (PHM), fetal tissue is present because the hydropic villi are vascular. The chromosome structure is of maternal origin, but the additional haploid chromosome is usually of paternal origin [22]. Generally, the oocyte is exposed to two sperm (dyspermy) [23]. Since the CDKN1C gene is of maternal origin, it can be detected as p57 positive and is located in the short arm of the 11th chromosome [5, 23]. Although the etiology of molar pregnancy is not clear, it is a disease associated with some changes in the implantation of trophoblastic tissue, and changes in the inflammatory response are observed in pathological examinations. The cellular immune response at the implantation site is higher in molar pregnancies than in the placenta of normal pregnant women. Studies have shown that the number of GrB- Tc cells and the number

of GrB+ NK cells in placental tissue are increased in molar pregnancies [24, 25]. On the other hand, the association between HM and the mutation in NLRP7 gene is clear, and this gene plays a role in the activation of inflammatory caspases [26]. These immunological changes and the response of placental tissue suggest viral infection. In 2002, Alex et al. published a study in which they suggested an association between human papillomavirus (HPV) infection and complete molar pregnancy and that the increase in proinflammatory markers was due to a viral etiology [8]. A common mechanism suggested was that infectious antigens activate endometrial lymphocytes and macrophages. However, other studies at HM have not demonstrated a clinical association with human immunodeficiency virus (HIV), cytomegalovirus (CMV), and herpes simplex virus (HSV) [8]. During the pandemic caused by COVID-19, Abbas et al. suspected a link between this virus and HM and that the possible pathophysiology is a low leukocyte count and an insufficient immunological response to cause molar pregnancy [20].

GTN, the invasive form of molar pregnancy that contains both benign and malignant components, belongs to a group of diseases that have an excellent prognosis and good clinical course when properly treated, unlike other malignancies [27]. Studies have also been conducted on the effects of viral infections on the progression of premalignant lesions to GTN. Of particular value is a study describing the association between human immunodeficiency virus (HIV) and GTN [27]. However, in such common viral infections, does this disease and its progression occur directly as an effect of the virus, or do the effects of medications, difficulties in the health care system, weakening of immunity, and increases in anxiety and stress trigger this situation indirectly? It is difficult to understand this and fully isolate the etiology.

This is the first study to examine in detail the association between COVID-19 and the incidence of PHM and progressive disease leading to GTN. The small sample size and retrospective design are the main limitations of our study. Another important limitation is that complete molar pregnancies could not be included in this study. There is a need for larger studies examining tissue diagnoses. Because our study was a clean hospital study, we cannot say that the direct effect of a COVID-19 positive patient is molar pregnancy; however, we found that there was an increase in the incidence of PHM that was indirectly influenced by many factors.

Thus, we had the opportunity to compare the 4-year period before the pandemic with the 2 years after the pandemic. Along with the increase in PHM incidence, the incidence of progression to GTN disease also increased. This is a remarkable finding that needs to be explored. More comprehensive studies examining biochemical and clinical parameters and long-term outcomes are needed.

Author contribution

Study conception and design: MCİ and YEÜ; data collection: MCİ and YAR; analysis and interpretation of results: MCİ, SYE, İÖU, KYY, Cİ, and YEU; draft manuscript preparation: MCİ, SYE, BSÜ, KYY, Cİ, and YEÜ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study protocol was approved by the Ethics Committee of Ankara Etlik Zubeyde Hanım Women's Health Training and Research Hospital (Protocol No. 02/28.01.2022).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Szulman AE, Surti U. The syndromes of hydatidiform mole: I. Cytogenetic and morphologic correlations. American journal of obstetrics and gynecology 1978; 131(6): 665-671.

[2] Nguyen NM, Bolze PA, Slim R. Hydatidiform moles. In: Hashkes PJ, Laxer RM, Simon A, eds. Textbook of Autoinflammation. Cham, Switzerland: Springer; 2019:485-497.

[3] Berkowitz RS, Goldstein DP. Molar pregnancy. New England journal of medicine 2009;360(16): 1639-1645.

[4] De Franciscis P, Schiattarella A, Labriola D, et al. A partial molar pregnancy associated with a fetus with intrauterine growth restriction delivered at 31 weeks: a case report. Journal of medical case reports 2019; 13(1): 1-5.

