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İSTANBUL KUZEY KLİNİKLERİ

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Original Article HISTOLOGY & EMBRYOLOGY

Urtica dioica can regulate autophagy pathway in the rat hippocampal tissue after STZ-induced neurodegeneration

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ABSTRACT

OBJECTIVE: Autophagy plays a crucial role in neuroprotection by helping to clear toxic substances, like misfolded proteins. In neurodegeneration, autophagy is impaired leading to the accumulation of harmful proteins that disrupt neuronal function, promote inflammation, and contribute to the degeneration of brain cells. Therefore, because of its anti-inflammatory and anti-oxidative actions, the effects of Urtica dioica (UD) on the proteins of autophagy signaling pathways was studied in the hippocampus of rats with streptozotocin-(STZ) induced neurodegeneration.

METHODS: Neurodegeneration model of rats was induced by intracerebroventricular injection of STZ (3 mg/kg) to observe both cognitive deficits and autophagic dysfunction. Then, the rats in the treatment group were consumed UD at the dose of 50 mg/kg/day for 4 weeks. At the end of 4 weeks, passive avoidance test was applied for cognitive functions and hippocampal tissue of rats were investigated to determine the changes in the proteins related to autophagy by western blotting and immunofluoresecence.

RESULTS: UD treatment slightly attenuated the STZ-induced memory deficiencies in the rats. In addition, an increase in the autophagy was noted by increasing the expression of Beclin, ATG5, and LC3β proteins in the STZ-UD group compared to the STZ group.

CONCLUSION: In summary, UD may be a candidate molecule as a therapeutic strategy to protect neurons in neurodegeneration through increasing autophagy to reduce toxic protein accumulation.

Keywords: Alzheimer's disease; autophagy; rat; streptozotocin; urtica dioica.

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Alzheimer's Disease (AD) represents a gradual and persistent neurodegenerative illness that leads to serious cognitive decline, memory disturbances, and noticeable behavioral changes [1]. Being a multifactorial disease, AD is mainly affected by genetic risk factors, aging, and oxidative stress [2]. The disease is

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thought to begin with neuronal degeneration in layer II of the entorhinal cortex, progressively extending to the hippocampus, temporal and frontoparietal cortices, and ultimately reaching the subcortical nuclei [3]. It is thought that oxidative stress has been strongly linked to cognitive decline and the progression of AD. Increased oxidative stress contributes to the accumulation of senile plaques and synaptic loss, ultimately leading to neurodegeneration [2].

Autophagy is a tightly regulated process that adapts to oxidative stress by breaking down cytoplasmic macromolecules and damaged organelles within lysosomes, thereby promoting cellular growth, development, and homeostasis. In addition to oxidative stress, autophagy can be triggered by cellular stress factors such as nutrient deprivation, energy shortages, hypoxia, toxins, radiation, DNA damage, and intracellular pathogens. This makes it a promising research target for preventing the progression of neurodegenerative diseases like AD [4]. Previous studies have shown that neuronal cell autophagy is markedly reduced in the onset of AD, and that sustaining autophagy in neuronal cells may help mitigate the progression of AD [5, 6]. Moreover, impaired autophagic flux has been associated with the accumulation of amyloid-beta (Aβ) peptides and hyperphosphorylated tau, which are hallmark pathological features of AD [7, 8]. Dysfunctional autophagy leads to the formation of autophagic vesicles that fail to mature and fuse with lysosomes, resulting in intracellular build-up of toxic protein aggregates [9]. Several key regulators of autophagy, including Beclin-1 and the mTOR signaling pathway, have been found to be dysregulated in AD brains, further implicating autophagic failure in the disease pathology [5, 10]. As such, enhancing autophagy through pharmacological or genetic approaches is being actively investigated as a potential therapeutic strategy in AD.

Urtica dioica (UD) is a plant which could have anti-inflammatory and anti-oxidative properties because it is rich in minerals and vitamins, such as pro-vitamin A and vitamin C. UD has been extensively studied as an antioxidant in protecting against hyperglycemia and diabetes [11]. Additionally, previous studies have demonstrated that this herbal extract treatment significantly improves learning and memory in an aged mouse model [2]. Furthermore, it has been shown that UD has potential therapeutic impacts against neuroinflammatory conditions due to its antioxidative effect [12]. Some of the constituents of UD, such as carvacrol, scopoletin, and rosiglitazone, have shown a neuroprotective effect against neurological impairments [13]. In one of the previous studies, it was shown that UD has an ameliorative effect on the midbrain dopaminergic neurons of animals with Parkinson's disease by restoring the autophagic machinery as a one of the therapeutic strategies [14]. Therefore, this study aimed to examine the effects of UD on the autophagic process in the hippocampus of rats with neurodegeneration, considering its potential therapeutic role in treating neurodegenerative diseases. In the present study, we employed an AD-like model induced by intracerebroventricular (i.c.v) injection of streptozotocin (STZ). This model closely mimics several pathological features of sporadic AD, including cognitive impairment, insulin resistance, oxidative stress, and neurodegeneration. Importantly, STZ-induced neurodegeneration models have also been shown to exhibit impaired autophagic flux, characterized by the accumulation of autophagic vesicles and altered expression of key autophagy-related proteins in the hippocampus [15, 16]. These features make it a suitable model for investigating autophagy-related mechanisms in the context of AD progression.

Highlight key points

- *Urtica dioica* slightly attenuates memory deficiencies.
- Neurodegeneration decreases autophagy in the hippocampus of rats.
- Urtica dioica increases the levels of Beclin, ATG5, and LC3β proteins to activate autophagy in neurodegeneration model.

MATERIALS AND METHODS

Experimental Subjects

Long-Evans Hooded rats (female, 6-month-old, weighing 350–400 g) taken from Bezmialem Vakif University Animal Research Center were used in the present study. Experimental procedures were followed in line with NIH guidelines (NIH publication No. 85-23, revised 1996) and ethical principles of the Declaration of Helsinki. Rats were free to food and water with housing in 12-hour light/dark cycles at 22°C temperature and 60% humidity. All procedures were authorized by the Animal Research Ethics Committee at Bezmialem Vakıf University (2019/266) and conducted in accordance with ARRIVE guidelines to minimize animal suffering.

Neurodegeneration Model by Streptozotocin (STZ) Injection and *Urtica Dioica* (UD) Treatment

At the start of the study, in a random manner, rats were divided to four experimental groups: the control group (without any experimental procedures, n=5), the Sham control group (injection of artificial cerebrospinal fluid (aCSF) via i.c.v., n=6), the STZ group (i.c.v. STZ injection, n=6), and the STZ+UD group (STZ injection via i.c.v. followed by UD treatment, n=5). The numbers of animals were determined according to a priori sample size calculation. To induce neurodegeneration, i.c.v. injections of STZ were administered following a protocol adapted from previous studies [17–19]. STZ (Sigma, St. Louis, MO) was dissolved in aCSF and injected bilaterally into the rats (coordinates: 0.8 mm anteroposterior, 1.5 mm mediolateral, 3.5 mm dorsoventral) for 48 hours apart at a total dose of 3 mg/kg per animal. For the sham control group, aCSF was injected (20 µl) similar to the STZ group.

After the second STZ injection, the rats stayed in their cages for 6-month to develop most of the AD-like phenotype as suggested in a previous study [20]. Subsequently, the STZ+UD group received UD treatment (50 mg/kg/day) via intragastric intubation for 4 weeks [21, 22]. The dose of UD used in our study (human equivalent dose was 10 mg/kg) falls within a range that could reasonably be expected to be achievable in human populations. This dose was calculated based on the literature [23] and the typical consumption levels of commercially available as herbal supplement capsules (500 mg/capsule) for humans [22].

UD was collected from a local Istanbul-Turkiye market and identified by Assoc. Prof. Dr. Cagla Kizilaslan Hancer, from the Department of Pharmaceutical Botany- Faculty of Pharmacy- Bezmialem Vakif University. The plants were cleaned and stripped of leaves in the laboratory to obtain healthy, uniformly colored, and sized leaves, with their stems and petioles removed. The nettle leaves were initially dried in a cool, dry, and shaded environment. Following the drying process, the leaves were manually ground into a fine powder using a pestle and mortar. For ultrasound-assisted extraction, 1 gram of the dried nettle powder was measured and placed into a screw-cap tube containing 15 mL of preboiled double-deionized water. The extraction was conducted in an ultrasonic bath at 65 °C for one hour, based on parameters established in previous studies [24, 25]. This temperature was also chosen to mimic the typical preparation method of nettle leaves, reflecting how they are usually consumed. After extraction, the mixture was filtered using Whatman No.1 filter paper to separate the solid residues from the liquid extract. The final extracts were stored in the dark at 5 °C until further analysis [26].

Passive Avoidance Test

This task was conducted at the day following the last UD treatment. The apparatus used for this task consisted of two compartments: one illuminated and the other dark. The apparatus comprised two chambers separated by a guillotine door. The test included an acquisition and a retention session. During acquisition, each rat was placed in the illuminated compartment for 20 seconds, after which the door opened automatically. Upon entry into the dark compartment, a 1 mA scrambled foot shock was delivered for 2 seconds. Animals were then returned to their cages. In the retention session, rats were reintroduced to the illuminated compartment; after 20 seconds, the door was opened, and step-through latency to the dark compartment was recorded. The maximum cutoff time for this session was 300 seconds.

Molecular Studies

Western Blotting

After the behavioral test, rats were decapitated using guillotine under anesthesia via ketamine and xylazine. Half of the brain (left) was dissected and quickly frozen for immunofluorescence studies. The right hippocampal tissue were dissected from each rat and was homogenized in RIPA lysis buffer containing metallic beads in an homogenizator after treated with 0.1 M phosphate-buffered saline (PBS). Then, homogenized tissues were centrifuged at 9000 x g for 15 min at 4 °C. After determination of protein concentration with Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham), equal amounts of protein (40 µg/µl) were diluted in Laemmli sample buffer (Bio-Rad Laboratories, Inc. U.S.A), boiled for denaturation of proteins and loaded onto 4–20% gradient of sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). At the end of the SDS-PAGE, the PVDF membranes were used to transfer proteins and the membranes were treated with blocking solution (5% milk powder in Tris-buffered saline-containing Tween 20 (TBST)). After an overnight incubation with primary antibodies including ATG5, Beclin, MAP LC3β (1:1000; Santa Cruz, USA), and NeuN (1:1000; Cell Signaling, Danvers, USA),

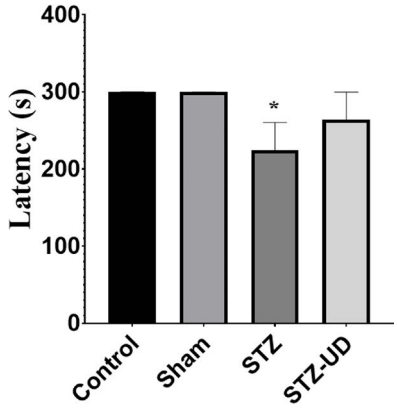

Figure 1. *Urtica dioica* (UD) treatment ameliorated the STZ-induced memory loss. The time to the entrance to the dark side of passive avoidance task for rats in the control (n=5), sham control (n=6), STZ (n=6) and STZ-UD (n=5) groups. Error bars denote SEM (standard error of mean). The degree of significance was denoted as * for p≤0.05.

membranes were incubated with secondary antibodies (1:1000; Cell Signaling, Danvers, USA). Luminol substrate (Advansta, San Francisco, USA) were used for determination of signal with Azure Western Blot Imaging System (Azure Biosystems, USA) containing CCD camera. Protein loading was normalized by β-actin (1: 1000; Cell Signaling, Danvers, USA). ImageJ analysis system (NIH; Washington, USA) were used to quantify the immunoreactive protein densitometrically.

Immunofluorescence Staining

Immunoflourescent analysis of rat brain tissue was done on 4 μm fresh frozen sections. The sections were fixed by 4% paraformaldheyhde (PFA) for 10 minutes at 4 °C. The permabilization was done by 10-min incubation with Triton X-100 (0.3%) at room temperature (RT). The blocking of sections were performed with 1% BSA in TBST for 1h at RT. The antibodies against NeuN (NBP1-92716, Novus) and Beclin (AC-0276RUO, Epitomics) were applied overnight at 4 °C. Negative controls were achieved by using T-TBS instead of the primary antibodies. The secondary antibody applied was Alexa Fluor 488 (ab150077) with 2 μg/ml concentration for 30-min at RT. The slides were covered by using Fluoroshield Mounting Medium with DAPI (ab104139, Abcam).

The sections were then examined under Zeiss Vert. A1 microscope and photographed by ZEN 2.6 application using Axiocam 503 color camera. The images taken was evaluated by QuPath [27]. In hippocampal CA1-CA3 regions, positive cells were counted for NeuN evaluation and H-scores were calculated for Beclin evaluation. The evaluations were done over the images taken three different sites of specified regions.

Statistical Analysis

The mean values and standard error of means were calculated for behavioral and molecular data. One-way ANOVA with Fisher's Least Significant Difference (LSD) test for pairwise comparisons was applied for behavioral and western blotting data to calculate the differences between groups. For analysis of data and representing the results as a graph, the Graphpad Prism 8 (GraphPad Software, Boston, USA) was used. The statistical significance value was accepted as p≤0.05.

RESULTS

Effect of UD Treatment on Cognitive Performance in STZ-Induced Neurodegeneration

During the retention session of the passive avoidance test, a shorter step-through latency were observed in the STZ group compared to both control groups, indicating impaired memory performance. This reduction was statistically significant only relative to the sham group (p=0.042). Conversely, administration of UD to STZ-exposed animals led to a noticeable improvement in retention performance, with latency values approaching those observed in the control groups (Fig. 1).

Effect of UD Treatment on Autophagy Protein Expression of STZ-Induced Neurodegeneration

The effect of STZ-injection and UD administration on the autophagy proteins including Beclin, ATG5, and LC3β were demonstrated in the hippocampal tissue (Fig. 2). Injection of STZ significantly declined the expressions of Beclin protein in the rats (Fig. 2A, p=0.0021), ATG5 (Fig. 2B, p=0.0078), and LC3β (Fig. 2C, p=0.0003) compared to that of the sham control group. In addition, an increase in the expression of these autophagy markers were noted in the sham control group compared to the control group (p<0.05). On the other hand, UD administration significantly increased the concentration of Beclin (p=0.0421) and LC3β (p=0.0038) compared to the STZ group approaching to the levels in the control groups. In contrast, the protein expression of ATG5 was not significantly changed due to UD treatment compared to the STZ group (Fig. 2B).

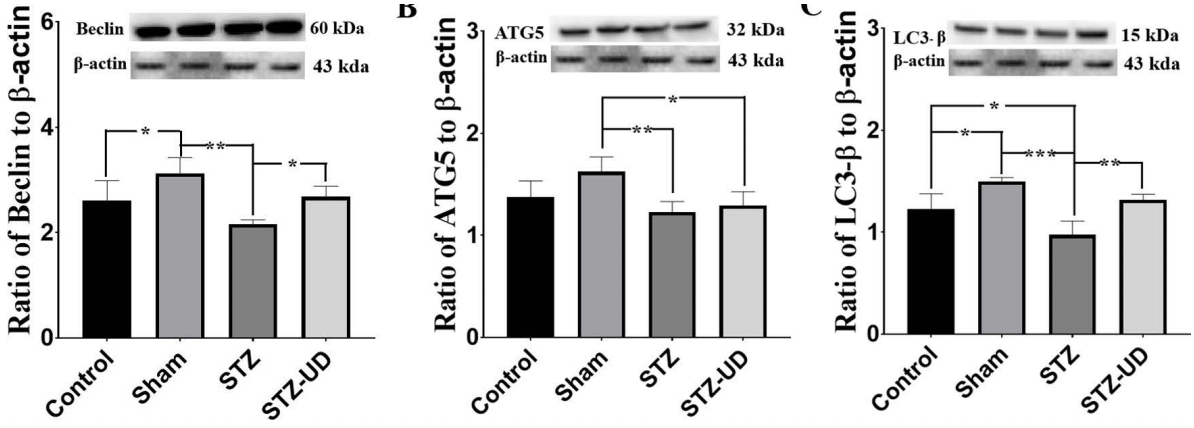

FIGURE 2. Representative pictures and relative amounts of (A) Beclin (B) ATG5, and (C) LC3β to β-actin which were analyzed by western blotting for control (n=5), sham control (n=6), STZ (n=6) and STZ-UD (n=5) groups. Error bars indicate SEM (standard error of mean). The degree of significance was denoted as * for p≤0.05, ** for p≤0.01, *** for p≤0.001. Lines show differences between two represented groups.

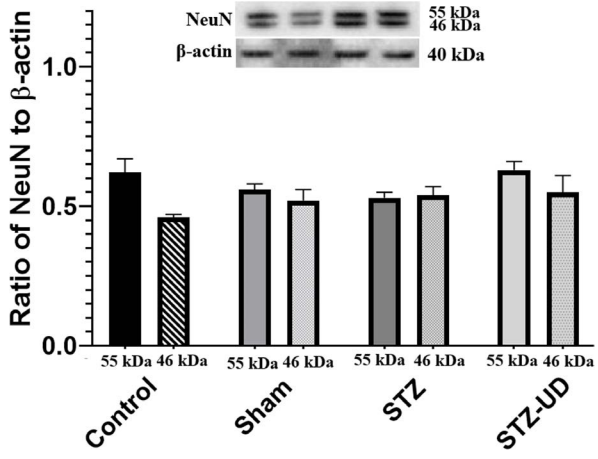

FIGURE 3. Representative pictures and relative amounts of NeuN protein to the to β-actin protein which were analyzed by western blotting for control (n=5), sham control (n=6), STZ (n=6) and STZ-UD (n=5) groups. Error bars indicate SEM (standard error of mean).

To observe effects of UD and the autophagy on the neuronal levels, we measured the expression of NeuN in the hippocampus of all studied groups (Fig. 3). Moreover, both STZ injection and UD treatment did not have any significant effect on the expression of NeuN protein compared to control animals (Fig. 3).

The Effect of UD Treatment on the Histological Parameters

To further detect and quantify NeuN positive cells over hippocampal CA1-CA3 subregions, immunofluorescence labeling was done (Fig. 4). In both CA1 and CA3 regions, the number of NeuN positive cells significantly decreased in the STZ group compared to the both control groups (Fig. 5, $p<0.01$). The UD-treatment group showed a statistically significant increase in both regions of the hippocampus compared to that of the STZ group ($p<0.05$).

The autophagy marker Beclin was also evaluated with immunofluorescence labeling for validation of western blotting data (Fig. 4). The STZ group had a significantly low Beclin H-Score compared to the both control and sham groups in hippocampal CA1-CA3 regions (Fig. 5, $p<0.001$). The UD treatment group, however, showed significantly high Beclin H-Score in both regions compared to the STZ group ($p<0.01$).

DISCUSSION

Neurodegenerative disorders are also defined as proteinopathies due to the accumulation of aggregates of insoluble proteins. Multiple risk genes related to neurodegenerative disorders, including *PICALM*, *PSEN1*, *TREM2*, *CLU*, etc. have been modulate autophagic flux [28]. Therefore, promoting autophagy to improve the removal of misfolded proteins is suggested as a potential therapeutic approach. Strategies targeting autophagy could offer a novel method for developing medications to combat neurodegenerative disorders, particularly phytotherapeutics [29]. Herbal drugs are considered as probable sources of antioxidants [30]. The most regular used forms of the herbal drugs are

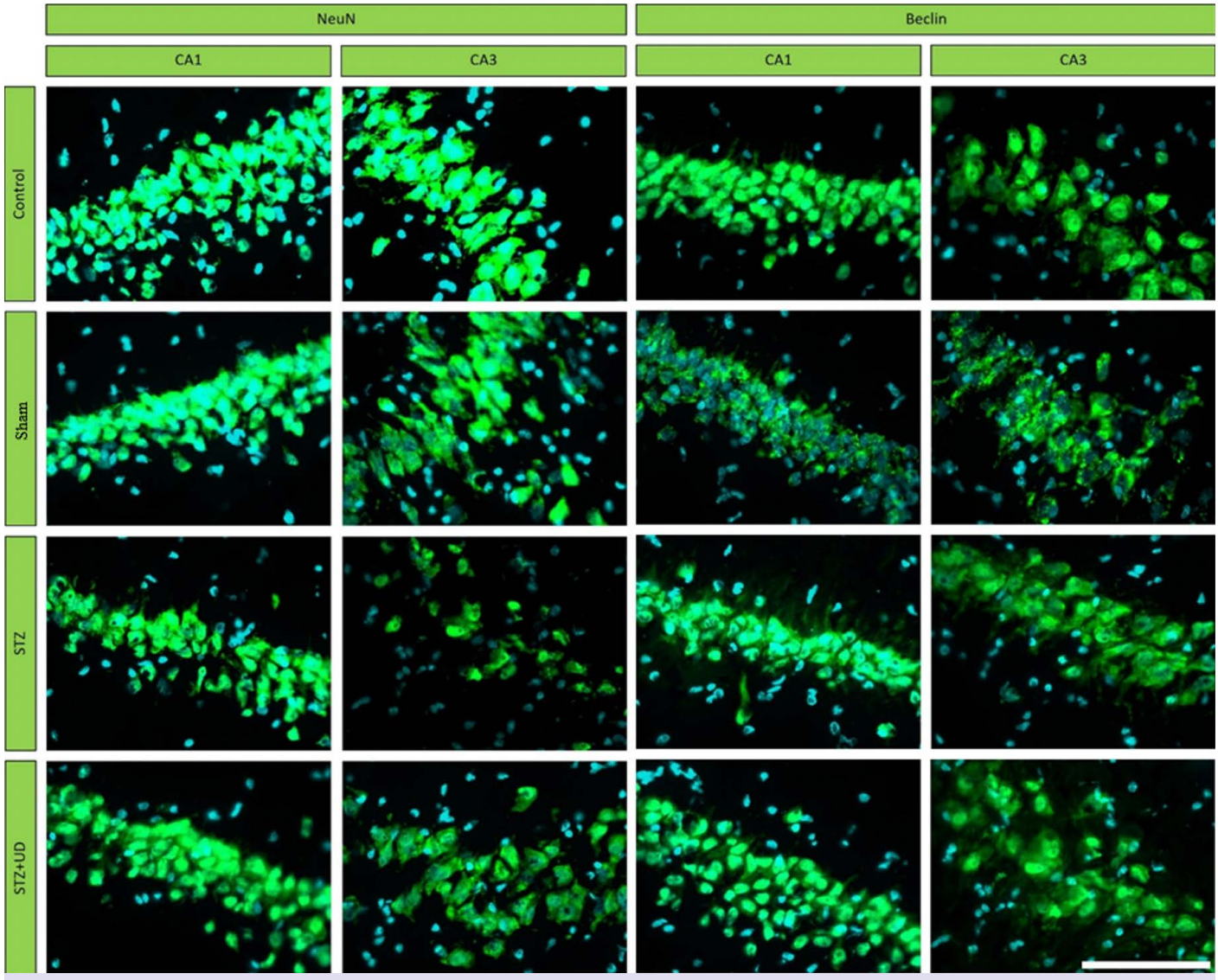

Figure 4. Immunoflourescence images from hippocampal CA1-CA3 subregions stained with NeuN and Beclin antibodies (Scale Bar:100 µm) X63.

plant extracts [31]. UD is a plant which has anti-infammatory and anti-oxidation properties with great promises for chronic inflammatory diseases [32, 33]. In the present study, we investigated the correlation between UD's protective role on the hippocampal neurons in regards to autophagy which remains to be elucidated for STZ-induced neurodegeneration.

In the present study, we observed the ameliorative role of UD on the STZ-induced cognitive impairment as previously noted in the rats with diabetic neuropathy [34, 35]. In addition to memory dysfunction in the diabetic animals, the healing effects of UD on memory function was also recorded in brain lesions, aging, and experimental memory deficiency models [12, 36–38]. In a previous study, when an herbal extract including UD was given to the rats with sporadic AD, an improvement in the spatial learning and memory was considered that this herbal extracts might have anti-dementia properties [2]. In these previous studies, the underlying molecular mechanism of this memory enhancing effect of UD might be related to the its antioxidative effects to increase some proteins that are crucial for memory formation and synaptic plasticity such as muscarinic cholinergic system, BDNF, NGF, and synaptophysin [2, 12, 36, 39].

On the other hand, the molecular parameters that activate cell survival and death in the hippocampal region are important for the alterations in the learning and memory. The two important physiological cell

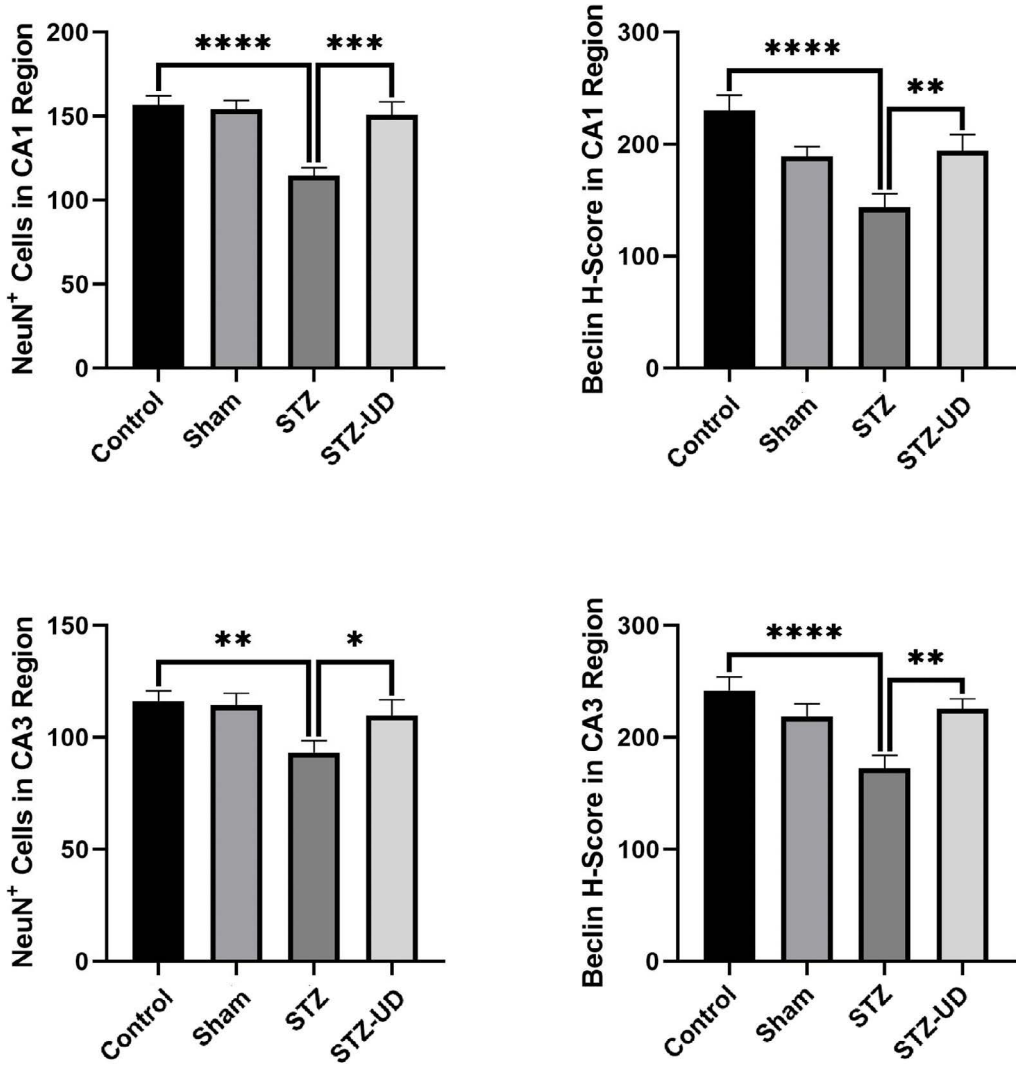

FIGURE 5. The levels of immunofluorescence of NeuN and Beclin from hippocampal CA1-CA3 subregions. The positive cells were counted for NeuN evaluation and H-scores were calculated for Beclin evaluation in the control (n=5), sham control (n=6), STZ (n=6) and STZ-UD (n=5) groups. Error bars indicate SEM (standard error of mean). The degree of significance was denoted as * for $p \leq 0.05$.

death mechanisms, autophagy and apoptosis, and their crosstalk between each other influence homeostasis of the cell, the clearance of cell debris, in addition to the action of therapeutics. Depending on the context, autophagy can either inhibit apoptosis or function in concert with it [40]. In our study, we investigated the alterations in the expression of proteins related to the autophagy to understand the neuroprotective effect of the UD on the STZ-induced neurodegeneration and its memory enhancing activity. As previously mentioned, we observed a significant decrease in the autophagy parallel to memory decline in the STZ-induced neurodegeneration [1, 4, 41, 42]. Impaired autophagy is recognized as a key contributor to cellular damage and neuronal death in neurodegenerative disorders, leading to abnormal protein aggregation, mitochondrial dysfunction, oxidative stress, and apoptosis [43, 44]. Therefore, the activation of autophagy has been suggested as a potential therapeutic approach for AD because of increasing evidence of it in maintaining neuronal function [45]. In our study, we observed a significant effect of UD on the autophagy by increasing the expression of Beclin, a crucial marker in the early stages of autophagy which is essential for autophagosome formation, and LC3β, a marker of the later stages of autophagy which is a membrane-bound protein asso-

ciated with autophagosomes, in the hippocampal tissue [46], while no variations were noted in autophagy levels of some other tissues such as ovarian cancer cells [47]. In addition, as seen in our study, ATG5 expression did not change due to UD supplementation in diabetic mice hippocampi [47]. As a validation of western blotting results, a significant increment in the immunofluorescence of Beclin protein were noted in the both CA1 and CA3 regions of hippocampus.

The downregulation of autophagy indices in the STZ-induced neurodegeneration has been associated with increased apoptosis in hippocampus suggesting a neuronal loss and cognitive impairment [35]. In the current study, while there was a slight decline in the NeuN protein expression of the hippocampal tissue which was determined by western blotting, the decrease in the NeuN positive cells in the both CA1 and CA3 regions was significant in the STZ-induced neurodegeneration model suggesting neuronal death in these parts of the hippocampus. Furthermore, parallel to the increase in the Beclin H-score, an UD-dependent increase in the NeuN positive cells of the both regions suggest the anti-apoptotic role of the UD through upregulating autophagy pathways in the hippocampus [48].

Conclusion

In the current study, we noted that UD may improve cognitive dysfunction by altering autophagy-related indexes in STZ-induced neurodegeneration model rats. This study aligns with previous research, further supporting the critical role of autophagy in the pathology of AD. Additionally, it suggests that autophagy may serve as a potential therapeutic target for AD, providing a foundation for future treatments with phytotherapeutics, like UD, that modulate autophagy as a strategic intervention offering new perspectives for AD treatment.

Ethics Committee Approval: The Bezmialem Vakif University Animal Experiments Ethics Committee granted approval for this study (date: 06.12.2019, number: 2019/266). Conducted in accordance with ARRIVE guidelines to minimize animal suffering.

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

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

Use of AI for Writing Assistance: The authors declared that artificial intelligence-supported technologies (such as Large Language Models [LLMs], chatbots or image generators, ChatGPT) were not used in the production of the study.

Authorship Contributions: Concept – SA, EC, BE; Design – SA, FB, BE; Supervision – SA, BE; Fundings – SA, SP, BE; Materials – BE, FB, EGD; Data collection and/or processing – SA, HIS, MS, AK, EGD, BE; Analysis and/or interpretation – SA, SP, BE; Literature review – SA, MS, AK, BE; Writing – SA, HIS, EC, BE; Critical review – SA, HIS, MS, AK, EC, EGD, FB, SP, BE.

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Original Article PHARMACOLOGY

Global bibliometric insights on prenatal exposures and pregnancy outcomes

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ABSTRACT

OBJECTIVE: Prenatal exposure to medications or environmental agents may contribute to adverse pregnancy outcomes and birth defects. This study provides a comprehensive bibliometric overview of the global research landscape on prenatal exposures and associated outcomes.

METHODS: A systematic literature search was conducted in the Web of Science database on November 11, 2024. Articles and reviews addressing prenatal exposures and pregnancy outcomes, indexed in the Science Citation Index, Science Citation Index-Expanded, or Emerging Sources Citation Index, were screened. Data were analyzed and visualized using VOSviewer, the R-package Bibliometrix, and Microsoft Excel 2021.

RESULTS: A total of 3,361 articles were analyzed. Publications on this topic have steadily increased over the past two decades, peaking in 2022. The United States emerged as the most productive country, followed by China and Canada. Gideon Koren was identified as the most prolific author, while Reproductive Toxicology published the highest number of articles. Among the keywords, "pregnancy" remained the most frequent overall; however, "placenta," "adverse pregnancy outcomes," and "systematic review" peaked in 2022, while "meta-analysis," "outcomes," and "stillbirth" peaked in 2021.

CONCLUSION: This bibliometric study highlights the global evolution of scientific research on prenatal exposures and pregnancy outcomes. The findings offer valuable insights for clinicians, researchers, and policymakers, enabling a better understanding of the dynamic trends and emerging areas in this field.

Keywords: Birth defects; prenatal exposure; pregnancy; research trends; women.

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The discovery of teratogenic agents in the early 20th century marked the beginning of modern teratology. Although early evidence suggested some drugs and environmental exposures cause birth defects, the belief in fetal protection persisted until the 1961 Thalidomide tragedy [1, 2]. This event raised global awareness about drug safety during pregnancy [3]. Nevertheless, medications are often essential for managing maternal conditions, while environmental exposure remains a concern. Ethical constraints limit randomized controlled trials (RCTs) in pregnant women, making observational studies the main source for guidance [4]. Preclinical research also contributes valuable insights.

Examining how the scientific community has addressed these challenges through research output is critical. Bibliometric analysis maps trends and identifies leading topics, authors, and institutions [5–7]. Despite increasing publications on teratogenicity, no bibliometric study has specifically focused on prenatal exposures. This study aims to fill this gap with a global bibliometric and visualization-based analysis of prenatal exposures and maternal-fetal outcomes.

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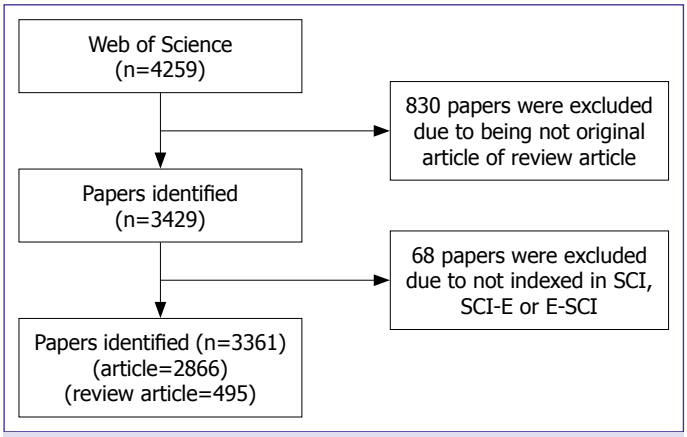

FIGURE 1. Flowchart of literature screening.

MATERIALS AND METHODS

Data Collection

A literature search was conducted in the Web of Science (WoS) database on November 11, 2024. The search formula was: "pregnancy" or "prenatal" or "maternal" (title)and "drug" or "treatment" or "exposure" or "use" (title) and "teratogenicity" or "teratogen" or "congenital malformation" or congenital malformations" or "birth defect" or "birth defects" or "pregnancy outcome" or "pregnancy outcomes" (topic). Inclusion criteria: articles and reviews indexed in Science Citation Index (SCI), Science Citation Index-Expanded (SCI-E), or Emerging Sources Citation Index (ESCI). Exclusion criteria: proceeding papers, early access, retracted publications, Social Sciences Citation Index (SSCI), Conference Proceedings Citation Index-Science (CPCI-S), and Conference Proceedings Citation Index-Social Science & Humanities (CPCI-SSH). The time frame included all WoS studies until November 11, 2024.

Data Analysis and Visualization

Bibliometric analysis was conducted using VOSviewer (v1.6.20), Bibliometrix R-package (v4.4.2) [8], and Microsoft Excel. VOSviewer enabled co-authorship, co-occurrence, and co-citation analyses, while Bibliometrix estimated indicators such as number of publications (NP), total citations (TC), local citations (LC), average citations, h-index, g-index, and total citations per year (TCpY), and visualized outputs by journal, author, and institution. Journal metrics (impact factor, "Journal Citation Indicator [JCI]," and "Journal Citation Report [JCR]" quartiles) were retrieved from WoS. Excel supported data organization. Rankings were based on NP; TC broke ties. TC reflects citations from all documents, whereas LC denotes citations within the dataset [9].

Highlight key points

- The USA leads in productivity, citations, and international collaboration within prenatal exposure research.
- Recent research trends highlight increasing focus on the placenta and maternal environmental exposures.
- Systematic reviews and meta-analyses are increasingly employed to evaluate prenatal exposures.

RESULTS

A comprehensive search in the WoS database on November 11, 2024, retrieved 4,259 records. After applying inclusion and exclusion criteria, 3,361 articles and reviews were included (Fig. 1). These publications included 16,632 authors from 4,128 institutions in 88 countries/regions and were published in 1,033 journals.

Publications Analysis

Among the 3,361 publications, 3,154 (93.8%) appeared in SCI-E journals, and 207 (6.2%) in ESCI journals. Most publications were in English (n=3,312; 98.5%), followed by French (n=16; 0.5%) and Spanish (n=12; 0.4%). Citation analysis is shown in Table 1, presenting the 10 most cited publications [10–19]. TC was 112,636; average citations per article were 33.45; and h-index was 137. Notably, nine articles each received over 500 citations. The top-cited article, "Effect of treatment of gestational diabetes mellitus on pregnancy outcomes," had 1,846 TC and highest TCpY (92.30). This RCT by Crowther et al.[10] assessed the effects of interventions on maternal and neonatal outcomes in gestational diabetes diagnosed at 24–34 weeks [10].

Annual NP indicates developmental patterns and trends [20]. Figure 2 depicts annual publication and citation trends. Publications remained under 100 annually until 2009, then increased steadily from 2010. The highest NP was in 2022 (n=237; 7.1%), followed by 2021 (n=232; 6.9%) and 2023 (n=208; 6.2%). Citation counts followed a similar pattern, peaking at 8,926 in 2021.

Table 1. Ranking of the most cited 10 publications

R	First author	Title	Journal	Type	LC	TC	TCpY	JCR quartile	JCI (2023)
1	Crowther, Caroline A. (2005)	Effect of treatment of gestational diabetes mellitus on pregnancy outcomes [10]	New England Journal of Medicine	Randomized controlled trial	0	1,846	92.30	Q1	25.31
2	Christensen, Jakob (2013)	Prenatal Valproate Exposure and Risk of Autism Spectrum Disorders and Childhood Autism [11]	Jama-Journal of The American Medical Association	Cohort	33	812	67.67	Q1	11.9
3	Skorpen, Carina Gotestam (2016)	The EULAR points to consider for use of antirheumatic drugs before pregnancy and during pregnancy and lactation [12]	Annals of The Rheumatic Diseases	Systematic literature review	31	702	78.00	Q1	5.1
4	Kutteh, William H. (1996)	Antiphospholipid antibody-associated recurrent pregnancy loss: Treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone [13]	American Journal of Obstetrics and Gynecology	Cohort	12	617	21.28	Q1	3.4
5	Park-Wyllie, Laura (2000)	Birth defects after maternal exposure to corticosteroids: Prospective cohort study and meta-analysis of epidemiological studies [14]	Teratology (Birth Defects Research)	Meta-analysis	0	614	24.56	Q4	0.45
6	Mitchell, Allen A. (2011)	Medication use during pregnancy, with particular focus on prescription drugs: 1976–2008 [15]	American Journal of Obstetrics and Gynecology	Article	57	574	41.00	Q1	3.4
7	Popova, Svetlana. (2017)	Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: a systematic review and meta-analysis [16]	Lancet Global Health	Review	13	554	69.25	Q1	6.28
8	Greer, Ian A. (2005)	Low-molecular-weight heparins for thromboprophylaxis and treatment of venous thromboembolism in pregnancy: a systematic review of safety and efficacy [17]	Blood	Review	17	552	27.60	Q1	3.58
9	Arbyn, M. (2008)	Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis [18]	BMJ-British Medical Journal	Meta-analysis	0	511	30.06	Q1	10.16
10	Haider, Batool (2013)	Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis [19]	BMJ-British Medical Journal	Meta-analysis	0	481	40.08	Q1	10.16

R: Rank; JCR: Journal citation report; JCI: Journal citation indicator; LC: Local citation; TC: Total citation; TCpY: Total citations per year.

TABLE 2. The top 10 most productive authors

Rank	Authors	NP	LC	TC	h-index	g-index	Total link strength	Articles fractionalized score
1	Koren, G.	102	398	6,337	45	78	228	24
2	Werler, MM.	46	248	2,378	23	46	210	7
3	Romitti, PA.	45	100	1,355	23	36	314	4.2
4	Langlois, PH.	37	47	909	18	29	281	3.2
5	Mitchell, AA.	36	286	2,587	26	36	100	6.9
6	Schaefer, C.	35	137	975	18	31	152	7.9
7	Li, Y.	32	1	418	11	20	131	4.5
8	Reefhuis, J.	30	82	1,610	18	30	179	3.4
9	Li, J.	30	11	216	9	13	173	3.3
10	Zhang, Y.	29	56	692	14	26	161	3.3

NP: Number of publications; LC: Local citation; TC: Total citation.

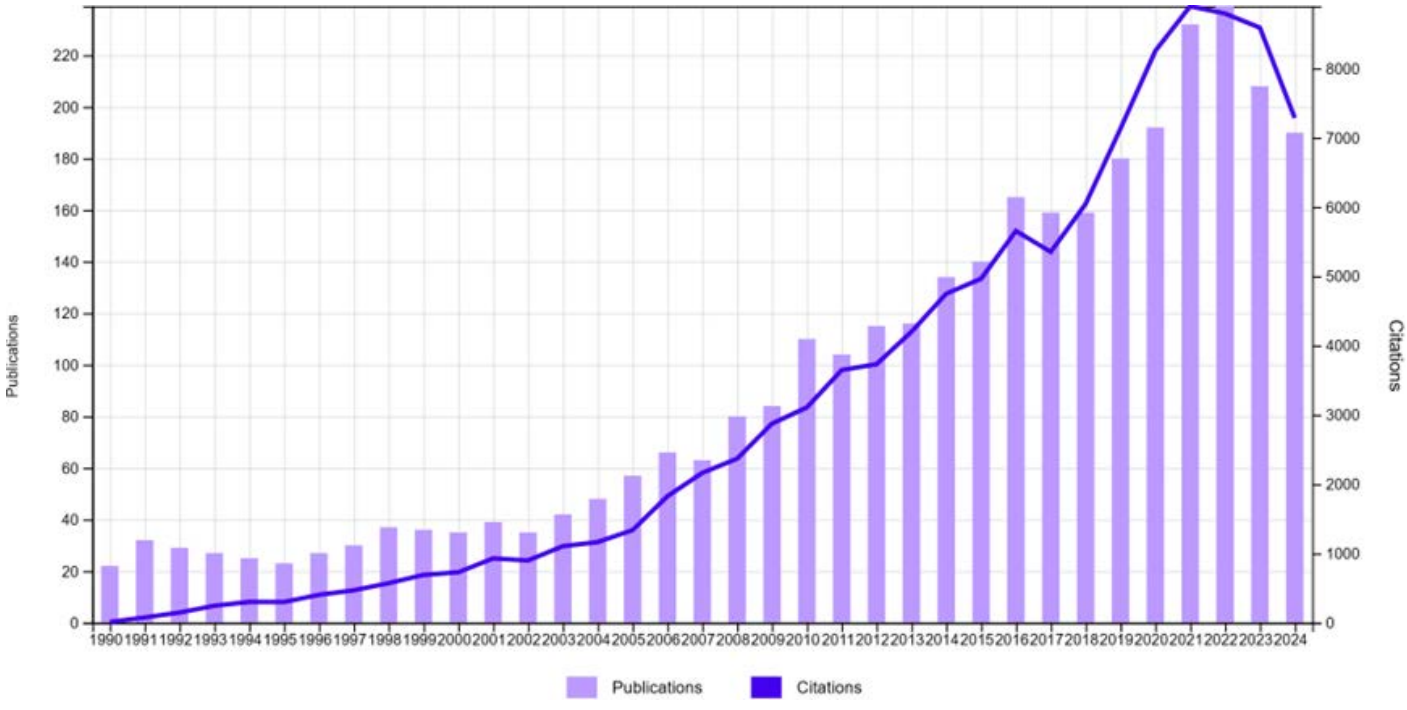

FIGURE 2. Annual citation and publication number trend chart.

Author Analysis

Most Productive Authors

A total of 16,632 authors studying prenatal exposures and pregnancy outcomes were systematically analyzed. Table 2 lists the top ten authors by NP. Leading contributors included Koren, G (n=102), Werler, MM (n=46), and Romitti, PA (n=45). The top three in LC were Koren, G (n=398), Mitchell, AA (n=286), and Werler, MM (n=248).

Koren, G had the highest h-index (45) and g-index (78), indicating strong productivity and citation impact. He was followed by Mitchell, AA (h-index 26; g-index 36) and Werler, MM (h-index 23; g-index 46).

Considering total link strength (collaboration intensity), Romitti, PA led (n=314), followed by Langlois, PH (n=281) and Koren, G (n=228).