[5] Sundara YT, Jordanova ES, Hernowo BS, Gandamihardja S, Fleuren GJ. Decidual infiltration of FoxP3+ regulatory T cells, CD3+ T cells, CD56+ decidual natural killer cells and Ki-67 trophoblast cells in hydatidiform mole compared to normal and ectopic pregnancies. Molecular Medicine Reports 2012; 5(1): 275-281.

[6] Hussein MR, Abd-Elwahed AR, Abodeif ES, Abdulwahed SR. Decidual immune cell infiltrate in hydatidiform mole. Cancer investigation 2009; 27(1): 60-66.

[7] Verit FF, Hilali NG. Increased insulin resistance and C-reactive protein in women with complete hydatidiform mole. Gynecological Endocrinology 2011; 27(10): 840-843.

[8] Alex S, Panikkar B, Shyamala PK, Balaram P. Viral etiology of Complete Hydatidiform Moles. Indian Journal of Biotechnology 2002; 1(2): 175-179.

[9] Bartas M, Brázda V, Bohálová N, et al. In-depth bioinformatic analyses of nidovirales including human SARS-CoV-2, SARS-CoV, MERS-CoV viruses suggest important roles of non-canonical nucleic acid structures in their lifecycles. Frontiers in microbiology 2020; 11: 1583.

[10] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The lancet 2020; 395(10223): 507-513.

[11] Ramljak D, Vukoja M, Curlin M, et al. Early Response of CD8+ T Cells in COVID-19 Patients. Journal of Personalized Medicine 2021; 11(12): 1291.

[12] Jin Y, Yang H, Ji W, et al. Virology, epidemiology, pathogenesis, and control of COVID-19. Viruses 2020; 12(4): 372.

[13] Bouachba A, Allias F, Nadaud B, et al. Placental lesions and SARS-Cov-2 infection: Diffuse placenta damage associated to poor fetal outcome. Placenta 2021; 112: 97-104.

[14] Celik OY, Çelen Ş, Üstün YE. Did the SARS-CoV-2 effect pregnancy complications? Ginekologia polska 2021;92(12): 872-877.

[15] Celik OY, Ozkan S, Erdogan K, Çelen Ş, Çağlar A, Ustun Y. Did Pregnancy Complications Increase During The COVID-19 Pandemic Period? Medical Records 2020; 2(3): 51-53.

[16] Hayashi K, Bracken MB, Freeman DH Jr, Hellenbrand K. Hydatidiform mole in the United States (1970-1977): a statistical and theoretical analysis. Am J Epidemiol. 1982; 115(1): 67-77.

[17] Gül T, Yilmaztürk A, Erden AC. A review of trophoblastic diseases at the medical school of Dicle University. European Journal of Obstetrics & Gynecology and Reproductive Biology 1997; 74(1): 37-40.

[18] Çetin M, Balta Ö, Duran B, Güvenal T, Yanar O. Kliniğimize Başvuran Mol Gebelik Olgularının Retrospektif İncelenmesi. CÜ Tıp Fakültesi Dergisi 2004; 26(1): 18-22.

[19] Gül A, Çelebi H. Yüzüncü Yıl Üniversitesi Tıp Fakültesi Kadın Doğum Kliniğindeki Trofoblastik Hastalıkların Değerlendirilmesi. Journal of Clinical Obstetrics & Gynecology 2000; 10(3): 192-195.

[20] Abbas AM, Ahmed OA, Shaltout AS. Hydatidiform mole in the era of COVID-19 pandemic. Is there an association? American Journal of Reproductive Immunology 2020; 84(1):13253.

[21] Aiob A, Naskovica K, Sharon A, Bornstein J. A possible association between hydatidiform mole and the COVID-19 pandemic: A retrospective cohort study. Gynecologic Oncology 2021; 161(2): 454-457.

[22] Abbas AM, Ahmed L, Salem AS et al. COVID-19 and hydatidiform mole. American Journal of Reproductive Immunology 2020; 84(5): 13310.

[23] Velicky P, Meinhardt G, Plessl K, Vondra S, Weiss T, Haslinger P, Pollheimer J. Genome amplification and cellular senescence are hallmarks of human placenta development. PLoS genetics 2018; 14(10): 1007698.

[24] Nagymanyoki Z, Callahan MJ, Parast MM, Fulop V, Mok SC, Berkowitz RS. Immune cell profiling in normal pregnancy, partial and complete molar pregnancy. Gynecologic oncology 2007;107(2), 292-297.

[25] Knoeller S, Lim E, Aleta L, Hertwig K, Dudenhausen JW, Arck PC. Distribution of immunocompetent cells in decidua of controlled and uncontrolled (choriocarcinoma/ hydatidiform mole) trophoblast invasion. American Journal of Reproductive Immunolog 2003; 50(1): 41-47.

[26] Fallahi J, Razban V, Momtahan M, Akbarzadeh-Jahromi M, Namavar-Jahromi B, Anvar Z, Fardaei M. A novel mutation in NLRP7 related to recurrent hydatidiform mole and reproductive failure. International Journal of Fertility & Sterility 2019;13(2): 135.

[27] Tayib S, van Wijk L, Denny L. Gestational trophoblastic neoplasia and human immunodeficiency virus infection: A 10-year review. International Journal of Gynecologic Cancer 2011; 21:1684-1691.

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CASE REPORT

A Case of Crimean-Congo Haemorrhagic Fever (CCHF) Mimicking the COVID-19 Disease

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ABSTRACT

A 74-year-old female from a rural district in Central Anatolia, Turkey, was admitted with persisting fever, malaise, cough, and vomiting. There is no abnormal finding on physical examination. There was no petechiae, purpura, ecchymosis, or bleeding in organ systems at any time. Laboratory findings showed increased D-dimer level and acute inflammation biomarkers such as C-reactive protein, ferritin, and thrombocytopenia with prolonged prothrombin time. Since the patient applied with clinic features indicating a viral infection and on a pandemic period, firstly, we focused on coronavirus disease 2019 (COVID-19)disease. However, the probability was reduced with negative chest imaging and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reverse-transcription polymerase chain reaction (RT-PCR) results. Any patient admitting with symptoms indicative of COVID-19 disease during the pandemic era should also be evaluated for other infections, especially endemic zoonotic infections such as brucellosis, Crimean-Congo hemorrhagic fever (CCHF), Q fever. In this case, serologic tests were all negative (Leptospira Toxoplasma, Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Rubella, Brucella, Coxiella, Hepatitis B, Hepatitis C). The patient was found to be IgM and RNA positive for the CCHF virus by ELISA and polymerase chain reaction (PCR) methods, respectively. In endemic areas, CCHF is one of the diseases which should be considered in the differential diagnosis. In addition, it is essential to accurately identify CCHF infection using proper medical tests to prevent misdiagnosis amid this COVID-19 pandemic. Hence, we need to keep an eye on the cases that come from endemic rural areas in the Central Anatolia region of Turkey. Herein, we present a case of CCHF patients initilally evaluated to have COVID-19.

Keywords: Crimean Congo Haemorrhagic Fever, zoonotic disease, endemic region, pandemic, COVID-19

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is an emerging zoonotic disease characterized by flu-like symptoms, fever, hemorrhage, and petechia. The CCHF virus is a member of the Bunyaviridae family and can be transmitted to humans by tick-bite. The disease is widely distributed from the Black Sea to southern Africa. It has been endemic in some parts of Turkey since 2002, with many fatalities, and it is considered a significant public health problem [1,2]. Laboratory findings may include thrombocytopenia, leukopenia, hyperbilirubinemia with elevated transaminases, prolongation of international normalized ratio, prothrombin time, and activated partial thromboplastin time. In addition, many of the initial nonspecific symptoms of CCHF can mimic other common infections [1,3,4].

Coronavirus disease 2019 (COVID-19) is a respiratory infectious disease in humans caused by a newly discovered virus known as the severe

acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 is characterized by fever, cough, dyspnea, myalgia, malaise, and fatigue symptoms like CCHF [1,3,5,6]. Also, laboratory findings are similar to CCHF, such as higher D-dimer, C-reactive protein (CRP), creatine kinase (CK), and lactate dehydrogenase (LDH) levels. Additionally, lymphopenia, thrombocytopenia, and pulmonary opacities on the chest CT [1,3,5,7].