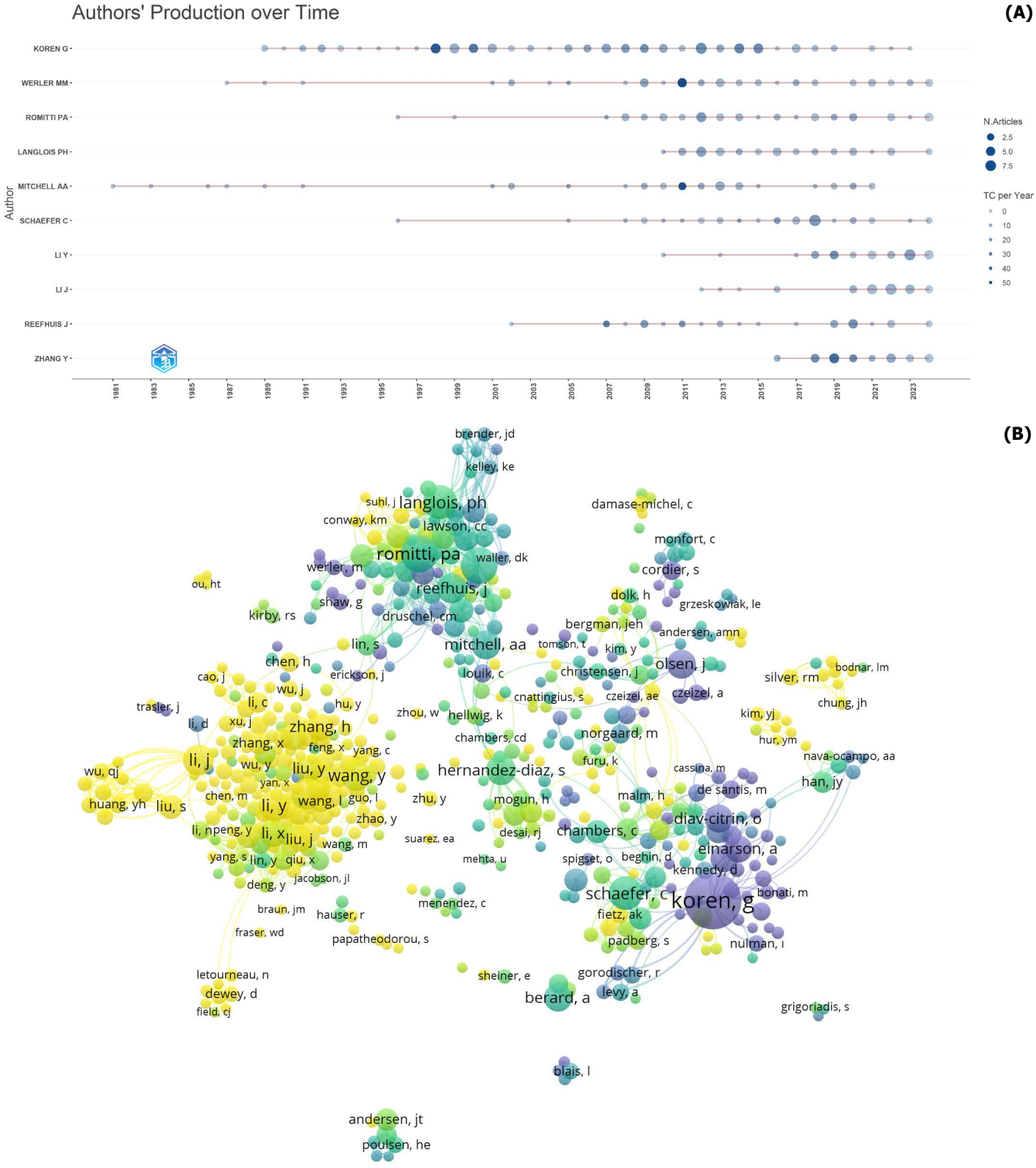

FIGURE 3. **(A)** Authors' production over time **(B)** Visualization co-authorship of authors that contributed to the papers by years **(C)** Collaboration network of author's **(D)** Visualization of co-citation of authors. Cont →

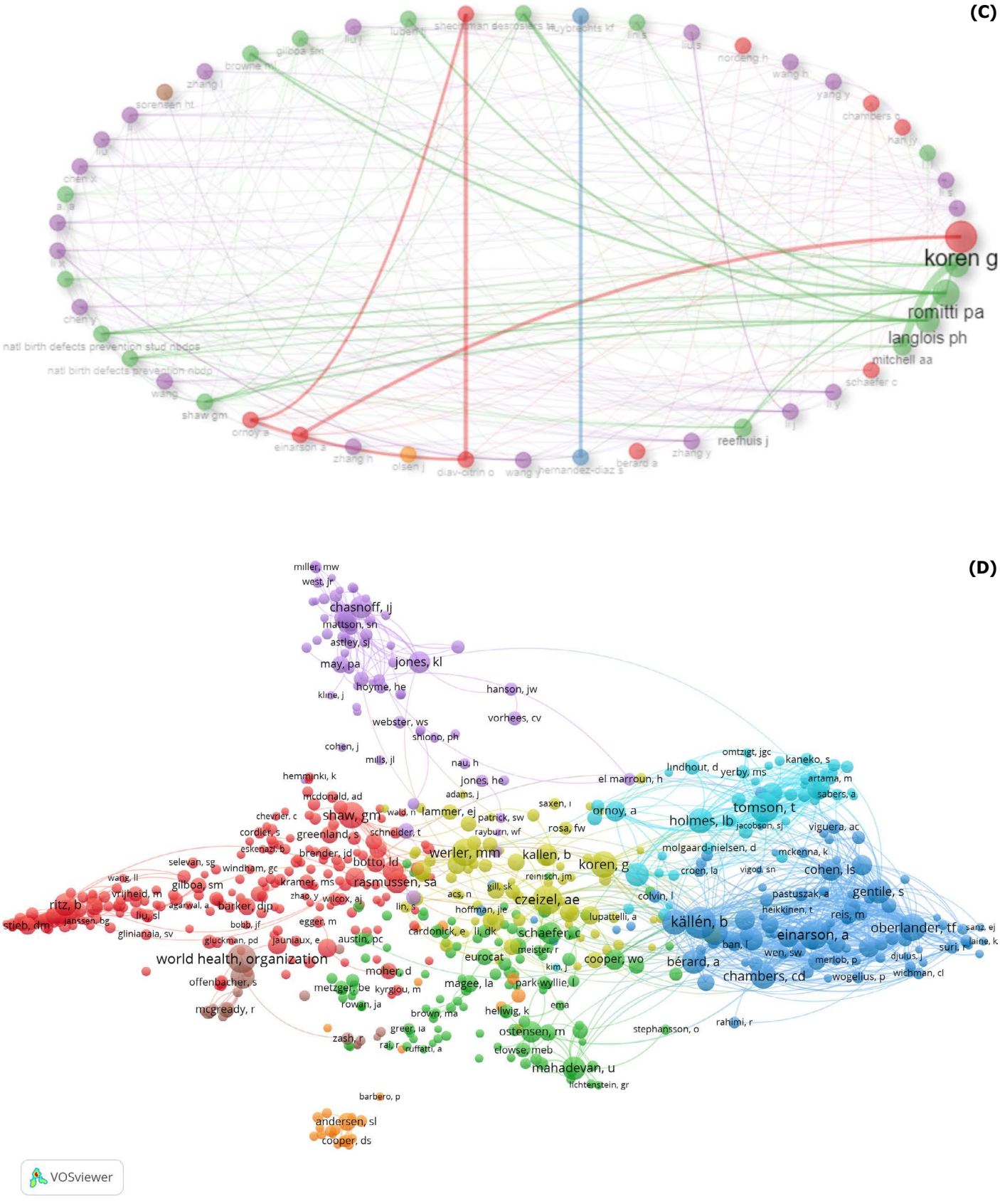
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FIGURE 3.

Table 3. The top 10 most productive journal

Rank	Journals	NP	LC	TC	h-index	g-index	Total link strength	JCR quartile	JCI (2023)
1	Reproductive Toxicology	96	1468	3051	32	52	353	Q2	1.00
2	American Journal of Obstetrics and Gynecology	70	3730	5787	43	70	219	Q1	3.4
3	Birth Defects Research Part a Clinical and Molecular Teratology	62	1493	229	27	46	329	Q3	–
4	Environmental Research	55	1306	1631	23	39	215	Q1	1.96
5	Plos One	49	1309	1644	21	40	96	Q1	0.88
6	Obstetrics And Gynecology	46	2702	2662	31	46	170	Q1	2.31
7	Pharmacoepidemiology And Drug Safety	43	1045	1211	20	34	206	Q3	0.62
8	Bmc Pregnancy and Childbirth	40	444	623	14	24	34	Q1	1.14
9	Birth Defects Research	38	1493	321	12	15	109	Q4	0.45
10	Drug Safety	37	418	1136	19	33	180	Q1	1.02

NP: Number of publications; LC: Local citation; TC: Total citation; JCR: Quartile journal citation report; JCI: Journal citation indicator.

Articles Fractionalized reflect individual author contributions to publications. This method divides a publication's contribution equally among its authors [21]. Based on this, Koren, G (n=24), Schaefer, C (n=7.9), and Werler, MM (n=7) had the highest individual contributions.

Annual productivity trends are shown in Figure 3a. Productive author-years included Schaefer, C (2018, NP=9, TCpY=19.57), Koren, G (2012, NP=8, TCpY=23.61), Li, J (2022, NP=8, TCpY=9.33), and Li, Y (2023, NP=8, TCpY=16.00).

Co-Authorship and Collaboration Network of Authors Analysis

Academic collaboration involves teamwork, with co-authorship reflecting this through joint publications. Similar names (e.g., Werler, M/Werler, MM) were consolidated during the analysis. Documents with a 'Maximum number of authors per document' greater than 25 were ignored. By setting the 'minimum number of documents of an author' to 4 and the 'minimum number of citations of an author' to 4 in VOSviewer, a total of 631 eligible authors were identified. The co-authorship and collaboration network among these authors is visually presented in Figures 3b, c.

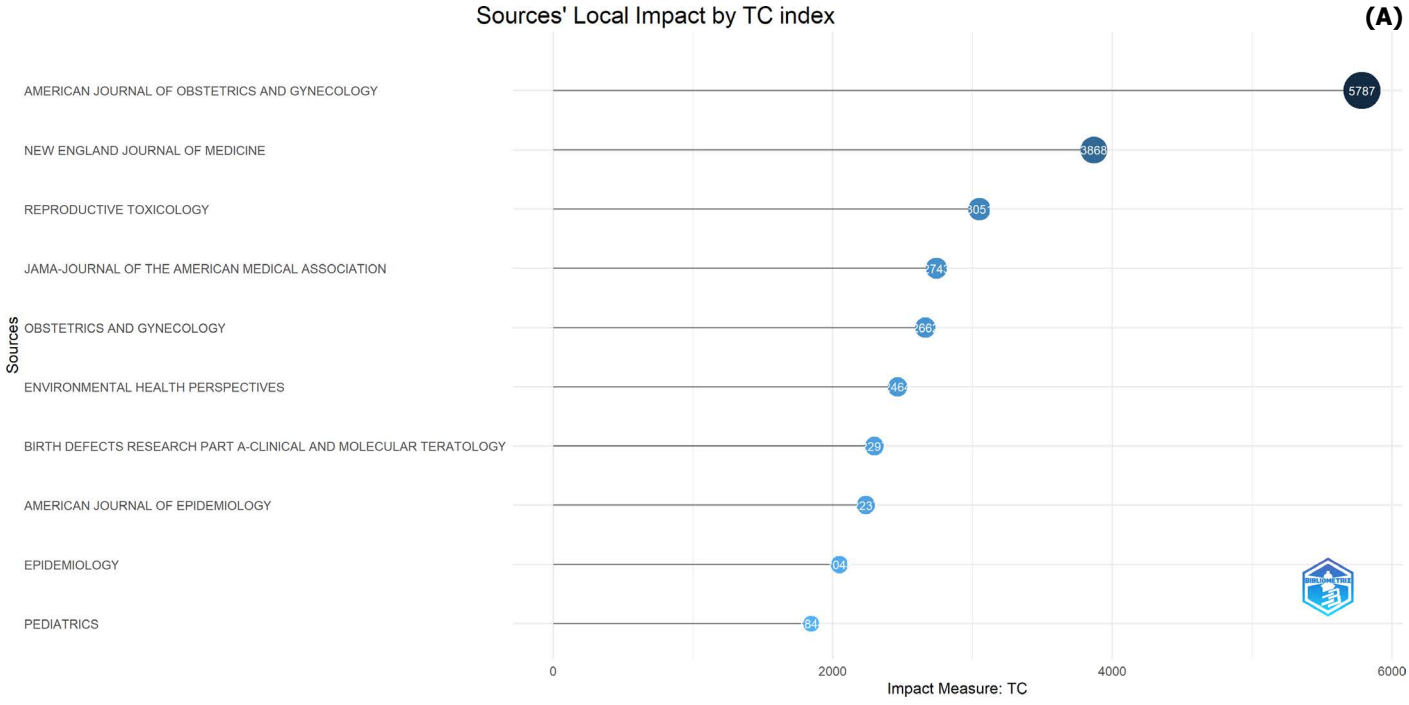
Co-Cited of Authors Analysis

Co-citation analysis is a science mapping technique assuming that publications frequently cited together are thematically related [22]. Author co-citation analysis identifies intellectual connections and research trends by highlighting relationships between frequently co-cited authors, revealing the structure of scientific collaboration and influence. The "minimum number of citations of an author" was set to 20 in VOSviewer, resulting in 683 authors out of 61,072 meeting this criterion. Kallen, B ranked highest on the list (Fig. 3d).

Journal Analysis

Most Productive Journals

A total of 1,033 journals were analyzed. Using VOSviewer with "The minimum number of documents of a source" set to 3 and "The minimum number of citations of a source" to 1, 254 journals qualified. Visualizations were performed in VOSviewer and Bibliometrix. Table 3 lists the top 10 journals, publishing 536 (16%) of 3,361 articles. "Reproductive Toxicology" led with 96 publications. "The American Journal of Obstetrics and Gynecology" ranked second by NP (n=70) but was highest in LC (3,730) and TC (5,787) (Fig. 4a). Other leading journals included "Birth Defects Research Part-A" (n=62), "Environmental Research" (n=55), and "PLOS ONE" (n=49). In impact metrics, "The American Journal of Obstetrics and Gynecology" led with h-index 43 and g-index 70, followed by Reproductive Toxicology (h-index 32; g-index 52). Among the top 10 journals, six were Q1, two Q3, one Q2, and one



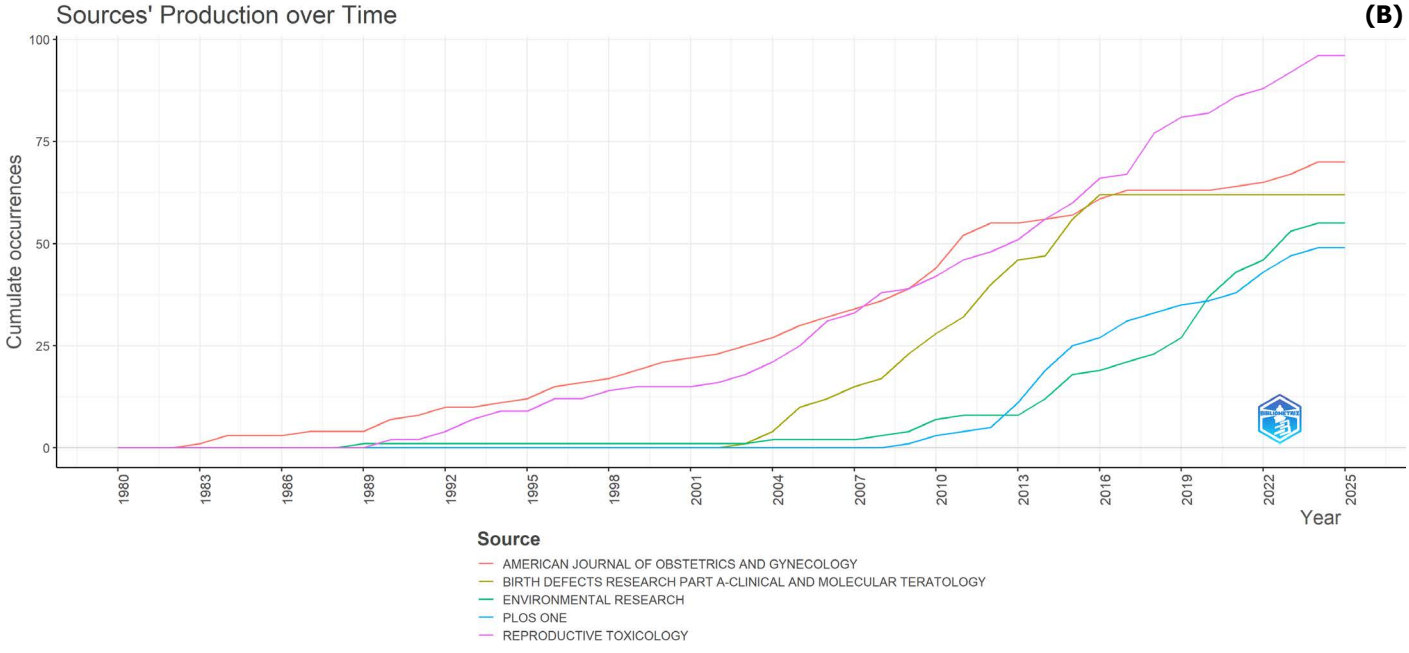


FIGURE 4. **(A)** Number of journal citations **(B)** Journals' production over time **(C)** Overlay visualization of the number of citations by time **(D)** The network visualization of the co-citation analysis at the journal level. Cont →

Q4. Figure 4b shows temporal trends: until 2013, "The American Journal of Obstetrics and Gynecology" led output; later, "Reproductive Toxicology" became dominant. Peak yearly outputs occurred in Reproductive Toxicology (NP=10, 2017), Environmental Research (NP=10, 2020), and Birth Defects Research Part A (NP=9, 2015).

Citation and Co-Citation Analysis of Journals

Citation counts over time were analyzed via overlay visualization, with node size showing citation frequency and node color indicating peak citation periods (Fig. 4c). Journals cited together in references are considered co-cited [23]. Using VOSviewer, with "minimum number of citations of a source" set to 50, 422 journals qual-

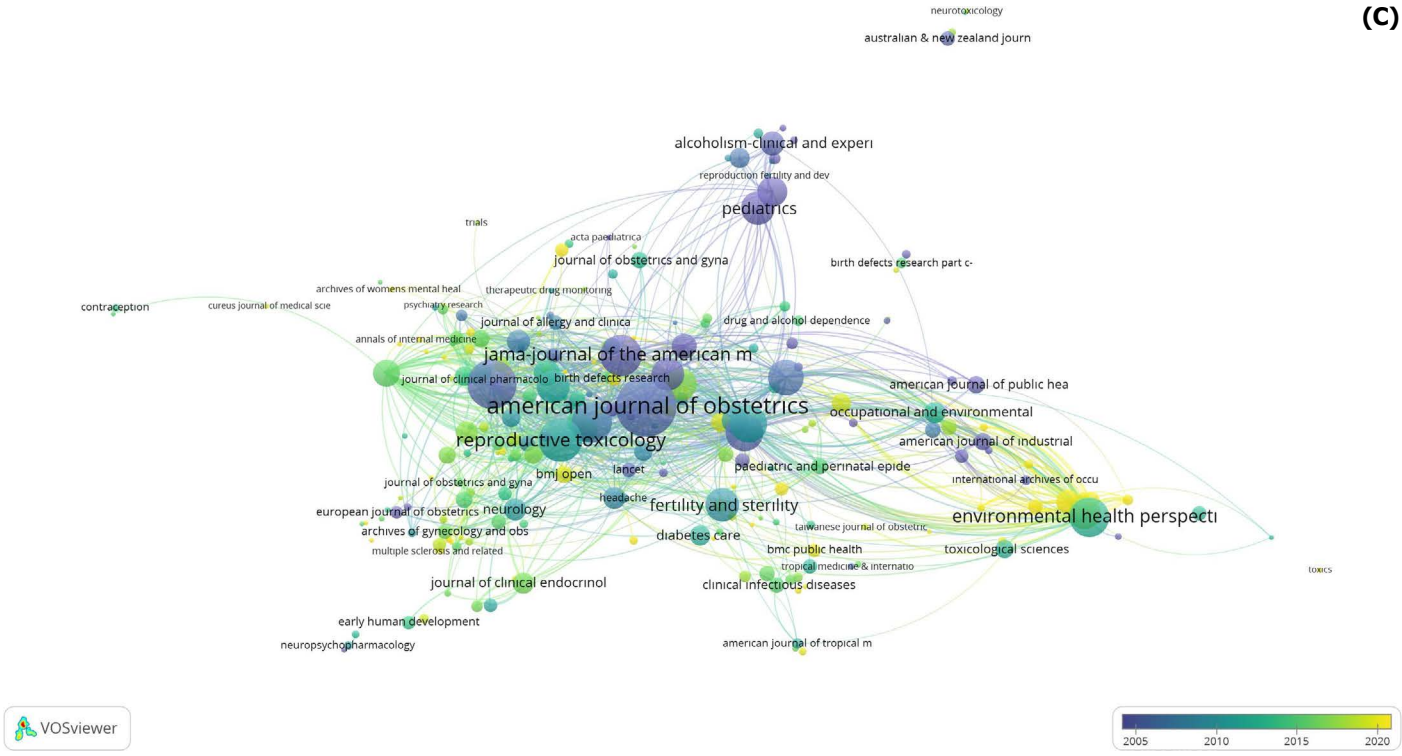

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FIGURE 4.

ified from 16,819. The co-citation network is in Figure 4d. "The American Journal of Obstetrics and Gynecology" had the highest co-citation frequency, followed by "Obstetrics and Gynecology" and "The New England Journal of Medicine."

Keywords Analysis

Most Frequent Keywords Analysis

Authors' keywords from publications on prenatal exposures and pregnancy outcomes were analyzed. The top keywords were "pregnancy" (n=1,135), "pregnancy outcome" (n=182), "birth defects" (n=171), "air pollution" (n=117), and "preterm birth" (n=97) (Fig. 5a).

Trend Topic of Keywords Analysis

Using a timespan from 2014 to 2024, with "minimum word frequency" set to 10 and "number of words per year" to 3, Bibliometrix identified trending keywords over the last decade (Fig. 5b). Keywords peaking in 2022 included "placenta" (n=34), "adverse pregnancy outcomes" (n=34), and "systematic review" (n=21). Keywords peaking in 2021 included "meta-analysis" (n=54), "outcomes" (n=35), and "stillbirth" (n=33).

Co-Occurrence of Keywords Analysis

VOSviewer was used for co-occurrence analysis to explore relationships among core concepts, identify major themes and trends, and analyze frequency and patterns of simultaneous keyword appearances [24]. Setting the "minimum number of occurrences of a keyword" to 5 yielded 401 keywords from 5,308. The co-occurrence network is shown in Figure 5c. Figure 5d presents a three-field plot of authors, keywords, and journals, visualized in Bibliometrix with the "number of items" set to 15.

Countries/Regions Analysis

Most Productive Country Analysis

This study covers 125 countries/regions, with corresponding authors from 88. The top 10 productive countries by corresponding authorship are listed in Table 4. The USA ranked first in NP (1,030) and NC (44,101). China was second in NP (372) but fifth in citations (5,229). The UK had the highest average citations. Publications were categorized as single- or multi-country. Figure 6a shows distribution by collaboration type, with the USA leading multi-country studies (n=163). UK (43.1%) and Spain (43.2%) had the highest multi-country collaboration proportions relative to total output.

Publication outputs by country over time (Fig. 6b) show the USA (5,225), China (1,933), and Canada (1,245) as most active, followed by the UK (716) and Australia (691). China's output surged after 2019; 25% of publications predate 2019, 75% appeared later.

Collaboration Network of Country Analysis

Figures 6c, d depict cross-country collaborations. The USA was central, collaborating most with the UK (n=63), Canada (n=62), China (n=58), and Denmark (n=38). The UK ranked second, frequently collaborating with the Netherlands (n=34), Italy (n=30), and France (n=26).

Institutions and Publishers Analysis

Institutions and publishers were analyzed by total NP (Table 5). The University of Toronto led with 229 documents (1.36%), followed by the Centers for Disease Control and Prevention (158, 0.94%) and Boston University (149, 0.89%). Elsevier was the top publisher (n=815, 24.78%), followed by Wiley (n=583, 17.73%) and Springer Nature (n=385, 11.71%).

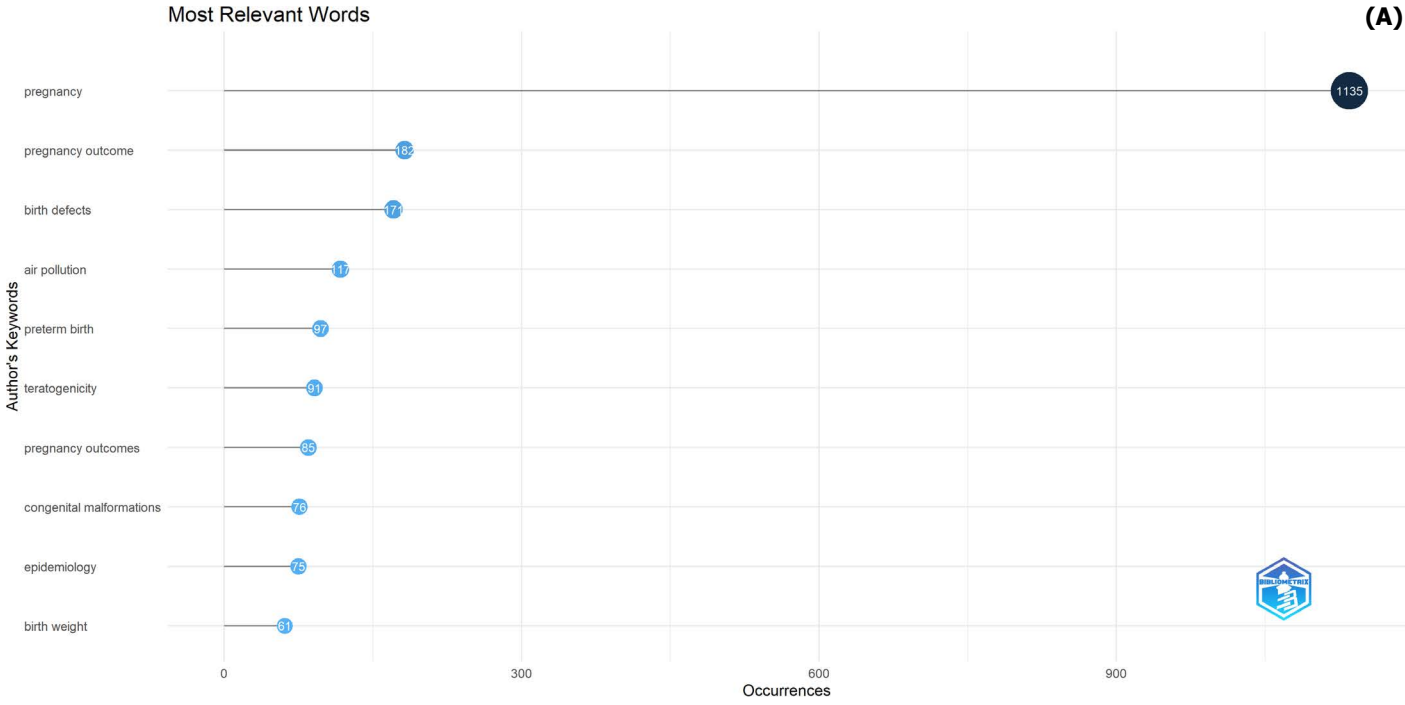
Boston University was the sole publisher until 1989. The University of Toronto nearly doubled its publications in 1998, from 17 to 31. By 2022, all leading institutions had over 100 publications. (Fig. 7a).

Funding Agencies

The most frequent funding sources were the United States Department of Health and Human Services (n=514, 16.27%), the National Institutes of Health (NIH), USA (n=420, 13.29%), and the National Natural Science Foundation of China (n=133, 4.21%) (Fig. 7b).

DISCUSSION

Bibliometric studies offer an overview of the literature in a specific field, reflecting current status and emerging trends, allowing researchers to rapidly track developments [25–27]. For newcomers, bibliometric analyses identify highly cited articles, key authors, leading countries, and collaboration patterns.



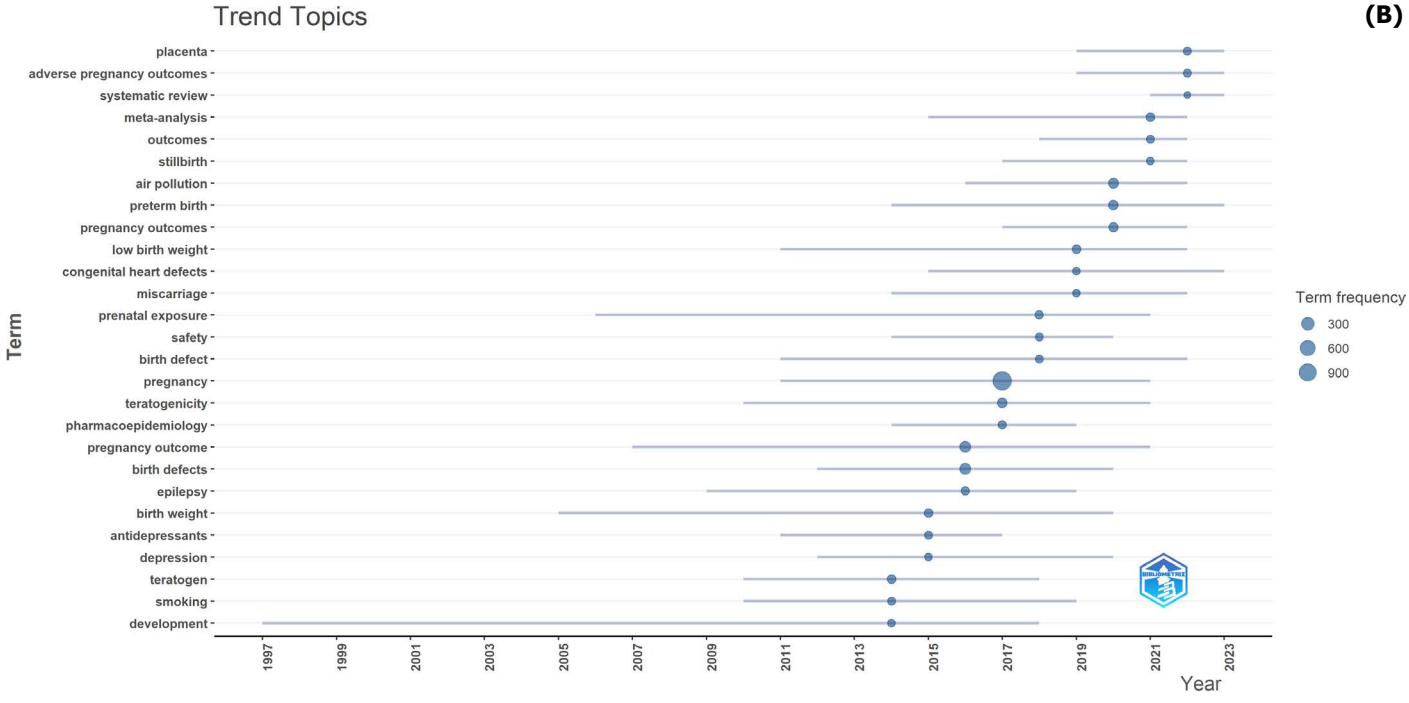


FIGURE 5. **(A)** The chart of the most frequently used keywords **(B)** Trend topic keywords in the last 10 years **(C)** Visualization of co-occurence of keywords **(D)** The relationship between authors-keywords-journals on the three-field plot. Cont →

AU: Authors; DE: Author's keywords; SO: Journals.

This study represents the first comprehensive worldwide bibliometric analysis focused on prenatal exposures and pregnancy outcomes. It analyzed 3,361 documents from the WoS database, mostly indexed in SCI-E. The most cited article was "Effect of Treatment of Gestational Diabetes Mellitus on Pregnancy Outcomes." NP increased notably after 2010. Among authors, Koren, G led in NP and academic metrics (h-

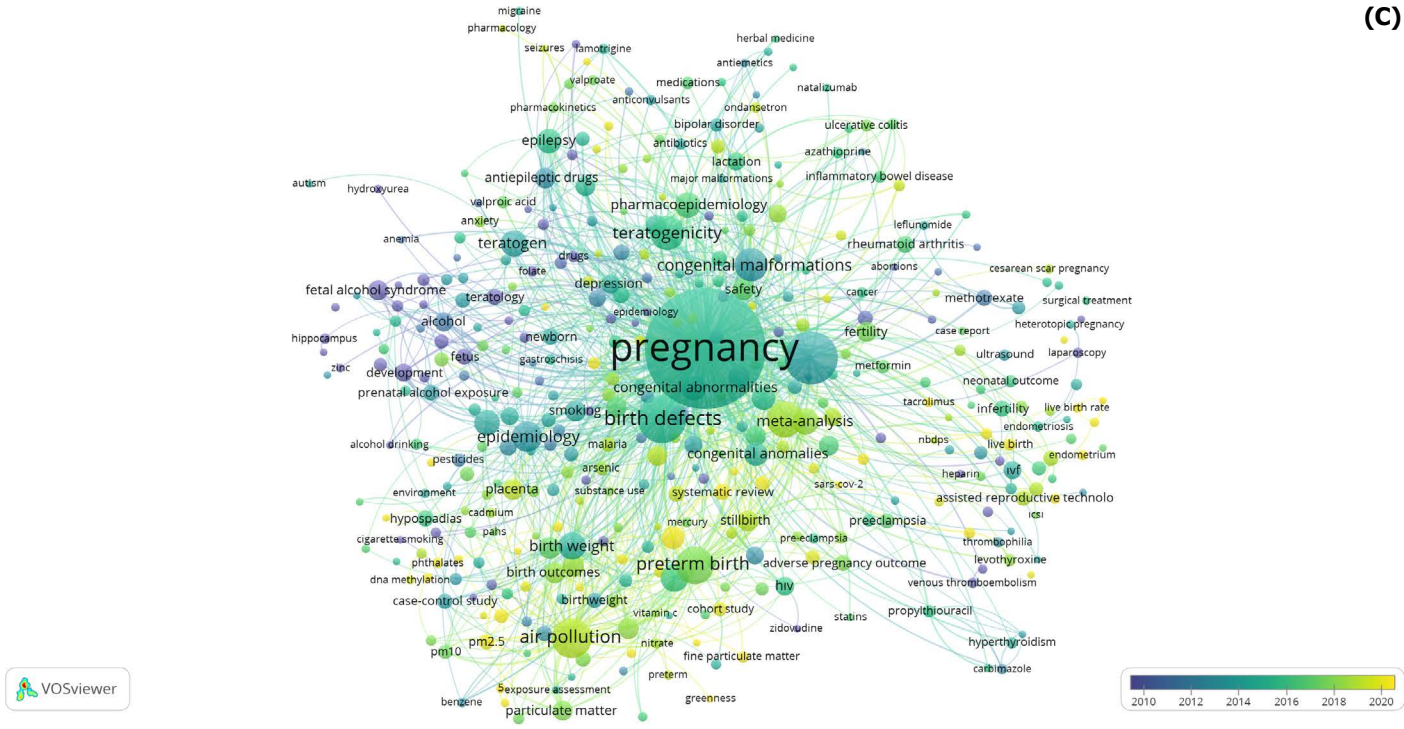

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FIGURE 5.

Table 4. The top 10 most productive corresponding author's countries/regions

Rank	Countries/regions	NP	TC	Average citations	SCP	MCP	MCP %
1	USA	1,030	44,101	42.80	867	163	15.8
2	China	372	5,229	14.10	308	64	17.2
3	Canada	250	11,234	44.90	176	74	29.6
4	United Kingdom	130	5,944	45.70	74	56	43.1
5	Australia	124	5,628	45.40	84	40	32.3
6	Denmark	120	4,504	37.50	85	35	29.2
7	France	95	2,692	28.30	75	20	21.1
8	Italy	95	2,575	27.10	76	19	20.0
9	Netherlands	87	3,056	35.10	54	33	37.9
10	Sweden	79	3,519	44.50	56	23	29.1

NP: Number of publications; TC: Total citation; SCP: Single country publication; MCP: Multiple countries publication.

Table 5. The top 5 most productive institutions and publishers

Rank	NP (%)[a]
Institutions	
1 University of Toronto	229 (1.36)
2 Centers for Disease and Prevent	158 (0.94)
3 Boston University	149 (0.89)
4 Washington University	137 (0.82)
5 The University of North Carolina	121 (0.72)
Publishers	
1 Elsevier	815 (24.78)
2 Wiley	583 (17.73)
3 Springer Nature	385 (11.71)
4 Lippincott Williams & Wilkins	203 (6.17)
5 Taylor & Francis	141 (4.29)

NP: Number of publications; a: Arranged according to the total number of publications of institutions/publishers.

index, g-index, TC, LC), while Schaefer, C ranked first in annual productivity. The highest publication activity by year occurred in Reproductive Toxicology (NP=10, 2017), Environmental Research (NP=10, 2020), Birth Defects Research Part A (NP=9, 2015). The most frequent keywords were "pregnancy," "pregnancy outcome," "birth defects," "air pollution," and "preterm birth." Trend analyses showed that in 2021 "meta-analysis," "outcomes," and "stillbirth" gained prominence; in 2022, "placenta," "adverse pregnancy outcomes," and "systematic review" led. The USA, China, and Canada had the highest NP; the USA led in TC and collaborations, partnering strongly with the UK, Canada, China, and Denmark. The UK followed closely, collaborating with the Netherlands, Italy, and France. The University of Toronto was the most productive institution.

The rise in annual NP post-2009 may be attributed to the creation of teratogenicity information services and regional-national databases, alongside increased sample sizes. Notably, OTIS, ENTIS, and TERIS—established by teratology experts in the 1980s–1990s—are key organizations providing specialized resources on teratogens [28–30]. Their sustained activity since the 2000s likely contributed to the growth of teratogenicity research.

The top-cited Crowther et al. [10] RCT (TC=1,846; TCpY=92.30) assessed dietary and insulin therapies' impact on gestational diabetes-related perinatal outcomes. The study's randomized controlled design and the challenge of conducting RCTs in pregnancy may explain its high citations [10]. Koren, G's leadership in productivity and impact metrics highlights significant scientific contributions. Articles Fractionalized scores further underscore contributions by Koren, Schaefer, Werler, and Mitchell. Romitti, PA showed highest academic collaboration network strength.

The most frequently occurring keywords were "pregnancy," "pregnancy outcome," "birth defects," "air pollution," and "preterm birth." The three-field plot showing

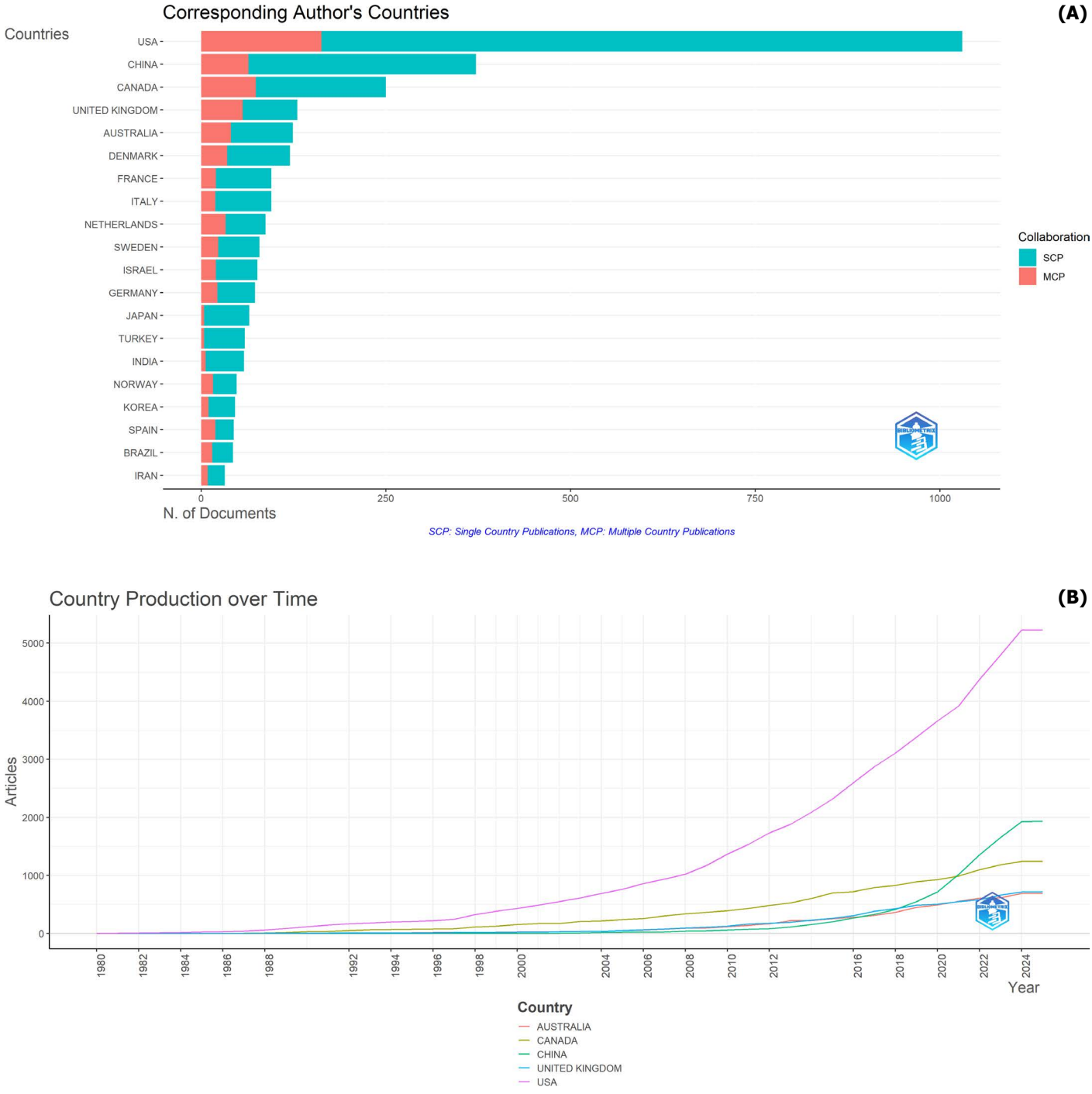

FIGURE 6. **(A)** The distribution of publication numbers in the countries **(B)** Countries' Production over time **(C)** Countries' collaboration network **(D)** Countries' collaboration world map

Cont →

MCP: Multiple countries publication; SCP: Single country publication.

relationships among authors, keywords, and journals confirmed "pregnancy" as the most recurrent keyword, with "air pollution" ranking third, consistent with its overall prominence. Journal-level analysis highlighted that publications focusing on environmental exposures, such as Environmental Research, which peaked in 2020, and Reproductive Toxicology and Birth Defects Research Part A in other years, showed high annual output. These findings reflect increasing research interest and growing clinical awareness of maternal environmental

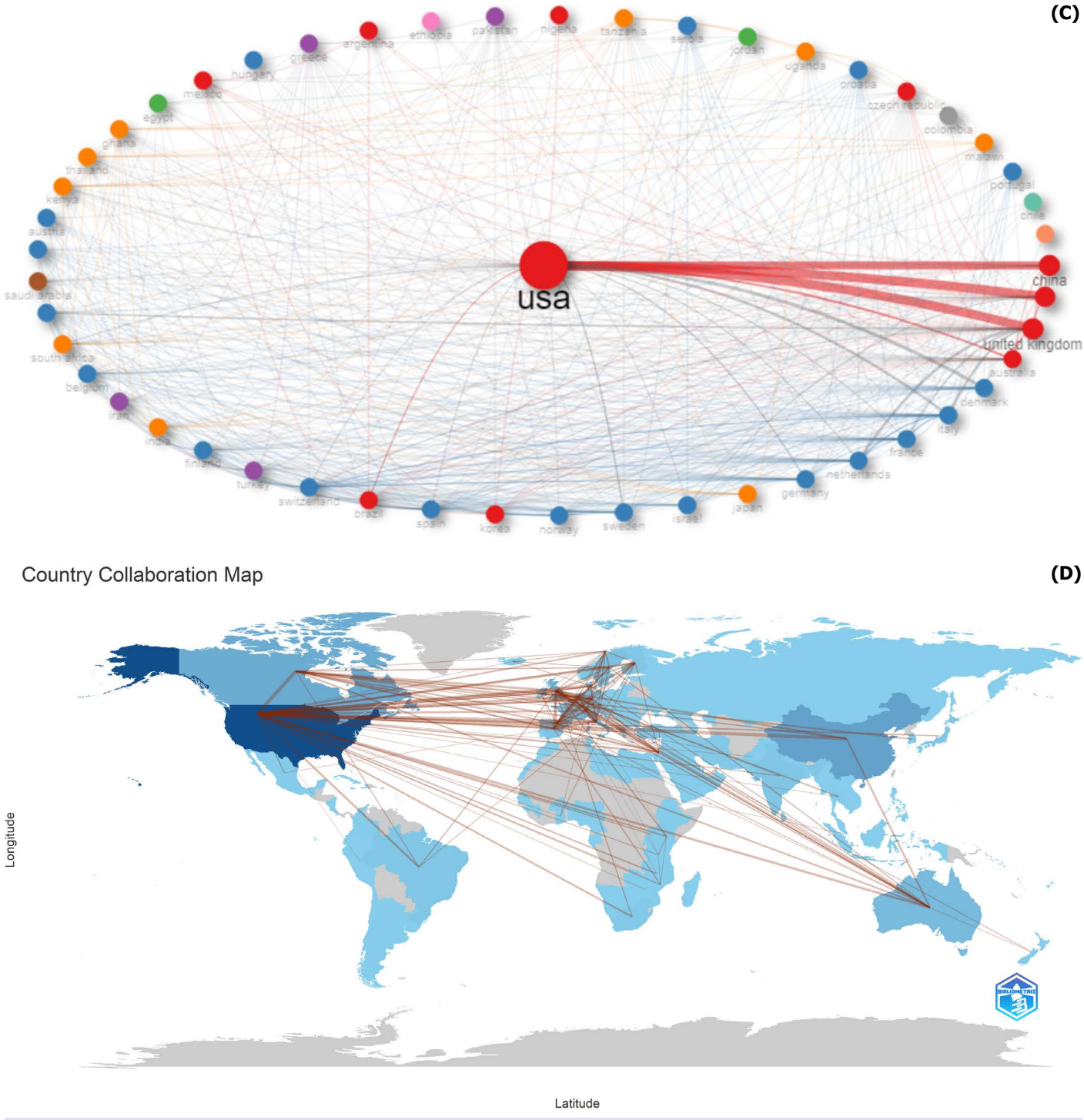

Figure 6.

exposures' impact on pregnancy outcomes, underscoring the importance of systematically evaluating these effects for maternal and fetal health.