Although CCHF is endemic in some countries, the main admission symptoms are fever, myalgia, malaise, and fatigue, so that it can be misinterpreted mainly due to the ongoing COVID-19 pandemic [9]. In this setting, a complete history and careful physical examinations must be followed by laboratory confirmation. The differential diagnosis should be broad enough when someone comes with a chief complaint of persisting fever, myalgia, and malaise [5,8,9].

Herein, we present some COVID-19 symptoms and their similarities with CCHF. In addition, we also describe similar laboratory findings of both diseases.

Case presentation

A 74-year-old female from a rural area in Central Anatolia, Turkey, was admitted with malaise, fatigue, persisting fever, cough, and vomiting for three days. She was hospitalized at the Infectious Diseases Service, Hacettepe University in Ankara, Turkey. On admission, she did not report any contact with a COVID-19 patient. There was no petechiae, purpuric lesions, or bleeding. Oxygen saturation was 96% in room air, respiratory rate 16/min, pulse 70/min and body temperature 36°C,and blood pressure 130/80 mm/Hg. There is no abnormal finding on physical examination. Complete blood count revealed Hb:12,5 g/dl, Leukocyte: 2.2 $x10^3$ µl, Lymphocyte: 0.35 $x10^3$/µl, Neutrophil 1.67 $x10^3$/ µl , Platelet: 86 $x10^3$/ µl. Other blood tests were; total CK: 152 U/L, LDH: 313 U/L, ALT: 23 IU/L, AST: 56 IU/L, Ferritin: 477,5 µg/L, D-dimer: 2,36 mg/L , International normalized ratio (INR): 1,6, CRP: 5,66 mg/dl. Bilirubin levels and other blood tests were normal. Chest X-ray and tomography were evaluted normal, without any pathological finding. SARS-CoV-2 RT-PCR from nasopharyngeal smear was negative in two subsequent occasions.

Lymphopenia resolved spontaneously within seven days, but thrombocytopenia and leukopenia continued. Also, platelet values decreased to its nadir of 25x10^3/ µl. The blood smear showed no signs of hemolysis or atypical cells. Abdominal ultrasound imaging was deemed to be normal. All serologic tests against an array of infectious agents were negative (leptospira, toxoplasma, CMV, EBV, rubella, brucella, coxiella, HBV, HCV, HIV, ANA, Anti-ds DNA, etc.). Although patient denied a history of a tick bite, CCHF was considered because the patient hailed from an endemic region and was engaged in animal husbandry. AntiCCHF IgM and RT-RNA were found to be positive.

The patient was followed up with supportive therapy in contact isolation. She did not develop petechiae, purpura, ecchymosis, or bleeding in organ systems at any time. Laboratory parameters normalized during follow-up and she was discharged home with close telemedicine observation.

DISCUSSION

Crimean-Congo hemorrhagic fever (CCHF) is an acute and fatal zoonotic disease caused by an RNA virus that is a member of the Bunyaviridae family. The fatality rate of the disease is 3%-30%. Hyalomma ticks transmit CCHF through direct contact with the blood and other bodily fluids of patients or infected animals [1,3]. The first Turkish case was reported in 2002. The CCHF disease is one of the most dangerous medically significant tick-borne disease affecting Turkish people and many countries at Africa, Eurasia, Central and Southwest Asia, and the Middle East [1,3]. CCHF is characterized by fever, myalgia, malaise, nausea, vomiting, headache, and hemorrhagic manifestations [1,3]. The laboratory findings of CCHF include anemia, thrombocytopenia, leukopenia, hyperbilirubinemia, elevated transaminases, CK, and LDH; and prolonged INR, higher fibrinogen and D-dimer levels [8,10]. These clinical features and laboratory findings may mimic various other infections [3,8].

COVID-19 infection is characterized by fever, cough, dyspnea, myalgia, malaise, fatigue symptoms, D-dimer elevation, elevated acute inflammation

biomarkers such as c-reactive protein, ferritin, thrombocytopenia, lymphopenia, and prolonged prothrombin time, and pulmonary infiltrate including ground-glass opacities on CT of the chest [5,11].