The rising prominence of the keyword "placenta" in 2022 indicates an increasing research focus on placental transfer and health within prenatal exposure and pregnancy outcomes. Studies on the transfer of drugs and substances through the placenta have significant clinical relevance, particularly for individualized drug safety assessments during pregnancy. Additionally, the growing use of "systematic review" and "meta-analysis" keywords in 2021 and 2022 reflects a methodological shift to-

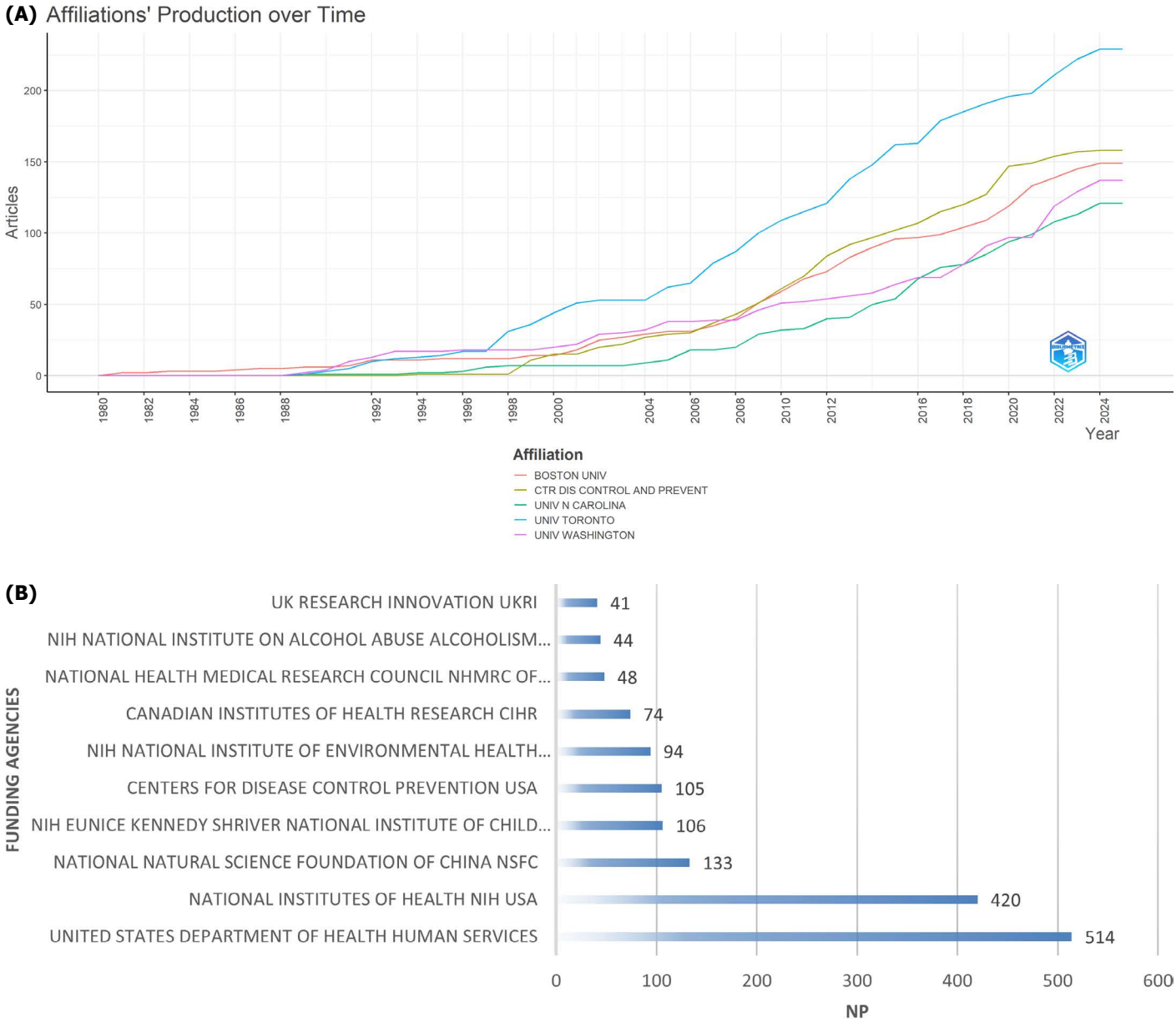

FIGURE 7. **(A)** Affiliations' production over time **(B)** The 10 most supportive funding agencies.

ward quantitative synthesis and comprehensive outcome evaluations. While this trend improves clinicians' access to current evidence, the methodological heterogeneity among these reviews necessitates cautious interpretation when applying findings in practice.

This study identifies the USA as the leading country in NP, citations, and collaborations on prenatal exposure and pregnancy outcomes research. China and Canada follow in NP rankings. The USA shows the highest collaboration rates, mainly partnering with the UK, Canada, China, and Denmark, while the UK ranks second and collaborates extensively with the Netherlands, Italy, and France. These findings reveal that international collaborations are largely concentrated among high-income countries, potentially limiting the generalizability and clinical relevance of results globally. To address this, expanding partnerships to include low- and middle-income countries is crucial for equitable dissemination and implementation of prenatal care advances worldwide.

Canada's productivity aligns with University of Toronto's leadership. Koren, G's tenure there and founding of the "Motherisk" program, which provides evidence-based teratogenic risk information during pregnancy and lactation, significantly boosted Canadian research output [31].

Conclusion

In conclusion, maternal exposure to drugs or substances can cause fetal abnormalities, posing significant challenges in prenatal care. Factors such as maternal age, chronic diseases, and environmental exposures have intensified research interest in this field. This bibliometric study reveals a growing body of literature focused mainly in developed countries, underscoring the need to include developing nations for a more comprehensive understanding. The increase in systematic reviews and meta-analyses reflects efforts toward standardizing research protocols and improving data sharing. Enhancing open access and fostering interdisciplinary collaboration are essential to better disseminate knowledge and translate findings into clinical practice. Ultimately, this study provides an overview of current research trends and offers guidance for future investigations aimed at improving maternal and fetal health outcomes.

Strengths and Limitations

This bibliometric study on prenatal exposures and outcomes guides future research. The search was limited to WoS to ensure data quality, possibly excluding studies from Scopus or PubMed, which may affect generalizability [32, 33]. Only SCI, SCI-E, and ESCI articles were included, ensuring rigor but possibly omitting some studies. Manual screening was avoided to reduce bias, though this may impact precision [34]. Non-English studies were included to reflect broader impacts. Productivity was measured by NP and Articles Fractionalized value, which may not perfectly capture individual contributions.

Ethics Committee Approval: This study is a bibliometric analysis based on data obtained from the Web of Science database. The study did not involve human participants, animals, or any personal/clinical data. Therefore, ethical approval and informed consent were not required.

Informed Consent: Not applicable. This bibliometric analysis was based on data retrieved from publicly available databases and did not involve human participants or personal data.

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

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Authorship Contributions: Concept – PK, EKA; Design – PK, EKA, AO; Supervision – PK, EKA; Data collection and/or processing – PK, EKA, AO; Analysis and/or interpretation – PK, EKA, AO; Literature review – PK, EKA; Writing – PK, EKA; Critical Review – PK, EKA.

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Original Article Ophthalmology

Identifying prognostic clues in CMV anterior uveitis: The role of corneal endothelitis and seasonal relapse patterns

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ABSTRACT

OBJECTIVE: To characterise the clinical spectrum, prognostic indicators and seasonal relapse patterns of cytomegalovirus anterior uveitis (CMV-AU), with particular emphasis on glaucoma risk and treatment outcomes.

METHODS: This retrospective study analysed 53 eyes of 52 immunocompetent patients diagnosed with CMV-AU between 2019 and 2023 at a tertiary referral centre. Diagnosis relied on characteristic clinical findings and, in selected cases, aqueous-humour polymerase chain reaction (PCR) testing. Demographic data, best-corrected visual acuity (BCVA), intraocular pressure (IOP), keratic precipitate (KP) patterns, anterior segment changes, treatment regimens and relapse rates were reviewed.

RESULTS: The cohort (67.3% male) had a mean diagnosis age of 34.3±11.1 years. Most eyes were Posner-Schlossmann-like (63.5%), followed by chronic non-specific AU (34.6 %) and Fuchs-like AU (1.9 %). KPs were chiefly solitary (58.5%), centrally located (50.9%) and sparse (< 5) in 66.0 %. Corneal endothelitis occurred in 39.6% of eyes and was an independent predictor of secondary glaucoma (OR=6.20, 95 % CI 1.04–36.86). Glaucoma developed in 56.6% of eyes; 13.2% required glaucoma surgery. Glaucoma was more frequent and more likely to require surgery in patients treated with oral valganciclovir compared to those treated with topical ganciclovir alone (p=0.049 and p=0.034, respectively). Having ≥ 1 winter relapse (OR=4.99, 95 % CI 1.14–21.86) and, in women, ≥1 spring relapse (OR=5.38, 95 % CI 1.11–25.96) independently predicted > 3 relapses. Overall relapse frequency peaked in winter and summer (p<0.05).

CONCLUSION: Corneal endothelitis appears to be an independent risk factor for secondary glaucoma in CMV-AU, warranting vigilant IOP monitoring and timely antiviral therapy. The observed seasonal pattern, with more frequent relapses in winter overall and an increased rate in spring among female patients, may assist clinicians in identifying high-risk groups and planning closer follow-up.

Keywords: Anterior uveitis; cytomegalovirus; endothelitis; ganciclovir; glaucoma; valganciclovir.

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Cytomegalovirus (CMV), a member of the Herpesviridae family, is highly prevalent in healthy adults and often establishes latency in myeloid progenitor cells. In immunocompetent individuals, CMV anterior uveitis (CMV-AU) presents unique clinical and therapeutic challenges [1]. CMV-AU manifests as recurrent acute hypertensive anterior uveitis resembling Posner-Schlossman syndrome (PSS), chronic anterior uveitis similar to Fuchs' uveitis syndrome (FUS), or anterior uveitis with iris atrophy [2]. These clinical manifestations may be accompanied by endothelitis (Fig. 1, 2) [3]. Glaucoma is a significant complication, with acute episodes causing ele-

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vated intraocular pressure (IOP), and prolonged disease increasing the risk of secondary glaucoma or vision loss.

Antiviral treatments such as oral valganciclovir or topical ganciclovir help manage inflammation and IOP, although optimal dosing and long-term treatment remain challenging [4, 5]. Diagnostic tools like polymerase chain reaction (PCR) and Goldmann-Witmer Coefficient (GWC) analysis confirm CMV in aqueous humor but have limitations, making clinical findings critical for diagnosis and guiding management, particularly when routine PCR testing is impractical [6, 7].

Literature on CMV-AU in Turkish populations is scarce, with only one case series previously reported [8]. To address this gap, we aimed to present the largest CMV-AU cohort in Türkiye, identify key prognostic indicators such as endothelitis and keratic precipitate (KP) patterns, evaluate clinical outcomes, and explore seasonal and gender-related variations in disease activity.

MATERIALS AND METHODS

This retrospective study included patients diagnosed with CMV-AU referred to the Uvea section of the Department of Ophthalmology, Kartal DR. Lütfi Kırdar City Hospital, between June 2019 and December 2023. The study was approved by the Clinical Research Ethics Committee of Kartal Koşuyolu Training and Research Hospital (Approval number: 2024/04/789, dated 20 February 2024), the study adhered to the Declaration of Helsinki. Only patients with at least 6 months of follow-up were included to ensure reliable outcomes.

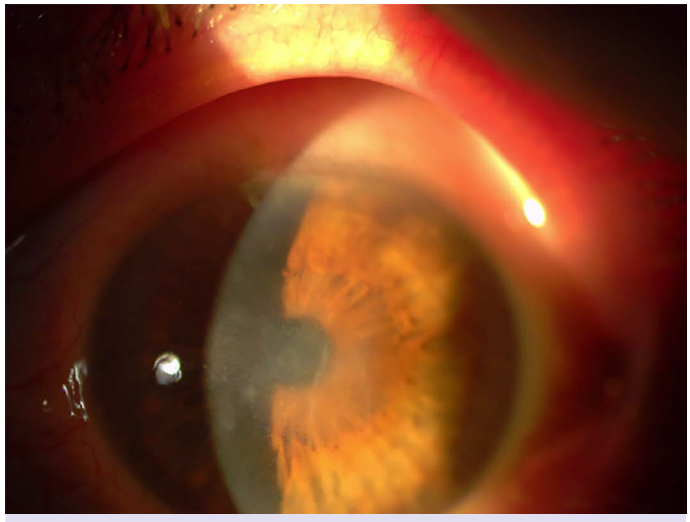

FIGURE 1. Anterior segment photograph demonstrating centrally to paracentrally localized corneal edema corresponding to the area of microgranulomatous keratic precipitates (KPs). The pattern and location of stromal haze support the diagnosis of CMV-associated corneal endothelitis.

Highlight key points

- Corneal endothelitis independently predicted secondary glaucoma in CMV-AU (OR=6.20), supporting vigilant IOP monitoring and prompt anti-CMV therapy.
- Relapses showed seasonality: any winter relapse, and in women, any spring relapse independently predicted >3 total attacks.
- In this largest Turkish CMV-AU cohort, topical ganciclovir ± oral valganciclovir reduced IOP and improved vision in real-world practice.
- Secondary glaucoma was frequent (56.6%) whereas surgery was uncommon (13.2%), reflecting effective surveillance and treatment.

Diagnostic Criteria for Presumed CMV Anterior Uveitis:

Patients were included if they met the clinical criteria for presumed CMV anterior uveitis, defined as presence of:

1. Recurrent unilateral hypertensive anterior uveitis in immunocompetent individuals
2. Acute intraocular pressure (IOP) elevation (>22 mmHg) during inflammatory episodes
3. Mild anterior chamber reaction
4. Presence of microgranulomatous or medium-to-large granulomatous KPs, including coin-shaped, inferior line-like, or scattered stellate forms

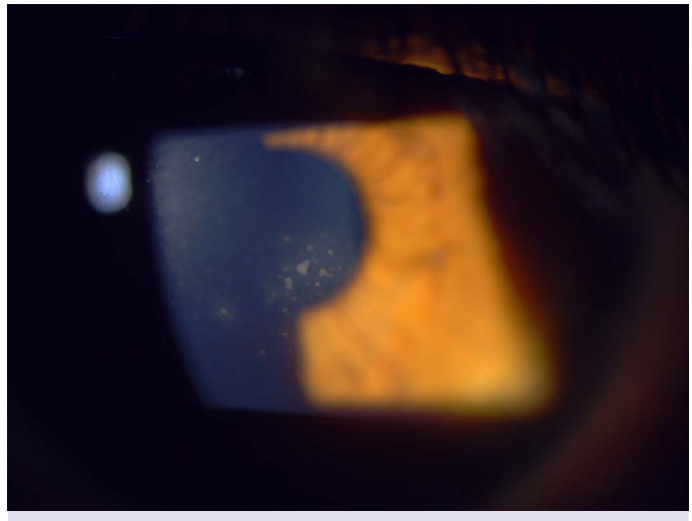

FIGURE 2. Coin-shaped KPs and endothelitis in a patient with cytomegalovirus anterior uveitis (CMV-AU).

5. Intact corneal sensation
6. Additional supportive findings included corneal endothelitis, diffuse iris stromal atrophy, and a round pupil without posterior synechiae [9].

Patients were excluded if they presented with clinical features suggestive of herpetic anterior uveitis, defined as the presence of any of the following:

1. Herpetic corneal epithelial involvement (e.g., dendrites, disciform keratitis, pseudodendrites)
2. A documented history of herpes zoster ophthalmicus
3. In the absence of corneal epithelial involvement, at least three of the following:
   - Recurrent unilateral attacks
   - Acute IOP elevation (>22 mmHg) during inflammatory episodes
   - Granulomatous KPs not confined to the Arlt triangle
   - Patchy or sectoral iris atrophy±transillumination defects
   - Distorted pupil or iris spiraling [10].

FUS was excluded based on the absence of diffuse stellate KPs, Koeppe's nodules, diffuse iris atrophy, heterochromia, vitreous involvement, posterior synechiae, or macular edema unless post-surgical [11].

Aqueous humor PCR testing was routinely recommended to all patients; however, following a selective testing strategy, it was performed only in those who consented, with particular emphasis on cases considered clinically ambiguous by the evaluating uveitis specialist (M.O., N.Z.K., or B.T.). Clinical diagnosis was established prior to anterior chamber tap or treatment initiation. Samples (~100 µL) were aseptically obtained using a 30-gauge needle and analyzed for CMV, HSV-1, HSV-2, and VZV DNA via real-time PCR [2]. Cases in which PCR was not performed but that showed characteristic clinical findings and a positive response to anti-CMV therapy (often after partial response to corticosteroids alone) were retained to reflect real-world clinical decision-making [7, 12, 13]. All participants were immunocompetent and underwent comprehensive uveitis workups, including complete blood counts and serological testing for fluorescent treponemal antibody absorption (FTA-ABS), hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). Tuberculosis screening was performed using either a tuberculin skin test or QuantiFERON-TB Gold assay to rule out infectious mimickers and confirm immunocompetent status.

Patient records were reviewed for demographics (age, gender), laterality, disease duration, clinical findings, prior treatments, and follow-up data. Participants were grouped by age: Group 1 (<30 years), Group 2 (30–44 years), and Group 3 (>45 years). Clinical data included BCVA, IOP (measured with Goldmann applanation tonometry), anterior chamber reaction graded according to the Standardisation of Uveitis Nomenclature (SUN) criteria [14], and slit-lamp findings (including KPs count, morphology, distribution, and corneal changes). Glaucoma was diagnosed through optic disc evaluation supported by visual field and retinal nerve fiber layer defects. Data on attack frequency, seasonal relapses, and complications (e.g., glaucoma, cataract) were analyzed. Treatment outcomes included BCVA improvement, IOP control, and recurrence rates.

Treatment strategies were tailored to inflammation severity, recurrence, and IOP levels. All patients received topical ganciclovir 0.15% gel (Virgan®, Laboratoires Théa, Clermont-Ferrand, France) five times daily, which was tapered over three months to three to four times daily [15]. In cases of more severe, resistant, or recurrent disease, oral valganciclovir (Valcyte®, Cheplapharm Arzneimittel GmbH, Greifswald, Germany) was incorporated into the treatment protocol. Full blood counts, liver, and renal function tests were monitored every four weeks. Topical steroids, including dexamethasone 0.1% (Maxidex®, Alcon Laboratories, Fort Worth, TX, USA) or prednisolone acetate 1% (Pred Forte®, Allergan, Irvine, CA, USA), were used for anterior uveitis and to resolve trabeculitis. Anti-glaucoma medications were prescribed as needed for elevated IOP or glaucomatous optic nerve damage. Glaucoma surgery was performed in eyes with persistently elevated IOP >28 mmHg with or without evidence of optic disc and visual field changes, or IOP >21 mmHg in the presence of progressive glaucomatous optic nerve or visual field damage, despite maximal topical therapy [16].

Statistical Analysis

Data analysis was conducted using SPSS® version 27.0 software (IBM Corp., Armonk, NY, USA). The normality of data distribution was evaluated with the Shapiro-Wilk test. Continuous variables were summarized as mean±standard deviation (SD) or median (interquartile range), depending on data distribution, while categorical variables were presented as frequencies (%). Paired and independent sample t-tests were used for normally distributed data, whereas the Wilcoxon signed-rank test and Mann-Whitney U test were applied for non-nor-

mally distributed data. For comparisons involving more than two groups, the Kruskal-Wallis test was used, with Bonferroni correction for post-hoc analyses when necessary. Categorical data were analyzed using the Chi-square test or Fisher's Exact Test for small sample sizes. Variables with a univariable association at $p<0.10$ were included in two separate multivariable binary logistic regression models using the Enter method: one to identify independent predictors of secondary glaucoma, and another to assess factors associated with having more than three uveitis attacks. Model calibration for both analyses was evaluated using the Hosmer–Lemeshow goodness-of-fit test. Statistical significance was set at $p<0.05$.

RESULTS

A total of 53 eyes from 52 patients diagnosed with CMV-AU based on clinical criteria were included, with 8 patients excluded due to incomplete data or unmet inclusion criteria. The cohort consisted of 35 males (67.3%) and 17 females (32.7%), with a mean age of 39.3±11.8 years (range: 16–68 years) and a mean age at diagnosis of 34.3±11.1 years. The mean follow-up duration was 35.1±31.2 months (range: 6–204 months), while the average disease duration before referral was 48.8±41.5 months.

Clinical Findings and Presentation

Most cases were PSS-like AU (33/52, 63.5%), followed by chronic non-specific AU (18/52, 34.6%) and FUS-like AU (1/52, 1.9%). Right-eye involvement was observed in 30 eyes (56.6%) and left-eye involvement in 23 eyes (43.4%), with one patient showing bilateral disease confirmed by CMV-PCR. Of 10 tested patients, 8 (80.0%) were PCR-positive for CMV, including the single FUS-like case. The mean CMV DNA load in PCR-positive samples was 2,650 copies/mL. Eighteen patients (34.6%) presented during their first documented uveitis attack at our center, while the remaining 34 (65.4%) had been followed or treated elsewhere prior to referral. None of the patients had a prior history of topical prostaglandin analogue or cyclosporine A use, intraocular surgery, or intravitreal injection before the diagnosis of CMV-AU.

All cases exhibited microgranulomatous or granulomatous KPs, either fine or medium-sized. Pigmented KPs were found in cases with longer symptom duration (mean: 9.56±2.60 days), while gray/white KPs were seen in earlier presentations (<3 days). Morphologically, 31 eyes (58.5%) had solitary/separate KPs, 17 (32.1%) had coin-like KPs (Fig. 2), and 5 (9.4%) showed linear patterns. KP distribution was central/paracentral in 27 eyes (50.9%) (Fig. 3), in the Arlt triangle/inferior cornea in 15 eyes (28.3%) (Fig. 4), and non-centralized and non-inferiorly distributed KPs, often located peripherally, perilimbally or scattered in 10 eyes (18.9%). FUS-like diffuse distribution was noted in 1 eye (1.9%). Sparse KPs (<5) were observed in 35 eyes (66.0%), while 18 eyes (34.0%) had numerous KPs (>5), significantly associated with coin-like and linear patterns ($p<0.001$). Over time, white/gray KPs became pigmented, resolving completely after treatment (Fig. 5, 6). Detailed characteristics of

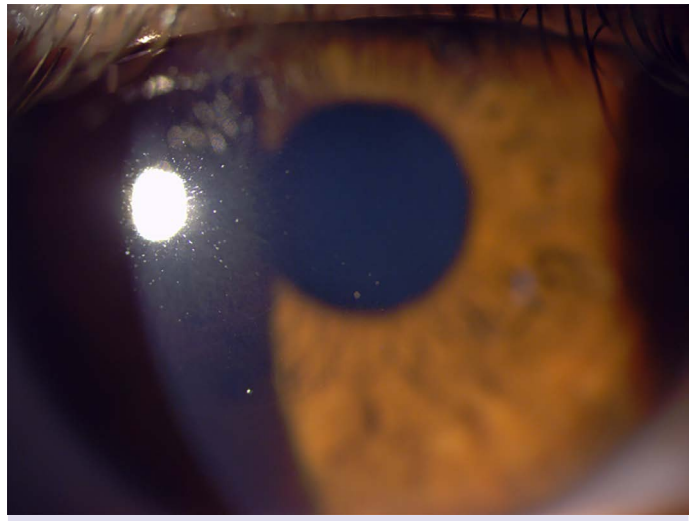

FIGURE 3. Central microgranulomatous KPs were observed in a patient with CMV-AU, indicating a characteristic inflammatory pattern associated with this condition.

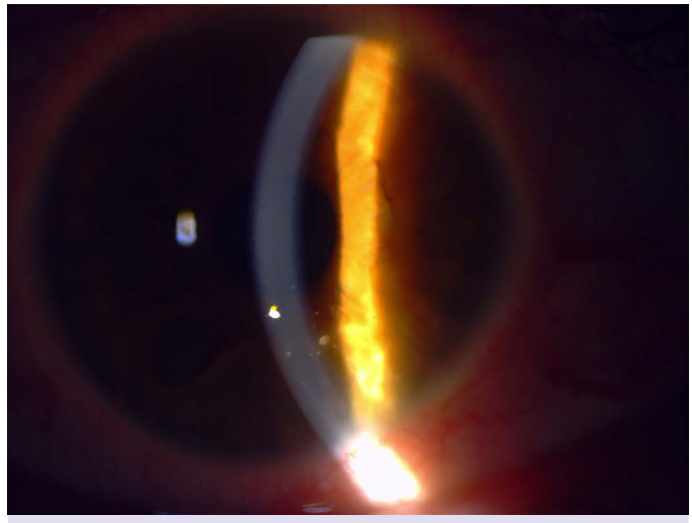

FIGURE 4. Nummular corneal endothelial lesions localized within the Arlt's triangle region in a case of CMV-AU.

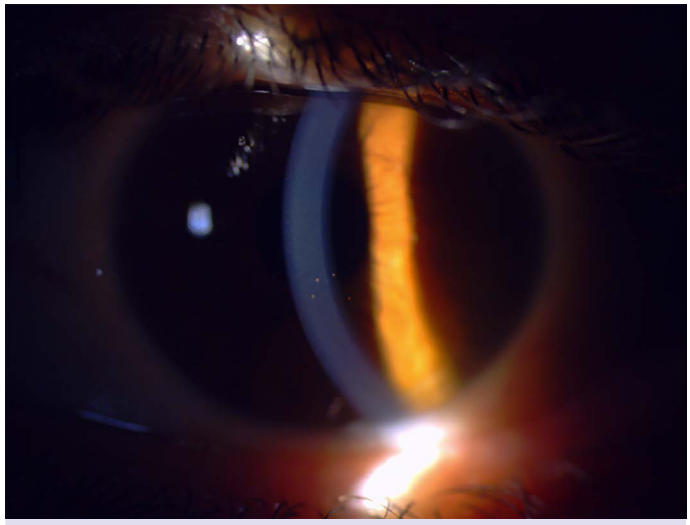

Figure 5. This image shows pigmented KPs that developed following treatment for CMV-AU. These pigmented KPs represent the transition from white or gray KPs to a more chronic state.

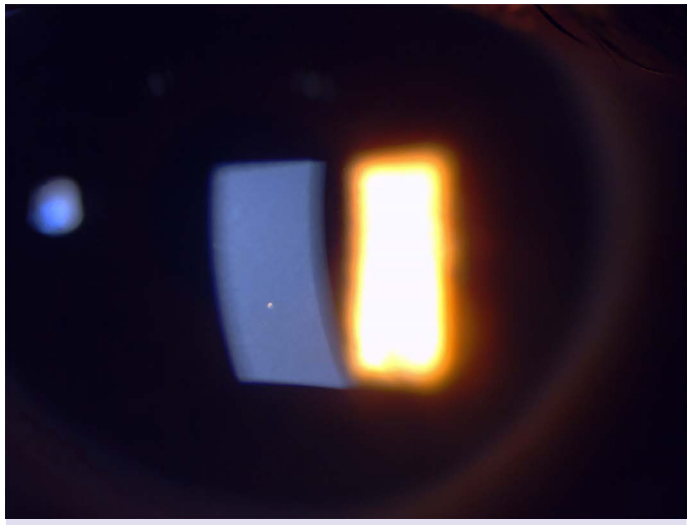

Figure 6. This image demonstrates resolving pigmented KPs after successful treatment, highlighting the gradual clearing of endothelial inflammation associated with CMV-AU.

KPs and anterior segment findings in CMV-AU patients treated with topical ganciclovir alone or in combination with oral valganciclovir are summarized in Table 1.

Anterior chamber reactions occurred in all eyes, graded as +1 in 41 (77.4%), +2 in 9 (17.0%), and +3 in 3 (5.6%). No posterior synechiae or vitritis were

Table 1. Comparison of keratic precipitate characteristics and anterior segment features between CMV-AU patients treated with oral valganciclovir and topical ganciclovir

Variable	Oral valganciclovir		Topical ganciclovir		p
	n	%	n	%	
KP morphology					0.088
Solitary or separate deposits	10	43.5	21	70.0	
Linear arrangements	2	8.7	3	10.0	
Coin-like shapes	11	47.8	6	20.0	
KP distribution					0.382
Inferior /lower half	8	34.8	7	23.3	
Central/paracentral	9	39.1	18	60.0	
Scattered	6	26.1	5	16.7	
KP count					0.249
<5	13	56.5	22	73.3	
>5	10	43.5	8	26.7	
Endothelitis					**0.010**[a]
Yes	14	60.9	7	23.3	
No	9	39.1	23	76.7	
Iris atrophy					**0.019**[a]
Yes	11	47.8	5	16.7	
No	12	52.2	25	83.3	

[a]: Fisher's Exact Test. CMV-AU: Cytomegalovirus anterior uveitis; KP: Keratic precipitate. (n) represents the number of treated eyes.

observed. Iris crypt loss and stromal atrophy were observed in 15 eyes (28.3%), including patchy (n=6) and diffuse (n=9) atrophy patterns. These findings were significantly associated with a higher number of uveitis attacks (4.2±1.7 vs. 2.3±1.5, p<0.001) and longer follow-up duration (69.0±47.7 vs. 20.4±19.0 months, p<0.001). Transillumination defects were not detected, except in one eye with patchy atrophy in which the defect developed after undergoing Gonioscopy-Assisted Transluminal Trabeculotomy (GATT) surgery. No heterochromia was observed during follow-up. Corneal endothelitis and stromal edema (excluding epithelial edema), identified by slit-lamp biomicroscopy in 21 eyes (39.6%), were significantly associated with glaucoma (p=0.026), oral valganciclovir use (p=0.019), coin-shaped KPs (p<0.001), scattered KP distribution (p=0.032), and higher KP counts (>5) (p=0.037). As endothelial cell counts were not routinely performed, the diagnosis of endothelitis was based on clinical examination findings. One patient with recurrent corneal endothelitis developed corneal decompensation and subsequently underwent Descemet membrane endothelial keratoplasty (DMEK).

Glaucoma, Treatment Outcomes, and Visual Acuity

Baseline IOP was elevated in 40 patients during attacks, with a mean IOP of 32.3±13.4 mmHg. All patients received topical ganciclovir as primary treatment, while 23 (44.2%) underwent combination therapy. The mean duration of valganciclovir therapy was 4.3±1.6 months. Two patients (8.7%) developed mild neutropenia during treatment; however, no therapy discontinuation was required. Antiviral treatments (oral and/or topical) were combined with intensive topical steroids, which were tapered over time. IOP-lowering agents were needed in 42 eyes (79.2%), with 21 eyes (39.6%) requiring ongoing anti-glaucoma medication. Endothelitis was significantly associated with a higher glaucoma risk (relative risk: 1.74, p=0.02), though not with higher baseline IOP (p=0.216). Multivariable analysis identified corneal endothelitis as the only independent predictor of secondary glaucoma (OR=6.20, 95 % CI 1.04–36.86; p=0.045). KP count, KP morphology, age at diagnosis, sex, and valganciclovir use were not statistically significant (p>0.10). The model displayed good calibration (Hosmer–Lemeshow p=0.624) and explained 26 % of the variance (Nagelkerke R^2=0.26), with an overall classification accuracy of 70 %.

Uveitic cataract developed in 8 patients (15.1%; mean age 36.8±15.2 years), and 5 of them (9.4%; mean age 39.8±16.4 years) underwent cataract surgery. Glaucoma surgery was required in 7 patients (13.2%; mean age 43.8 ±15.8 years), including GATT (n=1), trabeculectomy (n=4), and Ahmed valve implantation (n=2). Male patients accounted for 85.7% of glaucoma surgeries, but this was not statistically significant (p=0.404). Oral valganciclovir treatment was linked to a higher glaucoma surgery rate (p=0.034). Final IOP significantly improved to 16.4±6.6 mmHg (p < 0.001), with lower final IOP in surgical cases (12.43±4.16 mmHg) compared to non-surgical cases (17.04±6.72 mmHg, p=0.030). BCVA improved significantly from 0.10±0.17 to 0.04±0.09 logMAR (p=0.013). The comparison of clinical and treatment characteristics between CMV-AU patients treated with topical ganciclovir alone or combined with oral valganciclovir is summarized in Table 2.

Attack Frequency and Seasonal Variations

The mean number of uveitis attacks was 2.8±1.8, with recurrences in 35 eyes (66.0%) at an average rate of 1.63±1.31 per year. Attack frequency correlated significantly with age groups (p=0.039), with the 31–44 age group experiencing more frequent attacks (>3). Seasonal analysis showed significantly higher attack rates in winter and summer (3.83±1.85 and 3.82±1.65) compared to spring and autumn (3.15±1.78 and 3.24±1.84) (p < 0.001, p < 0.001, p=0.108, and p=0.206, respectively). Women were more likely to experience uveitis attacks during the spring compared to men (88.2% vs. 51.4%, p=0.014). Multivariable logistic regression showed that experiencing at least one attack during winter was independently associated with a higher likelihood of having more than three uveitis attacks per year (OR=4.99, 95% CI: 1.14–21.86; p=0.033). Similarly, female patients who had at least one spring attack were also at increased risk (OR=5.38, 95% CI: 1.11–25.96; p=0.036). The model demonstrated good fit (Hosmer–Lemeshow p=0.701) and explained 37% of the variance (Nagelkerke R^2=0.37).

DISCUSSION

This study examines the clinical features, risk factors, and outcomes of CMV-AU in a cohort of 53 eyes, focusing on key markers such as KPs morphology, seasonal variations, and treatment responses. While the prevalence

Table 2. Comparison of clinical and treatment characteristics between CMV-AU patients treated with topical ganciclovir alone or combined with oral valganciclovir

Variable	Oral valganciclovir Mean±SD Median (range)		Topical ganciclovir Mean±SD Median (range)		p
	n	%	n	%	
Age at diagnosis (years)	35.52±11.81		33.31±10.86		0.486a
Current age (years)	40.65±12.63		38.34±11.50		0.495a
Follow-up duration (months)	46.78±49.85 36 (6-204)		26.17±21.71 24 (6-96)		0.160[b]
Gender (n*)					
Male	17	73.9	18	62.1	0.393[c]
Female	6	26.1	11	37.9	
BCVA (LogMAR) before treatment	0.1±0.17 0.0 (0.0-0.52)		0.1±0.18 0.0 (0.0-0.82)		0.682[b]
BCVA (LogMAR) after treatment	0.04±0.95 0.0 (0.0-0.4)		0.04±0.99 0.0 (0.0-0.4)		0.966[b]
Number of attacks (n)	3.43±1.90		2.31±1.56		0.023[a]
Baseline IOP (mmHg)	33.91±13.16		30.93±13.86		0.435[a]
Final IOP (mmHg)	16.22±8.38		16.17±4.47		0.980[a]
Anti-glaucoma drops used during attacks (n)	1.52±0.99		1.59±1.09		0.826[a]
Anti-glaucoma drops currently used (n)	0.87±1.10		0.41±0.63		0.086[a]
Cup-to-disc ratio	0.59±0.20		0.58±0.20		0.950[a]
Presence of uveitic cataract (n)					1.000[c]
Yes	3	13.0	4	13.3	
No	20	87.0	26	86.7	
Glaucoma development (n)					0.049[c]
Yes	17	73.9	13	43.3	
No	6	26.1	17	56.7	
Anti-glaucoma drops at last visit (n)					0.778[c]
Yes	10	43.5	11	36.7	
No	13	56.5	19	63.3	
Underwent glaucoma surgery (n)					0.034[c]
Yes	6	26.1	1	3.3	
No	17	73.9	29	96.7	

[a]: Independent Samples t-test; [b]: Mann-Whitney U; [c]: Fisher's Exact Test. CMV-AU: Cytomegalovirus anterior uveitis; SD: Standard deviation; BCVA: Best corrected visual acuity; IOP: Intraocular Pressure. (n) represents the number of treated eyes, while (n*) represents the number of patients.

of CMV-AU across Asia, Europe, and America remains uncertain [17], most studies are from Asia, with limited data from Europe [13, 18, 19]. To our knowledge, this is the first Turkish cohort and the largest series of CMV-AU cases in immunocompetent patients. Previous Turkish studies addressed herpetic anterior uveitis [20], but this is the first focusing specifically on CMV-AU.

Consistent with prior research, our findings show that CMV-AU predominantly affects males [12], particularly within the 31–45 age range. While Tranos et al. [13] reported only 26.3% of cases as PSS-like, our study identified 63.5% of cases as PSS-like, aligning with Italian and French cohorts [18, 19]. Additionally, chronic-nonspecific AU cases accounted for 34.6% of our cohort, which is consistent with other European studies. In contrast, no FUS-like cases were identified in the French cohort [19], while only one case (1.9%) in our study presented with FUS-like features, similar to

the findings in the Belgian cohort [21], but significantly lower than those reported in the Greek (12.2%) and Italian (20%) cohorts [13, 18]. These findings highlight Turkey's geographical position bridging Asia and Europe, emphasizing the need for further research into how geography influences CMV-AU presentations.

CMV-AU is predominantly unilateral, with bilateral cases being rare [13]. Our findings support this, with only one case of bilateral CMV-AU confirmed by PCR. While bilateral viral uveitis can occur in specific contexts, such as iatrogenic immunosuppression or immunodeficiency (e.g., HIV), we excluded two such cases to maintain a homogenous group of immunocompetent patients. One involved an HIV-positive patient with bilateral CMV-AU and unilateral CMV retinitis, and the other had unilateral CMV-AU linked to localized immunosuppression from repeated dexamethasone injections for diabetic retinopathy.

Tranos et al. [13] reported PS in three eyes, suggesting a potential link to disease recurrence. However, PS is typically absent in CMV-AU, making this an unusual finding. No cases of PS were observed in our cohort, consistent with recent studies [7, 18, 19,], supporting its absence as a distinguishing feature of CMV-AU. Corneal endothelitis prevalence varies: Park et al. [22] reported 28.5% in Korea, Tranos et al. [13] observed 14% in Greece, and studies from Italy and Belgium reported 16.6% and 19.0%, respectively [18, 23]. A French study reported coin-shaped KPs in 83% of cases but no diffuse endothelitis or edema [19]. Our rate was 39.6%, strongly associated with coin-shaped KPs, scattered distribution, and higher counts. Iris atrophy prevalence (0–100%) correlates with disease duration [18]. In our cohort, this rate was 28.3%, with patchy and diffuse atrophy patterns observed in 6 and 9 eyes, respectively. Two cases exhibited pronounced peripupillary atrophy, and one developed iris transillumination defects after GATT surgery, which were absent at presentation.

Seasonal variations in uveitis incidence suggest environmental, hormonal, and immunological influences. Previous studies have reported increased anterior uveitis attacks in winter, correlating with cold or rainy conditions [24]. Gómez-Mariscal et al. [25] observed increased anterior uveitis attacks during winter in Madrid due to rainy and windy conditions, while Tan et al. [26] reported a 1°C temperature rise in China correlated with higher non-infectious uveitis cases, highlighting diverse climatic effects on uveitis trends. In our cohort, having at least one CMV-AU attack during winter was associated with an approximately five-fold increased likelihood of experiencing frequent relapses (>3 attacks). Notably, female patients who experienced at least one attack during spring also demonstrated a comparably elevated risk. To the best of our knowledge, this season and sex specific association has not been previously described in the CMV-AU literature, marking a novel contribution of the present study. These results point toward immuno-endocrine and environmental triggers such as estrogen-related immune modulation, or increased springtime UV exposure, that may amplify disease activity in women. Given its statistical significance, this finding warrants further investigation in larger prospective studies. Türkiye's unique geographic position, bridging Asia and Europe, may provide population-specific data with both regional and global relevance, thereby enriching the broader understanding of uveitis epidemiology. Comparative epidemiological studies across different regions are needed to elucidate how climatic, environmental, and demographic factors influence uveitis pathogenesis and to support the development of targeted preventive strategies.

The prevalence of secondary glaucoma in CMV-AU has been reported to range from 36.8% to 72% with 10.5%–68% of cases requiring filtration surgery [1, 19]. In our cohort, 56.6% of eyes exhibited glaucomatous changes, while the glaucoma surgery rate was relatively low at 13.2%. IOP-lowering agents were used in 79.2% of cases, with only 7 requiring surgery. Endothelitis cases were more likely to develop secondary glaucoma and require valganciclovir treatment. This increased glaucoma risk may result from CMV-induced TGF-β1 upregulation causing structural and inflammatory trabecular meshwork damage [27]. Higher valganciclovir use in endothelitis cases likely reflects its aggressive course, underscoring the importance of early identification and tailored treatment to prevent complications. Low glaucoma surgery rates are likely due to timely antiviral therapy and expert monitoring, ensuring effective IOP control and minimizing invasive procedures.

The SUN Group's criteria for CMV-AU emphasize the importance of combining clinical findings with PCR analysis for accurate diagnosis [28]. Thng et al. [29] reported 70% of uveitis specialists recommend aqueous humor sampling in suspected cases. While PCR and GWC analysis provide definitive diagnosis, their use is limited by high costs, accessibility, technical challenges, and potential patient refusal [6, 23]. Moreover, sev-

eral studies have observed that aqueous humour CMV PCR may yield false-negative results in approximately 20–30% of clinically suspected cases, particularly when the intraocular viral load is low [30]. Delays in diagnosis risk complications like corneal decompensation and glaucoma [19]. In our cohort, PCR positivity was 80.0%, exceeding prior reports (39–71%) [23, 31, 32], as testing was selectively performed in 10 patients to support clinical diagnosis. Repeated paracenteses may be needed for initial PCR-negative results to avoid misdiagnosis [33]. Different studies underscore varied diagnostic strategies for CMV-AU. Sobolewska et al. [5] emphasized recurrent episodes and typical clinical findings in acyclovir-resistant cases. Dheyab et al. [7] highlighted valganciclovir's efficacy in presumed CMV-AU based solely on clinical features only, while Hwang et al. [12] identified corticosteroid-resistant ocular hypertension and coin-shaped KPs as key screening tools. Our study advocates a practical approach by combining clinical and laboratory methods to improve diagnostic accuracy and optimize outcomes for CMV-AU.

Topical anti-CMV therapy offers reduced systemic side effects and cost-effectiveness, while systemic therapy achieves better inflammation control due to sustained ganciclovir levels in the aqueous humor [5]. A 900 mg oral valganciclovir dose twice daily has been shown to prevent glaucoma surgery in one-third of uncontrolled cases [34]. Although long-term valganciclovir therapy is more effective in reducing recurrences, its cost and potential side effects remain major limitations, especially in resource-limited settings [4, 5]. Our study supports the safety of long-term oral valganciclovir, with only two patients (8.7%) experiencing mild neutropenia—no discontinuation was required [4, 5, 21]. Importantly, none of the patients who adhered to a 450 mg twice-daily maintenance dose experienced flare-ups during treatment, consistent with the findings of Dheyab et al. [7]. Among 23 patients treated with oral valganciclovir, 4 remained flare-free after discontinuation, 3 experienced relapses, and 16 either continued therapy or lacked sufficient follow-up, reflecting a limitation of this retrospective study. Although a 6–9 month course was recommended, the mean treatment duration was limited to approximately four months, primarily due to out-of-pocket expenses, as the medication is only partially reimbursed in Türkiye. These findings underscore the urgent need to develop cost-reducing strategies and improve access to antiviral treatment in middle-income countries. Future studies should also aim to define the lowest effective dose and optimal duration for sustained remission while minimizing financial burden.

Our study's retrospective design introduces potential biases from incomplete data; its relatively small sample size further limits generalizability. In addition, PCR analysis was not performed in all cases because of practical and patient-related constraints. However, each case was assessed by at least one of three senior uveitis specialists, ensuring diagnostic consistency based on characteristic clinical findings. The absence of specular microscopy measurements before and after treatment represents another limitation. Nonetheless, the study identifies valuable prognostic markers and therapeutic insights. Larger prospective studies are required to validate these findings, investigate immunopathogenic mechanisms, and refine personalized treatment strategies for CMV-AU.

CONCLUSION

Key risk factors and clinical markers identified in this study provide fresh perspectives on CMV-AU management. Seasonal and gender variations highlight environmental influences on disease activity, emphasizing tailored treatments and early identification of high-risk cases. The significant glaucoma risk in endothelitis cases underscores the need for vigilant monitoring and timely antiviral therapy, such as oral valganciclovir, to prevent complications. As Türkiye's first cohort study on CMV-AU, these findings offer critical insights to optimize treatment protocols and address the financial challenges of long-term therapy.

Ethics Committee Approval: The Kartal Kosuyolu Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 20.02.2024, number: 2024/04/789).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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Authorship Contributions: Concept – MO, IS, ETK; Design – MO, IS, ETK; Supervision – MO; Materials – MO, IS, ETK; Data collection and/or processing – MO, IS, ETK; Analysis and/or interpretation – MO, NZK, BT; Literature review – MO, IS, ETK; Writing – MO, NZK, BT; Critical review – MO, NZK, BT.

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Original Article MEDICAL PHARMACOLOGY

Infection and infestation-related adverse events of ocrelizumab: A disproportionality analysis using FDA adverse event reporting system

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ABSTRACT

OBJECTIVE: The current study aims to describe the infection and infestation adverse events that may be associated with the use of ocrelizumab using real-world data.

METHODS: Infection and infestation adverse event reports with the generic name ocrelizumab as the primary suspect in the FAERS database from Q4 2003 to Q3 2024 were included in the study. The disproportionality analysis software package OpenVigil 2.1-MedDRA-v24, including the Reporting Odds Ratio (ROR) and Proportional Reporting Ratio (PRR) algorithms, was used to determine signal strength of infection and infestation adverse events associated with ocrelizumab. The signal intensity for ROR and PRR was classified as low, medium and strong according to signal strength.

RESULTS: The analysis of infection and infestation reports of the drug pharmacovigilance database on the use of ocrelizumab revealed 161 positive signals. The most common adverse event reported was COVID-19 (n=2287, ROR 24.303; PRR 22.681). According to the disproportionality analysis, the top six adverse events with the highest ROR and PRR were encephalitis enteroviral (ROR 101.831; PRR 101.809), meningitis enteroviral (ROR 76.019; PRR 76.005), fallopian tube abscess (ROR 51.225; PRR 51.221), nasal herpes (ROR 45.676; PRR 45.662), neuroborreliosis (ROR 28.563; PRR 28.559) and babesiosis (ROR 25.507; PRR 25.5).

CONCLUSION: Ocrelizumab may increase the risk of many infections and infestations, including enterovirus-related central nervous system infections, tick-borne infections, COVID-19-related disorders, genital tract infections and herpes infections, and therefore requires careful monitoring in clinical practice.

Keywords: Adverse event; adverse event reporting systems; infection; infestation; ocrelizumab.

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Anti-CD20 therapies have become the most widely used treatments for people with multiple sclerosis (MS) worldwide [1, 2]. Ocrelizumab is a cytolytic humanised second generation antibody directed against CD20. It is indicated for the treatment of relapsing forms of MS and primary progressive MS in adults [3]. Ocrelizumab was first approved for marketing in the United States of America (USA) on 28 March 2017 [4]. In Europe, the European Medicines Agency (EMA) granted marketing authorisation on 8 January 2018 [5].

Phase III clinical trials of ocrelizumab, ORATORIO and OPERA I/II have demonstrated efficacy and safety in the treatment of MS patients. In the ORATORIA, OPERA I and OPERA II studies, the rates of patients who discontinued the drug due to adverse events (AEs) were 4.1%, 3.2% and 3.8%, respectively [6, 7]. In a study of

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82 patients taking ocrelizumab, it was reported that half of the patients experienced at least one AE, and 4.8% of these patients experienced an AE requiring discontinuation of the drug [8]. In a comparison of ocrelizumab and rituximab, ocrelizumab had a higher rate of discontinuation due to AEs (6.8% vs 2.6%; p=0.026) [9]. Although the rates vary according to the studies, AEs are the main reasons for discontinuation of ocrelizumab [10].

Although anti-CD20 therapies, including ocrelizumab, are highly effective in controlling disease activity in MS, they have been associated with a clinically significant increase in the risk of infection with drug-induced hypogammaglobulinemia [11]. Compared with other drugs used in the treatment of MS (rituximab, cladribine), ocrelizumab was associated with a higher number of infection-related AEs [12, 13]. In a study evaluating the adverse drug effects of reports in the pharmacovigilance database until the last quarter of 2019, infections and infestations (21.93%) were the most reported reaction group [12].

The USA Food and Drug Administration's Adverse Event Reporting System (FAERS) is the most comprehensive database on AEs [14]. FAERS is a publicly accessible database of AE reports voluntarily submitted by physicians, patients and pharmaceutical companies and reflects the real-world occurrence of AEs [15]. In the FAERS database, all AEs are coded using the preferred terms (PTs) from the Medical Dictionary of Regulatory Activities (MedDRA) [16].

Disproportionality analysis has become an important method of drug safety monitoring in large databases of spontaneous AE reporting systems [17, 18]. In pharmacovigilance, the Proportional Reporting Ratio (PRR) and the Reporting Odds Ratio (ROR) are the most used methods for detecting AE signals [19, 20]. OpenVigil is an analysis tool that calculates ROR and PRR values using data from the FAERS database [21].

To date, the number of studies with real-world data evaluating adverse effects of ocrelizumab is limited. Reports of infections and infestations not reported in the literature may be available in FAERS spontaneous reporting databases. Although the availability of such reports does not indicate causality, statistical analysis of reports using disproportionality methods was used to identify signals of disproportionate reporting that may warrant further clinical investigation to understand the role of the drug in causing the event. The aim of this study is to describe the infection and infestation adverse effects that may occur with the use of ocrelizumab using real world data.

Highlight key points

- Infections are the most common adverse reactions to ocrelizumab in the FAERS database.
- Enterovirus-related central nervous system infections show particularly high ROR and PRR values.
- This study highlights new potential infection risks of ocrelizumab that clinicians should monitor closely.

MATERIALS AND METHODS

Data Source and Collection

In this study, a disproportionality analysis was performed to assess safety reports of infection and infestation in patients receiving ocrelizumab based on data from the FAERS database. This study was conducted using cases reported to FAERS from the fourth quarter of 2003 to the third quarter of 2024.

The OpenVigil 2.1 software application (Kiel, Germany), a tool for disproportionality analysis of FAERS data, was accessed online. OpenVigil is a tool that includes criteria filters and output filters for the analysis of spontaneous AE reports to FAERS. It is also a software programme that performs disproportionality statistics for signal detection.

The study included all adverse reaction reports where the reporters indicated ocrelizumab as the "Primary Suspect". AEs of infection and infestation in FAERS, named according to preferred terms (PTs) in the international MedDRA available in this tool, were individually selected and searched for assessment by the "Frequency" method. The OpenVigil tool contains data for reports up to 30 September 2024.

The human-related datasets used in this study are publicly available for any research programme. Patient consent was not obtained in this study due to the nature of the data source. This research did not require ethics committee approval as the authors had no knowledge of the data collection or the participants in the study.

Statistical Analysis

A disproportionality analysis was performed using OpenVigil version 2.1. PRR and ROR were used to detect safety signals. All algorithms are based on 2×2 contingency tables. In the context of this study, PTs with

a reported frequency of ≥3 were selected for the initial screening procedure. The PRR with chi-square and the ROR with 95% confidence interval (CI) were calculated. The risk signal strength assessment criteria for ROR and PRR were signal intensity (assessment criteria) classification as weak (2< ROR or PRR ≤10), medium (10< ROR or PRR ≤50) and strong (ROR or PRR >50) [22]. If signals met the criteria of both methods, they were classified as positive signal. It is widely accepted that a chi-square value exceeding 4 is statistically significant (https://openvigil.sourceforge.net/). The analysis was performed using Microsoft Excel 2024 software (Microsoft Corporation, Redmond, WA, USA).

RESULTS

Descriptive Analysis

The most common adverse drug reaction group in the FAERS database is infection and infestation for ocrelizumab. A search of the original data in the FAERS database identified 26358 reports for infection and infestation adverse effects of ocrelizumab. There were more females (17842 cases, 67.69%) than males (6828 cases, 25.90%), the predominant age group was the age group of 18 to 64 years (15452 cases, 58.62%), most cases were submitted in 2023 (5448 cases, 20.67%), and about half of the cases were from the USA (13175 cases, 49.98%) (Table 1).

Table 1. Basic information on infection and infestation adverse event reports related to ocrelizumab[a]

Category	Number of cases (n)	Percentage (%)
Gender		
Female	17842	67.69
Male	6828	25.90
Not specified	1668	6.33
Age (years)		
<18	36	0.13
18–64	15452	58.62
65–85	1752	6.65
>85	3	0.01
Missing	9115	34.58
Report year		
2008–2016	25	0.09
2017	265	1.01
2018	1298	4.92
2019	2257	8.56
2020	2543	9.65
2021	3460	13.13
2022	5643	21.41
2023	5448	20.67
2024[b]	5419	20.56
Reported countries		
United States of America (USA)	13175	49.98
Non-USA	13091	49.67
Not Specified	92	0.03

[a]: The information in this table includes information obtained from the FDA adverse reporting system; [b]: Except Q4 of 2024.

Disproportionality Analysis

Analysis of the infection and infestation reports reported to FAERS identified 36057 cases causing 357 different AEs (AEs with ≥3 cases). These AEs were searched in the OpenVigil tool. After data cleaning and deduplica-tion through OpenVigil quality and cleaning procedures, 13417 cases of 161 AEs with ≥3 cases were included in the study.

Common AE terms reported were COVID-19 (n=2287), urinary tract infection (n=1696), influenza (n=1080), nasopharyngitis (n=1041) and herpes zoster (n=691). According to their signal intensity, 3 strong, 26 medium and 132 weak signals were found. Encephalitis enteroviral (n=7, ROR 101.831; PRR 101.809), meningitis enteroviral (n=6, ROR 76.019; PRR 76.005) and fallopian tube abscess (n=3, ROR 51.225; PRR 51.221) had strong AE signal.

Tick-borne neuroborreliosis (ROR 28.563; PRR 28.559) and babesiosis (ROR 25.507; PRR 25.5) infections of which have medium signal intensity, are reported to occur because of the use of ocrelizumab. As well as the other tick-borne disease Lyme Disease (ROR 2.675; PRR 2.674) had a weak AE signal.

COVID-19 (ROR 24.303; PRR 22.681), COVID-19 pneumonia (ROR 24.303; PRR 22.681), coronavirus infection (ROR 15.188; PRR 15.118) and post-acute COVID-19 syndrome (ROR 13.660; PRR 13.659) had medium signal.

Genitourinary tract infections with medium signal intensity reported to occur as a result of the use of ocrelizumab are as follows: genital herpes (ROR 21.872; PRR 21.848), genital herpes simplex (ROR 18.267; PRR 18.265), streptococcal urinary tract infection (ROR 17.851; PRR 17.85), pyelitis (ROR 16.364; PRR 16.362), perineal infection (ROR 14.368; PRR 14.367), tuba-ovarian abscess (ROR 14.230; PRR 14.228), bac-

terial vaginosis (ROR 12.336; PRR 12.329), pelvic infection (ROR 11.408; PRR 11.406), uterine infection (ROR 10.575; PRR 10.572) and salpingitis (ROR 10.390; PRR 10.389).

Herpes infections (except genitourinary herpes infections) with medium signal intensity reported because of the use of ocrelizumab are as follows: nasal herpes (ROR 45.676; PRR 45.662), oral herpes (ROR 17.952; PRR 17.711) and herpes virus infection (ROR 15.023; PRR 14.975).

The results of the disproportionality analysis for the top 50 infections and infestation AEs according to signal intensity in ocrelizumab users are presented in Table 2. The full list of adverse events included in the study can be found in Appendix 1.

DISCUSSION

This study observed a wide range of drug-related adverse effect profiles for ocrelizumab. These included enterovirus-associated central nervous system infections, tick-borne infections, COVID-19-related disorders, genitourinary tract infections and herpes infections.

In the present study, although urinary tract infection ranked second in terms of the number of cases and nasopharyngitis ranked fourth in terms of the number of cases, they had weak signal strength (n=1696, ROR 8.508, PRR 8.121; n=1041, ROR 4.531, PRR 4.419, respectively). According to a review of 13 clinical trials of the adverse effects of ocrelizumab (excluding coronavirus disease 2019), nasopharyngitis, urinary tract infection and upper respiratory tract infection were found to be the three most common types of infection [23]. In this study, COVID-19 was the AE with the highest number of cases. In addition, many AEs associated with COVID-19 were found to have medium signal strength. In a prospective study of 305 patients with MS, the most common AE reported were infections (31.5%), including COVID-19 (11.5%) [24].

The high prevalence of MS, especially in female patients is evident from the fact that two-thirds of the case reports of AEs in the present study were from female [25]. The strong and medium risk of female genital tract infections in the present study reflects the females predominance. Female genital tract infections that attract particular attention due to their risk are fallopian tube abscess, perineal infection, tubo-ovarian abscess, bacterial vaginosis, uterine infection and salpingitis.

In the study by Caldito et al. [12] evaluating patients using ocrelizumab, oral herpes was found to have the highest signal strength among all AEs (PRR 44.35; ROR 45.34). In the present study, oral herpes (ROR 17.952; PRR 17.711) was found to have medium signal strength but ranked eleventh when ranked according to signal strength. Herpes virus infections other than oral herpes were also shown to have medium or weak signal strength (highest ROR 45.676; PRR 45.662; lowest ROR 4.443; PRR 4.438). Although ocrelizumab appears to increase the risk of herpes virus infection, routine antiviral prophylaxis is not required in these patients as most herpes infections are mild or moderate [23].

There are a small number of reported cases of encephalitis caused by ocrelizumab treatment in the literature. The infectious agents in these cases include West Nile virus, Coxsackie virus B5 and Herpes Simplex virus type 2 [26–29]. In the present study, enteroviral encephalitis was found to have strong signal strength despite the small number of cases. In addition, 'herpes simplex encephalitis', 'herpes zoster meningoencephalitis', 'encephalitis viral' and 'encephalitis' under the headings of encephalitis, although weak signal strength, draw attention to central nervous system cases, especially viral cases. Progressive multifocal leukoencephalopathy (PML) has a weak signal strength, although the number of cases is 28 (ROR 2.520, PRR 2.518). JCV infection resulting in PML has also been reported in patients treated with anti-CD20 antibodies and other MS therapies, including ocrelizumab [30].

Scientific literature has reported three cases of meningitis following the use of ocrelizumab. In one of these cases, ocrelizumab was used concomitantly with rituximab and it was reported to have occurred because of mycoplasma pneumonia [31]. In the other two cases of meningitis, it has been reported that one of them may be related to Lyme disease and the other may be aseptic meningitis [32]. Although the number of cases is low in the literature and in the present study, it is important to be careful about meningitis in terms of high risk and to keep enteroviruses in mind as the causative agent.

Numerous cases of babesiosis associated with ocrelizumab and other disease-modifying drugs have been reported to FAERS [8]. The finding of medium signal strength for babesiosis and neuroborreliosis in the present study demonstrates the importance of considering geographical risk factors that may expose

Table 2. PT signal for the 50 top AEs based on the number of ocrelizumab reports[a]

PTs	Reports (n)	ROR	95% CI	PRR	χ^2	Signal intensity
Encephalitis enteroviral	7	101.831	44.342; 233.853	101.809	477.521	Strong
Meningitis enteroviral	6	76.019	31.713; 182.222	76.005	311.757	Strong
Fallopian tube abscess	3	51.225	15.38; 170.618	51.221	89.932	Strong
Nasal herpes	10	45.676	23.727; 87.927	45.662	352.253	Medium
Neuroborreliosis	4	28.563	10.351; 78.821	28.559	75.082	Medium
Babesiosis	10	25.507	13.454; 48.359	25.5	198.574	Medium
COVID-19	2287	24.303	23.265; 25.386	22.681	44.934	Medium
Genital herpes	38	21.872	15.774; 30.328	21.848	696.503	Medium
Infection susceptibility increased	55	20.841	15.887; 27.339	20.807	966.179	Medium
Genital herpes simplex	4	18.267	6.703; 49.783	18.265	46.934	Medium
Oral herpes	466	17.952	16.35; 19.711	17.711	7020.58	Medium
Eye infection viral	7	17.853	8.37; 38.081	17.85	90.955	Medium
Streptococcal urinary tract infection	3	17.851	5.612; 56.779	17.85	30.915	Medium
COVID-19 pneumonia	134	16.518	13.891; 19.64	16.454	1852.433	Medium
Pyelitis	4	16.364	6.018; 44.494	16.362	41.587	Medium
Bacterial colitis	3	15.301	4.828; 48.493	15.3	26.027	Medium
Coronavirus infection	162	15.188	12.977; 17.775	15.118	2043.725	Medium
Herpes virus infection	112	15.023	12.436; 18.148	14.975	1393.888	Medium
Scarlet fever	6	14.547	6.439; 32.861	14.544	60.469	Medium
Perineal infection	3	14.368	4.54; 45.476	14.367	24.226	Medium
Tubo-ovarian abscess	5	14.230	5.83; 34.733	14.228	47.234	Medium
Post-acute COVID-19 syndrome	4	13.660	5.041; 37.02	13.659	33.916	Medium
Pharyngitis bacterial	4	13.543	4.998; 36.696	13.541	33.58	Medium
Ear infection fungal	5	12.588	5.167; 30.67	12.586	41.031	Medium
Bacterial vaginosis	20	12.336	7.904; 19.255	12.329	191.02	Medium
Pelvic infection	7	11.408	5.38; 24.189	11.406	54.841	Medium
Severe acute respiratory syndrome	3	10.614	3.371; 33.416	10.613	16.917	Medium
Uterine infection	7	10.575	4.991; 22.404	10.572	50.104	Medium
Salpingitis	5	10.390	4.275; 25.254	10.389	32.67	Medium
Diverticulitis intestinal perforated	4	9.818	3.64; 26.483	9.817	22.882	Weak
Pulpitis dental	9	9.739	5.026; 18.87	9.736	60.564	Weak
Papilloma viral infection	21	9.696	6.288; 14.951	9.69	151.345	Weak
Herpes zoster	691	9.616	8.91; 10.378	9.435	5.091	Weak
Hand-foot-and-mouth disease	9	9.606	4.958; 18.611	9.604	59.56	Weak
Vulvovaginitis	3	9.501	3.023; 29.865	9.501	14.735	Weak
Onychomycosis	40	9.294	6.791; 12.719	9.284	280.803	Weak
Herpes ophthalmic	17	8.905	5.506; 14.404	8.901	108.974	Weak
Vulvovaginal candidiasis	15	8.666	5.195; 14.456	8.662	92.103	Weak
Urinary tract candidiasis	3	8.663	2.759; 27.198	8.662	13.088	Weak
Urinary tract infection	1696	8.508	8.099; 8.939	8.121	10.435	Weak
Influenza	1080	8.125	7.643; 8.638	7.891	6.391	Weak
Herpes simplex encephalitis	5	8.081	3.333; 19.592	8.08	23.837	Weak
Joint abscess	3	8.015	2.555; 25.139	8.014	11.815	Weak
Herpes simplex reactivation	3	7.650	2.44; 23.984	7.65	11.01	Weak
Breast abscess	10	7.627	4.079; 14.262	7.625	50.141	Weak
Acne pustular	5	7.582	3.129; 18.371	7.581	21.925	Weak
Gastric infection	35	7.477	5.35; 10.448	7.47	186.178	Weak
Varicella	23	7.414	4.907; 11.201	7.409	118.941	Weak
Eyelid infection	4	7.307	2.717; 19.647	7.306	15.618	Weak
Ophthalmic herpes zoster	18	7.179	4.504; 11.445	7.176	87.986	Weak

[a]: The information in this table was analysed using the OpenVigil tool. CI: Confidence interval; χ^2: Chi-squared with Yates' correction; PRR: Proportional reporting ratio; PTs: Preferred terms; ROR: Reporting odds ratio.

patients to Babesia microti and Borrelia burgdorferi when considering or initiating ocrelizumab treatment for MS patients.

Recognizing the risk of infection associated with MS drugs is important for the prevention of infection. In this way, adverse effects caused by the treatment of infection and interruption of MS treatment are prevented [23]. However, there is a lack of strategies to minimize the risk of infection with anti-CD20 therapies [2]. The rates of infection as an AE in the ORATORIA, OPERA I and OPERA II studies were 71.4%, 56.9% and 60.2%, respectively (rates of serious infection were 6.2%, 1.2% and 1.4%, respectively) [6, 7]. In the phase IIIB ENSEMBLE study, which included 678 patients using ocrelizumab, it was reported that 95.4% of the patients had adverse effects; 15.5% of these were serious adverse effects. Infections were reported in 75.2% of these patients, of which 6.2% were serious infections [33].

Current studies and clinical trials show that ocrelizumab can cause infection, even serious infections. Serious infections may lead to treatment interruption because of drug discontinuation. For these reasons, knowledge of infections and infestations related to the use of ocrelizumab is valuable in continuing treatment, preventing adverse effects and early diagnosis of adverse effects.

As a spontaneous reporting system, the FAERS database has inherent limitations. These include lack of access to patients' medical history, disease severity and other relevant information. The signals of adverse reactions detected by signal detection methods indicate a statistical association between the drug and the AE rather than a definite causal relationship.

CONCLUSION

Although the mechanisms behind the association of ocrelizumab use with infections and infestations in this study are not understood, the immunosuppressive property of the drug may be an important reason. Although this study was based on spontaneous reports of adverse effects, it has been shown that ocrelizumab may cause many infections, especially viral and bacterial infections, or may increase the risk of possible infection and infestation adverse effects. Furthermore, this study has identified new potential AEs that require clinicians' attention. Further research is required to confirm these findings, which ensure the safe use of the drug.

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Appendix 1. Full list of infection and infestation adverse events included in the study

PTs	Reports	ROR (95%CI)	PRR	Chi- Squared with Yates' correction	Signal intensity
Covid-19	2287	24.303 (23.265; 25.386)	22.681	44.934	medium
Urinary tract infection	1696	8.508 (8.099; 8.939)	8.121	10.435	weak
Influenza	1080	8.125 (7.643; 8.638)	7.891	6.391	weak
Nasopharyngitis	1041	4.531 (4.258; 4.821)	4.419	2739.578	weak
Herpes zoster	691	9.616 (8.91; 10.378)	9.435	5.091	weak
Infection	602	3.203 (2.954; 3.473)	3.163	886.246	weak
Oral herpes	466	17.952 (16.35; 19.711)	17.711	7020.58	medium
Sepsis	403	2.836 (2.57; 3.13)	2.814	468.051	weak
Sinusitis	400	3.189 (2.889; 3.521)	3.163	586.916	weak
Upper respiratory tract infection	279	5.01 (4.45; 5.641)	4.976	872.791	weak
Cystitis	259	6.517 (5.761; 7.372)	6.473	1175.199	weak
Bronchitis	258	2.863 (2.532; 3.237)	2.848	306.229	weak
Fungal infection	213	4.717 (4.119; 5.402)	4.693	608.883	weak
Cellulitis	181	2.845 (2.457; 3.295)	2.835	212.072	weak
Coronavirus infection	162	15.188 (12.977; 17.775)	15.118	2043.725	medium
Lower respiratory tract infection	135	2.559 (2.16; 3.032)	2.552	125.304	weak
Covid-19 pneumonia	134	16.518 (13.891; 19.64)	16.454	1852.433	medium
Ear infection	127	3.796 (3.186; 4.522)	3.785	255.275	weak
Kidney infection	125	4.824 (4.043; 5.757)	4.81	369.155	weak
Respiratory tract infection	124	3.991 (3.343; 4.765)	3.98	271.159	weak
Herpes virus infection	112	15.023 (12.436; 18.148)	14.975	1393.888	medium
Staphylococcal infection	85	2.002 (1.617; 2.478)	2.0	41.32	weak
Clostridium difficile infection	81	3.544 (2.847; 4.412)	3.538	143.757	weak
Localised infection	80	2.648 (2.125; 3.301)	2.644	79.701	weak
Skin infection	76	5.797 (4.621; 7.273)	5.786	291.917	weak
Candida infection	74	2.942 (2.34; 3.699)	2.937	92.008	weak
Tooth infection	63	3.804 (2.967; 4.876)	3.798	125.947	weak
Urosepsis	60	5.324 (4.126; 6.87)	5.316	203.275	weak
Appendicitis	57	4.747 (3.655; 6.165)	4.74	162.588	weak
Infection susceptibility increased	55	20.841 (15.887; 27.339)	20.807	966.179	medium
Conjunctivitis	54	2.159 (1.652; 2.821)	2.157	32.201	weak
Osteomyelitis	50	2.145 (1.624; 2.833)	2.143	29.21	weak
Tuberculosis	50	2.629 (1.99; 3.472)	2.626	48.443	weak
Abscess	46	2.231 (1.669; 2.981)	2.229	29.801	weak
Bacterial infection	46	2.021 (1.512; 2.701)	2.02	22.569	weak
Oral candidiasis	45	3.076 (2.294; 4.125)	3.073	60.432	weak
Meningitis	44	4.705 (3.495; 6.335)	4.7	123.056	weak
Onychomycosis	40	9.294 (6.791; 12.719)	9.284	280.803	weak
Herpes simplex	40	4.443 (3.252; 6.068)	4.438	102.007	weak
Genital herpes	38	21.872 (15.774; 30.328)	21.848	696.503	medium
Vaginal infection	38	6.231 (4.522; 8.587)	6.225	158.982	weak
Wound infection	37	3.564 (2.578; 4.927)	3.561	65.041	weak

Appendix 1 (cont.). Full list of infection and infestation adverse events included in the study

PTs	Reports	ROR (95%CI)	PRR	Chi- Squared with Yates' correction	Signal intensity
Gastrointestinal infection	36	3.509 (2.527; 4.873)	3.506	61.473	weak
Eye infection	35	2.407 (1.726; 3.356)	2.405	27.192	weak
Pharyngitis streptococcal	35	2.504 (1.795; 3.491)	2.502	29.897	weak
Gastric infection	35	7.477 (5.35; 10.448)	7.47	186.178	weak
Pyelonephritis	34	3.276 (2.337; 4.592)	3.273	51.014	weak
Pharyngitis	34	2.157 (1.54; 3.022)	2.156	19.829	weak
Encephalitis	33	3.432 (2.436; 4.835)	3.429	53.922	weak
Respiratory syncytial virus infection	32	2.711 (1.915; 3.839)	2.709	32.606	weak
Pneumonia bacterial	30	2.783 (1.943; 3.987)	2.782	32.257	weak
Escherichia infection	30	3.013 (2.103; 4.315)	3.011	38.014	weak
Tooth abscess	29	2.216 (1.538; 3.193)	2.215	18.037	weak
Progressive multifocal leukoencephalopathy	28	2.52 (1.737; 3.654)	2.518	23.988	weak
Laryngitis	28	2.003 (1.381; 2.904)	2.002	12.989	weak
Atypical pneumonia	28	6.788 (4.671; 9.864)	6.783	130.087	weak
Streptococcal infection	25	3.399 (2.292; 5.039)	3.397	39.582	weak
Arthritis infective	24	4.018 (2.687; 6.007)	4.016	50.859	weak
Rhinitis	23	2.504 (1.661; 3.773)	2.503	19.155	weak
Abscess limb	23	4.503 (2.985; 6.793)	4.5	58.495	weak
Varicella	23	7.414 (4.907; 11.201)	7.409	118.941	weak
Tonsillitis	22	3.267 (2.147; 4.97)	3.265	32.075	weak
Fungal skin infection	22	4.106 (2.697; 6.251)	4.104	48.092	weak
Papilloma viral infection	21	9.696 (6.288; 14.951)	9.69	151.345	weak
Escherichia urinary tract infection	21	4.403 (2.863; 6.77)	4.401	51.259	weak
Bacterial vaginosis	20	12.336 (7.904; 19.255)	12.329	191.02	medium
Ophthalmic herpes zoster	18	7.179 (4.504; 11.445)	7.176	87.986	weak
Erysipelas	17	2.924 (1.814; 4.712)	2.923	19.479	weak
Herpes ophthalmic	17	8.905 (5.506; 14.404)	8.901	108.974	weak
Appendicitis perforated	17	5.288 (3.276; 8.534)	5.286	54.131	weak
Oral infection	16	2.977 (1.82; 4.87)	2.976	18.922	weak
Anal abscess	16	2.687 (1.643; 4.394)	2.686	15.186	weak
Mastitis	15	5.549 (3.333; 9.238)	5.547	50.725	weak
Vulvovaginal candidiasis	15	8.666 (5.195; 14.456)	8.662	92.103	weak
Cholecystitis infective	15	4.336 (2.606; 7.213)	4.334	34.825	weak
Vulvovaginal mycotic infection	14	2.528 (1.495; 4.277)	2.528	11.369	weak
Large intestine infection	14	7.033 (4.145; 11.932)	7.03	65.331	weak
Meningitis aseptic	14	2.489 (1.472; 4.211)	2.489	10.953	weak
Hordeolum	13	2.522 (1.461; 4.351)	2.521	10.388	weak
Chronic sinusitis	13	3.549 (2.055; 6.127)	3.548	21.109	weak
Varicella zoster virus infection	13	4.845 (2.804; 8.373)	4.843	35.455	weak
Myelitis	13	6.514 (3.765; 11.27)	6.511	54.348	weak
Suspected Covid-19	12	3.654 (2.069; 6.452)	3.653	20.35	weak
Lyme disease	12	2.675 (1.516; 4.72)	2.674	10.885	weak
Pneumonia viral	12	3.476 (1.969; 6.137)	3.475	18.584	weak

Appendix 1 (cont.). Full list of infection and infestation adverse events included in the study

PTs	Reports	ROR (95%CI)	PRR	Chi- Squared with Yates' correction	Signal intensity
Viral upper respiratory tract infection	12	2.074 (1.176; 3.659)	2.074	5.613	weak
Lung abscess	12	4.493 (2.543; 7.939)	4.492	28.843	weak
Urinary tract infection bacterial	10	3.442 (1.847; 6.416)	3.442	14.832	weak
Infected skin ulcer	10	4.279 (2.294; 7.98)	4.278	21.701	weak
Breast abscess	10	7.627 (4.079; 14.262)	7.625	50.141	weak
Nasal herpes	10	45.676 (23.727; 87.927)	45.662	352.253	medium
Respiratory tract infection viral	10	3.674 (1.971; 6.849)	3.673	16.714	weak
Babesiosis	10	25.507 (13.454; 48.359)	25.5	198.574	medium
Central nervous system infection	10	6.831 (3.656; 12.766)	6.829	43.334	weak
Tinea infection	10	6.928 (3.707; 12.947)	6.926	44.158	weak
Brain abscess	10	2.788 (1.496; 5.193)	2.787	9.669	weak
Pulpitis dental	9	9.739 (5.026; 18.87)	9.736	60.564	weak
Intervertebral discitis	9	4.174 (2.164; 8.05)	4.173	18.452	weak
Periodontitis	9	3.297 (1.711; 6.355)	3.297	12.09	weak
Hand-foot-and- mouth disease	9	9.606 (4.958; 18.611)	9.604	59.56	weak
Tinea pedis	8	4.075 (2.031; 8.179)	4.075	15.447	weak
Oral fungal infection	8	2.502 (1.248; 5.014)	2.501	5.746	weak
Latent tuberculosis	8	2.909 (1.451; 5.833)	2.909	8.139	weak
Sinusitis bacterial	8	6.066 (3.017; 12.196)	6.065	28.511	weak
Infected cyst	8	5.571 (2.772; 11.196)	5.57	25.234	weak
Genital infection fungal	7	5.391 (2.557; 11.366)	5.39	20.539	weak
Uterine infection	7	10.575 (4.991; 22.404)	10.572	50.104	medium
Pelvic infection	7	11.408 (5.38; 24.189)	11.406	54.841	medium
Eye infection viral	7	17.853 (8.37; 38.081)	17.85	90.955	medium
Encephalitis enteroviral	7	101.831 (44.342; 233.853)	101.809	477.521	strong
Meningitis viral	6	3.202 (1.434; 7.15)	3.201	6.953	weak
Encephalitis viral	6	3.927 (1.757; 8.778)	3.927	10.218	weak
Scarlet fever	6	14.547 (6.439; 32.861)	14.544	60.469	medium
Meningitis enteroviral	6	76.019 (31.713; 182.222)	76.005	311.757	strong
Infected bite	6	4.396 (1.966; 9.831)	4.396	12.381	weak
Salmonellosis	6	2.802 (1.255; 6.255)	2.802	5.225	weak
Bacterial vulvovaginitis	5	3.256 (1.35; 7.853)	3.256	5.671	weak
Acne pustular	5	7.582 (3.129; 18.371)	7.581	21.925	weak
Skin bacterial infection	5	5.547 (2.295; 13.411)	5.547	14.155	weak
Tubo-ovarian abscess	5	14.23 (5.83; 34.733)	14.228	47.234	medium
Groin abscess	5	3.733 (1.547; 9.007)	3.733	7.382	weak
Salpingitis	5	10.39 (4.275; 25.254)	10.389	32.67	medium
Herpes simplex encephalitis	5	8.081 (3.333; 19.592)	8.08	23.837	weak
Beta haemolytic streptococcal infection	5	2.953 (1.225; 7.118)	2.953	4.613	weak
Acarodermatitis	5	3.262 (1.353; 7.866)	3.262	5.69	weak
Cellulitis orbital	5	6.234 (2.577; 15.083)	6.233	16.769	weak
Ear infection fungal	5	12.588 (5.167; 30.67)	12.586	41.031	medium
Syphilis	5	5.293 (2.19; 12.793)	5.292	13.192	weak

Appendix 1 (cont.). Full list of infection and infestation adverse events included in the study

PTs	Reports	ROR (95%CI)	PRR	Chi- Squared with Yates' correction	Signal intensity
Pulmonary sepsis	5	2.931 (1.216; 7.065)	2.931	4.537	weak
Post-acute Covid-19 syndrome	4	13.66 (5.041; 37.02)	13.659	33.916	medium
Pyelitis	4	16.364 (6.018; 44.494)	16.362	41.587	medium
Genital herpes simplex	4	18.267 (6.703; 49.783)	18.265	46.934	medium
Mastoiditis	4	3.314 (1.239; 8.867)	3.314	4.315	weak
Gastroenteritis salmonella	4	4.212 (1.572; 11.281)	4.211	6.767	weak
Haemophilus infection	4	3.46 (1.293; 9.26)	3.46	4.705	weak
Bronchitis bacterial	4	4.376 (1.633; 11.724)	4.375	7.226	weak
Neuroborreliosis	4	28.563 (10.351; 78.821)	28.559	75.082	medium
Ophthalmic herpes simplex	4	4.62 (1.724; 12.382)	4.62	7.914	weak
Chlamydial infection	4	3.767 (1.407; 10.085)	3.767	5.539	weak
Eyelid infection	4	7.307 (2.717; 19.647)	7.306	15.618	weak
Pharyngitis bacterial	4	13.543 (4.998; 36.696)	13.541	33.58	medium
Herpes zoster oticus	4	6.742 (2.509; 18.116)	6.742	13.987	weak
Body tinea	4	5.712 (2.129; 15.33)	5.712	11.023	weak
Gastrointestinal bacterial overgrowth	4	3.705 (1.384; 9.918)	3.705	5.369	weak
Diverticulitis intestinal perforated	4	9.818 (3.64; 26.483)	9.817	22.882	weak
Vulvovaginitis	3	9.501 (3.023; 29.865)	9.501	14.735	weak
Conjunctivitis bacterial	3	6.473 (2.068; 20.26)	6.473	8.793	weak
Vulval abscess	3	5.95 (1.903; 18.609)	5.95	7.772	weak
Urethritis	3	4.514 (1.446; 14.088)	4.514	5.004	weak
Severe acute respiratory syndrome	3	10.614 (3.371; 33.416)	10.613	16.917	medium
Cervicitis	3	5.719 (1.829; 17.88)	5.719	7.323	weak
Bronchitis viral	3	3.994 (1.281; 12.455)	3.993	4.024	weak
Herpes simplex reactivation	3	7.65 (2.44; 23.984)	7.65	11.01	weak
Perineal infection	3	14.368 (4.54; 45.476)	14.367	24.226	medium
Urinary tract infection pseudomonal	3	4.331 (1.388; 13.515)	4.331	4.658	weak
Bacterial colitis	3	15.301 (4.828; 48.493)	15.3	26.027	medium
Herpes zoster meningoencephalitis	3	6.81 (2.175; 21.324)	6.81	9.452	weak
Joint abscess	3	8.015 (2.555; 25.139)	8.014	11.815	weak
Streptococcal urinary tract infection	3	17.851 (5.612; 56.779)	17.85	30.915	medium
Fallopian tube abscess	3	51.225 (15.38; 170.618)	51.221	89.932	strong
Urinary tract candidiasis	3	8.663 (2.759; 27.198)	8.662	13.088	weak

CI: Confidence interval; χ^2: Chi-squared with Yates' correction; PRR: Proportional reporting ratio; PTs: Preferred terms; ROR: Reporting odds ratio.

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Original Article General Surgery

Comparison of administration methods in adipose tissue-derived stem cell therapy in rats with colitis: An experimental study

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ABSTRACT

OBJECTIVE: Stem cell studies have been increasing in recent years owing to the healing effect of stem cells on colitis. This study aimed to compare the effects of locally and intra-arterially administered adipose tissue-derived stem cells (ATDSCs) in a colitis model induced by acetic acid (AA).

METHODS: Five groups were established, each consisting of six Wistar-Albino male rats. Group 1, colitis control group; Group 2, local phosphate-buffered saline (PBS) group; Group 3, local stem cell group; Group 4, intra-arterial PBS group; Group 5, intra-arterial stem cell group. Colitis was induced by the rectal administration of acetic acid. All rats were weighed on the day colitis was induced, on the day of stem cell administration (day 7), and on the day of sacrifice (day 14). All groups were evaluated for weight loss, histopathology, blood tumor necrosis factor-α-stimulated gene/protein-6 (TSG-6) levels, macroscopic laparotomy findings, and fluorescence microscopy.

RESULTS: Significant weight loss was observed in all the animals with colitis ($p<0.05$). Histopathological examination revealed less mucosal damage and greater healing in the intra-arterial group ($p<0.05$). Blood TSG-6 levels were also significantly higher in the intra-arterial group than in the other groups ($p<0.05$). Immunofluorescence microscopy showed that intra-arterially administered stem cells exhibited a more uniform and widespread distribution throughout the colon.

CONCLUSION: We believe that intra-arterially administered ATDSCs spread to the colon more effectively than locally administered ATDSCs, and that intra-arterial ATDSCs application may be a safe option for treating colitis with interventional angiographic techniques. Further experimental and clinical studies are needed to assess the efficacy of this treatment.

Keywords: Colitis; green fluorescent protein; stem cells.

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Immune, genetic, and environmental factors can cause inflammatory bowel disease (IBD). The incidence of this disease has increased in recent years and it affects the young population as a group of chronic inflammatory conditions. Environmental factors are thought to contribute to an abnormal immune response against normal intestinal flora in genetically predisposed individuals, leading to IBDs [1–3]. These chronic condition is classified as ulcerative colitis (UC) and Crohn's disease (CD) [1, 2]. Although the pathophysiology of IBD has not been fully elucidated, it is thought that the abnormal interaction between the patient and intestinal flora due to genetic and environmental factors, immunopathology, and microbiota differences leads to epithelial dysfunction and triggers inflammation with an abnormal immune response [4–7]. The inflammation-regulating effect of stem cells is known, and due to the healing effect of stem cells on colitis, studies on stem cells have been increasing in recent years [7–9].

The ease of isolating mesenchymal stem cells (MSCs), their self-renewal capacity, and their multipotency make them a promising option for stem cell studies and treatment [10–13]. MSCs, adipose tissue-derived stem cells (ATDSCs), and bone marrow-derived stem cells (BMDSCs) are the most commonly used [12–14]. MSCs are known for their immunomodulatory properties, positive healing effects, and angiogenesis, as shown in various studies. The immunomodulatory activity of stem cells can be assessed by measuring TNF-induced gene 6 (TSG-6) levels in the blood [15–19]. MSCs can be obtained from many tissues and applied to diseased areas using various techniques [20–22]. However, studies have identified some limitations associated with these application methods. Locally applied stem cells are not distributed homogeneously, whereas intravenously applied stem cells are dispersed systemically and cannot effectively reach the diseased area [22–24]. If it has been shown that ATDSCs can be administered intra-arterially to the diseased colon segment, and it may be possible to apply ATDSCs to the diseased intestine selectively, in low doses and without systemic spread, with interventional radiology in IBD patients [25–27]. Our study aims to compare the local and intra-arterial effects of ATDSCs in the colitis model induced by asetic acid (AA).

Highlight key points

- Intra-arterial administration of adipose-derived stem cells resulted in significantly better mucosal healing in the colitis model.
- Higher systemic TSG-6 levels were detected in the intra-arterial stem cell group, indicating stronger immunomodulatory activity.
- Stem cells administered intra-arterially showed more homogeneous and widespread distribution in colon tissue under fluorescence microscopy.
- Intra-arterial delivery of stem cells appears to be a feasible and effective method using interventional radiological techniques.

MATERIALS AND METHODS

The study was approved by the Research Board and the Local Ethics Committee for Animal Experiments with the decision numbered 22/13 dated 21/03/2022. This experimental study was conducted in accordance with the principles outlined in the Helsinki Declaration. Wistar-Albino male rats, 6–9 months old, weighing 300–450 grams (average 370 grams) were used.

Study Model

The animals were divided into five groups, with six rats per group. Six rats were used to harvest stem cells. Colitis was induced in all groups. To induce colitis, the bowels of all animals were emptied by stimulating the defecation reflex. A 1.5 mm thick, 6 cm long catheter was placed in the anal canal on a 45 degree inclined table and 1.5 cc of 4% AA was administered and waited for 20 seconds (Fig. 1). Group 1 was designated the control group. On day 7, 0.15 cc phosphate-buffered saline (PBS) solution without stem cells was injected into the intestinal wall of group 2 via laparotomy. Group 3 received 3x106 stem cells (in 0.15 cc PBS solution) into the intestinal wall on day 7 via laparotomy. On day 7, laparotomy was performed in group 4, both main iliac arteries were clamped (25–30 seconds), and 0.15 cc PBS was administered via cannulation of the inferior mesenteric artery (IMA) via 32 gauge needle. Group 5 received 3x106 stem cells in a similar manner on day 7. All animals were weighed on day 0, day 7, and day 14 using the Weightlab Instrument device, model number WI-6002. All groups were sacrificed on day 14. Blood was collected for TSG-6 level analysis prior to sacrifice. The sigmoid colon was removed and the colon wall was macroscopically evaluated. It was set aside for the pathological examination and tracking of stem cells under a fluorescence microscope. The macroscopic appearance of the colon wall was evaluated using the classification by Campos et al.[28]. Serum TSG-6 levels were measured using an ELISA assay. For histopathological evaluation, a pathologist was assigned to each group to microscopically evaluate mucosal

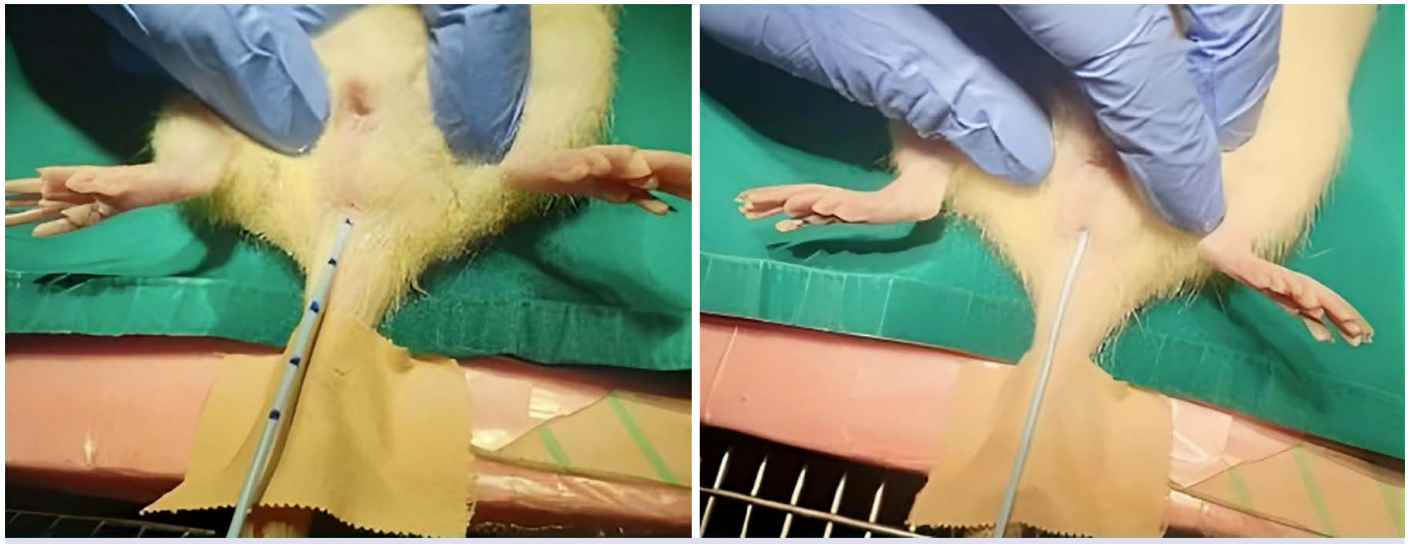

Figure 1. Application of 4% acetic acid via rectal enema.

structural loss, ulceration, inflammation severity, cryptitis, crypt abscess, depth of mucosal damage, and regeneration. Green fluorescent protein (GFP)-labeled stem cells were tracked using fluorescence microscopy. The pathologist was blinded as to which group of animals he was assigned.

Preparation and Transfection of Adipose-Derived Mesenchymal Stem Cells with Green Fluorescent Protein

To procure ATDSCs, tissues from 6 rats were used. A total of 90 g of fat tissue was obtained from both inguinal regions through a midline incision following anesthesia with intraperitoneal injection (0.04 mg/g ketamine and 0.005 mg/g xylazine) , according to the Ogawa protocol [29]. The harvested stem cells were prepared and suspended in PBS. The cells were transfected with GFP and seeded into 12 cell culture dishes for 12 animals at 6×10^5 cells per dish. Cells were incubated until 3×10^6 stem cells were obtained from each dish. ATDSCs were drawn into 1.5 cc syringes with PBS for preparation.

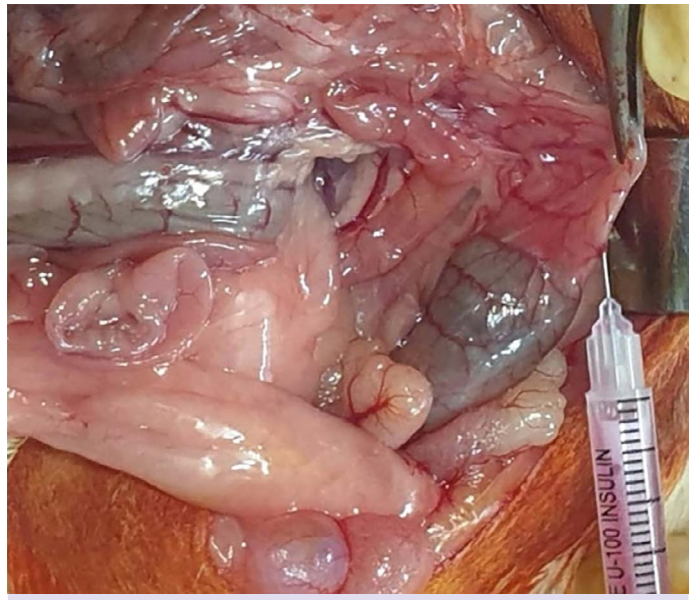

Figure 2. Local PBS/stem cell application to the sigmoid colon wall.