COVID-19 and CCHF are hard to distinguish because they share similar clinical features [6,8]. Cytokine storms are the underlying reason of increased morbidity and mortality in both diseases. While CCHF results in hemorrhage, COVID-19 can cause thrombosis [6].

To our knowledge no CCHF case mimicking the COVID-19 clinical features and laboratory findings have been reported before. Especially at the beginning of the summer, the incidence of CCHF gradually increases in the rural areas due to the increased contact with infected insects and animals. Our patient presented with myalgia, malaise, fatigue, persisting fever, cough, and vomiting. As Turkey is an CCHF-endemic region, CCHF should be kept in mind in patients especially those that come from the endemic regions ie. Corum, Yozgat, and Kırsehir. Since this patient applied with a clinic resembling a viral infection during the pandemic period, COVID-19 infection was considered firstly. However, COVID19 was ruled out with chest imaging and negative SARS CoV-2 RT-PCR. Subsequently, we perfomed an array of infectious disease work-up to diagnose causative disease. CCHF was confirmed with positive RT-PCR and specific IgM and IgG tests [1,3,8,12]. Besides, extensive laboratory work-up ruled out other possible infectious casuses

If this case has not been labeled as COVID-19, the CCHF could be dignosed earlier and not been overlooked. In a large epidemiologic study including more than 1800 cases of CCHF, nearly one third of cases denied prior history of tick bite or tick contact, 62 %reported close contact with animals, and 10 percent had a history of direct contact with animal body fluids or tissue. Hemorrhagic findings were detected in 23.0% of the patients [11]. In endemic areas, regardless of the history of bleeding and tick bites, especially in viral infection-like diseases accompanied by thrombocytopenia, CCHF should be kept in mind [7,8,11].

CONCLUSION

The CCHF cases hailing from rural areas apply to emergency departments overcrowded by COVID19 pandemic. Despite the pandemic constraints, any patient admitting with symptoms indicative of COVID-19 during the pandemic era should also be evaluated for other infections, particularly endemic zoonotic infections. In endemic areas, CCHF is one of the diseases which should be considered in the differential diagnosis. In addition, it is essential to accurately identify CCHF infection using proper medical tests to prevent misdiagnosis amid this COVID-19 pandemic. Hence, we need to keep an eye on the cases that come from endemic rural areas in the Central Anatolia region of Turkey.

Author contribution

Study conception and design: ZT, MCS, and MA; data collection: ZT, GTD, ACI, and OU; analysis and interpretation of results: MCS, OU, and MA; draft manuscript preparation: ZT, MCS, and GTD. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Ergonul O. Crimean-Congo hemorrhagic fever virus: new outbreaks, new discoveries. Curr Opin Virol. Apr 2012;2(2):215-20. doi:10.1016/j.coviro.2012.03.001

[2] Yilmaz GR, Buzgan T, Irmak H, et al. The epidemiology of Crimean-Congo hemorrhagic fever in Turkey, 2002-2007. Int J Infect Dis. May 2009;13(3):380-6. doi:10.1016/j.ijid.2008.07.021

[3] Ertem G, Sönmezer M, Temoçin F, Ataman Hatipoğlu Ç, Tülek N, Oral B. The efficacy of oral ribavirin on clinical and laboratory parameters inCrimean-Congo hemorrhagic fever: an observational study from Turkey. Turk J Med Sci. Nov 17 2016;46(5):1407-1414. doi:10.3906/sag-1506-92

[4] Heydari Aa. Acute Complicated Brucellosis Mimicking Crimean-Congo Hemorrhagic Fever (CCHF) and Vice Versa. Review article. Journal of Medical Microbiology and Infectious Diseases. 2019;7(1):1-5. doi:10.29252/JoMMID.7.1.2.1

[5] Sonmezer MC, Inkaya AC. COVID-19: Virology, pathogenesis, Clinical Features, Treatment. Turkey Clinics specail issue COVID-19. 2020. p. 1-8. Online ISBN: 978-625-401-397-3.