PBS: Phosphate-buffered saline.

Surgical Procedure

Groups 2 and 3 were anesthetized by intraperitoneal injection on day 7 and underwent laparotomy. A 1.5 cc solution of PBS, with and without stem cells, was injected into the sigmoid colon wall (Fig. 2). Groups 4 and 5 were anesthetized by intraperitoneal injection on day 7 and underwent laparotomy. Because the fragile IMA and cannulation were not feasible, both iliac arteries were temporarily clamped. A 32-gauge needle was used to cannulate the aorta, and a 1.5 cc solution of PBS, with and without ATDSCs, was administered over the IMA (Fig. 3). The iliac arteries were then released and hemostasis was achieved in the aorta. After the procedure, the midline fascia was closed with 3–0 polyglactin and the skin was closed with 5–0 propylene in all groups. 0.02 mcg/kg fentanyl was administered subcutaneously for analgesia. The animals were fed after awakening. On day 14, laparotomy was performed in all groups, the sigmoid colon was resected and sent for histological analysis, blood was collected intracardially, and the animals were sacrificed by cervical dislocation.

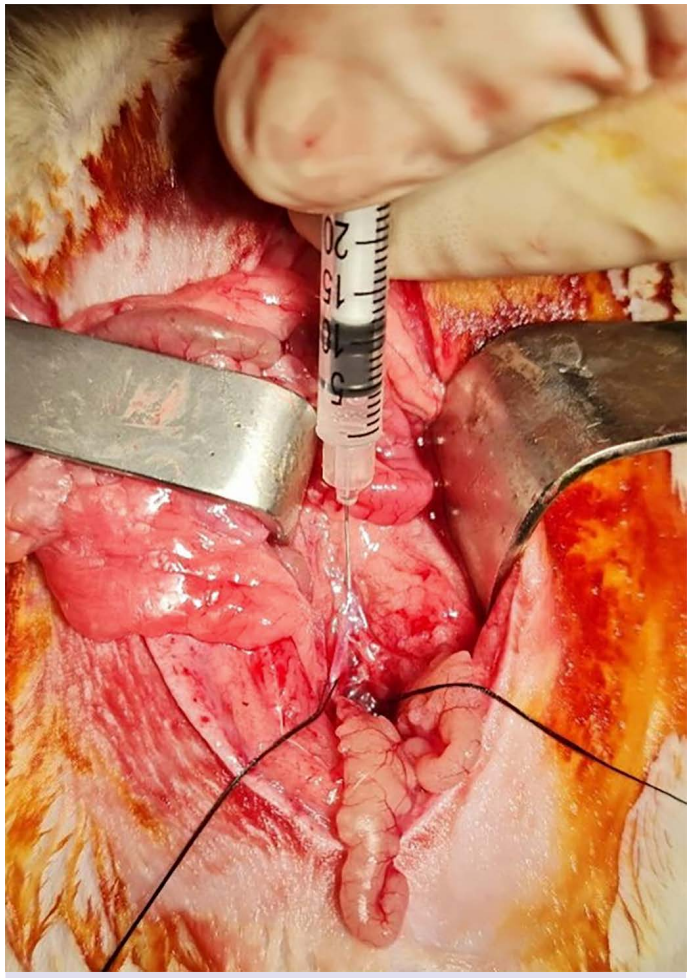

FIGURE 3. Returning both iliac arteries, cannulating the aorta, and applying stem cells/PBS.

PBS: Phosphate-buffered saline.

Statistical Analysis

Statistical analyses were performed using SPSS version 25.0 (IBM inc. Armonk, New York). The suitability of variables for normal distribution was examined using histogram graphics and the Shapiro-Wilk test. Descriptive analyses were performed using the mean, standard deviation, median, minimum, and maximum values.

The Kruskal–Wallis test was used for nonparametric variables when evaluating differences between more than two groups. Differences between groups were determined using the Bonferroni Method. The Friedman Test was used to determine time-dependent changes in the weights of the rats on day 0., 7., and 14. The Wilcoxon Signed-Rank Test was used to identify measurements that differed. The relationship between categorical variables was assessed using Fisher's Exact Test. Results were considered statistically significant when the p-value<0.05.

RESULTS

Evaluation of Weight Loss

In group 1, the weights of the rats on days 7th and 14th days were significantly lower than those on day 0 ($p<0.05$). Groups 1, 2, and 4 experienced significant weight loss on day 7. on day 14, the rats gained weight compared to that on day 7, but this increase was insignificant. Groups 3 and 5 experienced significant weight loss on day 7; however, by day 14, a significant weight gain was observed compared to day 7 (Table 1).

TABLE 1. Depending on the time, the weight change (in grams) of animals in each group

	Day 0	Day 7	Day 14	p
1	371.67±54.31	346±53.42	349.17±52.78	**0.009**
	384 (304-454)[a]	349 (276-424)[b]	354.5 (279-420)[b]	
2	343.67±25.88	324.5±23.99	325.5±28.65	**0.009**
	352.5 (303-372)[a]	331.5 (291-353)[b]	332.5 (290-360)[b]	
3	378.5±9.95	351.33±14.67	366±15.21	**0.002**
	380 (360-388)[a]	355 (325-366)[b]	370.5 (339-379)[c]	
4	357.33±19.93	332.5±23.26	335.17±25.47	**0.009**
	361 (333-385)[a]	336.5 (302-365)[b]	340 (299-369)[b]	
5	359.67±24.04	339±22.82	349±24.04	**0.006**
	355 (340-405)[a]	331.5 (320-380)[b]	350 (320-389)[c]	

Friedman Kruskal Wallis Test Letters indicate significant differences over time in each row, b, c. [Mean±SD |Median(Min.-Max.)]. SD: Standard deviation

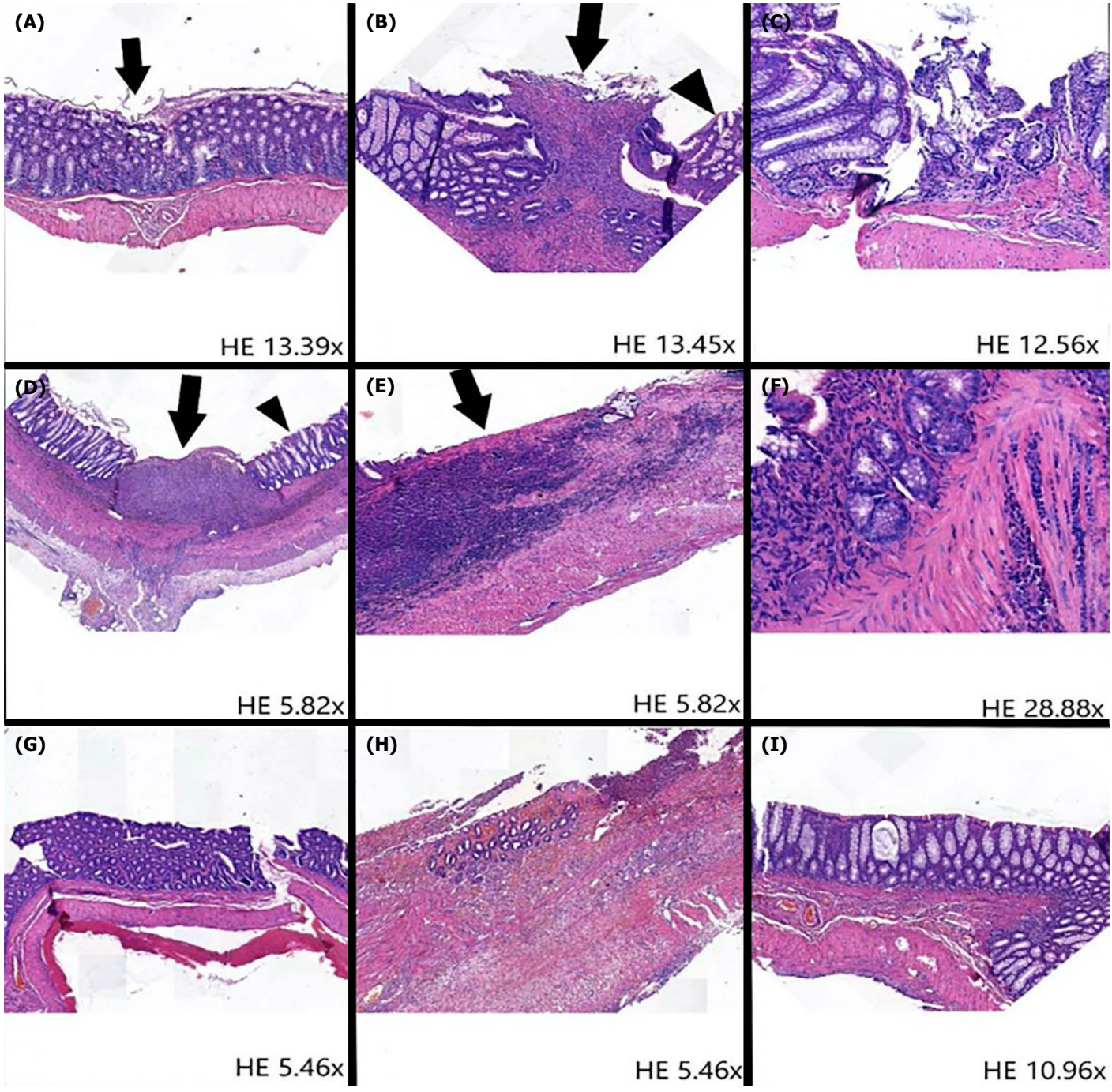

Figure 4. **(A)** Near complete regeneration (arrow indicates the area where regeneration is intense), **(B)** superficial ulceration (arrow) epithelialization starting from the edge (arrowhead), **(C)** mild mucosal loss and minimal inflammation, **(D)** superficial ulceration (arrow) moderate mucosal loss (arrowhead), **(E)** widespread mucosal loss (arrow points to the luminal side), **(F)** inflammation involving the mucosa and submucosa, **(G)** complete regeneration of epithelialization in the tissue accompanied by minimal inflammation, H: Full-thickness colon wall damage and full-thickness severe inflammation, I: Moderate inflammation characterized by inflammatory infiltration located in the mucosa and lamina propria.

Histopathological Evaluation

Mucosal loss was significantly lower in group 5 (Fig. 4). No significant relationship was found between the mucosal damage depth and regeneration among the experimental groups. The ulceration rate in group 1 was significantly higher. The absence of crypt abscesses was more

TABLE 2. Relationship between Group and Pathology variables

	Group 1		Group 2		Group 4		Group 3		Group 5		p
	n	%	n	%	n	%	n	%	n	%	
Mucosal loss											
None	–	–	–	–	1	16.67	1	16.67	5	83.33	**0.039**
Mild	4	66.67	4	66.67	1	16.67	3	50.00	–	–	
Moderate	1	16.67	1	16.67	1	16.67	1	16.67	1	16.67	
Severe	1	16.67	1	16.67	3	50.00	1	16.67	–	–	
Ulceration											
None	2	33.33	5	83.33	3	50.00	4	66.67	5	83.33	0.447
Yes	4	66.67	1	16.67	3	50.00	2	33.33	1	16.67	
Depth of mucosal damage											
None	–	–	1	16.67	1	1.67	1	16.67	–	–	0.837
Mucosa	2	33.33	4	66.67	2	33.33	1	16.67	3	50.00	
Submucosa	3	50.00	1	16.67	1	16.67	3	50.00	2	33.33	
Trasnmural	1	16.67	–	–	2	33.33	1	16.67	1	16.67	
Inflammation											
None	–	–	1	16.67	–	–	–	–	–	–	0.611
Mild	3	50.00	2	33.33	1	16.67	4	66.67	4	66.67	
Moderate	2	33.33	3	50.00	3	50.00	1	16.67	2	33.33	
Severe	1	16.67	–	–	2	33.33	1	16.67	–	–	
Regeneration											
None	–	–	–	–	–	–	–	–	–	–	0.539
Epithelization starting from the edge	1	16.67	–	–	2	33.33	2	33.33	1	16.67	
There is epithelization, and there is crypt loss	1	16.67	1	16.67	–	–	1	16.67	–	–	
Almost complete epithelization	3	50.00	4	66.67	1	16.67	1	16.67	1	16.67	
Complete epithelization	1	16.67	1	16.67	3	50.00	2	33.33	4	66.67	
Cryptitis											
None	1	16.67	4	66.67	3	50.00	3	50.00	3	50.00	0.587
Yes	5	83.33	2	33.33	3	50.00	3	50.00	3	50.00	
Crypt Abscess											
None	6	100.0	6	100.0	6	100.0	5	83.33	3	50.00	0.090
Yes	–	–	–	–	–	–	1	16.67	3	50.00	

Fisher's Exact Chi-square test

likely in Group 5 compared to the other groups; however, this difference was not statistically significant (Table 2).

Laparotomy Findings

No significant differences were found between the groups in terms of edema of the colon and peritonitis. The proportion of normal macroscopic mucosa in group 5 was significantly higher than that in groups 1, 2, and 4. No hematomas occurred in any animal. Peritonitis on day 14 significantly increased in groups 2 and 4 (Table 3).

Fluorescence Microscope Findings

GFP-labeled ATDSC could be selected in the muscle layer, submucosa, and mucosa (Fig. 5). When examining the local ATDSCs group, it was observed that GFP-labeled ATDSCs had a heterogeneous distribution. The administered ATDSCs generally penetrated the tissue in areas close to the needle entry points, whereas areas far from the needle entry were poor in ATDSCs. Intraarterially administered ATDSCs spread diffusely and homogeneously (Fig. 6).

Table 3. Relationship between experimental groups and day 14 laparotomy findings

	1		2		4		3		5		p
	n	%	n	%	n	%	n	%	n	%	
Edema in the colon											
None	–	–	–	–	–	–	–	–	–	–	0.975
Mild	4	66.67	4	66.67	4	66.67	3	5.00	5	83.33	
Moderate	2	33.33	2	33.33	2	33.33	3	50.00	1	16.67	
Severe	–	–	–	–	–	–	–	–	–	–	
Peritonitis											
None	2	33.33	2	33.33	3	5.00	3	50.00	4	66.67	0.917
Yes	4	66.67	4	66.67	3	50.00	3	50.00	2	33.33	
Ischemia											
None	6	100.0	6	100.0	5	83.33	6	100.0	5	83.33	1.000
Yes	–	–	–	–	1	16.67	–	–	1	16.67	
Macroscopic Mucosa											
Normal	–	–	–	–	–	–	2	33.33	5	83.33	**0.009**
Erythematous areas	3	50.00	4	66.67	5	83.33	3	50.00	–	–	
Linear ulcer	3	50.00	2	33.33	1	1.67	1	16.67	1	16.67	
Ulcer between 15–45 mm	–	–	–	–	–	–	–	–	–	–	
Ulcer over 45 mm	–	–	–	–	–	–	–	–	–	–	

Fisher's Exact Chi-square test

Blood TSG-6 Level

The TSG-6 blood levels in groups 1 and 5 were significantly higher than those in groups 3 and 4 ($p<0.05$).

DISCUSSION

To our knowledge, no other study has compared the local and intra-arterial effects of ATDSCs in an AA-induced colitis model. IBDs are thought to arise due to an abnormal communication disorder between the immune system and mucosal epithelium [2–4]. The primary pathological process of IBD is inflammation. Inflammation was induced using various models, and experimental studies were conducted [4]. Many parameters, such as submucosal edema, cryptitis, crypt abscess, inflammatory cell density, and goblet cell loss, have been examined microscopically in animals with colitis [20–22]. Based on these studies, we also created the colitis model with AA to evaluate mucosal structure loss, ulceration, the severity of inflammation, cryptitis, crypt abscess, depth of mucosal damage, and regeneration.

The number of treatment methods has increased over the years, and studies on IBD have continued. Stem cell studies have been increasing in recent years owing to the healing effect of stem cells on colitis. The regeneration and differentiation abilities of stem cells distinguish them from those of other cells. Stem cells produce these effects through resistance to apoptosis, the release of growth factors and hormones, and an increase in the microvascular structure [19, 21, 30]. MSCs can be obtained from various tissues, such as the umbilical cord, endometrial polyps, bone marrow, and fat tissue [11–13]. MSCs are used in many fields, such as tissue engineering, immunosuppression, and cell and gene therapies [13, 14, 23, 26]. MSCs, ATDSCs, and BMDSCs are the most commonly used for this purpose [14, 26]. ATDSCs stimulate macrophage immunity and induce angiogenesis by inhibiting T cells and dendritic cells. In addition, these cells reduce apoptosis and trigger anti-inflammatory responses [26]. Because ATDSCs are more accessible than BMDSCs and have differentiating capacities, they have been used in many studies, and their effects and benefits have been demonstrated [26, 31, 32]. For these reasons, we preferred ATDSCs in our study.

Weight loss during the active phase of the disease is evident in IBD patients [2–4]. The animals in our study experienced significant weight loss by day 7. We

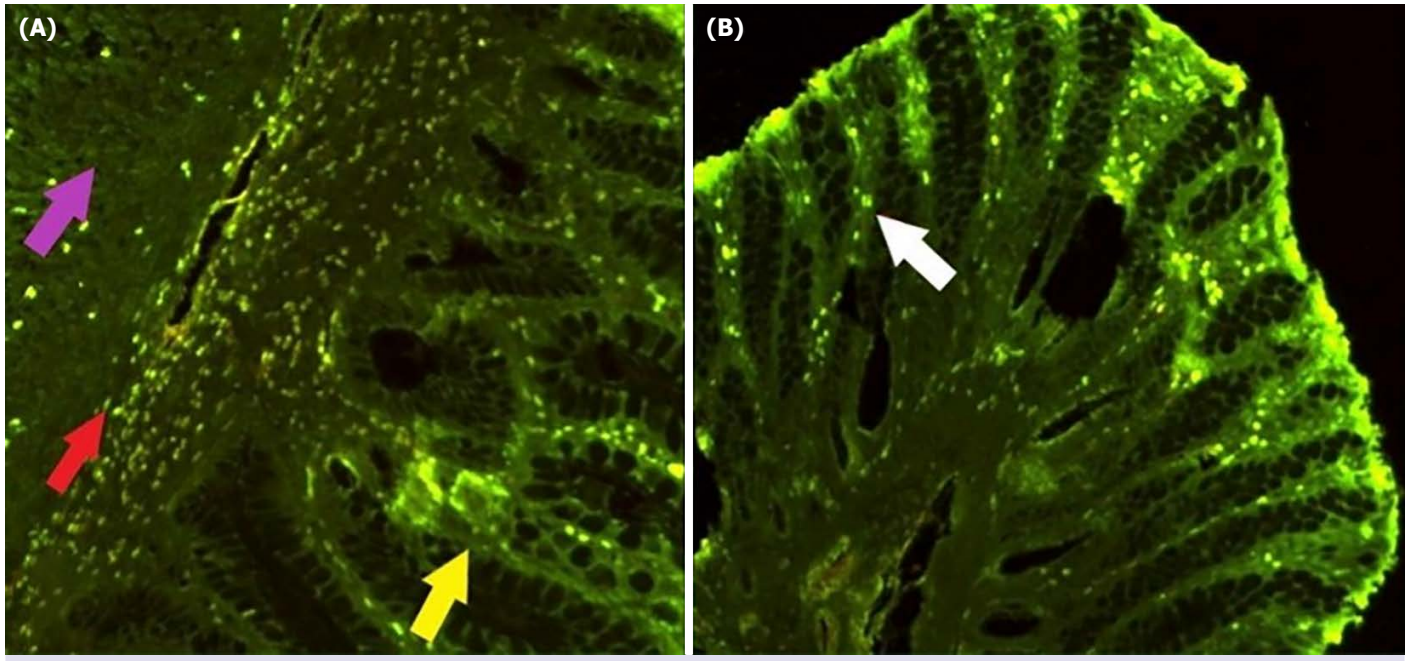

FIGURE 5. **(A)** Muscle layer (purple arrow), submucosa and infiltrating GFP-labeled stem cells (red arrow), GFP-labeled stem cells between epithelial cells (yellow arrow), **(B)** fluorescence under the microscope due to GFP between epithelial cells (white arrow).
GFP: Green fluorescent protein.

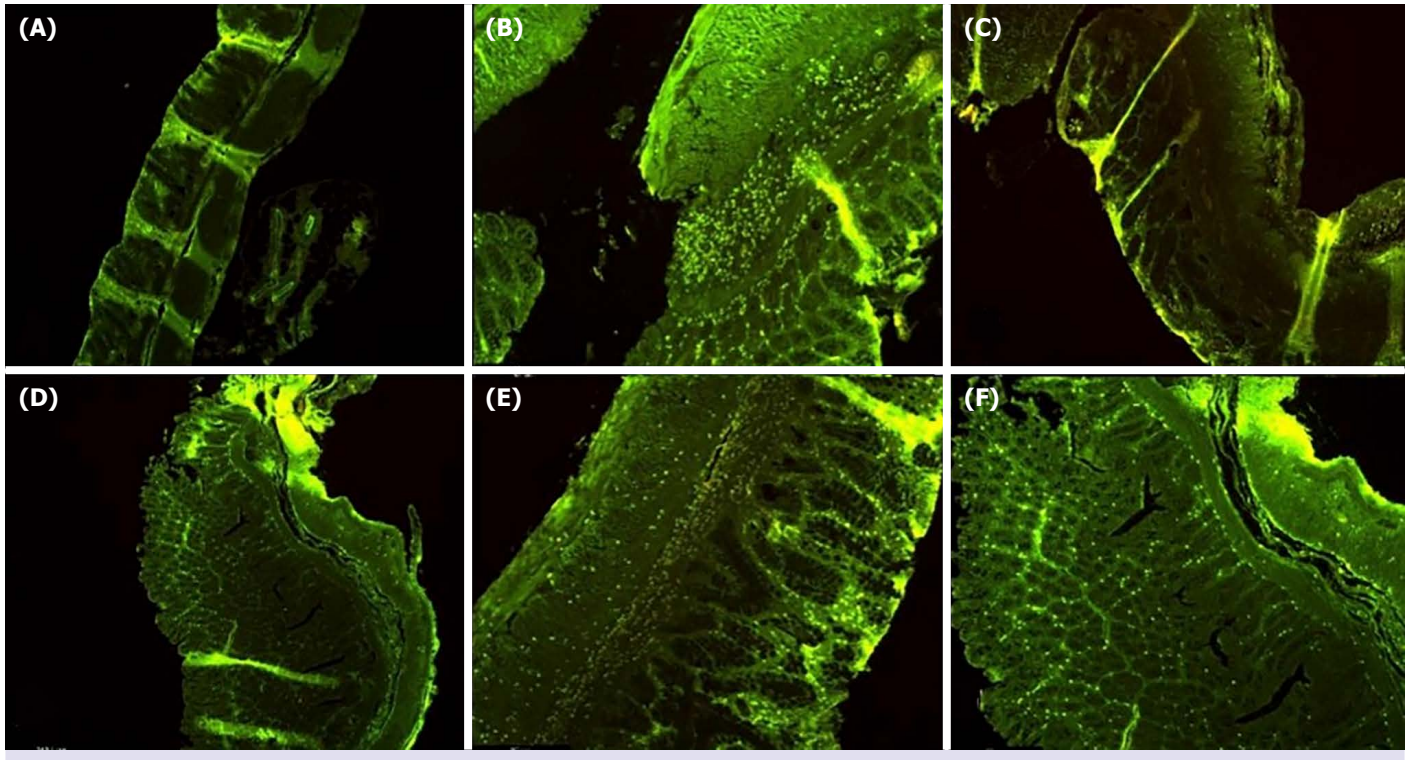

FIGURE 6. **(A)** In the local stem cell group, stem cells cannot be detected in some areas (heterogeneous distribution), **(B)** in the local stem cell group, it can be seen that the stem cells are concentrated in the injection site, **(C)** heterogeneous distribution can be detected in the local stem cell group, **(D-F)** in the mucosa in the intra-arterial group, stem cells distributed homogeneously and in high density between the submucosa and the muscle layer can be selected.

consider this to be a symptom of colitis that occurs in all animals. Although not significant, an increase in weight was observed on day 14 compared with day 7 in the groups that received stem cell application. We attributed the lack of significance in this weight gain to the short follow-up period.

TSG-6 is believed to play a key role in the immunomodulatory and protective effects of MSCs [33, 34]. In the experimental colitis model, colitis was decreased in rats administered TSG-6 [34]. A study showed that this is due to the diffuse and homogeneous application of stem cells to the diseased area and the faster adaptation of stem cells delivered via the vascular axis for immunomodulation [35]. In a study by Yang et al.,[36] they reported that TSG-6 regulates the migration of leukocytes, lymphocyte adhesion, and endothelial migration stimulated by chemokines and also increases the anti-inflammatory effect by regulating the cyclooxygenase enzyme. In our study, TSG-6 levels were significantly higher in the group that received intra-arterial ATDSCs. We believe that this is because ATDSCs were applied diffusely and homogeneously to the diseased area, and ATDSCs via the vascular axis adapted more quickly for immunomodulation. TSG-6 levels were also higher in the colitis control group than in the other groups. We suggest that the animals in this group did not undergo laparotomy on day 7, which affected the TSG-6 level. Tissue damage and immunosuppression caused by surgery may be suppressed by TSG-6.

The absence of ischemia during laparotomy and the absence of hematoma and death due to bleeding in any animal indicates that the intra-arterial ATDSCs technique does not lead to any complications.

Histopathological evaluation showed significantly less mucosal loss in the intra-arterial group. However, there were no differences in other parameters between the groups. Although not statistically significant, the intra-arterial group exhibited reduced ulceration and mucosal damage depth compared to the control group, with twice as many animals showing full-thickness regeneration.

Fluorescence microscopy confirmed that the locally administered ATDSCs reached the diseased area. However, multiple injections into the colon wall are required, and we believe that trauma caused by the needle leads to a secondary traumatic process in the colon. Additionally, the dense concentration of ATDSCs around the injection site and the sparse distribution in areas far from the site indicate a heterogeneous distribution. In the intra-arterial ATDSCs group, it was observed that the ATDSCs administered from the IMA spread throughout the entire sigmoid colon. This distribution was predominantly homogeneous in the submucosa where the vascular network was dense. This technique showed that ATDSCs were distributed effectively and at high concentrations throughout the colon.

Our study has certain limitations. The use of an animal model may limit the generalizability of the findings to human applications. Additionally, our study is restricted to short-term effects; further research is needed to evaluate the long-term safety and efficacy of stem cell therapy.

CONCLUSION

This study showed that intra-arterial ATDSC administration histologically reduced mucosal loss and significantly increased mucosal healing. Although not significant, the severity of inflammation was lower and the regeneration capacity was higher. It showed that intra-arterial ATDSC application does not cause ischemia and hematoma, and that intra-arterial ATDSC injection can be an effective and safe treatment method for IBD, depending on the affected part of the colon. ATDSC administration through interventional angiography in humans by cannulating the colic arteries could be a permanent and effective treatment method. Further experimental and clinical studies are required to validate this treatment method.

Ethics Committee Approval: The Baskent University Research Board and the Local Animal Experiments Ethics Committee granted approval for this study (date: 21.03.2022, number: 22/13).

Informed Consent: Written informed consent was not applicable as this was an experimental animal study.

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

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Original Article CARDIOVASCULAR SURGERY

Effects of CAPE on biochemical, histopathological and cardiac parameters in doxorubicin induced cardiotoxicity

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ABSTRACT

OBJECTIVE: In this study, the protective effect of Caffeic acid phenethyl ester (CAPE) against doxorubicin (DOX)-induced cardiotoxicity was investigated by evaluating oxidative stress parameters, ECG changes, *matrix metalloproteinase 2* (*MMP-2*) gene expression, troponin I level and histopathology in Wistar Albino rats.

METHODS: Forty rats were divided into 4 groups (n=10) including control (saline (vehicle for DOX) and 2.5% ethanol (vehicle for caffeic acid phenethyl ester), CAPE only (10 μmol/kg bw), DOX only (10 mg/kg bw) and CAPE+DOX groups. Molecular, biochemical and histopathological analyses were performed on blood and heart tissues.

RESULTS: No alterations were observed in oxidative stress parameters and *MMP-2* gene expression of DOX and CAPE+DOX groups compared to control. Troponin I levels were higher in DOX and CAPE+DOX groups than in the control. Variable ECG changes were observed in the experimental groups such as increased systolic blood pressure, decreased QRS and QT interval in DOX group compared to the control without any ameliorative effect of CAPE. The presence of dense degenerative cardiomyocytes in the myocardium of the DOX group was noted. DOX caused damage to cardiomyocytes. It was observed that CAPE showed a significant decrease in histopathological changes and histopathological scoring in the CAPE+DOX group compared to DOX group.

CONCLUSION: CAPE treatment ameliorated histopathological changes induced by DOX while other parameters including oxidative stress, *MMP-2* gene expression, Troponin I and ECG studied in our study were not altered remarkably.

Keywords: CAPE; cardiotoxicity; doxorubicin; rat.

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Doxorubicin (DOX), an antineoplastic agent used in cancer treatment, is obtained from the bacterium *Streptomyces peucetius*. This antineoplastic agent exhibits remarkable efficacy in the treatment of various malignancies, encompassing leukemia, lymphomas, breast carcinoma, uterine carcinoma, pulmonary carcinoma, soft tissue sarcomas, and solid neoplasms [1–4]. Anthracycline derivatives such as DOX, epirubicin, idaru-

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bicin, daunorubicin, and mitoxantrone are employed in clinical practice, with DOX being the most extensively utilized. Although the cytotoxic effects of DOX are different, it mostly causes cardiotoxicity and hepatotoxicity [2, 5]. Despite their highly beneficial effects against cancer, anthracyclines have been reported to show severe cardiotoxicity in clinical use. Anthracyclines cause both acute and chronic cardiotoxicity, which is an important problem limiting the use of these drugs. Acute cardiotoxicity also presents with rhythm problems, hypotension and impaired cardiac function. DOX-associated chronic cardiotoxicity has been shown to lead to progressive left ventricular dysfunction and, in some cases, can result in fatal congestive heart failure [6, 7]. Reports indicate that patients administered DOX and its derivatives may suffer from cardiac complications for many years post-chemotherapy [8]. The use of DOX causes loss of contractility, heart rate (HR) and blood pressure differences. It has also been shown that DOX cardiotoxicity causes vacuolization, sarcomere damage, microtubule damage, loss of myofibrils, mitochondrial damage, and disruption of myocyte structure [9, 10]. Research suggests that DOX-induced cardiotoxicity may stem from a range of mechanisms, including endoplasmic reticulum-mediated apoptosis, damage to DNA/RNA, disruption of calcium homeostasis and autophagy, oxidative stress, and lipid peroxidation [11–13]. The hypothesis that doxorubicin is a mitochondrial toxin has been postulated on the basis that the drug induces cardiac dysfunction and cell death by damaging the mitochondrial membrane. To survive and contract, cardiomyocytes need large numbers of healthy and functional mitochondria that can generate adequate ATP. Overproduction of DOX-induced free radicals is predicted to cause various molecular damage, leading to cardiomyocyte toxicity and cell death [14, 15]. Due to free radicals causing doxorubicin-induced toxicity, the use of various antioxidants and substances with antioxidant properties on DOX toxicity has been studied previously [12, 16, 17].

Matrix metalloproteinases (*MMPs*) are endopeptidases that facilitate the degradation of structural components of the intercellular matrix, including laminin, collagen, and proteoglycan [18]. *MMP* activation, particularly *MMP-2* activation, has been reported to be an acute marker of DOX-induced cardiotoxicity. Structural dysregulation of the cardiac extracellular space and increased expression of *MMP-2* and *MMP-9* have been described in doxorubicin cardiotoxicity [19–21]. Therefore, they can be used as degeneration markers in heart tissue.

Highlight key points

- Doxorubicin administration did not cause any change in some oxidative stress parameters and MMP-2 gene expression.
- Variable ECG changes such as increased systolic blood pressure and decreased QRS and QT intervals were seen with doxorubicin administration and this was not affected by CAPE.
- CAPE administration with doxorubicin reduced doxorubicin-induced histopathological changes and scoring.

Caffeic acid phenethyl ester (CAPE) is a potent phenolic compound with anti-inflammatory, antioxidant, and antineoplastic properties, derived from plant extracts gathered by bees [22–24]. The high cell permeability of CAPE is attributable to its phenolic nature and the presence of an ester bond, which facilitates its entry into the cell. Thereafter, it is subject to breakdown by intracellular esterases, resulting in the release of effective caffeic acid. CAPE has various potent therapeutic activities by inducing apoptosis and is currently utilized in the treatment of different disorders [25].

CAPE has been shown to reduce the toxicity of chemotherapy by suppressing free radicals [26] and inhibiting NF-κβ activation [27]. CAPE has been demonstrated to inhibit the 5-lipoxygenase-mediated oxygenation of linoleic acid and arachidonic acid [23]. It has been reported that CAPE reduces DOX-induced damage to the heart muscle by regulating the dysfunction in the endoplasmic reticulum and by helping proteins to fold correctly [28]. Karaboga [29] demonstrated that CAPE prevents tissue damage by reducing NF-κβ and inducible nitric oxide synthase inflammatory mediators. It has been demonstrated that the biochemical and histological alterations in tissues generated by cisplatin's oxidative and nitrosative damage are partially prevented by CAPE [30].

In this study, the mechanisms of the possible protective effects of CAPE in the prevention of cardiotoxicity induced by doxorubicin were evaluated. These mechanisms were investigated by studying *MMP-2* gene expression, changes in Troponin I, electrocardiography (ECG) alterations, oxidative stress parameters, tissue damage, and histopathology.

MATERIALS AND METHODS

The present study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, as well as the institutional guidelines for the use of an-

Table 1. Primer sequences and expected product size for *MMP-2* and *β-actin*

Genes	Primer sequences	NCBI reference sequence	PCR product size (bp)
β-Actin	F: 5′-CTGGCTCCTAGCACCATGA-3′ R: 5′-TAGAGCCACCAATCCACACA-3′	NM_031144.3	76
MMP-2	F: 5′-CACCACCGAGGATTATGACC-3′ R: 5′-CACCCACAGTGGACATAGCA-3′	NM_031054.2	71

β-Actin: Beta Actin; MMP-2: Matrix metalloproteinase-2; F: Forward, R: Reverse; NCBI: The National Center for Biotechnology Information; PCR: Polymerase Chain Reaction; bp: base pair

imals in research. This commitment to animal welfare is evidenced by the approval of the research protocol by the Inonu University Animal Experiments Local Ethics Committee on 06/01/2022 (approval no: 2022/1-2). Rats were kept in at 21°C, 55–60% humidity, on a 12 h light/12 h dark cycle and divided into 4 groups randomly (n=10). Saline (vehicle for DOX) and 2.5% ethanol (vehicle for caffeic acid phenethyl ester (CAPE, Lot. No: 1016880, Bachem-Switzerland) were administered to the control group, while CAPE at 10 µmol/kg body weight (bw) was applied to the CAPE group intraperitoneally (ip) for 10 days. Doxorubicin (Adriamycin-Deva Holding A.Ş.-Turkey) at 10 mg/kg bw was given ip to DOX group for the last 3 days (a total of 30 mg/kg). In CAPE+ DOX group, CAPE at 10 µmol/kg body weight was given ip for 10 days, and DOX at 10 mg/kg bw was applied for the last 3 days of the study. Rats were assessed for mean arterial pressure (MAP) and heart rate (HR) on the final day of the experiment while under anesthesia induced by 1.2 g/kg of urethane (Ethyl carbamate, CAS: 51-79-6). Under anesthesia, ECG signal activity was monitored for a duration of at least one minute using disposable electrodes on the rat's thorax. Furthermore, high-precision measures of duration and variations in pulse rate, PR, QRS, and QT interval variability across groups were also examined.

Tissue and Sample Collection

The rats were sacrificed by exsanguination under xylazine/ketamine anesthesia. Serum samples were saved at -80 °C for biochemical analyses. The hearts were collected and bisected longitudinally to obtain the atrial and ventral cardia. One half was chopped into small pieces and placed in RNA saving solution and then saved at -80 °C for molecular analyses or freshly used for biochemical analyses. The remaining half was preserved for histological analysis in 10% neutral buffered formalin.

mRNA Expressions of *MMP-2*

Total RNA was extracted from heart tissue using the "EasyPure® RNA Kit" (Trans, Lot no: L41223). Subsequently, cDNA synthesis was carried out using the "Evoscript Universal cDNA Master Kit" (Roche, Ref no: 07912439001). RT-qPCR analysis was subsequently performed on a real-time PCR machine (Roche) using the "Fast Start Essential DNA Probes Master Kit (Ref no: 06402682001)" and the "Real Time Ready Assay "*MMP-2* (Config no: 100143836.)" and "*β-Actin* (Config no: 100129896.)" (Table 1). The PCR protocol followed these conditions: initial denaturation at 95°C for 10 min, followed by 55 cycles consisting of 10 s of denaturation at 95°C and 30 s of annealing at 60°C, and concluding with a final extension at 72°C for 1 s. The $2-\Delta\Delta Ct$ technique was used to calculate mRNA expressions [31].

Heart Biochemical Analyses

Tissue samples were homogenized, and the subsequent homogenates were used to measure the levels of Glutathione (GSH), Malondialdehyde (MDA), Catalase (CAT), and Copper Zinc Superoxide Dismutase (CuZn-SOD). GSH levels were measured using Ellman's method (1961), with results expressed as nmol/g wet tissue (gwt) [32]. MDA levels were quantified at 535 nm/520 nm, then expressed as nmol/gwt [33]. CAT activity in heart tissue samples was measured at 240 nm and expressed as K/g protein [34]. CuZn-SOD activity was detected using the Sun et al. [35] method and read spectrophotometrically at 560 nm. Results were expressed as U/g protein.

Quantification of Troponin in Serum

Concentrations of Troponin-I were then quantified using the Elabscience troponin-I type-3 ELISA assay

Table 2. Group comparison findings on biochemical analyses for the heart

Parameters*	Groups**				p
	CAPE (n=10)	CAPE+DOX (n=10)	DOX (n=10)	Control (n=10)	
MDA nmol/gwt	46.79[a] (8.35)	46.92[a] (4.46)	50.23[a] (2.10)	47.68[a] (3.69)	0.435
GSH nmol/gwt	1456.87[a] (192.93)	1320.37[ab] (148.31)	1294.12[ab] (568.31)	1197[b] (326.81)	0.045
CuZn SOD U/g protein	652.52[a] (116.52)	616.47[a] (58.88)	645.99[a] (40.18)	649.21[a] (102.35)	0.929
CAT K/g protein	37.66[a] (4.91)	35.06[a] (11.28)	33.95[a] (9.36)	29.82[a] (7.69)	0.218
MMP-2 gene expression	0.002[a] (0.023)	0.002[a] (0.068)	0.004[a] (0.005)	0.016[a] (0.026)	0.425
Troponin I pg/ml	275.23[a] (102.23)	524.462[b] (248.75)	461.577[b] (175)	217.538[a] (106.442)	0.011

*: Data are summarized as median (interquartile range); **: There is a statistically significant difference in group categories that do not contain the same letter in each line. CAPE: Caffeic acid phenethyl ester; DOX: Doxorubicin; MDA: Malondialdehyde; gwt: Gram wet tissue; GSH: Glutathione; CuZn SOD: Copper Zinc Superoxide Dismutase; CAT: Catalase; *MMP-2: Matrix Metalloproteinase-2*.

(E-EL-R1253, lot no. KL17H68T9583). The reaction yielded a yellow-colored product. Its proportionality to the Troponin-I present in the sample was then determined. The 96-well plate was read at OD 450 nm at room temperature. Results were expressed as pg of cTnI per milliliter of total protein.

Histopathological Analysis

The heart samples were fixed in 10% formalin, dehydrated using alcohols, cleared in xylene and embedded in paraffin. Myocardial damage was evaluated for interstitial oedema and cardiomyocyte degeneration (eosinophilic cytoplasm, vacuolization, and pyknotic nuclei) in various heart areas with hematoxylin and eosin (H-E).

Statistical Analysis

From the calculations, it was determined that a minimum of 7 subjects per group was required to detect a statistically significant difference, with a predicted effect size of 0.05 Type I error (alpha), 0.8 test power (1-beta) and 1.80 GSH (nmol). Therefore, a total sample size of at least 28 is necessary. The Shapiro-Wilk test was employed to evaluate the conformity of the quantitative data to the normal distribution. However, the data did not demonstrate a normal distribution; consequently, they were presented with a median and interquartile range. The Kruskal-Wallis H test was utilized for intergroup comparison of data, and the Conover test was employed for post-hoc analysis. All analyses were performed using IBM SPSS Statistics 28.0 for Windows (New York; USA).

RESULTS

A statistically significant difference was observed between the groups with regard to GSH levels ($p<0.05$). However, no such difference was observed for MDA, Cu-Zn SOD and CAT ($p>0.05$). GSH tissue levels were higher in the CAPE group ($p<0.05$), but not in the other groups ($p>0.05$). Table 2 provides a comprehensive overview of the alterations in biochemical and molecular analyses of the heart. The study found no significant difference between the groups in terms of *MMP-2* gene expression ($p>0.05$). Furthermore, a significant statistical difference in Troponin I levels (pg/ml) was detected among the groups ($p<0.05$). No significant difference was observed between the CAPE+DOX and DOX groups ($p>0.05$), but Troponin I levels were higher in the CAPE+DOX and DOX groups compared to the Control and CAPE groups ($p<0.05$).

Table 3 shows a statistically significant difference between the groups in terms of HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), QR, QRS and QT waves ($p<0.05$). The CAPE+DOX group had a higher HR than the CAPE group ($p<0.05$). However, there were no statistically significant differences in the other groups ($p>0.05$). The SBP was higher in the DOX group than in the CAPE and control groups ($p<0.05$). However, there was no significant difference between the DOX and CAPE+DOX groups ($p>0.05$). The DBP increased in the CAPE+DOX group compared to the CAPE and Control groups ($p<0.05$). There were no significant differences between the DOX and CAPE+DOX groups or between DOX and the control group ($p>0.05$). The MBP was

TABLE 3. Electrocardiographic changes in the treatment groups

Parameters*	Groups**				p
	CAPE (n=10)	CAPE+DOX (n=10)	DOX (n=10)	Control (n=10)	
HR (beats/min)	234[a] (45.5)	296.5[b] (65.75)	251[ab] (45.75)	258[ab] (29.25)	0.048
SBP (mm-Hg)	86[a] (4)	101.5[b] (8.25)	102.5[b] (7.75)	84.5[a] (13.5)	<0.001
DBP (mm-Hg)	45.5[a] (9.75)	72.5[b] (13.75)	70.5[bc] (10.25)	60.5[c] (15.25)	<0.001
MBP (mm-Hg)	57.5[a] (15.25)	85.5[b] (10.25)	85.5[b] (8.5)	73[c] (14)	<0.001
PR interval (ms)	46[a] (8.5)	45[a] (5)	48[ab] (5)	52[b] (5)	0.018
QRS duration (ms)	53[a] (15.5)	46[b] (3.5)	44[b] (5.5)	66[a] (28)	<0.001
QT interval (ms)	92[a] (5.5)	86[bc] (6.5)	81[b] (4)	90[ac] (27)	0.007

*: Data are summarized as median (interquartile range); **: There is a statistically significant difference in group categories that do not contain the same letter in each line. CAPE: Caffeic acid phenethyl ester; DOX: Doxorubicin; HR: Heart rate; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MBP: Mean blood pressure

significantly elevated in the CAPE group compared to the other two groups ($p<0.05$), yet no significant difference was observed between the CAPE+DOX and DOX groups ($p>0.05$). The PR interval was higher in the control group than in the CAPE+DOX and CAPE groups ($p<0.05$). No significant difference was observed between DOX and the other groups ($p>0.05$). The QRS interval increased significantly in the control group compared to the CAPE+DOX and DOX groups ($p<0.05$), while no significant difference was observed between the control-CAPE and CAPE+DOX-DOX groups ($p>0.05$). In addition, the QRS interval was significantly higher in the CAPE group than in the CAPE+DOX and DOX groups ($p<0.05$). The QT interval was significantly increased in the CAPE group compared to the CAPE+DOX and DOX groups ($p<0.05$), whereas no significant differences were observed among the Control-CAPE, Control-CAPE+DOX, and CAPE+DOX-DOX groups ($p>0.05$). There was no arrhythmia in the subjects in the groups (100%). ST depression was only seen in 1 (10%) subject in the DOX group. ST elevation was seen in 7 (70%) subjects in the DOX group, in 2 (20%) subjects in the CAPE group, and in 3 (30%) subjects in the CAPE+DOX group. T negativity was seen in 1 (10%) subject in each of the DOX, CAPE and CAPE+DOX groups. While block was observed in 4 (40%) subjects in the control group, it was not observed in the other groups. Table 4 shows the descriptive statistics regarding the qualitative variables in the ECG.

The myocardium in the control and CAPE groups exhibited a typical normal histological structure, as depicted in Figures 1a and 1b. Normal cardiomyocytes with eosinophilic cytoplasm and euchromatic nuclei were seen in the cardiac tissue of these groups. A thin connective tissue (the interstitial tissue) was seen between cardiomyocytes. However, the presence of intensely degenerative cardiomyocytes was marked in the myocardium of the DOX group. In the DOX group, more edematous interstitial connective tissue was also seen between cardiomyocytes (Fig. 1c). The histopathological alterations that occurred in the DOX group were clearly reduced in the CAPE+DOX group (Fig. 1d).

DISCUSSION

Oxidative damage plays a significant role in DOX toxicity. It has been reported that DOX increases lipid peroxidation in plasma by causing excessive production of naturally occurring ROS in the cellular environment [36]. Yarmohmmadi et al. [37] reported that DOX causes cardiomyopathy by inducing cardiac dysfunction, increasing the levels of oxidative stress products, proinflammatory cytokines and proapoptotic factors. Oxidative stress is described as an oxidative balance disorder in which the balance between the increase in ROS formation and the antioxidant system that detoxifies them is disrupted in favor of reactive species [38]. During oxidative stress, reactive species alter and destroy various intracellular components such as DNA, RNA, lipids, and proteins. This occurs when the balance between pro-oxidants and antioxidants shifts in favor of oxidants. Biological membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid (DNA) can all be adversely impacted by the

Table 4. Descriptive statistics for qualitative data on ECG

Parameters	Category	Control		DOX		CAPE		CAPE+DOX	
		n	%	n	%	n	%	n	%
Arrhythmia	Absent	10	100.00	10	100.00	10	100.00	10	100.00
	Exist	0	0.00	0	0.00	0	0.00	0	0.00
ST depression	Absent	10	100.00	9	90.00	10	100.00	10	100.00
	Exist	0	0.00	1	10.00	0	0.00	0	0.00
ST elevation	Absent	10	100.00	3	30.00	8	80.00	7	70.00
	Exist	0	0.00	7	70.00	2	20.00	3	30.00
T negativity	Absent	10	100.00	9	90.00	9	90.00	9	90.00
	Exist	0	0.00	1	10.00	1	10.00	1	10.00
Block	Absent	6	60.00	10	100.00	10	100.00	10	100.00
	Exist	4	40.00	0	0.00	0	0.00	0	0.00

ECG: Electrocardiography; DOX: Doxorubicin; CAPE: Caffeic acid phenethyl ester

damaging process of oxidative stress [39, 40]. Because membrane phospholipids are key targets of ROS, lipid peroxidation is a vital indicator of oxidative damage [41]. MDA is commonly used to evaluate lipid peroxidation and oxidative stress levels [42]. The findings of present study indicate that there is no alteration in MDA levels among the groups suggesting that no lipid peroxidation is present in the cardiac tissues of DOX -treated group. Antioxidants inhibits oxidative damage to the target molecules by reacting with free radicals and neutralizing the free radicals [43]. The body has developed two categories of endogenous antioxidant defense mechanisms: enzymatic and non-enzymatic. The enzymatic defense system includes various endogenous enzymes, such as glutathione reductase, CAT, glutathione peroxidase, and SOD. Additionally, a non-enzymatic defense system, like GSH, is also present [44]. In this study, Glutathione levels were significantly higher in the CAPE group compared to the control group ($p<0.05$). However, no statistically significant differences were found in the pairwise comparisons of the other groups. Additionally, there was no significant difference in SOD and CAT activities between groups, indicating that CAPE did not affect the antioxidant status of the cardiac tissue in the rats across all groups. Troponin I is suggested to be a suitable method for the detection of myocardial necrosis. It seems to be as sensitive to cardiac damage as CK-MB, and it has a high specificity for myocardium [45]. Troponin I levels were significantly elevated in the DOX group compared to the control group, indicating myocardial damage in the DOX-treated group. On the other hand, CAPE treatment in DOX group (CAPE+DOX) did not affect Troponin I level compared to DOX group.

Matrix metalloproteinases plays a crucial part in the processes of tissue remodeling because of their high affinity for extracellular matrix (ECM) components. The ECM regulates the alignment of cardiac cells and the overall stability of the myocardium. In a healthy heart, the balance between the synthesis and degradation of the ECM is tightly controlled [46]. A variety of developing cardiovascular disease states have recently been found to have altered *MMP* expression patterns and myocardium undergoes changes in collagen structure and content, which may have an impact on left ventricular geometry [47]. Myocyte alignment is disturbed as a result of increased metalloproteinase activity, which also speeds up the remodeling of the left ventricle in congestive heart failure [46]. Polegato et al. [48] reported that *MMP-2* gene expression is increased in acute DOX toxicity, and cardiac dysfunction is associated with myocardial *MMP-2* activation. Similarly, Shaker and Souror [46] reported that DOX significantly increased the *MMP-2* level in the plasma of DOX treated rats. In our study, there were no alterations in *MMP-2* gene expressions among the groups.

SBP increased in DOX-treated rats compared to control animals, without any change in HR. The HR remained unchanged in the DOX group when compared to the control and CAPE+DOX groups. SBP was elevated

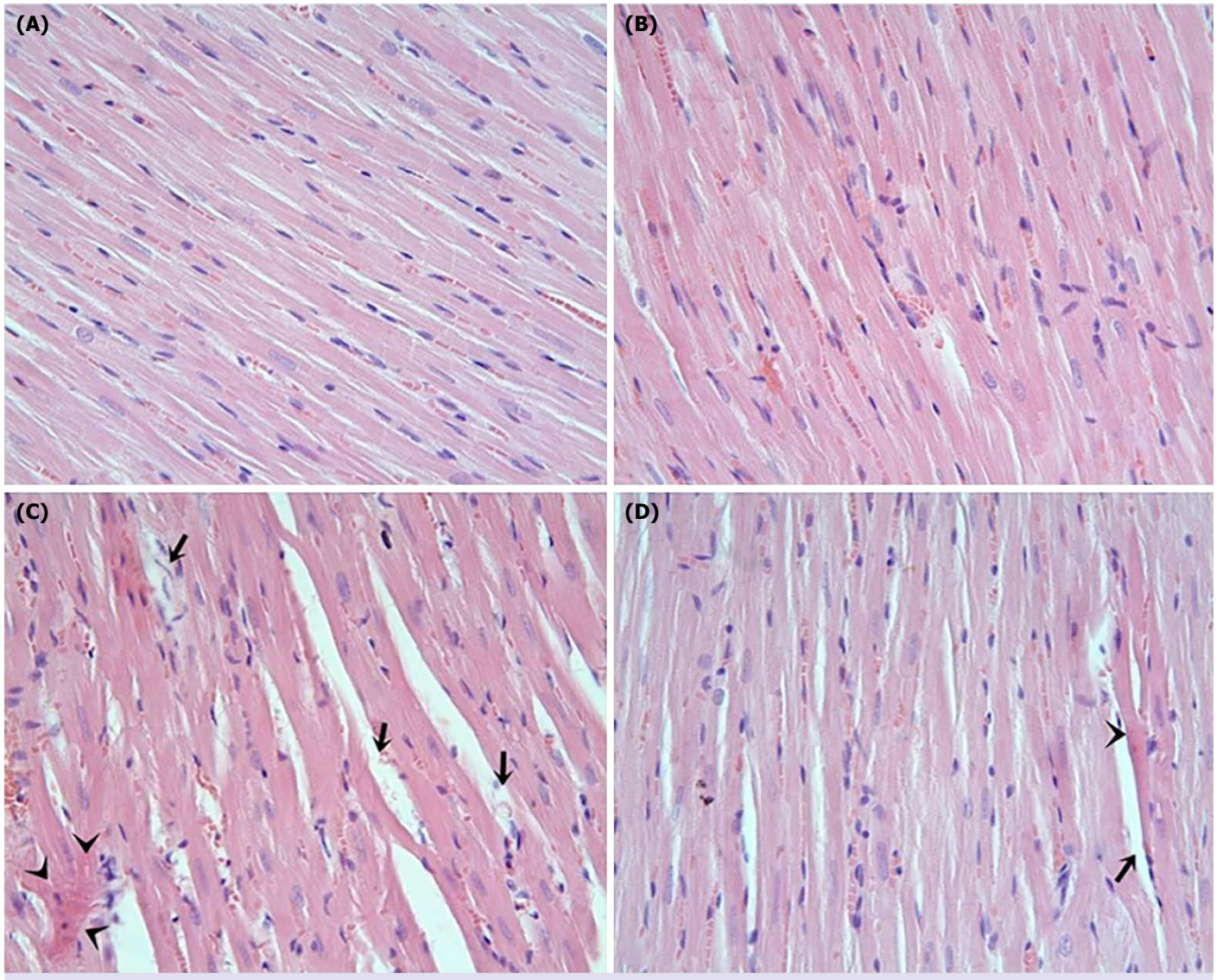

FIGURE 1. Photomicrographs of the longitudinal section of the myocardium **(A-D)**. Control **(A)** and CAPE **(B)** groups exhibit normal histological appearance. DOX group **(C)** draws attention to the presence of degenerate cardiomyocytes (arrowheads) and interstitial edema (arrows). CAPE+DOX group **(D)** demonstrates a prominent decrease in the intensity of degenerate cardiomyocytes (arrowheads) and interstitial edema (arrows) and marked improvement in the myocardial structure (H-E, x40).

CAPE: Caffeic acid phenethyl ester; DOX: Doxorubicin.

in both the DOX and CAPE+DOX groups compared to the control, while DBP showed no difference between the DOX group and the control. We also evaluated the ECG changes in the treatment groups. While duration of PR wave was not changed between DOX group and control, duration of QRS and QT waves was decreased in DOX group compared to control. These alterations may reflect intraventricular conduction defects as a result of myocardial ultrastructural changes. However, ECG parameters appear to be not affected by CAPE treatment meaningfully to report a clear conclusion from our results. Villani et al. [49] reported that DOX causes prolongation of QRS complex in a dose dependent manner. Similarly, Shekari et al. [50] reported that DOX treatment resulted in significant prolongation of QRS complex and QT interval compared to the control group. However, no alterations in the ST segment were observed between the treated groups and the control group. It has been reported that prolongation of the QT interval, widening of the QRS and flattening of the T-wave were observed in rats given DOX as well as causing body weight loss and death during drug treatment [37].

In terms of histopathological alterations in the present study, the presence of dense degenerative cardiomyocytes in the myocardium of the DOX group was noted. Furthermore, increased interstitial connective tissue and edema were seen between cardiomyocytes in the DOX group. Our results showed that DOX causes damage to cardiomyocytes. It was observed that CAPE showed a significant decrease in histopathological changes and scoring in the CAPE+DOX group compared to DOX group. According to Shekari et al. [50] DOX caused loss of myofibrils, inflammation with mononuclear cell infiltration, perinuclear and cytoplasmic vacuolization and hypertrophy of myocardium. Yarmahmoudi et al. [37] reported similar changes including mononuclear inflammation and cytoplasmic vacuolization in the cardiomyocytes of DOX-treated rats. A study investigating the protective effect of CAPE on DOX-induced cardiotoxicity reported that CAPE pretreatment significantly reduced DOX-induced cardiac damage Biochemical parameters and electron microscopy results revealed a significant protection where DOX caused swelling of mitochondria and cristae disappearance and matrix clear out ultrastructurally. However, CAPE treatment ameliorated these ultrastructural alterations [51]. In general, DOX 6 times every other day at 2.5 mg/kg led to the infiltration of leukocyte cells, inflammation, and unevenly spaced myocardium fiber [52].

Limitations

The absence of Western blot analysis, immunohistochemistry findings, echocardiographic analysis and levels of B-type natriuretic peptide may be one of the limiting factors of the study. Another limiting factor is that CAPE was administered for as short as 10 days. In our study, longer CAPE administration instead of 10 days could have positively changed many parameters.

CONCLUSIONS

CAPE treatment ameliorated histopathological changes induced by DOX while other parameters including oxidative stress, *MMP-2* gene expression, Troponin I and ECG studied in our study were not altered remarkably. CAPE did not affect these parameters significantly. Improved histopathological alterations due to CAPE treatment may be associated with other mechanisms that were not studied in this study. Although histologically the benefits of cape are seen, it has been observed that it causes changes in the ECG and leads to changes in the interventricular conduction system. Therefore, further research is needed for cape in terms of arrhythmia and resynchronization disorder.

Ethics Committee Approval: The Inonu University Animal Experiments Local Ethics Committee granted approval for this study (date: 06.01.2022, number: 2022/1-2).

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

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Peer-review: Externally peer-reviewed.

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Original Article INTERNAL MEDICINE

Liver fibrosis scores can also be used as a marker of cardiovascular risk in non-alcoholic fatty liver disease

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ABSTRACT

OBJECTIVE: This study was designed to evaluate the relationship between Atherogenic Plasma Index (API), a cardiovascular event risk marker, and hepatic fibrosis level in non-alcoholic fatty liver disease (NAFLD) patients.

METHODS: 129 people over the age of 18 with NAFLD were included in the study. Fasting blood glucose (FBG), HOMA-IR, HbA1C, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), albumin, triglyceride (TG), total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), nonHDL-cholesterol (nonHDL-C), direct bilirubin, TC/HDL-C, LDL-C/HDL-C and TG/HDL-C ratios were calculated. NAFLD fibrosis score (NFS), FIB-4, APRI scores were determinated. It was classified as low, high and intermediate. The API was calculated and its relationship with the level of hepatic fibrosis was evaluated.

RESULTS: The API level in the high group of NFS is higher than that in the low group. Similarly, the API level in the high group of FIB-4 is higher than that in the low group. The API in the low group of APRI are lower than the ID group. There is a positive correlation between API and NFS, FIB-4, APRI scores in all NAFLD subjects. There is a positive correlation between API and NFS, FIB-4, APRI scores in all NAFLD subjects in the study.

CONCLUSION: In this study, we found that there was a relationship between FIB-4, NFS, APRI scores, API and dyslipidemia in people with NAFLD, and that as the level of fibrosis increased, the risk of cardiovascular event also increased. Liver fibrosis scores can also be used as cardiovascular risk markers.

Keywords: Atherogenic plasma index; FIB-4 score; non-alcoholic fatty liver disease.

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Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the world, with a prevalence of approximately 25% in the adult population. The disease is becoming a public health problem in the world with each passing day [1]. NAFLD is often accompanied by obesity, type 2 diabetes mellitus (DM), hyperlipidemia, hypertension and metabolic syndrome. NAFLD patients have a higher mortality than the general population. The likelihood of a non-fatal cardiovascular disease or death from cardiac events is twice as high as that of the general population. NAFLD patients' rise in morbidity and mortality is not solely caused by liver diseases, but also by cardiovascular diseases (CVD) [2–4].

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Epidemiological studies have shown that NAFLD and increased CVD risk are connected.

The histology cohort study revealed that biopsy-proven NAFLD is linked to major adverse cardiovascular events and is associated with ischemic heart disease, stroke, and congestive heart failure [1]. According to a meta-analysis, NAFLD is a factor that raises the risk of myocardial infarction, ischemic stroke, atrial fibrillation, and heart failure [5]. The relationship between NAFLD and CVD is bidirectional [6]. Considering the close relationship of NAFLD with metabolic syndrome components, CVD risk is expected to increase in those with NAFLD. However, it has also been suggested that NAFLD may be a cause of CVD independent of classical cardiovascular risk factors [7]. In addition to traditional cardiovascular risk factors, there are several secondary risk factors, such as hyperuricemia, hypoadiponectinemia, proinflammatory cytokines, and endothelial dysfunction, that are believed to be behind the increased incidence of CVD in NAFLD [8]. In the cardiovascular system in NAFLD cases; coronary artery disease (CAD), ischemic stroke, arrhythmias, diastolic dysfunction, heart failure with preserved ejection fraction, and valvular diseases [9–11].

The level of liver fibrosis is a strong finding indicating the progression of NAFLD's clinical course and the mortality associated with the liver [12, 13]. The most important finding that determines the natural history of NAFLD is this hepatic fibrosis level. In the natural course of the disease in any time period; It can be progress, stagnation or regression. The existence of risk factors increases the progression of fibrosis. It is thought that there may be a relationship between hepatic fibrosis and cardiovascular complications [14]. Liver biopsy is the gold standard for evaluating liver fibrosis. Non-invasive tests are preferred because they are easier and more economical. To evaluate hepatic fibrosis, noninvasive tests such as NFS, FIB -4, APRI, Forns are used in clinical practice.

The atherogenic Plasma index (API), a new quantitative index used to evaluate lipid levels, is a well-known predictive and prognostic biomarker for atherosclerotic cardiovascular diseases [15, 16]. The initial description of AIP was given by Dobiárco and Fröhlich in 2001 [17]. API has been shown to be a strong predictor in diseases that cause major cardiovascular death, such as CAD and acute coronary syndrome. Many studies have shown that API has a high level of accuracy in estimating the risk of atherosclerosis, CAD, myocardial infarction [18, 19] and hyperuricemia [20, 21].

Highlight key points

- There is a relationship between FIB-4, NFS, APRI scores, and API in people with NAFLD.
- There is a positive correlation between API and APRI, FIB-4, NFS.
- As the level of fibrosis increases in NAFLD, the risk of cardiovascular events also increases.
- Fibrosis scores may also predict the risk of cardiovascular events.

Although numerous studies recently examined the relationship between NAFLD and API, no studies examined the relationship between liver fibrosis level and API [22]. This study was designed to examine the correlation between the level of hepatic fibrosis and the API to determine cardiovascular risk.

MATERIALS AND METHODS

Study Population

Between January 2019 and September 2022 in the internal medicine department of our hospital, 129 people over the age of 18 with ultrasonographic fatty liver were included in the research. While determining the people to be included in the study, as the exclusion criteria; viral hepatitis, autoimmune hepatitis, toxic hepatitis, cirrhosis, biliary tract diseases, pregnancy, lactation, cancer, those who use drugs that will affect lipid profile and liver enzymes, and those who use alcohol (20 g/day for women, 30 g/day for men) were determined. This study was conducted in accordance with the Declaration of Helsinki, ethics committee approval was obtained from the Istanbul University of Health Sciences Umraniye Training and Research Hospital Clinical Research Ethics Committee (date: 24.11.2022, number: 2022/354).

Measurements

Demographic information (age, gender, etc) of the participants in the study was recorded. The use of standardized equipment was employed to measure weight and height. Body mass index (BMI) was calculated. A venoz blood sample was taken after a 12-hour fast, and the required parameters were analyzed by using the Abbott i8000 device and Abbott kits in our central laboratory.Fasting blood glucose (FBG) levels were measured

by hexokinase method and aspartate aminotransferase (AST), alanine aminotransferase (ALT) were evaluated by enzymatic method. Triglyceride (TG), and HDL-cholesterol (HDL-C) were evaluated by photometric method. The insulin levels were studied using the Abbott i16000 device and the chemiluminescent microparticle immunoassay (CMIA) method.

HOMA-IR was calculated using the formula:

HOMA-IR=[fasting insulin (μIU/mL)×fasting glucose (mg/dL)]/405] [23].

Liver Fibrosis Scores

The fatty liver was determined by a skilled radiologist using ultrasonography. To determine the level of NAFLD, four parameters (vascular blur, deep attenuation, liver brightness, echo contrast) in the scoring system proposed by Hamuguchi et al. [24] were examined.

The NAFLD fibrosis score (NFS) was determined using the formula.

NFS: -1.675+0.037 × age (years) + 0.094×BMI (kg/m^2)+1.13 pre-diabetes /diabetes (yes=, no=0)+ 0.9×AST [IU/L]/ALT[IU/L]−0.013×platelet count (109/L)−0.66×albumin(g/dL) [25].

The probability of fibrosis was low NFS<-1.445.

The probability of fibrosis was high NFS>0.676.

The probability of fibrosis was Indeterminated NFS -1.445- 0.676.

The FIB-4 score was determined using the formula.

FIB-4 score: (Age [years]×AST[U/L])/(platelet [109] X√ALT [U/L]) [26].

The probability of fibrosis was low FIB-4<1.30.

The probability of fibrosis was high FIB-4>2.67.

The probability of fibrosis was indeterminate FIB-4 between 1.30-2.67.

The APRI score was determined using the formula.

APRI score: [(AST/upper limit of normal)/Platelet Count (109/l)]×100.

The probability of fibrosis was low APRI<0.5.

The probability of fibrosis was high APRI>1.5.

The probability of fibrosis was indeterminate APRI between 0.5-1.5.

Atherogenic Plasma Index (API)

API was calculated [log(TG/HDL-C)] by taking the logarithm of the ratio of TG to HDL-C [27].

Statistical Analysis

Statistical analysis was performed using the MedCalc® Statistical Software version 19.7.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). The Shapiro-Wilk test was used for continuous variables. Frequency (n) and percentage (%) were used to express categorical variables. The Ki-Square (or Fisher's Precise Test, where appropriate) test was used to compare categorical variables. The Kruskal Wallis test was used to compare more than two independent non-normal distribution group continuous variables. The Bonferroni-tuned Mann Whitney test was used for post-hoc comparisons. An ANOVA test was used to compare two or more independent normally distributed group continuous variables. For Post Hoc Comparisons Bonferroni adjusted Tamhane's T^2 test was used. The correlation between two normally distributed variables was used by Pearson Correlation coefficient, while for non-normal data, spearman Correlation coefficient was used.

RESULTS

Of the 129 participants in the study, 68.2% (n=88) were female and 31.8% (n=41) were male. Their mean age was 53.06 (53.6+10.9) years. According to their blood glucose, 58.9% them were normal, 28.7% were prediabetic, and 12.4% were DM (Table 1). According to the participants' FIB-4 scores, 17% are in the high fibrosis(HF) groups, 31% Indeterminated fibrosis (IDF) groups, and 52 % low fibrosis (LF) groups.

According to the NFS score, 17.1% HF, 28.7% IDF, and 54.3% LF group. According to the APRI score, 7% are in the IDF group and 93% are in the LF group. There are no participants with a High group. (Table 2).

In terms of all parameters, all three groups determined by FIB-4 scoring were compared.

The HF group has a higher level of API, TG, TC, non HDL-C, non HDL-C/ HDL-C, TC /HDL-C, LDL-C/HDL-C compared to the LF group (p<0.001).

In terms of all parameters, all three groups determined by NFS classification were compared. The HF group has a higher level of API, TG, TC, non HDL-C, non HDL-C/ HDL-C, TC /HDL-C, LDL-C/HDL-C compared to the LF group(p<0.001).

In terms of all parameters, all three groups determined by APRI classification were compared. Significantly lower API and TG were found in the LF group than in the IDF group (p<0.01) (Table 3).

Table 1. Demographic characteristicsand laboratory parameters

	n	%
Gender		
Female	88	68.2
Male	41	31.8
DM status		
Normal	76	58.9
Prediabetes	37	28.7
DM	16	12.4
	Mean±SD	Med (min-max)
Age (year)	53.6±10.9	54 (27–84)
BMI (kg/m^2)	28.9±4.3	27 (22.5–43)
FBG (mg/dl)	104±25.5	98 (77–241)
AST (IU/l)	25.4±10.9	22 (7–60)
ALT (U/l)	25.5±13.8	21 (8–62)
Platelet (×10^9/l)	255.4±63.4	255 (118–500)
Albumin (gr/dl)	4.7±2.9	4.5 (3.4–37)
Homa-IR	3.9±3.9	2.8 (1.4–38)
HBA1c (%)	5.8±1	5.5 (4.6–11)
API	0.6±0.3	0.6 (0–1.3)
TG (mg/dl)	204.5±113.3	165 (51–536)
TC (mg/dl)	204.6±39.6	203 (102–299)
Non-HDL-C	158.4±41.7	157 (54–242)
HDL-C (mg/dl)	45.8±10.5	44 (25–77)
Non-HDL/HDL	3.7±1.4	3.6 (0.7–8.5)
TC/HDL-C	5.2±5.3	4.5 (1.7–62.5)
LDL-C (mg/dl)	125.8±33.7	124 (46–211)
LDL-C /HDL-C	2.9±1.1	2.9 (0.9–6.7)
GGT (U/l)	43.3±51.9	26 (7–336)

DM; Diabetes mellitus; SD: Standard deviation; BMI:Body mass index; FBG: Fasting blood glucose; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; API : Atherogenic plasma index; TG: Triglyceride; TC: Total cholesterol; Non-HDL-C: Non-HDL- cholesterol; HDL-C: HDL- Cholesterol; LDL-C: LDL-cholesterol; GGT: Gamma glutamil transferase

Table 2. Fibrosis scoring and classifications (n=129)

FIB-4, mean+SD	1.2+0.7
	%
High	17
ID	31
Low	52
NFS, mean+SD	-1.6+1.6
	%
High	17.1
ID	28.7
Low	54.3
APRI, mean+SD	0.3+0.2
	%
High	-
ID	7
Low	93

SD: Standard deviation; FIB-4: <1.3low, 1.3-2.67 ID, >2.67 high; NFS: <-1.445 low -1.445-0.676 ID, >0.676 High; APRI: <0.5low, 0.5-1.5I D, >1.5 high; NFS: NAFLD fibrosis score

The correlation between fibrosis scores of people with NAFLD and API was investigated. There is a positive, high correlation between API and FIB-4 score (r=0.825, p<0.001) (Fig. 1). There is a positive, high correlation between API and NFS (r=0.753 p<0.001) (Fig. 2). There is a positive, moderate correlation between API and APRI (r=0.666, p<0.001) (Fig. 3).

DISCUSSION

In this study, we found that there is a relationship between FIB-4, NFS, APRI scores, API and dyslipidemia in people with NAFLD. In other words, as the level of fibrosis increases, the risk of CVD also increases. Liver fibrosis scores can also be used as cardiovascular risk markers.

The most common liver disease in the world is NAFLD, with a prevalence of approximately 25% in among the adults. The disease is becoming a public health problem in the world with each passing day [1]. The increase in morbidity and mortality in NAFLD patients is not just due to liver diseases, but also to CVD [2–4].

The relationship between NAFLD and CVD is bidirectional [6]. Considering the close relationship of NAFLD with metabolic syndrome components, it is expected that CVD risk will increase in those with NAFLD. However, it has also been suggested that NAFLD may be a cause of CVD independent of classical cardiovascular risk factors [7]. In addition to traditional cardiovascular risk factors like DM, hypertension, and dyslipidemia, there are several secondary risk factors like hyperuricemia, hypoadiponectinemia, proinflammatory cytokines, and endothelial dysfunction that are believed to be behind the rise in the frequency of CVD in

TABLE 3. Fibrosis scores and parameters

	FIB- 4 Mean±SD				NFS Mean±SD				APRI Mean±SD			
	High (n=22)	ID (n=40)	Low (n=67)	p	High (n=22)	ID (n=37)	Low (n=70)	p	ID (n=9)	Low (n=120)	p
API	1.1±0.1	0.7±0.1	0.4±0.2	**<0.001**[1]	1±0.1	0.7±0.2	0.4±0.2	**<0.001**[1]	0.9±0.3	0.6±0.3	**0.011**[2]
TG	393.6±69	242±64	120.1±31.8	**<0.001**[1]	380.5±82.8	226.8±87	137.4±56	**<0.001**[1]	334.7±150	194.8±105	**0.010**[2]
TC	222±35	211.6±36	194.8±40.7	**0.012**[1]	222.3±35	206.2±38	198.3±41	**0.041**[1]	209.2±34.9	204.3±40	0.721[3]
Non-HDL	187.4±36	167.8±34	143.2±41.5	**<0.001**[1]	187.5±35.6	162.6±36	147±42	**<0.001**[1]	168.7±33.3	157.6±42	0.222[3]
HDL-C	34.6±6	43.8±6.5	50.7±10.6	**<0.001**[1]	34.8±5.8	43.7±8	50.4±10	**<0.001**[1]	40.6±8.4	46.2±11	0.154[2]
Non-HDL/HDL	5.6±2	3.9±0.9	3±1	**<0.001**[1]	5.6±1.5	3.8±1	3.1±1	**<0.001**[1]	4.4±1.1	3.7±1.4	0.117[2]
TC/ HDL	6.6±2	6.3±9.2	4±1	**<0.001**[1]	6.6±1.5	6.4±10	4.1±1	**<0.001**[1]	5.3±1.2	5.1±6	0.164[2]
LDL	135.6±34	131.2±30	119.3±34.6	0.071[1]	136.1±33.7	126.3±32	122.3±34	0.247[1]	124.4±32.2	125.9±34	0.902[3]
LDL/ HDL	4±1	3±0.8	2.4±0.8	**<0.001**[1]	4±1.2	3±0.9	2.5±0.8	**<0.001**[1]	3.1±0.9	2.9±1	0.548[2]

[1]: Kruskal Wallis Test; [2]: Mann-Whitney U; [3]: Independent Samples T-Test. API: Atherogenic plasma index; TG: Triglyceride; TC: Total cholesterol; Non-HDL: Non-HDL cholesterol; HDL-C: HDL cholesterol; LDL: LDL cholesterol

FIGURE 1. There is a positive, high and statistically significant correlation between API and FIB-4 score.

API: Atherogenic plasma index.

FIGURE 2. There is a positive, high and statistically significant correlation between API and NFS.

FIGURE 3. There is a positive, moderate and statistically significant correlation between API and APRI score.

NAFLD [8]. It should not be forgotten that NAFLD is closely associated with cardiovascular mortality as well as liver-related mortality [9–11].

NAFLD's progression depends on the level of hepatic fibrosis.The level of hepatic fibrosis is a reliable determinant of the course of NAFLD and liver-related mortality [12, 13]. It is also thought that there may be a relationship between hepatic fibrosis and cardiovascular complications [28]. In this research, we examined the correlation between hepatic fibrosis level (NFS, FIB-4, APRI scores) and cardiovascular event risk in NAFLD. As a result, we found that the risk of cardiovascular events increased as the degree of hepatic fibrosis increased. Chen et al. [14] suggested in a study that there is an association between liver fibrosis and cardiovascular mortality in people with coronary artery disease. In another meta-analysis, it was reported that there was a correlation between NAFLD and subclinical atherosclerosis, including coronary calcification, arterial stiffness and endothelial dysfunction. In the research of Neri et al. [29], a positive relationship was determined between the stages of liver fibrosis and intima-media thickness. Possible mechanisms of this increased atherosclerosis during NAFLD may be related to the secretion of certain cytokines and molecules, including adropine, selenoprotein P, fibroblast growth factor 21, sex hormone-binding globulin, and retinol-binding protein 4 [14].

API is a well-known predictive and prognostic biomarker for atherosclerotic CVD [15, 16]. API has been shown to be a strong predictor in diseases that cause major cardiovascular death, such as coronary artery disease and acute coronary syndrome.Various studies have demonstrated that API has a high level of accuracy in forecasting the risk of various situations such as atherosclerosis, CAD, myocardial infarction [18, 19] and hyperuricemia [20].

API has greater sensitivity in reflecting the relationship between atherogenic and protective lipoproteins. In a study by Frohlich et al. [21] it was stated that the API value was inversely related to the diameter of LDL-C particles and showed the sdLDL particle size. Therefore, it is thought that API can be accepted as an economical and reliable indicator of CAD. As a result of numerous studies in recent years, it is suggested that the API is a strong predictor of CAD risk [30]. In this study, we used API as a marker of CVD risk.

Obesity and dyslipidemia are the main risk factors for the development of NAFLD [31]. The relationship between traditional lipid parameters such as TC, LDL-C, TG and CAD is known. In addition to these, TG/HDL-C, TC/HDL-C, LDL-C/HDL-C ratios are thought to be predictors for CAD. In a study of more than 12 million people, it was emphasized that there was a relationship between lipid indices, especially TC/HDL-C, and the prevalence of NAFLD [30, 31]. In a study conducted by Nobili et al. [32], a positive correlation was shown between NAFLD fibrosis scores and TG/HDL-C TC/HDL-C, LDL-C/HDL-C ratios in 118 children who underwent a biopsy. We also found a significant relationship between fibrosis scores and lipid parameters in this study.

According to NFS and FIB-4 scoring, we found that HF group had higher TC, TG, nonHDL-C, non HDL/HDL-C, LDL/HDL-C and lower HDL-C than LF group. In other words, we determined that dyslipidemia was higher in the group with higher fibrosis. According to APRI scoring, we found that the TG levels of the IDF group were higher than LF group.

CONCLUSIONS

NAFLD is an important disease with a rapidly increasing prevalence in the world day by day. Noninvasive fibrosis scores are widely used to determine liver damage. In this study, we found that there is a relationship between FIB-4, NFS, APRI scores, API and dyslipidemia in people with NAFLD. In other words, as the level of fibrosis increases, the risk of CVD also increases. We think that these fibrosis scores in NAFLD patients can be used to evaluate the risk of cardiovascular events as well as to show liver damage.

Ethics Committee Approval: The Istanbul University of Health Sciences Umraniye Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 24.11.2022, number: 2022/354).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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Original Article HEMOTOLOGY

Overview of blood transfusion appropriateness with one-day point prevalence: Right decision? Right product? Right amount?

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ABSTRACT

OBJECTIVE: Blood and blood products are a scarce resource and thus, they should be used efficiently. Inappropriate use of blood leads not only to waste of valuable medical resource, but also increases the risks of blood transfusion and economic burden.

METHODS: This study is a prospective, observational one-day single-center point prevalence study in which transfusion activities performed in our hospital. It was aimed to determine the prevalence of patients receiving blood products in hospitals, types of blood products, characteristics of recipients, distributions of blood group, appropriateness of blood transfusion and the cost of these products, using the one-day standardized prevalence method for the first time in our country.

RESULTS: One hundred seventy blood transfusions were performed in 102 patients. It was found that 64 (37.6%) of 170 blood products used according to the guidelines were non-evidence-based and inappropriate blood transfusions. It was established that the frequency of inappropriate use of blood products was significantly higher in surgical divisions (n=31 48.4%) compared to internal medicine divisions (n=20, 31.3%) ($p<0.0001$). The amount corresponding to 64 (37.6%) units transfused inappropriately according to the guidelines was found for one day. When we estimated the results of this study at the level of yearly use of blood products, we found that transfusion of approximately 28 000 units of blood products was being performed yearly in our hospital and a total of 8.435.006 TL/281.166 USD was being spent yearly considering 10 523 units were being transfused inappropriately.

CONCLUSION: This study shows that the condition in our country is only the tip of the iceberg. Examination of the indications for use of blood products by transfusion committees in hospitals and generalizing use of national guidelines could be the primary applications to prevent inappropriate use of blood products.

Keywords: Blood cost; point prevalence; transfusion indications.

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Blood transfusion is one of the important, irreplaceable and most commonly used clinical treatment methods worldwide. Recently, blood transfusion has reached considerably high levels of safety due to advancements in science and legal regulations [1, 2]. Advancements in the quality of blood transfusions have been achieved with establishment of Hospital Transfusion Committees, development of education programs and guidelines, and application of cost saving strategies [3].

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However, there are still important potential risks related to blood transfusion despite such advancements. Many transfusion-related adverse events, including immunological reaction and infections, have been reported [4–7].

The basic objective of modern transfusion services is to provide a supply of blood components for therapeutic use in an adequate, safe and efficient manner. Increased pressure on both supply and blood request has drawn attention to appropriate clinical use of present blood components. Transfusion safety is based not only on appropriate selection, preparation and application of blood products, but also on the ability to properly interprete the appropriate time of such an intervention. Considering the risks, scarcity and high cost, indications for use of blood products should be appropriate in order to prevent wastage and minimize the risks [8]. In this context, it is estimated that 5–58% of blood transfusions are "unnecessary or inappropriate" [9–12]. An indication for blood transfusion is generally based on clinical application guidelines or the recommendations of expert panels, but rarely supported by clinical researches [13, 14]. Therefore, blood transfusion is a complex treatment method that should be based on clinical evidence and adjusted according to cardiovascular risk factors and laboratory test results.

Use of risk reduction methods, development and application of new technologies for donation and transfusion have caused an important increase in the cost of blood transfusion [15]. Currently, 1% of the total hospital costs is attributed to blood transfusion, but this may change by disease and procedure. For treatments such as liver and bone marrow transplantation, for example, blood transfusion plays an important financial role that represents 5–9% of total hospital costs [16].

Hospital blood transfusion surveillance, which is defined as systematic collection, analysis, and interpretation of data for the objective of controlling use of blood products in hospitals, enables knowlegde about the magnitude of the problem related to blood use in hospitals, monitoring tendencies and changes in time, comparison between hospitals and continuous education of healthcare personnel. For surveillance studies to achieve success, opportunities should be reviewed and evaluated in detail, and a clear model with a specific objective should be presented. Blood use in a hospital may be examined with prevalence surveillance. Prevalence studies are cross-sectional studies and involve individuals who are receiving blood products at the time of the study; these studies are also named one-day (point) prevalence surveys. These surveys are useful for rapidly determining blood use in hospitals and for specifying its limits. In addition, it may be considered a good alternative method to be used in large hospitals with limited resources.

Highlight key points

- According to national guidelines, 37.6% of blood products were used inappropriately in a non-evidence-based manner.
- The highest transfusion rate was observed in internal medicine divisions.
- The rate of inappropriate blood product use was significantly higher in surgical divisions than in internal medicine units.
- Inappropriate transfusions resulted in an unnecessary annual cost exceeding 281166 USD for 10523 units.

In this study, it was aimed to demonstrate use of blood products in hospitals, which has been defined as one of the important health problems in recent years and to which much effort has been devoted, in different dimensions. With this objective, it was aimed to determine the types of blood products used for transfusion in our hospital, characteristics of recipients, blood group distributions, appropriateness of blood transfusion and the cost of these products using one-day standardized prevalence method for the first time in our country.

MATERIALS AND METHODS

Our hospital is a tertiary city hospital in Istanbul with 758 beds, 27 operation rooms, and 96 intensive care beds, in which education-training activities are being conducted. This study is a prospective, observational, one-day, single-center point prevalence study in which transfusion activities in our hospital were investigated in a one-day follow-up period. All transfusions performed in our hospital in a period of 24 hours on the date specified were included in the study. No transfusion was excluded from the analysis. This study was conducted in compliance with the ethical standards of the Helsinki Declaration of Human Rights. The study was approved by Istanbul Medeniyet University Goztepe Training and Research Hospital Ethics Committee (Decision number: 2020/0503) on 12.08.2020.

The patients' demographic data, diagnoses, clinics where they were followed up, type of the blood product transfused, blood groups, transfusion period, transfusion dose, if the product was processed additionally (radiation, cleaning, filtration), if any reaction developed against the blood products, the objective of use of blood product, if the blood product was given for the appro-

Table 1. Indications for transfusion of blood and blood components

Indication for transfusion of blood and blood components	
Erythrocyte concentrate (E)	Platelet concentrate (P)
E1 Acute hemorrhage **E2** Stabile patient with Hb ≤ 7 g/dL, **E3** Cardiovascular disease or hematologic malignancy with Hb ≤8 g/dL **E4** Chronic anemia requiring transfusion **E5** Exchange Transfusion **E6** Preoperative preparation of patients **E7** Risk for peroperative hemorrhage **E8** Other (explain):.....	**P1** Prophylaxis to prevent spontaneous hemorrhage **P1A** Platelets <10×10^9/L; reversible bone marrow failure **P1B** Platelet 10-20×10^9/L; sepsis/hemostatic anomaly **P1C** Platelet <30×10^9/L; confirmed coagulopathy **P2** Prophylaxis befor invasive procedure or preoperative prophylaxis **P2A** Platelet <20×10^9/L; central venous catheter **P2B** Platelet <40×10^9/L; before lumbar puncture/spinal anesthesia **P2C** Platelet <50×10^9/L before liver biopsy/major surgery **P2D** Platelet <80×10^9/L; epidural anesthesia **P2E** Platelet <100×10^9/L; before surgery on a critial region, for example: central nervous system, eye **P3** Therapeutic use to treat hemorrhage (WHO bleeding grade 2 or above) **P3A** Platelet <50×10^{9}L; major hemorrhage **P3B** Platelet <100×10^9/L; critical region hemorrhage, CNS/ traumatic brain damage **P3C** Platelet <30×10^9/L; clinically prominent hemorrhage **P4** Specific clinical conditions **P4A** Before procedure or if bleeding is present in disseminated intravascular coagulation. **P4B** Primary immune thrombocytopenia (severe hemorrhage). **P5** Platelet dysfunction **P5A** Critical hemorrhage arising from use of anti-platelet drug **P5B** Hemorrhage in hereditary platelet disorders **P6** Other (explain).....
Fresh frozen plasma (P)	
P1 Major Hemorragia **P2** INR>1.5 with hemorrhage **P3** PT/INR>1.5 before procedure **P4** Liver disease or preprocedural period with PT/INR >2 **P5** FFP/Plasma Exchange **P6** Replacement of a Deficient Coagulation Factor **P7** Preoperative preparation of patient **P8** Risk of peroperative hemorrhage **P9** Other (explain).....	
Cryoprecipitate (C)	
C1 Clinically significant hemorrhage and Fibrinojen <1,5g/L (for obstetric hemorrhage <2 g/L) **C2** Preprocedural Fibrinojen <1 g/L **C3** Hemorrhage related to thrombolytic treatment **C4** In conditions where fibrinogen concentrate can not be reached in hereditary hypofibrinogenemia	

priate indication and the costs were recorded in the form prepared. These data were obtained by using patient files and nursing documentations and by inteviewing with physicians. The patients' complete blood counts were reviewed primarily. Thereafter, the patients' diagnoses and other medical records were reviewed in the hospital information system. Additional evidence, including medical history, the patient's condition (symptoms and signs etc.) and blood loss, was searched in the medical records. Appropriate use of blood products (appropriate indication, appropriate dose, appropriate period) was evaluated by a hematologist considering the "Appropriate Clinical Use of Blood Products 2020 Guideline" and "National Hemovigilance 2020 Guideline" which were put into effect by the Ministry of Health [17, 18]. The criteria that were used to evaluate the indications for transfusion decisions are explained in Table 1. Inappropriate use of blood was specified considering absence of an indication, inappropriate dose, inappropriate period of use, unnecessary or deficient additional procedure and lack of appropriate filling of follow-up forms. Presence of one or more criteria was considered inappropriate use.

For analysis of the direct costs of the blood products applied to the patiens, a calculation was made according to the retail prices used by the Turkish red crescent and Social Security Institution (SSI) prices and Commu-

Table 2. Types of inappropriateness in use of blood products

Types of inappropriateness	%
Inappropriate indication	21.2
Inappropriate period	14.7
Inappropriate dose	7.1
Inappropriate fiiling of blood monitoring form	19.4
Presence of at least one inappropriateness	37.6

Table 3. Rates of appropriate use by blood product type

Type of product	Appropriate use n=106	Inappropriate use n=64	Total number of products n=170
	%	%	n (%)
Erythrocyte suspension	82.1	59.4	125
Platelet suspension	8.5	6.3	13
Fresh frozen plasma	9.5	34.4	32

nique on Healthcare Practices (CHP) prices on the day of the study considering the costs directly related to processing of the blood products (selection of blood donor, collection, analysis, degradation, storage, additional procedure and compliance test) without considering annual inflation rate. Conversion from Turkish lira (TL) to USA dollar (USD) was performed according to the Exchange Rate Related to Pay off USA Dollar Debts in Türkiye published by the Turkish Central Bank on the day when the study was conducted.

Statistical Analysis

SPSS 22 (*Statistical Package for Social Science Inc., Chicago, USA*) was used for statistical analysis of the data obtained at the end of the study. Compatibility of the measurement values obtained in the study to the normal distribution was examined using the "Shapiro-Wilk Test". Descriptive statistics for continuous numerical variables were expressed as mean±standard deviation and median [Interquartile range (IQR) in 95% confidence interval] and categorical variables were expressed as numbers (n) and percentages (%). The "Student's t-testi" and "Mann-Whitney U Test" were used for comparison of continuous numerical variables between the groups, and "Chi-square Test" and "Fischer's Exact test" were used for comparison of categorical variables. A p value of <0.05 was considered statistically significant for statistical decisions.

RESULTS

On the day when the point prevalance study was performed in our hospital, 170 blood transfusions were performed in 102 patients and multiple blood products were used in 51 patients. It was found that 4 units of blood were used maximally for one patient. Among the patients who underwent transfusion, 52 (51%) were female, 50 (49%) were male, and the median age of the patients was found to be 61.5 (28–73) years. When the diagnoses were examined in terms of the indications of transfusion, it was found that transfusion was performed because of malignancy in 35 (34.3%) patients, because of operation in 19 (18.6%) patients, because of anemia in 13 (12.7%) patients, because of thalassemia in 13 (12.7%) patients, because of coagulation disorder in 10 (9.8%) patients, because of renal failure in 8 (7.8%) patients and because of gastrointestinal bleeding in 4 (3.9%) patients.

The most commonly used blood product was erythrocyte suspension (ES, n: 125, 73.5%). The most commonly transfused blood type was A Rh positive. Mild allergic reaction was observed only in one patient. When the indications for transfusion of blood products were examined, it was found that the most common diagnosis code was chronic anemia for ES, thrombocytopenia ($<30\times10^9$/L) and confirmed coagulopathy (T1C) for platelets (PLT) and an INR value >1.5 associated with bleeding for fresh frozen plasma (FFP).

When the blood products transfused were examined, it was found that 125 of these blood products were ES (73.5%), 32 were FFP (18.8%) and 13 were PLT (7.6%). According to the "Appropriate Clinical Use of Blood Products 2020 Guideline" and "National Hemovigilance 2020 Guideline", it was found that 64 units (37.6%) among 170 blood products were used inappropriately in a non-evidence-based manner and 106 units (62.4%) were used appropriately (Table 2). The rate of inappropriate transfusion was found to be 59.4% for ES transfusions, 43.4% for FFP transfusions and 6.3% for PLT transfusions. ESs had the highest rate of inappropriate transfusion (Table 3).