[6] Pazarlı AC, Parlak Z, Ekiz T. COVID-19 and Crimean-Congo Hemorrhagic Fever: Similarities and Differences. Heart Lung. Nov-Dec 2020;49(6):892-893. doi:10.1016/j.hrtlng.2020.05.013

[7] Kara SS, Kara D, Fettah A. Various clinical conditions can mimic Crimean-Congo hemorrhagic fever in pediatric patients in endemic regions. J Infect Public Health. Sep-Oct 2016;9(5):626-32. doi:10.1016/j.jiph.2016.01.007

[8] Tasdelen Fisgin N, Doganci L, Tanyel E, et al. Initial high rate of misdiagnosis in Crimean Congo haemorrhagic fever patients in an endemic region of Turkey. Epidemiol Infect. Jan 2010;138(1):139-44. doi:10.1017/s0950268809990318

[9] Butt MH, Ahmad A, Misbah S, Mallhi TH, et al. Crimean-Congo hemorrhagic fever and Eid-Ul-Adha: A potential threat during the COVID-19 pandemic. J Med Virol. Feb 2021;93(2):618-619. doi:10.1002/jmv.26388

[10] Zayet S, Kadiane-Oussou NJ, Lepiller Q, et al. Clinical features of COVID-19 and influenza: a comparative study on Nord Franche-Comte cluster. Microbes Infect. Oct 2020;22(9):481-488. doi:10.1016/j.micinf.2020.05.016

[11] Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, et al. Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. Travel Med Infect Dis. Mar-Apr 2020;34:101623. doi:10.1016/j.tmaid.2020.101623

[12] Onguru P, Dagdas S, Bodur H, et al. Coagulopathy parameters in patients with Crimean-Congo hemorrhagic fever and its relation with mortality. J Clin Lab Anal. 2010;24(3):163-6. doi:10.1002/jcla.20383

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CASE REPORT

Coexistence of Geographic Tongue and Palmoplantar Pustulosis: A Case Report

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ABSTRACT

Palmoplantar pustulosis is strongly suggested the involvement of oral bacteria, but the cause of geographic tongue remains unknown. A 20-year-old female patient described a 1-2 year history of geographic tongue with irregular, smooth, red patches on parts of the tongue. She also presented several pustules, accompanied by delimited erythematous skin patches on both soles. One month after the first visit, when she returned for follow-up, the skin condition had progressed further, and pustules and scales became more prominent. Tongue rinses with commercial mouth washes were tried, but the condition of the lesions fluctuated between improvement and exacerbation. This report presents a rare case of the coexistence of geographic tongue and palmoplantar pustulosis in a young female patient.

Keywords: Geographic tongue, palmoplantar pustulosis, oral bacteria

INTRODUCTION

Geographic tongue (G tongue) [1] or benign migratory glossitis is characterized by irregular, smooth, red patches with filiform papillae atrophy, producing a map-like aspect on different parts of the tongue. The lesions change in shape and size with time. The condition is harmless and affects approximately 1% to 3% of the people at any age [1]. Various factors [1], including hormonal disturbances and emotional stress, may be associated with the development of the disease, however, the precise cause remains unknown. No specific treatment for symptomatic benign migratory glossitis [2] has been found.

Palmoplantar pustulosis (PPP) [3] is a unique, chronic inflammatory skin disease that specifically affects the palms and soles, with prominent intraepidermal pustules accompanying with erythematous plaques. Lesions vary in severity and the symptoms may persist for many years. PPP is very difficult to treat, but topical steroids and psoralen plus ultraviolet A light (PUVA) [3] have long been used. Metal allergy and/or bacterial infections have been strongly suspected as its causes, but until recently, the fact that dental metal allergy is not a cause of PPP has been reported [4]. There has been a recent report [5] that strongly suggests the involvement of oral bacteria in PPP.

This report presents the case of a 20-year-old female patient with the coexistence of G tongue and PPP. Whether G tongue and PPP exhibit coexistence or association, will be discussed.

CASE PRESENTATION

A 20-year-old female patient described a 1-2 year history of G tongue (Figure 1A) with irregular, smooth, red patches on parts of the tongue. The patches had a fine white margin. She had no dental caries or gingivitis, and fungus was not detected on microscopic examination of a tongue scraping sample. She also presented several almost-symmetrically scattered pustules (Figure 1B (arrows)), accompanied by delimited erythematous

skin patches, forming plaque-like features on both soles (Figure 1B). One month after the first visit, when she returned for follow-up, the skin condition had progressed further, and pustules and scales became more prominent (Figure 1C (arrows)), which are the typical features of PPP [1]. So far, skin biopsy was not conducted without problems. Other than plantar lesions, no other skin lesions were detected. She is a non-smoker and reported no systemic symptoms, such as joint pain. The skin lesions were present for about 4 months and treated at another clinic using betamethasone ointment without improvement. Only zinc ointment was applied for the sensation of dryness on her soles.

Although G tongue is thought to be caused by oral bacteria [6], there are no specific treatments. Tongue rinses with commercial mouth washes containing sterilization agent were tried and continued for several months. However, the condition of the lesions fluctuated between improvement and exacerbation, and, unfortunately, neither the G tongue nor PPP lesions were completely cured.

DISCUSSION

Recent reports strongly attribute PPP to oral bacteria [5] such as periodontopathic bacteria. The involvement of oral bacteria in G tongue has also been suggested [6], as certain specific bacteria may be present in the lingual microbiota of a patient. The pattern of G tongue changes over time, suggesting that moving microorganisms, which can penetrate the mucosa, may be involved. However, many different types of oral bacteria [6] are present in the oral cavity, and it would be difficult to determine the specific bacteria causing both G tongue and PPP. In the present circumstances, no particular micro-organism has been consistently found in association with G tongue. Identifying the precise cause of G tongue requires additional investigation, including whether oral bacteria are involved in this disease.

On the other hand, the association between G tongue and psoriasis, an inflammatory skin disease characterized by clinical features such as plaques, guttate, pustular, and erythrodermic lesions [7], has been reported in many studies [8]. There are many cases in which the features of psoriasis and G tongue do not overlap. Histopathological and other similarities also exist between G tongue and psoriasis [9]. Histological findings of G tongue [9] show inflammatory features including the occasional presence of small spongiform pustules and Munro's microabscesses due to inflammatory cell infiltration by the neutrophils. The histological appearance of G tongue closely resembles those of psoriasis [9]. G tongue has been suggested to

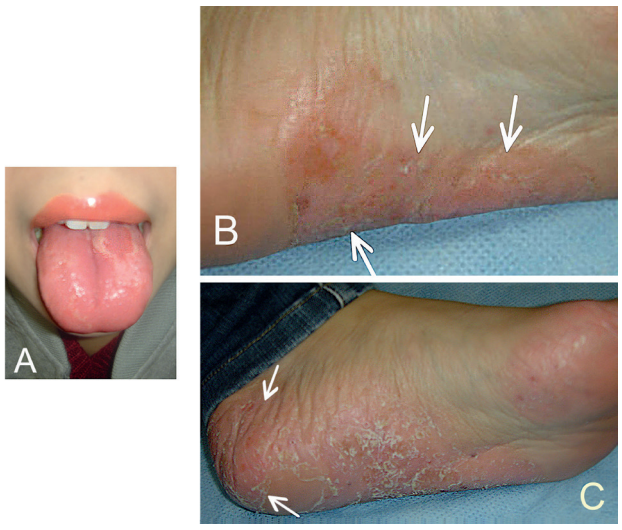

Figure 1. A Appearance of the geographic tongue at first visit. **B** At the first visit, several scattered pustules (points with arrows) accompanying erythematous and thickened patches forming plaques were noted on the soles. **C** At the time of consultation, from the mid-plantar surface of the foot to the plantar arch, there were many scales mixed with pustules and prominent disseminated tiny pustules (points with arrows) accompanying the erythema with scales on the heel, almost symmetrically on both soles, with typical PPP clinical features being noted. In addition, there was erythema on the hallux region.