The frequencies of blood product transfusion were examined by the hospital wards. The highest transfusion

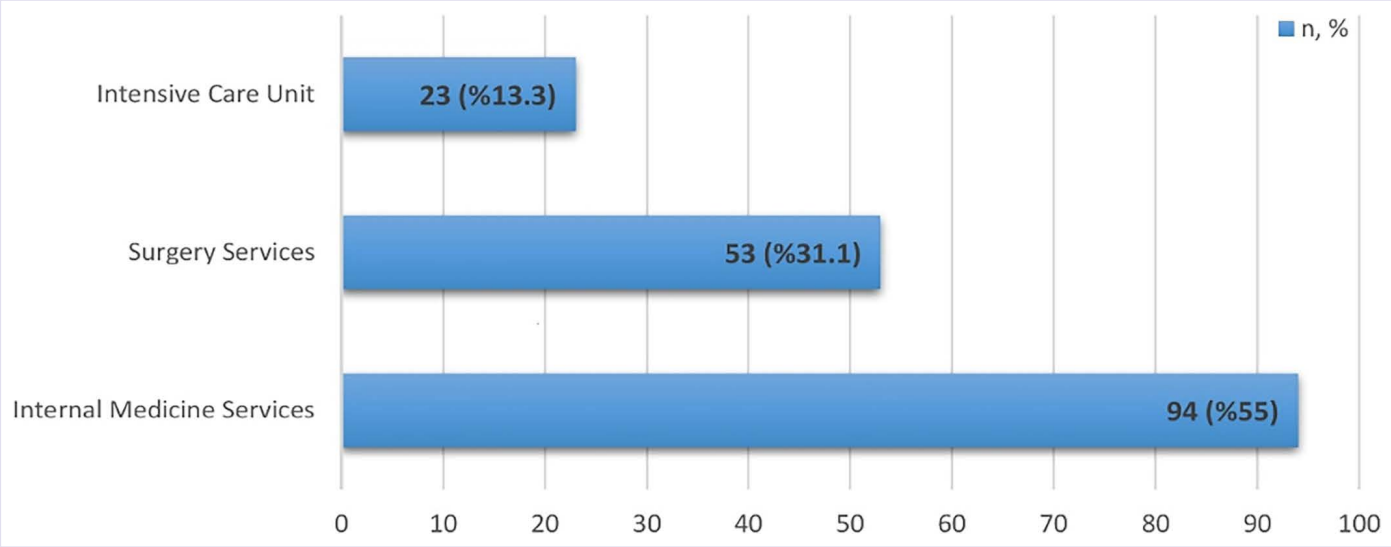

Figure 1. Blood transfusion numbers and percentages by services.

rate was found in internal medicine divisions (55%, 94 units), while this rate was found to be 31.1% (53 units) in surgical divisions and 13.5 % (23 units) in intensive care units (Fig. 1). Although the highest number of blood products was used in internal medicine divisions, the rate of inappropriate use of blood product was found to be significantly higher in surgical divisions (n=31, 48.4%) compared to internal medicine units (n=20, 31.3%) (p<0.0001) (Table 4).

According to the results, the approximate daily cost of 170 units included in this study was 136.440 TL which corrresponded to 4 553 USA dollars. The daily cost corresponding to 64 units (37.6%), which were transfused inappropriately according to the guidelines, was found to be about 51.301 TL, 1 710 dollars. When we estimated the results of this study at the level of yearly use of blood products, we found that transfusion of approximately 28 000 units of blood product was being performed in our hospital yearly and a total of 8.435.006 TL/281 166 USD was being spent yearly considering 10 523 units were transfused inappropriately.

DISCUSSION

Since blood is a limited resource depending only on the devotion of individual donors, it is essential to rationalize and optimize transfusion applications. Transfusion has risks and costs as well as benefits. Appropriateness of use of blood products has become an issue that has been emphasized in recent years [19–22].

The rate of inappropriate blood transfusion found in our study (37.6%) was in the mid range (4–66%) of the results reported in other studies [23]. Different study periods, application guidelines, study designs and especially specific appropriateness criteria probably contribute to the variation in the results reported, and make it difficult to compare absolute inappropriate transfusion levels between studies [24].

In the literature, the rate of inadequate indications was reported to be 74% for eryhtrocyte suspension (ES), 96.2% for FFP and 13% for PLT [25, 26]. In a study conducted on appropriate blood transfusion indications in Spain, the rate of inappropriate use was observed to be 13%, 48% and 67%, respectively, for erythrocyte suspension, platelet concentrate and fresh frozen plasma [27].

Table 4. Distribution of inappropriate use of blood products

Use of blood product	Divisions where blood products were used			p
	Internal medicine services n=94 (%)	Surgical services n=53 (%)	Intensive care units n=23 (%)	
Inappropriate use	31.3[a,b]	48.4	20.3	<0.001
Appropriate use	69.8	20.8	9.4	

[a]: Statistical difference by surgical wards p<0.001; [b]: Statistical difference by intensive care units p=0.001

In our study, the rate of inappropriate use was found to be 59.4% for ES, 43.4% for FFP and 6.3% for PLT.

In another study conducted in Brazil to evaluate the appropriateness of blood product requests, it was found that 85.57% of transfusions were appropriate and the rate of appropriate use was 81.4% for intensive care unit and 71.42% for surgical wards [28]. Studies have emphasized the importance of introducing blood bank awareness-raising campaigns related to rational use of blood products, applying strategies directed to more efficient use of blood products and establishing mandatory tranasfusion committees [29].

It is possible to at least partially explain the variation observed in the levels of inappropriate transfusion between different clinical specialties with the typical characteristics of the patients treated in these specialities. Therefore, the possibility of surgical patients to receive inappropriate erythrocyte transfusion is higher. Although the highest number of transfusions (94 units, 55%) was found in the internal medicine patients in our study, the rate of inappropriate use of blood products was found to be significantly higher in surgical divisions (n=31 48.4%) compared to internal medicine dividions (n=20, 31.3%) and this was similar to previous studies [30].

Another objective of this study was to determine the effect of inappropriate blood transfusion on the cost. Continuously increasing financial pressure and limited resources necessitate critical review of transfusion applications. In a retrospecitve study conducted by Juarez-Rangel et al. in 2004 [26], it was reported that 63.3% of blood products were transfused inappropriately and the annual cost corresponded approximately to 95 775 USD. Our study, which was conducted to evaluate transfusion costs in our hospital, is the first study in which inappropriate use of transfusion and its cost were evaluated in our country. As a result of the assessment based on the guidelines in our country, it was found that more than one third of transfusions were used inappropriately and this caused an unnecessary total annual cost above 281 166 USD for 10 523 units. However, only the direct costs related to blood product processing were calculated when performing cost analysis, and indirect costs that could increase transaction costs in divisions (labour cost, equipment amortization, facility maintenance costs etc.) or annual fluctuation were not considered. These figures would be higher if indirect costs were considered. In addition, this cost arising from inappropriate use of blood products signifies a serious burden for the national economy. Considering this financial aspect of transfusion of blood products, reducing inapproprate use of blood products is important in terms of decreasing this economic burden. Application of the guideline principles that will justify use of blood products and periodical inspections by transfusion committees will provide a reduction in the rate of inappropriate use of blood products.

CONCLUSION

In this study, which was conducted to evaluate use of blood products, shows that the condition in our country is only "the tip of the iceberg". A protocol related to appropriate clinical use of blood products should be prepared, such that surgical divisions are prioritized, in order to reduce the rates of inappropriate use of blood products. Education studies related to appropriate use of blood products and adjustments that would increase the efficiency of transfusion committees are needed. The primary interventions include examination of the indications for use of blood products by hospital transfusion committees and extension of use of national guidelines.

Ethics Committee Approval: The Medeniyet University Goztepe Training and Research Hospital Ethics Committee granted approval for this study (date: 12.08.2020, number: 2020/0503).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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ORIGINAL ARTICLE PEDIATRICS

Anemia, inflammation, circadian rhythm and season effects in febrile convulsions

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ABSTRACT

OBJECTIVE: Our aim in the study is to investigate circadian rhythm, season, age, gender, and hemogram parameters in children diagnosed with febrile convulsions.

METHODS: The data of a total of 478 children, consisting of 160 patients with febrile convulsions (90 male/70 female) and 318 control groups (healthy, convulsiyon free- febrile), were compared.

RESULTS: The average age of all patients was 25.7±14.7 (minimum 6-maximum 60) months, and the most common convulsion age was 13-18 months. Circadian rhythm was similar, application seasons were similar. The male/female ratio was 1.3. MPV in winter was lower than other seasons (p=0.002). At younger ages, lymphocyte count was higher (p=0.048) and NLR was lower (p=0.036). NLR was lower in patients with febrile convulsions with anemia than in patients with febrile convulsions without anemia (p=0.029). NLR was lower in patients with febrile convulsions with anemia than in the convulsion-free febrile control group with anemia (p=0.001). In patients with febrile convulsions, MPV was lower (p=0.033) and NLR was higher (p=0.001) than in the convulsion-free febrile control group. One third of the patients had hypocapnia.

CONCLUSION: Young age, anemia, inflammation, and hypocapnia may facilitate the occurrence of febrile convulsions. MPV and NLO, which are hemogram parameters, can guide us about inflammation. Blood gases may provide information regarding hypocapnia. These rapid examinations may help elucidate the etiology. Prospective studies are needed for circadian rhythm in febrile convulsions.

Keywords: Circadian rhythm; febrile convulsions; hemogram parameters; hypocapnia.

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Febrile convulsions (FC) are convulsions that occur at a body temperature above 38°C without central nervous system infection, trauma, metabolic abnormality, electrolyte imbalance, drug intake, and history of afebrile convulsions [1, 2]. FC is very common in childhood. The age of FC is between 6–60 months. It is most common around 2 years of age [2].

Body temperature is regulated by cell clusters in the hypothalamus, showing circadian rhythm variation. The body has a circadian rhythm. Thanks to the circadian rhythm, body temperature can be controlled for 24 hours [3]. The circadian rhythm develops from approximately the age of five [4]. Circadian rhythm in children is affected by internal-external factors. [3–5]. The circadian rhythm is

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associated with many agents in the central nervous system. Melatonin is the pineal hormone. It can inhibit the central nervous system. Day-night differences in melatonin levels have been demonstrated during convulsions. The production of kynurenine pathway metabolites (proconvulsant/anticonvulsant) in convulsion is different. Arginine vasopressin modulates the thermoregulatory response, and arginine vasopressin level increases at high fever [4].

Body temperature may vary according to gender, age, outdoor temperature, metabolic rate, and time of day [3–5]. Body temperature is about 36.5 ºC in the early morning and 37.5 ºC in the evening [3]. Body temperature reaches its highest level in the afternoon [3–5]. There is a physiological increase in body temperature in the evening [4].

High fever causes changes in neuronal activity in the anterior hypothalamus, hippocampus, and cerebral cortex due to infection or non-infection reasons, and is a defense mechanism [3–5].

A one-degree increase in body temperature can cause an increase in breathing rate of 3.7 breaths per minute, which can cause a change in blood gas and a decrease in PCO_2 [5]. pH changes have a central role in the brain. pH changes control convulsion activity. It has been shown in animal (mouse) experiments that alkalosis increases neuronal excitability and facilitates epileptic activity. Hyperthermia causes hyperventilation. Intracerebral hypocapnia and alkalosis resulting from hyperventilation may cause convulsions [6, 7].

Anemia causes an energy metabolism disorder. Changes in the synaptic neurotransmitter system and delay in myelin development may occur due to anemia [8].

Fever, age and genetic predisposition, positive family history, central thermoregulation disorders, iron, and zinc deficiency, humoral immune system disorders, hyperventilation and respiratory alkalosis due to hyperthermia, changes in cytokines, daily and seasonal changes, changes in melatonin levels, and many other conditions have been investigated and associated with febrile convulsions [5–7, 9, 10]. The cause of febrile convulsions is not fully understood [3, 7].

In our study, we aimed to investigate the etiological importance of circadian rhythm, season, age, gender and hemogram parameters in children diagnosed with febrile convulsions.

Highlight key points

- Young age, anemia, inflammation, and hypocapnia appear to facilitate the development of febrile convulsions.
- MPV and NLR values may serve as quick hemogram markers reflecting inflammatory activity in febrile convulsions.
- Circadian rhythm and seasonal variation showed no significant influence on febrile convulsion occurrence.

MATERIALS AND METHODS

It is a descriptive study conducted retrospectively on patient records. The university medical sciences ethics committee gave permission for the study with the decision numbered 07/09 taken at the session numbered 07 (Ethics committee approval, date of receipt 25/06/2019). This study was conducted in accordance with the Declaration of Helsinki. It was done between 2012–2019. Patients who applied to the pediatric emergency outpatient clinic of the research hospital due to febrile convulsions were retrospectively analyzed.

Patient Group Selection

The anamnesis of the pediatric patients aged between 6 and 60 months who had a febrile convulsion was reviewed from the records of patients. Previous diagnoses, medications, radiographs, brain images and examination results were checked. Time of admission, season, age, additional diagnosis of febrile convulsion, hemogram parameters, CRP level, gender, and recurrent febrile convulsions, if any, were recorded.

Patients with severe anemia (those with Hb levels that would indicate insufficiency such as tachypnea, tachycardia), neurometabolic disease, younger than six months, older than sixty months, with central nervous system infection or inflammation, acute systemic metabolic abnormality that may cause convulsions, history of afebrile convulsions or head trauma, respiratory distress, severe diarrhea-vomiting (those with moderate to severe dehydration, those with fluid electrolyte imbalance, those with acidosis), hemodynamic disorders, who applied to the hospital long after their convulsions, and patients who had previously been diagnosed with mental-motor retardation, malignancy, or rheumatological disease were excluded from the study. The patients with complex FC, acute gastroenteritis and lower respiratory tract infections were excluded from this study.

Control Group Selection

Healthy control group: The records of patients who were brought to the control for healthy child examinations or referred by the family doctor for control were examined. Children with hemogram examination in

their files were recorded as the control group. The anamnesis of the patients in this control group was analyzed from the records. Previous diagnoses, medications, radiographs, brain images and examination results, if any, were checked, and those with a previous diagnosis of chronic or acute disease or drug use were excluded from the study.

Convulsion-free febrile control group: Patients of similar age and gender, without a diagnosis of chronic disease, only diagnosed with fever and/or fever. Children with hemogram examination in their files were recorded as the control group. The anamnesis of the patients in this control group were analyzed from the records. Previous diagnoses, medications, radiographs, brain images and examination results, if any, were checked, and those with a previous diagnosis of chronic or acute disease or drug use were excluded from the study.

Evaluation of laboratory parameters: From the hemogram parameters, white blood cell count (WBC), platelet (PLT) counts, mean platelet volume (MPV), hemoglobin (HGB), red cell distribution width (RDW), mean corpuscular volume (MCV), red blood cell (RBC) and neutrophil and lymphocyte counts were recorded. The neutrophil-to-lymphocyte ratio (NLR) was calculated. The NLR result was written by dividing the absolute neutrophil counts by the absolute lymphocyte counts. Anemia was accepted if hemoglobin was lower than 10.5 g/dL in children aged 6 months to 2 years and if hemoglobin was lower than 11.5 g/dL in children aged 2–5 years and if MCV and RBC were low for age [11]. The reference range accepted by our hospital laboratory was used. Reference ranges of hemogram parameters were WBC 4000–11000 mm^3, neutrophil 1500–8000 mm^3, lymphocytes 2000–8000 mm^3, RDW 11–14%, MPV 9–12 femtoliter (fl), PLT 150–450×10^3 ml. Reference ranges of biochemical parameters were glucose 60–100 mg/dl, sodium 135–145 mmol / L, potassium 3.5–5.1 mmol / L, calcium 8.5–10.8 mg/dl, CRP 0–5 mg / L. Reference ranges of blood gas parameters were pH 7.35–7.45, pCO_2 35–45 mmHg, HCO_3 22–26 mmol/L. Blood gases were studied in some of the patients. The patients whose blood gas was taken were also evaluated within themselves.

Evaluation of circadian rhythm: The day was divided into six-hour periods to evaluate for circadian rhythm. Between 06:00 and 12:00 was considered as morning, between 12:00 and 18:00 was considered as noon, between 18:00 and 24:00 as evening, and between 24:00 and 06:00 was considered as night. The time when the patients first applied to the outpatient clinic was recorded.

Statistical Analysis

We performed the statistical analysis using IBM SPSS 21.0 (Armonk, NY: IBM Corp.) program. We presented the results as mean±standard deviation for continuous variables, median/interquartile range for medians, and minimum-maximum n (%) for categorical variables. We confirmed the normality of the distribution in continuous variables with the Shapiro-Wilk test. We used the Student's t-test or the Mann-Whitney U test, depending on whether the statistical hypotheses were fulfilled in the comparison of independent continuous variables between the two groups. We used paired samples t-test and Wilcoxon signed-rank test when evaluating dependent continuous variables. We accepted a p-value of <0.05 as statistical significance.

RESULTS

The number of patients diagnosed with febrile convulsions in our study was 160, of which 90 (56.3%) were male and 70 (43.7%) were female. 160 healthy control groups of the same age and gender and 158 febrile control groups without convulsions of the same age and gender were also included in this study. The incidence in male was 1.3 times that of females. The mean age was 25.7±14.7 (minimum 6- maximum 60) months, and 58.1% of these patients had febrile convulsions between 6–24 months. In these, the most common age of convulsion was found to be 13–18 months. When we divided the day into 6 hours, 63 (39.4%) patients were admitted to the pediatric outpatient clinic (emergency or general outpatient clinic) at night and in the morning, and 97 (60.6%) patients were admitted afternoon and evening. The most frequent application was between 12:00–18:00. The highest number of patient admissions was in the summer months with 26.9%. Upper respiratory tract infection (94.4%) was the most common diagnosis accompanying febrile convulsions (Appendix 1).

Anemia was present in 13 of 160 patients (8.1%). Hemoglobin levels were lower in children first two years of age than those at other ages ($p<0.001$) (Appendix 1). MPV value was lower in winter ($p=0.002$) (Appendix 1). Lymphocyte levels were higher in children aged 6–12 months than in children aged 25–30/37–60 months ($p=0.048$), and NLR was higher in children aged 49–60 months than other months ($p=0.036$) (Appendix 2). Biochemical parameters were glucose: 122.5±32.2 mg/dl, sodium: 134.5±2.8 mmol/L, potassium: 4.2±0.5 mmol/L, calcium: 9.5±0.5 mg/dl. CRP was studied in 143 of 160 patients, of which 79 (49.4%) were CRP neg-

Table 1. Relationship between gender, age groups, and pH, PCO_2, HCO_3 of patients with febrile convulsion

	n	pH^+	p	pCO_2^+ (mmHg)	p	HCO_3^+ (mmol/L)	p
Total	82	7.32±0.09		38.8±9.0		19.2±2.6	
Gender							
Girls	33	7.30±0.89	0.123	40.8±7.4	0.093	19.2±2.4	0.081
Boys	49	7.33±0.10		37.4±9.8		19.1±2.7	
Age groups							
6–12 m	13	7.32±0.09	0.119	38.1/18.7 (16.7–55.1)	0.169*	18.6±2.7	0.204
13–18 m	18	7.30±0.09		41.1/11.5 (22.3–54.2)		18.8±2.1	
19–24 m	15	7.36±0.10		31.1/10 (20.4–55.6)		19.3±1.6	
25–30 m	10	7.35±0.07		36.3/11.1 (24.3–46.3)		19.9±2.4	
31–36 m	8	7.33±0.04		41.4/9.5 (34.2–49.9)		20.9±1.19	
37–48m	11	7.31±0.09		37.9/15.7 (31.9–53.6)		19.3±3.9	
49–60 m	7	7.24±0.11		42.9/17.9 (31.3–60.9)		17.4±3.4	

+: Results are presented as Mean±SD and/or Median/IQR (minimum-maximum); *: Kruskal- Wallis test. pH: Power of hydrogen; PCO_2: Partial carbon dioxide pressure; HCO_3: Bicarbonate; SD: Standard deviation; IQR: interquartile range for medians; m: Mont

ative, 38 (23.8%) had moderate CRP elevation, and 26 (16.3%) had very high CRP. The blood gas parameters of 82 patients were evaluated. The value ranges of pH, pCO_2, HCO_3 among the blood gas parameters included in the study were as follows, respectively: pH 7.30±0.09, pCO_2 38.8±9.0 mmHg, HCO_3 19.2±2.6 mmol/L. There was no relationship between gender, age groups and pH, PCO_2 and HCO_3 (Table 1). There were 46 (56.1%) patients with pH<7.35, 30 (36.6%) patients with pH=7.35–7.45, and 6 (7.3%) patients with pH>7.45. There were 29 (35.4%) patients with pCO_2<35 mmHg, 34 (41.5%) patients with pCO_2=35–45 mmHg, and 19 (23.2%) patients with pCO_2>45 mmHg. There were 70 (85.4%) patients with HCO_3<22 mmol/L, 12 (14.6%) patients with HCO_3=22–26 mmol/L, and zero patients with HCO_3>26 mmol/L.

When the hemogram parameters of the febrile convulsion patient group-the control groups were compared: There were differences between the patient (FC) groups and the control groups in terms of Hb (p=0.006), MPV (p=0.033), WBC (p=0.001), lymphocyte (p=0.001), neutrophil-lymphocyte ratio (p=0.001) (Table 2). Anemia was found in 13 (8.1%) of 160 patients, 31 (19.6%) of 158 in the convulsion-free febrile control group and 21 (13.1%) of 160 in the control group (p=0.011) (Table 3, 4).

Twenty-seven (16.9%) of 160 patients diagnosed with FC had recurrent convulsions. Of those who had recurrent convulsions, 14 (51.9%) were boys and 13 (48.1%) were girls, with a male-female patient ratio of 1.07. Eighteen (66.7%) of 27 patients had their first convulsion before the age of two and six (22.2%) before the age of one. Twenty-seven patients with recurrent convulsions had a mean convulsion recurrence rate of 2.4±1.08 and had a history of at least one recurrent convulsion and at most four recurrent convulsions.

DISCUSSION

The childhood period between six and sixty months, when febrile convulsions are seen, is the age range in which infections are most common [8, 11]. In this age group, the sensitivity and neuronal excitability of the brain tissue to a sudden increase in body temperature is high [3, 12]. 50–70% of children with FC may have convulsions in the first 24 months and most frequently in 18–24 months [2]. In our study, FC was detected in approximately half of our patients in the first 24 months, the most common age range was 13–18 months, and our study was compatible with the literature. In some studies on the circadian rhythm in the formation of febrile convulsions, it has been reported that FC is five times or seven times higher between 18:00 and 24:00 in the evening, and in some studies, body temperature is higher in the afternoon and evening [3, 4, 9, 13, 14]. It has been suggested that the circadian rhythm does not change the tendency of convulsions and that exceeding the convulsion threshold

TABLE 2. Hemogram parameters in the control groups and patients with febrile convulsion

	Patient+	Convulsion-free febrile control group+	Control group+	p
HGB (gr/dL)	11.9±0.9 11.9/1.1 (8.6–14.4)	11.4±1.2 11.7/1.4 (8–16.1)	12.1±1.0 12.1/1.3 (8.1–14.0)	**0.006**
RDW (%)	14.6±2.3 14.0/2.2 (11.9–32.0)	15.0±2.3 14.2/2.9 (11.7–24)	14.3±1.7 13.9/1.8 (11.4–22.0)	0.062*
MPV (fl)	8.4±1.1 8.4/1.6 (4.6–11.1)	8.8±1.2 9.0/1.8(6.1–11.5)	8.7±1.2 8.7/1.7 (6.2–11.7)	**0.033***
PLT (×10³ ml)	323±112 302/121 (135–835)	308±125 290/134 (116–1087)	342±86 337/104 (176–683)	**0.001***
WBC (mm³)	12611±5516 11200/6875 (2800–35700)	11715±6219 10650/8100 (1800–37500)	8693±1849 8800/2775 (4200–14500)	**0.001***
Neutrophil (mm³)	7522±4997 6652/6093 (223–30345)	6414±4899 5675±5799 (316–31205)	2930±1499 2622/1682 (523–10686)	**0.001***
Lymphocyte (mm³)	3684±2204 3414/2575 (88–14081)	4092±2434 3551/2744 (343–12486)	4822±1406 4690/2065 (1881–8353)	**0.001***
NLR (Neutrophil /lymphocyte)	2.9±3.0 1.9/2.4 (0.1–19.3)	2.2±2.5 1.3/1.7 (0.1–16.4)	0.7±0.6 0.5/0.5 (0.1–4.6)	**0.001***

+: Results are presented as Mean±SD and/or Median/IQR (minimum-maximum); *: Kruskal-Wallis test. HGB: Hemoglobin; red blood cell distribution width (RDW) reference range: 11–14%; mean platelet volume (MPV) reference range: 9–12; PLT: Platelet reference range: 150–450×10³ ml; white blood cell count (WBC) reference range: 4000–11000 mm³; neutrophil 1500–8000 mm³; lymphocytes 2000–8000 mm³; femtoliter (fl); SD: Standard deviation; IQR: interquartile range for medians

according to environmental or genetic sensitivity in situations that facilitate an increase in body temperature such as infection causes convulsions [9]. In our study, most of our patients came in the afternoon. But there wasn't statistical significance. This result cannot suggest that circadian rhythm may have an effect on FC formation. It has been reported in many studies that febrile convulsions occur most frequently in winter and in January, and this may be associated with a higher incidence of febrile illness in winter [4, 9, 13, 14]. In some studies, febrile convulsions were reported to be associated with seasonal viral outbreaks [15]. The prevalence of febrile convulsions was found to be high from late spring to summer [15]. For our patients, the most frequent application month was September, and the most frequent application season was summer. Frequency rates were similar in all seasons. In our study, upper respiratory tract infection was found to be the most common cause in children with FC.

Studies have also shown that patients with febrile convulsions have hyperventilation and respiratory alkalosis due to hyperthermia, and that intracerebral hypocapnia and alkalosis may cause convulsions [6, 7]. In a study comparing patients who applied to the pediatric department with fever without gastroenteritis or lower respiratory tract infection (LRTI), it was suggested that hypocapnia may be one of the main mechanisms in the onset and maintenance of febrile convulsions [10]. One-third of our patients had hypocapnia, which supports the literature.

There are studies that anemia lowers the convulsion threshold and increases the risk of FC [8, 16]. Iron participates in the structure of some enzymes in many neurotransmitter metabolism in the central nervous system. Iron deficiency anemia is common in developing countries and in children aged 6 to 24 months. Iron deficiency anemia in children with FC is reported to be more common in children than in controls. It is suggested that iron deficiency anemia facilitates the occurrence of convulsions. The iron levels of our patients could not be checked. The frequency of anemia was more common in the the convulsion-free febrile control group and in the healthy control group than in patients with febrile convulsions. In patients with febrile convulsions with anemia, RDW was higher and NLR was lower than in patients with febrile convulsions without anemia. in the with anemia in the without anemia. In the convulsion-free febrile control group RDW and NLR were higher

Table 3. Anemia and hemogram parameters in the control groups and patients with febrile convulsion

	Anemia	%	Age (months)	Hb+ (gr/dL)	RDW+ (%)	Plt+ (×10³ ml)	MPV+ (fl+)
Patient with FC n=160	Yes	8.1	22.4±12.5	10.2±0.8	17.5±4.9	329±170	8.4±0.9
			25/20.5 (6–45)	10.3/1.5 (8.6–11.4)	16.0/4.7 (13.3–32)	278/112 (187–835)	8.6/1.4 (7–10)
	No	91.9	25.9±14.9	12.0±0.8	14.4±1.8	323±107	8.4±1.2
			21/20 (6–60)	12.0/1.1 (10.3–14.4)	14.0/1.9 (11.9–21)	308/122 (135–706)	8.4/1.6 (4.6–11.7)
Convulsion free-febrile	Yes	19.6	23.5±13.7	10.0±0.9	17.0±3.0	378±190	8.3±1.5
			19/23 (6–60)	10.1/1.3 (8.0–11.5)	16.4/3.8 (13.1–24)	329/125 (186–1087)	8.3/2.4 (6.2–11.4)
control group	No	80.4	25.6±14.8	12.0±0.8	14.5±1.9	290±97	8.8±1.1
			21/22 (6–59)	12.0/1.0 (10.6–16.1)	13.8/2.2 (11.7–21.1)	271/135 (116–579)	9.0/1.6 (6.1–11.5)
n=158	Yes	13.1	18.1±11.5	10.2±0.7	14.3±1.9	344±96	8.7±1.0
			12.0/18.0 (6–47)	10.3/0.8 (8.1–11.2)	13.6/2.3 (12.3–19.6)	327/153 (211–552)	8.8/1.3 (6.7–10.8)
Healthy control group	No	86.9	26.9±14.9	12.3±0.8	14.3±1.7	342±84	8.6±1.2
			21.5/21.3 (6–60)	12.3/1.2 (10.5–14)	13.9/1.8 (11.4–22)	338/100 (176–683)	8.7/1.8 (6.2–11.2)
n=160	p	**0.011****	0.740	**0.001**	**0.001**	**0.001***	**0.044***

+: Results are presented as Mean±SD and/or Median/IQR (minimum-maximum); *: Kruskal- Wallis test; **: Pearson Chi-Square; Hb: Hemoglobin; red blood cell distribution width (RDW) reference range: 11–14%; white blood cell count (WBC) reference range: 4000–11000 mm³; platelet (PLT) reference range: 150–450×10³ ml; mean platelet volume (MPV) reference range: 9–12; femtoliter (fl); neutrophil 1500–8000 mm³; lymphocytes 2000–8000 mm³; FC: Febrile convulsion; SD: Standard deviation; IQR: interquartile range for medians

in those with anemia than in those without anemia. In the healthy control group RDW and NLR were similar in the with anemia in the without anemia. It may suggest that anemia may lower the convulsion threshold in people prone to febrile convulsions.

A link between FC and inflammation has been demonstrated [17]. Overproduction of proinflammatory cytokines (interleukin -6, interleukin-1β, tumor necrosis factor-α) during infections may lower the convulsion threshold in children [18]. Publications have shown that neutrophil counts increase and lymphocyte counts decrease in patients with FC compared to control groups [19]. In our study, neutrophil count was found similar in all groups of patients with FC (Gender, age, cause of fever, season, circadian rhythm, anemia).

Neutrophil count, lymphocyte count, MPV, PLT and RDW are inexpensive indicators that can be detected easily and quickly in inflammatory reactions [19, 20]. It has been suggested that NLR is found to be significantly higher in patients with FC than in patients without FC, and that NLR may have a synergistic effect on the formation of FC [19]. In our study, NLR, neutrophil counts were significantly higher in FC patients than in the control groups, which is consistent with the literature.

It has been reported that red blood cell distribution (RDW) is an inflammatory marker, there is more inflammation in the brain in patients with complex FC, and RDW is found to be higher than patients with simple FC [19, 20]. Approximately half of our patients had high RDW, but the RDW level was similar in patients with FC and the control group.

MPV reflects the platelet volume and the rate of platelet production in the bone marrow. It can also be used as an indicator of platelet activation and inflammation severity [21]. Activated platelets secrete a large number of substances that are an important mediator of inflammation [22]. In studies, it was shown that the platelet count was significantly lower in children with FC and the MPV was significantly higher than in the control groups without FC [19]. In our study, platelet count was lower in

Table 4. Anemia and hemogram parameters in the control groups and patients with febrile convulsion

	Anemia	%	WBC⁺ (mm³)	Neutrophil⁺ (mm³)	Lymphocyt⁺ (mm³)	NLR⁺
Patient with FC n=160	Yes	8.1	10992±4297	5724±3474	4259±1642	1.6±1.3
			10500/7100 (5600–20300)	4437/4600 (1713–14210)	4146/2542 (1498–7455)	1.0/1.2 (0.5–5.6)
	No	91.9	12754±5600	7681±5088	3634±2244	3.0±3.0
			11500/6900 (2800–35700)	6825/6227 (223–30345)	3336/2761 (88–14081)	2.0/2.7 (0.1–19.3)
Convulsion free-febrile control group n=158	Yes	19.6	15277±7823	9262±7003	4324±2580	3.5±4.0
			14400/9800 (5100–37300)	7761/9428 (651–31205)	3640/2644 (525–12322)	1.9/1.3 (0.1–16.3)
	No	80.4	10846±5453	5719±3969	4036±2404	1.8±1.8
			10100/7600 (1800–25500)	5289/5653(316–19933)	3484/2762 (343–12486)	1.2/1.5 (0.1–11.9)
Healthy control group n=160	Yes	13.1	8152±1752	2643±850	4524±1510	0.6±0.5
			7400/2800 (4900–10700)	2781/1418 (1332–4388)	4577/3055 (2183–7158)	0.5/0.3 (0.3–2.0)
	No	86.9	8775±1856	2973±1572	4867±1390	0.7±0.6
			8900/2700 (4200–14500)	2602/1824 (523–10686)	4747/2014 (1881–8353)	0.5/0.5 (0.1–4.7)
	p	**0.011****	**0.001***	**0.001***	**0.001***	**0.001**

+: Results are presented as Mean±SD and/or Median/IQR (minimum-maximum); *: Kruskal- Wallis test, **: Pearson Chi-Square; White blood cell count (WBC) reference range: 4000–11000 mm³; neutrophil 1500–8000 mm³; lymphocytes 2000–8000 mm³; NLR: Neutrophil lymphocyte ratio; FC: Febrile convulsion; SD: Standard deviation; IQR: interquartile range for medians

patients with FC compared to the control group, but unlike other studies, MPV was statistically lower than the control group.

CRP is one of the important indicators of inflammation. CRP reaches peak values 24–48 hours after inflammatory events. Because inflammatory events progress very rapidly in FC, FC occurs before CRP reaches high values. Therefore, CRP was found to be lower in children with FC than in children without FC [23]. In our patients, CRP was mostly negative or not very high, which is consistent with the literature.

CRP is one of the important indicators of inflammation. CRP reaches peak values 24–48 hours after inflammatory events. Because inflammatory events progress very rapidly in FC, FC occurs before CRP reaches high values. Therefore, CRP was found to be lower in children with FC than in children without FC [23]. In our patients, CRP was mostly negative or not very high, which is consistent with the literature.

Children with FC may have mild electrolyte disturbances and slightly low serum sodium levels [24]. In febrile convulsions, blood glucose levels may rise temporarily as a result of acute stress-induced elevation of catecholamines, cortisol, and glucagon [25]. Electrolyte values of our patients were normal in our study, since those with electrolyte disorders that could cause convulsions were excluded from our study.

FC is slightly more common in boys than in girls. There are studies in Turkey with a male/female ratio of 1.36:1 to 1.01:1 [2]. In our study, the M/F ratio was found to be 1.3 and was consistent with the literature. Febrile convulsions may recur especially in the first two years of life (15–70%) [17, 26]. Due to the decreasing convulsion threshold with age, the probability of recurrence is high in patients younger than one year of age [17]. Family history, genetic factors, and environmental factors are risk factors for recurrence. Recurrence is more common in boys [17, 26]. There was no family history, genetic result or environmental factor in the patients' emergency clinic files. Therefore, no information could be provided on this subject. Recurrence was seen in 16.9% of our patients, in accordance with the literature, Patients with recurrence were similar in age and gender.

CONCLUSION

Young age, anemia, hypocapnia and inflammation were important in febrile convulsion. The combination of young age, inflammation and anemia may facilitate the occurrence of convulsions in children with fever. In FC patients, increased NLR and decreased platelet, decreased MPV, increased lymphocyte counts, neutrophil counts and WBC counts levels are important indicators of inflammation. In patients with FC, ıt was observed that the lymphocyte level was high and MPV was low in the winter season. It was observed that the lymphocyte count was higher and the NLR rate was lower in anemic children with febrile convulsions at younger ages. In FC, NLR and neutrophil count were higher and MPV was lower than in the control group. This situation may support that inflammatory conditions facilitate the formation of febrile convulsions. Anemia may lead to febrile convulsion even at low NLR in children with a predisposition to febrile convulsion. We did not find seasonal and circadian rhythm differences in our study. More prospective studies are needed to elucidate these issues.

Evaluation of multiple parameters such as circadian rhythm, low hemoglobin levels and inflammation parameters, blood gas parameters to elucidate the etiology of febrile convulsions are the strengths of the study. The limitations of this study are that it is a retrospective and single-center study.

Ethics Committee Approval: The Erzincan Binali Yıldırım University Clinical Research Ethics Committee granted approval for this study (date: 25.06/2019, number: 07/09).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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Appendix 1. Relationship between gender, cause of fever, age groups, season, circadian rhythm and hemoglobin, red blood cell distribution, mean platelet volume, platelet of patients with febrile convulsion

	n	%	Age+ (months)	p	Hb+ (gr/dL)	p	RDW+ (%)	p	Plt+ (×10³ ml)	p	MPV+ (fl+)	p
Total	160	100	25.7±14.7		11.9±0.9		14.6±2.3		323/120 (135–835)		8.4±1.1	
Gender												
Girls	70	43.7	21/20 (6–60)	0.695	11.8±1.0	0.303	14.5±1.6	0.428	309/149 (135–835)	0.216*	8.4±1.1	0.486
Boys	90	56.3	21/21 (6–60)		12.0±0.9		14.8±2.7		289/111 (139–561)		8.3±1.2	
Age groups												
6–12 m	29	18.1	10.1 ±1.9	**0.001**	11.3±0.9	**0.001**	14.8±2.1	0.402	316/227 (187–835)	0.180*	8.1±1.1	0.546
13–18 m	39	24.4	15.4±1.7		11.6±1.1		15.1±3.4		320/99 (139–577)		8.5±1.2	
19–24 m	25	15.6	21.1±1.6		11.9±0.6		14.4±1.8		323/143 (135–453)		8.1±1.2	
25–30 m	20	12.5	27.8±1.8		12.3±1.1		15.1±1.9		299/106 (168–537)		8.2±1.1	
31–36 m	11	6.9	33.9±1.7		12.0±0.8		14.7±1.5		261/129 (145–433)		8.8±1.1	
37– 48 m	19	11.9	42.6±3.8		12.0±0.5		14.5±1.9		308/147 (164–488)		8.4±0.9	
49–60 m	17	10.6	55.8±4.6		12.8±0.6		13.5±1.0		262/47 (193–518)		8.4±1.2	
Cause of fever												
URTI	151	94.4	21/21 (6–60)	0.346*	11.9±0.9	0.275	14.7±2.3	0.261	306/122 (132–835)	0.736	8.4±1.1	0.318
Other	9	5.6	24/39 (6–60)		12.2±1.2		13.8±1.3		289/110 (162–509)		8.0±1.4	
Season												
Winter	40	25.0	20/28 (6–60)	0.232	12.0±1.1	0.285	14.5±3.1	0.374	313/146 (139–537)	0.720	7.8±1.0 6	**0.002**
Spring	41	25.6	18/14 (8–60)		11.9±0.8		15.1±1.7		316/123 (168–706)		8.7±1.16*	
Summer	43	26.9	24/24 (6–60)		11.6±0.9		14.7±1.9		278/140 (135–835)		8.6±1.0 6*	
Autumn	36	22.5	24/25 (9–60)		12.1±0.9		14.2±2.2		300/124 (193–561)		8.2±1.2	
Circadian rhythm												
Morning	35	22.9	29/31 (8–60)	0.231*	12.0±0.9	0.292	14.8±2.2	0.781	308/135 (148–835)	0.615*	8.4±1.0	0.978
Afternoon	52	32.8	19/19 (6–60)		11.9±1.0		14.8±3.0		310/127 (145–706)		8.4±1.0	
Evening	45	28.1	21/25 (6–60)		12.0±0.9		14.4±1.5		286/142(135–675)		8.3±1.2	
Night	28	16.1	18/10 (12–60)		11.6±1.0		14.3±2.1		297/111 (181–538)		8.4±1.3	
Anemia												
Yes	13	8.1	25/21 (6–45)	0.409	12.0±0.97	**0.001**	17.5±4.88	**0.001**	278/112 (187–835)	0.849	8.3±1.1	0.797
No	147	91.9	21/20 (6–60)		10.0±0.87*		14.5±1.88*		308/122 (135–706)		8.4±0.9	

+: Results are presented as Mean±SD, Median/ IQR (minimum-maximum); *: Kruskal- Wallis test; Hb: Hemoglobin; RDW: Red blood cell distribution width; Plt: Platelet; MPV: Mean platelet volume; fl: Femtoliter; m: Months; URTI: Upper Respiratory Tract Infection; IQR: interquartile range for medians

Appendix 2. Relationship between gender, cause of fever, age groups, season, circadian rhythm and WBC, neutrophil, lymphocyte, neutrophil-lymphocyte ratio of patients with febrile convulsion

	n	WBC⁺ (mm^3)	p	Neutrophil⁺ (mm^3)	p	Lymphocyt⁺ (mm^3)	p	NLR⁺	p
Total	160	11200/6875 (2800–35700)		6652/6093 (223–30345)		3414/2575 (88–14081)		1.9/2.4 (0.1–19.3)	
Gender									
Girls	70	10900/6325 (2800–29100)	0.102	6457/5867 (418–22756)	0.182	3334/2723 (683–14081)	0.399	1.9/2.4 (0.1–9.5)	0.518
Boys	90	12600/7225 (2900–35700)		6968/6282 (223–30345)		3662/2672 (88–13855)		1.8/2.6 (0.2–19.3)	
Age groups									
6–12 m	29	11800/6100 (6000–22000)	0.984	6630/6198 (1092–13690)	0.439	4171/3166 (337–14081)	**0.048***	1.3/2.1 (0.2–19.3)	**0.036***
13–18 m	39	11200/6800 (4800–28600)		5720/4644 (223–23428)		3744/2890 (456–7117)		1.6/1.4 (0.2–7.5)	
19–24 m	25	10500/7445 (5700–29100)		6349/4316 (1827–22756)		3225/2202 (1113–13855)		1.8/3.7 (0.2–7.3)	
25–30 m	20	13500/8025 (3500–25300)		7417/7761 (418–20872)		3178/2303 (875–8840)		2.5/2.2 (0.1–6.7)	
31–36 m	11	13500/10410 (2800–26900)		6674/10908 (1276–18991)		3902/3876 (1086–8245)		1.6/2.2 (0.9–4.4)	
37–48m	19	11000/5000 (5000–35700)		8040/5325 (2545–30345)		2786/2468 (88–6400)		2.7/3.5 (0.4–10.6)	
49–60 m	17	14000/7450 (5100–25600)		9010/8953 (2458–24704)		1872/2592 (128–8540)		3.2/8.1 (0.4–19.3)	
Cause of fever									
URTI	151	11200/6790 (2800–35700)	0.137*	6630/5831 (418–30345)	0.981*	3483/2404 (88–14081)	**0.026***	1.8/2.4 (0.1–19.3)	**0.240***
Other	9	9400/7350 (4300–18800)		7325/10196 (223–16111)		1272/2331 (456–6249)		2.5/9.3 (0.5–19.2)	
Season									
Winter	40	10900/6725 (5000–35700)	0.601	5711/6582 (418–30345)	0.783	3224/2771 (710–10191)	0.940	1.9/3.7 (0.1–19.2)	0.749

Appendix 2 (cont.). Relationship between gender, cause of fever, age groups, season, circadian rhythm and WBC, neutrophil, lymphocyte, neutrophil-lymphocyte ratio of patients with febrile convulsion

	n	WBC+ (mm^3)	p	Neutrophil+ (mm^3)	p	Lymphocyt+ (mm^3)	p	NLR+	p
Spring	43	12200/7350 (3500–29100)		7626/5436 (223–22756)		3586/2771 (456–7480)		1.9/2.2 (0.2–12.1)	
Summer	41	11000/5700 (2800–21900)		6825/6036 (1092–16111)		3476/2764 (88–14081)		2.0/3.1 (0.2–10.6)	
Autumn	36	11000/7075 (5600–26900)		5740/7098 (1713–24704)		3710/2241 (128–13855)		1.7/2.5 (0.2–19.3)	
Circadian rhythm									
Morning	35	12300/6800 (5100–25300)	0.837	7539/6111 (1092–20872)	0.088	3224/3142 (88–8540)	0.466	2.5/3.7 (0.2–12.1)	0.202
Afternoon	52	11050/6475 (2800–28600)		5789/6889 (223–23652)		3432/3525 (456–14081)		1.7/2.4 (0.2–7.5)	
Evening	45	12200/7250 (5000–35700)		6844/6293 (418–30345)		3476/2373 (128–10191)		2.1–2.9 (0.1–19.3)	
Night	28	10450/7222 (5600–22000)		5644/4671 (1193–16111)		3720/2443 (1116–6780)		1.4/1.5 (0.2–9.5)	
Anemia									
Yes	13	10500/7100 (5600/20300)	0.271	4437/4600 (1713–14210)	0.177	4146/2542 (1498–7455)	0.328	1.0/1.2 (0.46–5.6)	**0.029***
No	147	11500/6900 (2800–35700)		6825/6227 (223–30121)		3336/2761 (88–14081)		2.0/2.7 (0.1–19.3)	

+: Results are presented as Median/ IQR (minimum-maximum); *: Kruskal-Wallis test; 1>1*. WBC: White blood cell count; NLR:Neutrophil-lymphocyte ratio; m: Months; URTI: Upper Respiratory Tract Infection; IQR: Interquartile range for medians

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Original Article PEDIATRICS

A reference centre experience in central Anatolia in terms of causes, severity and treatment of childhood anaphylaxis

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ABSTRACT

OBJECTIVE: Anaphylaxis is a life-threatening reaction in every age groups. The causative factors, incidence, and severity of anaphylaxis differ between children and adults. Literature on anaphylaxis during childhood is insufficient. We believe that the data of this study will make important contributions to the literature.

METHODS: A retrospective analysis was conducted on patients aged 0–18 years who were admitted to the Pediatric Allergy Outpatient Clinic at Erciyes University between 2015 and 2021 and diagnosed with anaphylaxis.

RESULTS: The majority of the patients were male. Females, however, exhibited greater prevalence during adolescence ($p<0.001$). Of the total number of patients, 153 (86.9%) had atopy, whereas 25.56% had non-allergic chronic diseases. Food-induced anaphylaxis was identified in 49 (27.84%) patients, drug-induced anaphylaxis in 41 (23.29%), venom-induced anaphylaxis in 62 (35.22%) and idiopathic anaphylaxis in 19. The study observed that anaphylaxis affected several systems, including the skin/mucosa (91.47%), respiratory (72.15%), GI (40.34%), cardiovascular system (20.45%), and central nervous system (17.04%). It was also found that drug-induced anaphylaxis increased the severity of symptoms ($p=0.003$). Additionally, it was statistically significant that antibiotic-induced anaphylaxis ($p=0.002$) and wasp sting-induced anaphylaxis ($p=0.003$) developed within the first minute. Patients received intramuscular adrenaline in 95.46% of cases, while 4.54% did not receive appropriate treatment.

CONCLUSION: Anaphylaxis is a completely reversible reaction when diagnosed correctly and treated promptly. Therefore, it is essential to recognize the common triggers, know the risk factors, and provide patients with appropriate treatment. We believe that this study will contribute to better recognition of deficiencies in the management of anaphylaxis by highlighting important information for anaphylaxis.

Keywords: Adrenaline; anaphylaxis; childhood anaphylaxis; severity of anaphylaxis; trigger.

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Anaphylaxis is a rapidly developing, life-threatening and unpredictable reaction. If recognized early and treated properly, it's completely reversible [1].