be a psoriatic tongue lesion [9]. However, even if the tissue response resembles psoriasis, it can not explain the geographic patterns in many patients with G tongue and without psoriasis. Treatment with tacrolimus [10] (immunosuppressant FK506) has been sporadically attempted and the findings suggest that G tongue is a disease associated inflammatory immune responses. Based on these collected findings, the author believes that some bacteria may cause psoriasis-like local tissue reactions in the affected tissues. Overall, evidence supporting the possibility that G tongue is caused by oral bacteria is increasing. Also from the dental field, it has been reported that the indigenous bacteria of the tongue resemble those of the periodontal flora [11] and it has been pointed out that indigenous tongue bacteria are a source of periodontal bacteria [11]. It can not be ruled out that the G tongue may be the causative lesion in some cases of PPP and psoriasis; further verification is required.

The homology between PPP and psoriasis is still debatable [12], but the two should be considered separate entities owing to the specificity of the site of development of PPP. The specificity of the site of the lesion to the palms and soles indicates the unique pathomechanism of PPP [3] and its difference from that of psoriasis [7]. If PPP is a disease related to psoriasis [12], then an association with G tongue and PPP would also be likely, as G tongue is sometimes associated with psoriasis [8]. It has been pointed out that the cause of psoriasis is also oral bacteria [13].

In conclusion, to the best of my knowledge, cases of G tongue coexisting with PPP have not been previously reported. Determining the cause of this unexplained idiosyncratic disease, G tongue will open the way for treatment.

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Author contribution

The author is solely responsible for the content of this manuscript. The author reviewed the results and approved the final version of the manuscript.

Ethical approval

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Conflict of interest

The author declares that there is no conflict of interest.

Patient's consent

Informed consent was obtained verbally from the patient for this publication and accompanying images.

REFERENCES

[1] Pass B, Brown RS, Childers EL. Geographic tongue: literature review and case reports. Dent Today. 2005; 24(8):54, 56-57.

[2] Gushiken de Campos W, Esteves CV, Fernandes LG, et al. Treatment of symptomatic benign migratory glossitis: a systematic review. Clin Oral Investig. 2018; 22(7):2487-2493.

[3] Olazagasti JM, Ma JE, Wetter DA. Clinical features, etiologic factors, associated disorders, and treatment of palmoplantar pustulosis: The Mayo Clinic experience, 1996-2013. Mayo Clinic Proc. 2017; 92(9):1351-1358.

[4] Masui Y, Ito A, Akiba Y, et al. Dental metal allergy is not main cause of palmoplantar pustulosis. J Eur Acad Dermatol Venereol. 2019; 33(4): e180-e181.

[5] Horiuchi Y. Palmoplantar pustulosis treated with oral rinse using ozone nanobubble water: A case series. Dermatol Ther. 2020; 33(6):e13924.

[6] Dafar A, Bankvall M, Çevik-Aras H, et al. Lingual microbiota profiles of patients with geographic tongue. J Oral Microbiol. 2017; 9(1):1355206.

[7] Langley RG, Krueger GG, Griffiths CE. Psoriasis: epidemiology, clinical features, and quality of life. Ann Rheum Dis. 2005; 64(Suppl 2):ii18-23. P-10.

[8] Femiano F. Geographic tongue (migrant glossitis) and psoriasis. Minerva Stomatol. 2001; 50(6):213-217.

[9] Picciani BLS, Domingos TA, Teixeira-Souza T, et al. Geographic tongue and psoriasis: clinical, histopathological, immunohistochemical and genetic correlation - a literature review-. An Bras Dermatol. 2016; 91(4):410-421.

[10] Ishibashi M, Tojo G, Watanabe M, et al. Geographic tongue treated with topical tacrolimus. J Dermatol Case Rep. 2010; 4(4):57–59.

[11] Göhler A, Samietz S, Schmidt CO, et al. Comparison of oral microbe quantities from tongue samples and subgingival pockets. Int J Dent. 2018; 2018: 2048390.

[12] Brunasso AMG, Massone C. Psoriasis and palmoplantar pustulosis: an endless debate?. J Eur Acad Dermatol Venereol. 2017; 31(7):e335-e337.

[13] Sharma A, Raman A, Pradeep AR. Association of chronic periodontitis and psoriasis: periodontal status with severity of psoriasis. Oral Dis. 2015; 21(3):314-319.