Large-scale studies reveal an epidemiology of 50–112 cases per 100,000 people per year [2]. The incidence of anaphylaxis has shown a 4.3% increase annually between 2001–2010 [3]. While anaphylaxis can affect individuals of all ages, it's more prevalent in the 0–4 age group [4].

Cause of anaphylaxis varies based on age and country of residence. As anaphylaxis can have various causes, in certain cases, the triggering factor of the reaction cannot be identified. Hence, while anaphylaxis with un-

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known cause's rare in childhood, it has been found to be the most frequent trigger in some studies conducted with adults [5].

Allergic diseases, uncontrolled asthma, chronic conditions, medication usage can elevate the risk of anaphylaxis development [6].

The classical way for anaphylaxis to occur is IgE-mediated anaphylaxis. Mast cells and basophils plays important role in this pathway [7–9]. While there's evidence of anaphylaxis reactions involving IgG and immune complexes, non-allergic anaphylaxis can also occur in cases where the immune system isn't involved or the cause is unclear [7, 10].

There is no definitive laboratory test for diagnosing anaphylaxis. Diagnosis relies solely on clinical observations [11, 12]. If clinical findings indicate anaphylaxis, treatment should be initiated immediately, as this reaction can be mistaken for other diseases and can worsen rapidly [12]. The reaction typically occurs within 1 hour of exposure to the anaphylaxis agent. There are also potential difficulties in cases of biphasic anaphylaxis and late onset anaphylaxis [13].

While children typically experience skin/mucosal involvement the most, the respiratory, cardiovascular, gastrointestinal, and nervous systems may also be impacted. Urticaria is the most frequent symptom [14]. Reaction can be categorized as mild, moderate or severe, depending on symptom severity [15].

The indispensable administration in the treatment of anaphylaxis is always epinephrine to be administered intramuscularly without delay after diagnosis. The recommended dose of epinephrine is 0.01 mg/kg, administered into the vastus lateralis muscle [12].

The objective of the research is to ascertain the causative agents of anaphylaxis among children, the clinical manifestations, system participation, and the features of the observed reaction.

Highlight key points

- Atopy was detected in 86.9% of patients diagnosed with anaphylaxis.
- Skin reactions are the most common manifestation of anaphylaxis. The absence of skin involvement in 8.53% of patients should not delay the diagnosis of anaphylaxis.
- Drug-induced reactions are more severe.
- Reactions associated with antibiotic use and wasp stings occur more rapidly.
- The cause of anaphylaxis varies by age and gender. Among all patients, venom-associated anaphylaxis is the most common.

MATERIALS AND METHODS

Patiens

In this study, patients between the ages of 0–18 who were followed up with for anaphylaxis at Erciyes University Faculty of Medicine Pediatric Allergy Polyclinic between 2015–2021 were examined. Based on the data in the hospital information system and the hospital archive, an evaluation was made on the data of 176 patients who met the anaphylaxis diagnostic criteria. The study was conducted in accordance with the declaration Helsinki.

This single center retrospective cross-sectional study was conducted after the Erciyes University Clinical Research Ethics Committee with approval (approval number 2022/36, date 5/1/2022).

Prick Test

In the skin prick test, it was required that at least 4–6 weeks had passed since anaphylaxis before the test. Care was taken to ensure that the patients had not taken antihistamine medication in the last week. 23 parameters were evaluated in the prick test. Histamine was used as positive control. Physiological serum was used as negative control. Compared to negative control, those with an induration diameter of over 3 mm were considered positive.

Statistical Analysis

The data analysis was performed using the statistical software TURCOSA (Turcosa Analytics Ltd Co, Türkiye www.turcosa.com.tr). Numerical variables are presented as medians and quartiles, while categorical variables are presented as numbers and percentages. The data distribution was assessed using hypothesis tests (Shapiro Wilk, Kolmogorov-Smirnov normality test) and graphical methods (histogram, Q-Q plot, etc.). Objectivity and comprehensibility were prioritized in the presentation of the findings. The Mann-Whitney U test was employed to compare non-parametric data between groups, while categorical data was analyzed using the Pearson Chi-square and Fisher's exact tests. The level of significance was set at $p<0.05$.

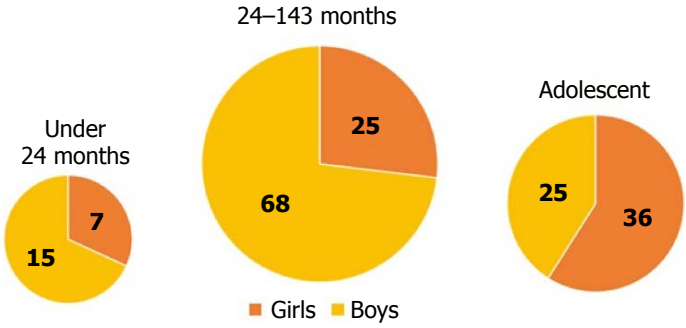

Figure 1. Number of the patients in the age groups.

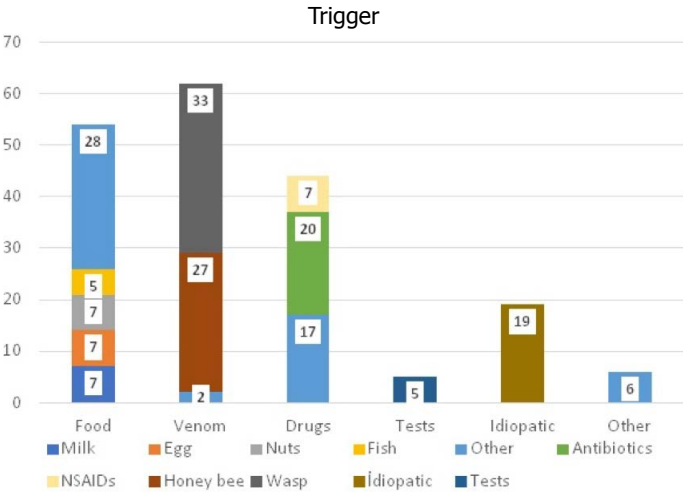

Figure 2. Trigger of the anaphylaxis reaction.

RESULTS

The mean age of 176 patients was 8.32±5.33 years. Of these patients, 108 (61.3%) were male, 68 (38.9%) were female. The patients were classified into three groups according to age-related risk factors, namely those under 24 months, those between 2–12 years, and adolescents (12 years and older). Of the patients under 24 months, 15 (68.18%) were male and 7 (31.81%) were female. Amongst the adolescent group, 25 (40.98%) were male and 36 (59.02%) were female. Among the two groups, boys accounted for 68 (73.11%) and girls 25 (26.88%). By age, the adolescent group was predominantly female ($p<0.001$) (Fig. 1).

Of the patients, 21 (11.93%) had obesity. There was no significant link between obesity and anaphylaxis severity ($p=0.49$) or recurrent anaphylaxis ($p=0.26$).

Atopy was present in 153 patients (86.9%), shown in Table 1.

Table 1. Atopy of the patients (n=176)

	%
Food allergy	31.81
Pollen allergy	23.29
Wasp venom allergy	18.75
Seasonal allergic rhinitis	17.61
Asthma	17.05
Honeybee venom allergy	15.3
Drug allergy	12.5
Cat allergy	7.38
House dust mites allergy	6.81
Atopic dermatitis	5.68
Mold allergy	3.4
Dog allergy	1.13

The rate of individuals with non-allergic chronic diseases was found 25.56%.

52 patients (29.54%) had recurrent anaphylaxis. There was no significant correlation between age groups and recurrent anaphylaxis ($p=0.21$).

In 49 patients (27.84%), anaphylaxis was triggered by food. Eggs, milk and nuts were triggered in 7 patients each (3.97%) and fish in 5 patients (2.84%).

41 patients (23.29%) presented drug-induced anaphylaxis. In 20 (11.36%) of these cases, antibiotics were the cause, while NSAIDs were responsible for 7 (3.97%) cases.

27 patients (15.34%) were diagnosed with anaphylaxis due to honeybee sting, whereas 33 patients (18.75%) had wasp sting-induced anaphylaxis.

Additionally, 19 patients (10.79%) were determined to have idiopathic anaphylaxis.

A total of 5 (2.84%) patients presented anaphylaxis following allergy test application. while rupture of hydatid cyst caused anaphylaxis in 1 patient (0.56%). In 5 patients (2.84%), anaphylaxis was associated with contact with cats (Fig. 2).

Patient's symptoms during anaphylaxis are listed in Table 2.

Affected systems were as follows: 161 patients (91.47%) had skin/mucous membrane involvement, while 127 (72.15%) presented with respiratory symptoms. 71 (40.34%) had GI symptoms, 30 (17.04%) suffered from

TABLE 2. Symptoms of anaphylaxis

	%
Urticaria	72.72
Shortness of breath	62.5
Angioedema	56.81
Vomiting	29.54
Hypotension	13.36
Nausea	9.09
Abdominal pain	7.38
Tachycardia	5.11
Cough	4.54
Cyanosis	4.54
Dizziness	4.54
Wheezing	4.54
Dysphagia	3.4
Sleepiness	2.84
Blackout	2.27
Throat itchiness.	2.27
Confusion	1.7
Sweating	1.7
Diarrhea	1.13
Itchy eyes	1.13
Seizure	1.13
Tingling in the mouth	1.13
Weakness	1.13
Arrest	0.56
Flushing	0.56
Shock	0.56
Sneezing	0.56

central nervous system (CNS) involvement, 36 (20.45%) reported cardiovascular system (CVS) symptoms (Fig. 3).

Mild, moderate and severe anaphylaxis were observed in 22 (12.5%), 105 (59.65%) and 49 (27.84%) patients. No statistically significant relationship was found between age-related risk factors and the severity of anaphylaxis or the presence of asthma (p=0.80, p=0.88).

The severity of anaphylaxis increased in cases of drug-induced anaphylaxis (p=0.003) (Table 3).

Biphasic anaphylaxis was observed in 7 patients (3.97%).

Upon retrospective analysis of anaphylaxis treatment, it was found that adrenaline was administered to 168 patients (95.46%), while 8 patients (4.54%) were given only pheniramine and dexamethasone.

TABLE 3. Drug induced anaphylaxis and severity of anaphylaxis (n=176)

	Drug induced anaphylaxis		
	No	Yes	Total
Anaphylaxis severity			
Mild	18	4	22
Moderate	88	17	105
Severe	29	20	49
Total	135	41	176

Pearson's Chi-Square. P=0.003.

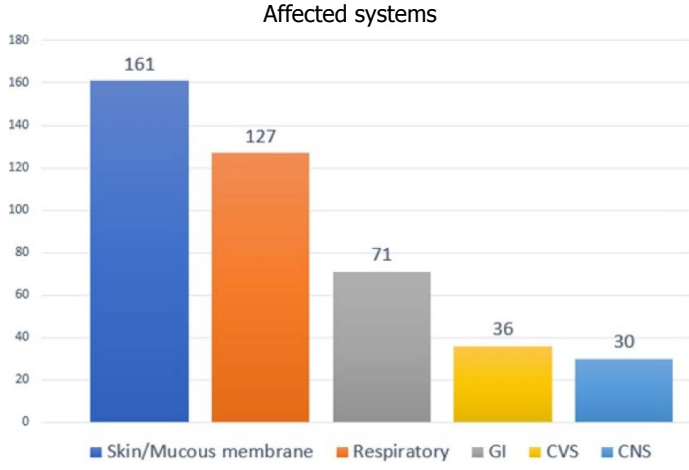

FIGURE 3. Affected systems.

When evaluating reactions within the first minute of exposure to the agent, it was significant that anaphylaxis induced by antibiotics (p=0.02) and wasp stings occurred within the first minute (p=0.03).

Eight patients (4.5%) who did not receive appropriate treatment were not treated with epinephrine and instead received glucocorticoids with antihistamines. All of these patients had multisystem involvement. One of these patients was classified as having severe anaphylaxis, one as having mild anaphylaxis, and the remaining six as having moderate anaphylaxis (Table 4).

DISCUSSION

Anaphylaxis is a potentially life-threatening reaction, and its prevalence is on the rise. Currently, there's a world-

Table 4. Patients who have not received appropriate treatment

Patient	Affected system	Severity of anaphylaxis	Treatment
1	Skin, respiratory	Moderate	Antihistamine + Glucocorticoid
2	Skin, gastrointestinal	Mild	Antihistamine + Glucocorticoid
3	Skin, respiratory	Moderate	Antihistamine + Glucocorticoid
4	Skin, respiratory	Moderate	Antihistamine + Glucocorticoid
5	Skin, respiratory	Moderate	Antihistamine + Glucocorticoid
6	Skin, respiratory, gastrointestinal	Moderate	Antihistamine + Glucocorticoid
7	Skin, CNS	Moderate	Antihistamine + Glucocorticoid
8	Skin, respiratory, CVS	Severe	Antihistamine + Glucocorticoid

CNS: Central nervous system; CVS: Cardiovascular system.

wide insufficiency of studies investigating anaphylaxis in pediatric populations, highlighting the need for a greater quantity and quality of research publications.

Anaphylaxis is more prevalent among women in adulthood, while in childhood, it's more prevalent in girls after puberty. 108 patients (61.3%) were male, 68 (38.9%) were female. Although males were more common in the study, the female gender was predominant in the adolescent period. In a comprehensive study including all age groups, 62% of 601 cases were females [16]. A study covering all age groups found that 62% of the 601 cases were female. A review comprehensively demonstrated that anaphylaxis was prevalent in males under the age of 10 years [17]. Another review indicated that girls experienced more anaphylaxis above 15 years of age [18]. Results aligned with the literature on the age and gender relationship.

The average age of the patients was 8.32±5.33 years. Categorized by age groups, 22 patients (12.5%) were below 24 months, 61 patients (34.65%) were adolescents, and 93 patients (52.84%) were between 2 and 12 years of age. The distribution was consistent with the multicenter study carried out in Türkiye [6].

Atopy was present in 86.9% of the patients. 17.61% of patients had seasonal allergic rhinitis, and 17% had asthma. Honey bee venom allergy was seen in 15.3% while wasp venom allergy was seen in 18.75%. The proportion of children with food allergy was 31.81%. Among food allergies, the most common allergy was to nuts with 16.47%. Drug allergy was present in 12.5% of patients and pollen allergy in 23.29%. In a large European study, asthma was associated with 22.9% of patients, seasonal allergic rhinitis was associated with 22.9% of patients and food allergy was the most common associated allergy in 55.43% of patients [19].

A history of atopy in first-degree relatives was present in 34.09% of the patients. In a study on pediatric anesthesia, family history was reported as a significant risk factor for anaphylaxis in such cases. However, many publications, including this study, had inadequate sharing of data regarding family history of anaphylaxis/atopy [20]. In a multicenter study conducted in Türkiye, the prevalence of family history of atopy was found to be 34.3%, which is similar [6].

The rate of individuals with non-allergic chronic diseases was 25.56%. Chronic diseases both activate inflammatory mechanisms and constitute a risk factor for anaphylaxis due to frequent drug use in the case of chronic disease. Unfortunately, the rates of non-allergic chronic diseases aren't reported in the literature.

Recurrent anaphylaxis was seen in 29.54% of patients. In a 2021 European review, recurrent anaphylaxis was seen in one in three patients [21].

A history of venom-induced anaphylaxis was present in 35.22% of patients.

Honeybee venom triggered anaphylaxis in 15.34% of patients, while wasp venom caused anaphylaxis in 18.75% of patients. 27.84% of patients experienced anaphylaxis due to food, with 3.97% developing it with eggs, milk, and nuts, respectively, and 2.84% with fish.

Drugs triggered anaphylaxis in 23.29% of patients, and 11.36% of all patients developed anaphylaxis after taking antibiotics.

The cause of anaphylaxis could not be identified in 10.79% of all patients.

In a study conducted in Türkiye, venom-induced anaphylaxis was the most frequently occurring factor, accounting for 32.3% of cases, followed by food-induced anaphylaxis at 31.3%, drug-induced anaphylaxis at 27.1%, and 8.3% of cases where the cause couldn't be determined. The study findings exhibit similarity [22]. In a study conducted in multiple centers in Türkiye, 38.4% of the participants experienced anaphylaxis due to food, 37.5% due to venom, and 21% due to drugs. 0.9% had idiopathic anaphylaxis [6]. Although foods and venoms were observed at similar rates, venom was found to be the most common trigger of anaphylaxis in some studies. The best example of how geographical structure affects anaphylaxis factors is a 5-year retrospective case review in Australia, in which 85% of patients had food-related anaphylaxis, drugs were the cause of anaphylaxis in 6% and insect stings in 3% [23]. In a study including both adult and pediatric data, idiopathic anaphylaxis was found to be the most frequent cause of anaphylaxis at 59%. This suggests that the causes of anaphylaxis vary according to age [16].

Skin/mucous membrane involvement was present in 91.47% of patients, respiratory system symptoms occurred in 72.15%, gastrointestinal system in 40.34%, cardiovascular system in 20.45% and CNS involvement in 17.04%. Similar results were obtained in previous studies conducted in Türkiye and Korea [22, 24].

Severe anaphylaxis was observed in 27.84%, mild in 12.5% and moderate in 59.65% of the patients. Although similar results have been observed in previous studies, mild anaphylaxis is expected to be the most common. This discrepancy between the data and the expectation is thought to be due to the fact that mild cases weren't admitted to the hospital or anaphylaxis wasn't recognized in these cases. In the study, no significant relationship was found between age groups and anaphylaxis severity. However, in a Korean study, it was reported that the severity of anaphylaxis increased with age. [24] This may be attributed to the different anaphylaxis triggers according to age. In the study, more severe reactions were seen in anaphylaxis triggered by drugs, especially antibiotics.

Biphasic anaphylaxis was seen in 3.97% of patients, and biphasic anaphylaxis was more common in NSAID-induced anaphylaxis. In a study including adult patients, the rate of biphasic anaphylaxis was 7.2% [13]. In another study, the rate of biphasic reactions was 11% and 75% of these reactions were food-related [25]. The incidence of biphasic reactions in adults reaches up to 20%, but 6% in children [26].

Patients diagnosed with anaphylaxis were evaluated, adrenaline was administered in 95.46% of the patients. In a multicentre study in Türkiye, adrenaline was administered to only 32.3% of patients, and it was noteworthy that 93.7% of patients received antihistamines and 83.5% received corticosteroids [6]. In another Turkish study, adrenaline was administered to 44.4% of patients [22]. In a Korean study, adrenaline was administered to 63.8% of patients [24]. In a Portuguese study, the rate of adrenaline administration was only 46% [27]. An analysis of 20 years of data from a center in Italy showed that intramuscular adrenaline was administered to only 18% of patients [28].

Although the skin is the most commonly involved system in anaphylaxis and all patients who did not receive appropriate treatment in this study had multiple system involvement including the skin, it was observed that adrenaline was not administered to these patients. Adrenaline administration should not be delayed after diagnosis.

Conclusion

Anaphylaxis is a serious reaction with sudden onset that can lead to death if not treated appropriately. Therefore, accurate diagnosis and treatment are essential. Although the frequency of the anaphylaxis is increasing, deficiencies of the diagnosis and treatment are noteworthy in the literature. It is imperative to increase the level of knowledge about anaphylaxis worldwide. We think the aim should be to recognize all patients in the early period, to manage cases appropriately and to administer intramuscular adrenaline, the most important treatment of anaphylaxis, in all patients. We believe that this study will contribute to better recognition of deficiencies in the management of anaphylaxis by giving important information for anaphylaxis.

Ethics Committee Approval: The Erciyes University Clinical Research Ethics Committee granted approval for this study (date: 05.01.2022, number: 2022/36).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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Use of AI for Writing Assistance: The authors declared that artificial intelligence was not used in the study.

Authorship Contributions: Concept – TG, FT; Design – TG, FT; Supervision – FT; Data Collection and processing – TG, HE, SG; Analysis and interpretation – FT, HE, SG; Literature search – TG, HE, SG;Writing – TG, HE, SG; Critical review – FT, HE.

Peer-review: Externally peer-reviewed.

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Original Article NEONATOLOGY

Is the P-wave dispersion a valuable tool in determining the atrial arrhythmia of newborn babies with sepsis?

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ABSTRACT

OBJECTIVE: This study aimed to ensure that newborns receive early diagnosis through P-wave dispersion (PWD) in terms of possible atrial arrhythmias on the provocative basis of sepsis.

METHODS: In this prospective study twenty term sepsis and twenty preterm sepsis patients were compared with control groups with their own characteristics in terms of PWD by electrocardiography.

RESULTS: The P-wave dispersion value in patients with term sepsis was determined to be statistically significant (p=0.03). There was no difference in PWD between the patient and control groups in preterms (p<0.05). Heart rate in preterm patients with sepsis was found to be statistically significantly higher than in the control group (p=0.001).

CONCLUSION: Our study revealed a tendency for atrial arrhythmias in term newborn infants with sepsis. These babies should be followed by pediatric cardiology with cardiac monitoring throughout their treatment.

Keywords: Atrial arrhythmias; newborn; P-wave dispersion; sepsis.

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Neonatal sepsis is a clinical syndrome caused by the spesific pathogens in which systemic signs and symptoms of infection are observed in the first month of life [1]. Neonatal sepsis can be categorized into two types: early onset, which manifests as respiratory distress within 72 hours after birth due to perinatal risk factors, and late onset, which is diagnosed after 72 hours and develops secondary to nosocomial risk factors [2]. Despite the developments in the neonatal field, it is still an important cause of morbidity and mortality [3]. The mortality rate due to sepsis can be up to 24.4%, but this rate can be up to 30% in babies born between 25 and 28 weeks, and up to 54% in babies born between 22 and 24 weeks [4]. The lack of specific findings related to sepsis and the similar signs and symptoms of non-infectious clinical conditions frequently encountered in the neonatal period create diagnostic difficulties.

In sepsis, upregulation of pro- and antiinflammatory pathways leads to system-wide release of cytokines, and activated cascades lead to myocardial dysfunction [5]. Hyperinflammation, the combination of myocardial dysfunction and extra fluid volume with resuscitation, leading to the development of stress in the left atrium [6]. These pathological changes of myocardium may lead to rhythm disturbances in patients with sepsis. The existence of various cardiac arrhythmias in septic patients has been demonstrated by various clinical reports and studies [7–9]. Troponin values in newborns are variable and no pathology may be observed on echocardiography un-

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til the decompensated period [10]. Thus, damage to the conduction pathways and/or myocardium can be detected practically and non-invasively by electrocardiography (ECG). P-wave dispersion (PWD) is a measure of the likelihood of developing abnormal heart rhythms, which is determined via a non-invasive method that involves analyzing a surface electrocardiogram. An elevation in PWD is presumed to be linked to an escalation in the likelihood of both first and recurring episodes of atrial arrhythmias. Assessing the occurrence of atrial arrhythmias in newborns with sepsis might be a useful approach.

This study aimed to ensure that newborns, who are already prone to atrial arrhythmias, receive early diagnosis through PWD in terms of possible atrial arrhythmias on the provocative basis of sepsis. This will be the first study designed in this way in the neonatal patient group in the literature to date.

Highlight key points

- P wave dispersion (PWD) is a measure of the likelihood of developing abnormal heart rhythms.
- An increase in PWD is thought to be associated with an increased likelihood of both initial and recurrent episodes of atrial arrhythmia.
- The PWD value was significantly higher in term infants with sepsis. Therefore, these infants are prone to atrial arrhythmia. These infants should be monitored by pediatric cardiology with cardiac monitoring during treatment.

MATERIALS AND METHODS

The study was conducted prospectively from June 2023 to December 2023 in Ministry of Health Konya Dr. Ali Kemal Belviranlı Obstetrics and Gynecology Hospital, Department of Neonatology. 20 preterm and 20-term newborns with sepsis verified by clinical or laboratory testing, whom we monitored in the neonatal intensive care unit of our hospital, and 20 preterm and 20-term babies without sepsis as a control group were included in the study. The demographic and laboratory findings of the patients were recorded in the hospital information system. The patients with congenital cardiac defects, newborns of mothers with systemic lupus erythematosus, those with metabolic illnesses, and those with documented arrhythmias were excluded from the study.

The parents/legal guardians of the children provided informed consent.

Electrocardiogram Analysis

Electrodes were placed in anatomical places following standard protocol, and electrocardiography (ECG) strips were recorded for a duration of 10 seconds using a standard instrument. The 12-lead electrocardiogram (ECG) was obtained using a Cardiofax C machine manufactured by Nihon Kohden in Tokyo, Japan. The electrocardiogram (ECG) was obtained using a paper speed of 25 millimeters per second and an amplitude of 10 millimeters per millivolt, while the patient was in a supine posture. The ECG strips were evaluated in a manner that the pediatric cardiologist, who had over ten years of experience in the area, did not have access to any identifying information.

The initiation of the P wave was determined as the moment when positive waveforms first deviated upwards from the baseline, or when negative waveforms first deviated downwards from the baseline. The return to the starting state was acknowledged as the conclusion of the P wave. The length of the P-wave was calculated using data from all leads. The maximum, minimum, and mean durations of the P-wave, as well as the P-wave dispersion, were determined by subtracting the minimum P-wave duration from the maximum P-wave duration in 12 leads. This calculation was performed using one randomly selected beat in a steady state. Simultaneously, an average of 7–12 beats were calculated for a duration of 10 seconds for each ECG measurement. P-wave lengths and P-wave dispersion were computed using the same approach (P-wave dispersion equals the highest P-wave duration minus the shortest P-wave duration). In order to eliminate daily fluctuations, we collected electrocardiography recordings from all working groups within a consistent time frame of 10–12 hours.

Statistical Analysis

All statistical analyses were performed using SPSS 22.0 (Armonk, New York: IBM Corp.) program. Data were presented as count (n) and standard deviation. An independent sample t-test was performed. A p-value less than 0.05 is considered statistically significant. This study was conducted in accordance with the Declaration of Helsinki.

RESULTS

20 preterm healthy and 20 preterm sick babies, 20 term healthy and 20 term sick babies were included in this study. Table 1 shows demographic data, maternal ages, Agpar 1st and 5th minute scores, and peak heart rates of the patient and control groups.

Table 1. Characteristics of term and preterm patients

Variables	Group	n	Mean±SD	p
Birth week (week)	Preterm sepsis	20	31.15±2.96	>0.05
	Preterm control	20	32.45±2.48	
	Term sepsis	20	39.00±1.41	>0.05
	Term control	20	38.584±.73	
Weight (gram)	Preterm sepsis	20	1713.25±558.36	>0.05
	Preterm control	20	1799.00±488.87	
	Term sepsis	20	3240.45±658.35	>0.05
	Term control	20	3273.55±525.72	
Maternal age (year)	Preterm sepsis	20	26.35±6.20	p>0.05
	Preterm control	20	26.50±5.82	
	Term sepsis	22	27.00±6.60	p>0.05
	Term control	20	29.20±6.26	
Apgar 1 score	Preterm sepsis	20	6.60±1.53	**0.043**
	Preterm control	20	7.70±1.78	
	Term sepsis	22	790±1.37	
	Term control	20	8.70±0.57	**0.022**
Apgar 5 score	Preterm sepsis	20	7.50±1.10	**0.008**
	Preterm control	20	8.45±1.05	
	Term sepsis	20	8.72±1.03	>0.05
	Term control	20	9.20±0.41	

SD: Standard deviation.

Table 2. Characteristics of the term and preterm sepsis groups related to sepsis

Variable	Preterm sepsis	Term sepsis
Clinic sepsis	9	15
Klebsiella pneumoniae	6	2
Staphylococcus epidermidis	3	1
Candida	1	0
Staphylococcus haemolyticus	1	1
Streptococcus agalactiae	0	2
Staphylococcus aureus	0	1
C-reactive protein (mg/dl)	49.68±39.36	57.78±47.77
Length of stay (day)	45.05±27.63	16.59±13.93
Platelet count (10^9/L)	165700.07±153692.71	176154.55±109020.04
White blood cell count (10^9/L)	12969.47±9509.44	213112.27±33823.57

The term and preterm patient and control groups were similar in terms of gender and mode of delivery.

Details of sepsis in the term and preterm sepsis groups are given in Table 2.

Table 3. Comparison of ECG findings between patients

Variables	Group	n	Mean±SD	p
P-wave dispersion [milliseconds (ms)]	Preterm control	20	0.01±0.00	>0.05
	Preterm sepsis	20	0.02±0.03	
	Term control	20	0.01±0.00	**0.03**
	Term sepsis	20	0.01±0.00	
P-wave dispersion minumum (ms)	Preterm control	20	0.05±0.00	>0.05
	Preterm sepsis	20	0.05±0.00	
	Term control	20	0.07±0.00	>0.05
	Term sepsis	20	0.10±0.14	
P-wave dispersion maximum (ms)	Preterm control	20	0.06±0.00	>0.05
	Preterm sepsis	20	0.07±0.00	
	Term control	20	0.08±0.00	>0.05
	Term sepsis	20	0.11±0.14	
Heart rate (beats per minute)	Preterm control	20	146.10±19.41	**0.01**
	Preterm sepsis	20	159.70±8.82	
	Term control	20	144.42±14.19	>0.05
	Term sepsis	20	143.30±16.36	

SD: Standard deviation.

When ECG findings (Table 3) of the study group were compared with the control group, it was found that average PWD value of the patients in the preterm control group was 0.013050±0.0033791 seconds. In patients with preterm sepsis, this value was determined to be 0.020409±0.0313531 seconds on average. No statistically significant difference was found between the groups (p>0.05).

The average heart rate of the patients in the preterm control group was determined to be 146.1053±19.41046, and this value was found to be 157.0455±13.43583 in patients with preterm sepsis. There was a statistically significant disparity in heart rates between the two groups (p=0.01).

While the average PWD values of the patients in the term control group were 0.011000±0.0031464 seconds, the average PWD values of the patients in the term sepsis group were 0.013667±0.0046726 seconds. A significant statistical difference was observed between the groups (p=0.03).

The correlation analysis between C-reactive Protein (CRP) and PWD did not yield any statistically significant results. (p=0.064).

DISCUSSION

Neonatal sepsis remains a significant contributor to morbidity and mortality and it has become a global health challenge [11]. Delayed treatment and prematurity often lead to adverse outcomes. It is known that sepsis increases the likelihood of neurodevelopmental complications such as cerebral palsy, psychomotor and mental developmental delay, especially in babies with low birth weight [12]. In addition, overdose antibiotic use can lead to the presence of multidrug-resistant organisms and candida infections. Sepsis can result in the failure of several organs. One of the primary causes of this failure is the impairment of the heart's function, known as cardiac dysfunction [13]. In experimental animal models of sepsis, the heart's production of ATP is predominantly achieved by the oxidation of fatty acids and glucose. However, both of these processes are significantly reduced [14]. On the other hand, impaired β-adrenergic signaling that leads to reduced cardiac contractility is also present in sepsis [15].

It is widely recognized that infants are susceptible to life-threatening irregular heart rhythms, which can hap-

pen in babies with a healthy heart or in those with cardiac abnormalities. The incidence of neonatal arrhythmia is reported to be 1%–5% in all neonates [16]. Turner et al. [17] retrospective study spanning two decades revealed an arrhythmia rate of 0.02% in 662,698 live births. However, Badrawi et al. [18] determined this rate as 8.5% in benign arrhythmias and 1.5% in malignant arrhythmias in patients in the neonatal intensive care unit. These data prove the increased tendency for arrhythmias in sick babies. Previous studies have shown that atrial arrhythmias, especially premature atrial contractions, can be seen with a frequency of up to 51% even among normal births [19]. Therefore, it is possible to say that newborns are more prone to atrial arrhythmias. Previous studies have reported that increased PWD is a strong indicator of the likelihood of atrial fibrillation [20–22]. In our study investigating sepsis and atrial arrhythmia in newborns, we found that the PWD value was significantly higher in term babies with sepsis. Nevertheless, we did not observe any supraventricular arrhythmia when administering therapy to these individuals. Similar to our study, Özdemir et al. [23] found that PWD was significantly higher in the sepsis group compared to the control group in pediatric intensive care unit patients with sepsis. Similar to the results of our study, they did not detect supraventricular arrhythmia in these patients. However, Walkey et al. [8] found that the risk of developing arterial fibrillation in adults with sepsis who were treated in the hospital was 6 times higher than in those without sepsis. Even in a study, the atrial fibrillation caused by sepsis rate was between 2–26%, and in cases of septic shock, this rate could increase up to 40% [24–26].

We did not detect a correlation between CRP, an important indicator of inflammation, and PWD in our term patients with sepsis. In our study, we did not find a connection between the severity of sepsis and the tendency to atrial arrhythmia. While Özdemir et al. [23] did not find a correlation CRP and PWD in their study, they showed a positive correlation with Tp-e interval and Tp-e/QTc ratio. They associated this with ventricular tachyarrhythmias based on myocardial fibrosis.

Neonates in the neonatal intensive unit are susceptible to sepsis, especially preterm neonates and low birth weight neonates [27]. In our study, we did not find any difference in PWD between the sepsis and control groups in preterms, whose hemodynamics and physiology are different from term babies. We attributed this difference in babies with preterm sepsis to lack of maturation in every system of their body. However, heart rate was found to be higher in these babies with sepsis. The gold standard for diagnosis is the presence of a positive blood culture. But, the initiation of treatment is not dependent on it since the results are often delayed. So timely treatment of antibiotics will decrease the length and intensity of the illness. Deviation from normal heart rate patterns is linked to the onset of sepsis and might be crucial in promptly identifying and treating high-risk infants. In sepsis, the adrenergic system is activated, and overproduction of catecholamines leads to tachycardia [28]. There are even studies showing that early recognition of sepsis by observing heart rate variability with the use of the Hemoaccess Reliable Outflow device reduces mortality in newborns [29, 30].

The study has some limitations. Increasing the sample size would enhance the accuracy of our result interpretation. Evaluation of ECG parameters indicating ventricular arrhythmias would provide a stronger chance of predicting arrhythmias in patients with sepsis.

Conclusion

Our study revealed a tendency for atrial arrhythmias in term newborn infants with sepsis. These babies should be followed by pediatric cardiology with cardiac monitoring throughout their treatment.

Ethics Committee Approval: The Selcuk University Clinical Research Ethics Committee granted approval for this study (date: 19.12.2023, number: 2023/592).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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Authorship Contributions: Concept – FHY, DA; Design – FHY; Supervision – FHY; Materials – FHY, DA; Data collection and/or processing – FHY, CC, DA; Analysis and/or interpretation – FHY, CC, DA; Literature review – FHY, CC, DA; Writing – FHY, CC, DA; Critical review – FHY.

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Original Article Geriatrics

Prognostic nutritional index and 28-day mortality in elderly septic patients: A retrospective analysis based on the MIMIC database

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ABSTRACT

OBJECTIVE: Peripheral blood lymphocyte count and serum albumin level are crucial predictors of mortality across various diseases, including sepsis. However, previous studies have focused primarily on individual indicators (lymphocyte count or albumin) in relation to sepsis prognosis. Given the limitations of these single indicators, we investigated the relationship between the Prognostic Nutritional Index (PNI)—a composite indicator combining serum albumin level and lymphocyte count—and 28-day all-cause mortality in elderly septic patients.

METHODS: This retrospective study analyzed data from elderly septic patients in the Medical Information Mart for Intensive Care (MIMIC-IV, v2.2) database from 2008 to 2019. Patients were categorized into survival and mortality groups based on 28-day outcomes, and baseline data were compared between the groups. Restricted cubic spline (RCS) analysis was used to determine PNI values at which the hazard ratio (HR) for 28-day mortality was 1. Patients were then classified into extreme (PNI<29.24 or PNI>47.77) and moderate (29.24≤PNI≤47.77) PNI groups. Kaplan-Meier survival curves were used to analyze cumulative 28-day survival rates, and Cox regression models assessed the relationship between PNI and 28-day outcomes.

RESULTS: The study included 2,121 patients. PNI values were significantly lower in the mortality group compared to the survival group ($p<0.05$). RCS analysis indicated a nonlinear relationship between PNI and 28-day all-cause mortality risk ($X^2<0.001$, $p<0.001$), with mortality risk decreasing as PNI increased at lower PNI values. Kaplan-Meyer survival curves revealed that patients in the extreme PNI groups had significantly lower cumulative 28-day survival rates than those in the moderate PNI group ($p<0.001$). Cox regression models further confirmed that extreme PNI values (either extremely high or extremely low) were independent risk factors for 28-day all-cause mortality (HR=1.349, $p=0.004$).

CONCLUSION: To our knowledge, this study is the first to reveal a nonlinear relationship between PNI values and 28-day all-cause mortality in elderly septic patients. Our findings associated extreme PNI values with increased mortality risk, and suggest that PNI may serve as an effective tool for prognostic risk stratification.

Keywords: All-cause mortality; elderly sepsis patients; MIMIC-IV; prognostic nutritional index.

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Sepsis is a common systemic inflammatory response syndrome and a leading cause of mortality among intensive care unit (ICU) patients. It is characterized by a dysregulated host response to infection, resulting in life-threatening organ dysfunction [1]. Despite significant advancements in critical care that have improved the

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understanding and treatment of sepsis and have reduced mortality rates, certain patient populations, particularly elderly patients with prolonged ICU stays, complex comorbidities, and immune dysregulation continue to experience clinical deterioration and poor outcomes [2, 3]. Therefore, the early assessment of disease severity and aggressive treatment are critical for improving outcomes in elderly septic patients.

Numerous studies have confirmed the relationship between C-reactive protein (CRP) and procalcitonin (PCT) levels and the severity and prognosis of sepsis [4]. However, these markers exhibit low diagnostic specificity and predictive value. Multiple studies have associated sepsis with other biomarkers that include complement component proteins, cytokines, chemokines, damage-associated molecular patterns (DAMPs), cell membrane receptors, and cellular proteins [5–7]. However, these markers lack sufficient sensitivity. Thus, the exploration of additional indicators holds significant clinical value.

The Prognostic Nutritional Index (PNI) accurately and comprehensively assesses patient nutritional and immune status [8]. By combining peripheral blood lymphocyte counts and serum albumin levels, PNI reflects the impact of inflammation and related signaling pathways on nutrition and metabolism. In septic patients, invading pathogens initiate immune responses through pathogen-associated molecular patterns and pattern recognition receptors, stimulating the release of numerous inflammatory mediators. Excessive levels of pro-inflammatory cytokines accelerate catabolism, energy and nutritional loss, and protein-energy malnutrition [9]. Consequently, anti-inflammatory and nutritional therapies are imperative to treat sepsis and improve clinical outcomes. Initially used to predict immune status and risks before gastrointestinal surgery, PNI is closely related to the prognosis of various diseases, including cirrhosis and various cancers [10–13]. To our knowledge, no previous studies have applied PNI as a biomarker of sepsis in elderly patients.

Based on the above background, we hypothesized that the PNI is significantly associated with 28-day all-cause mortality in elderly septic patients and that abnormal fluctuations in PNI levels (either elevated or decreased) may be related to higher mortality risks. Therefore, this study aimed to explore the relationship between PNI and 28-day all-cause mortality in elderly septic patients by interrogating data from the MIMIC-IV database, with the goal of providing a new prognostic assessment tool to identify high-risk patients and to optimize treatment strategies.

Highlight key points

- This study aims to explore the relationship between the prognostic nutritional index (PNI) and the prognosis of elderly septic patients.
- In comparison with traditional prognostic indices for the elderly, the PNI introduced in this study places a greater emphasis on immune-inflammatory responses, making it more aligned with clinical practice for elderly patients with sepsis.
- The study pioneers the investigation of PNI's correlation with elderly sepsis prognosis using a large, real-world dataset, offering precise PNI cutoffs for early detection of high-risk patients.

MATERIALS AND METHODS

Data Source

The MIMIC-IV (v2.2) database, developed by the Laboratory for Computational Physiology at the Massachusetts Institute of Technology, provided all data used in this study. The database, available at https://mimic.mit.edu/, contains detailed ICU-specific data from all patients treated at the Beth Israel Deaconess Medical Center between 2008 and 2019. To protect patient privacy, all personal identifiers have been replaced with random numbers. Therefore, this study was exempt from ethical review and informed consent requirements.

Population Selection and Inclusion/Exclusion Criteria

The MIMIC-IV database includes data from 180,733 patients admitted to the ICU between 2008 and 2019. Using a retrospective study design, elderly patients with sepsis in MIMIC-IV were enrolled in our research. The diagnosis of sepsis was confirmed according to the Sepsis-3 definition [13], which identifies sepsis as an acute increase in sequential organ failure assessment (SOFA) score by ≥2 points attributable to infection. Inclusion criteria were: (1) Age≥65 years old; (2)First admission to the ICU; (3) Meeting Sepsis-3 criteria. Exclusion criteria included: (1) ICU stay <24 hours; (2) Missing albumin or lymphocyte count data.

Ultimately, 2,121 patients were included in the study.

Data Extraction and Grouping

Main study variable

The PNI was the primary study variable. Based on previous literature [14], PNI was calculated using the formula: albumin (g/L)+5×lymphocyte count (10^9/L).

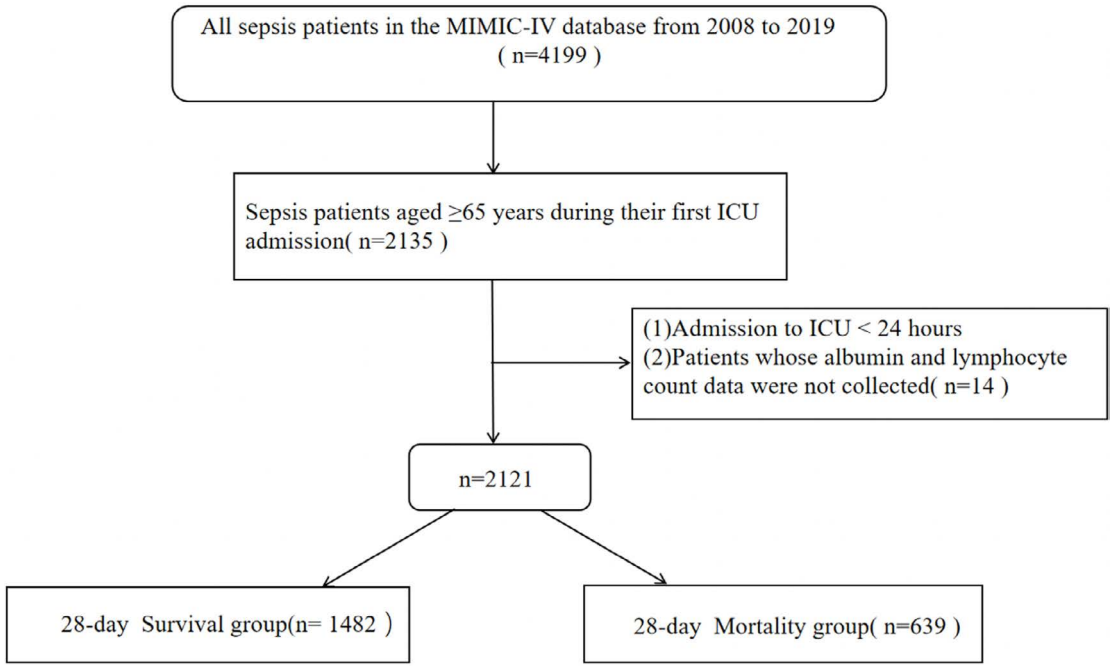

FIGURE 1. Flow chart of the study.

MIMIC-IV: Medical Information Mart for Intensive Care; ICU: Intensive care unit

The first laboratory measurements after ICU admission were selected to minimize potential interference from treatment. Data were extracted from the MIMIC-IV database using PostgreSQL's SQL language. Extracted variables included age, gender, weight, Acute Physiology and Chronic Health Evaluation (APACHE) III score, Sequential Organ Failure Assessment (SOFA) score, laboratory test results, and ICU length of stay. Laboratory data included white blood cell count; red cell distribution width; hemoglobin level; platelet count; lymphocyte count; and serum albumin, creatinine, blood urea nitrogen, and potassium levels.

Grouping

Patients were divided into two groups based on 28-day outcomes: the survival (n=1,482) and mortality (n=639) groups. Additionally, based on RCS analysis, patients were categorized into extreme (PNI<29.24 or PNI>47.77, n=726) and moderate (29.24≤ PNI≤ 47.77, n=1,395) PNI groups (Fig. 1).

Study Outcomes

The primary outcome was 28-day all-cause mortality after hospital admission.

Statistical Analysis

Data analysis was performed using Stata 14.0 and R software. Continuous variables were expressed as mean±standard deviation (mean±SD) for normally distributed data and as median (interquartile range) (M [QL, QU]) for skewed distributions. Categorical variables were expressed as percentages (%). Intergroup comparisons were made using t-tests for continuous variables, rank-sum tests for skewed data, and chi-square tests for categorical variables. Kaplan-Meier curves were used for survival analysis, with log-rank tests for comparisons. Cox proportional hazards regression models were employed to assess whether PNI was an independent risk factor for mortality within 28 days, with results presented as hazard ratios (HR) and 95% confidence intervals (CI). A p-value<0.05 was considered statistically significant.

RESULTS

Baseline Demographic and Clinical Characteristics

A total of 2,121 elderly septic patients were included, with 1,482 in the survival group and 639 in the mortality group. There were no significant intergroup differences in gender and weight (p>0.05). However, the mortality group had significantly higher ages; SOFA

Table 1. Patient characteristics

	Total population (n=2121)	Survival group (n=1482)	Death group (n=639)	t/Z/X^2	p
Demographics					
Age, years	77.0 (70.0, 83.0)	76.0 (70.0, 83.0)	77.0 (71.0, 84.0)	-1.977	0.048
Gender, n (%)					
Male	1131 (52.9)	801 (53.83)	330 (51.08)	1.367	0.242
Female	990 (47.1)	681 (46.17)	309 (48.92)		
Weight (kg)	73.7 (62.1, 37.9)	73.8 (62.2, 87.8)	73.4 (61.5, 87.0)	-0.497	0.620
Scoring systems					
SOFA	1 (0, 3)	1 (0, 2)	2 (1, 4)	-9.158	<0.001
APACHE III score	60 (47, 76)	55 (44, 69)	73 (58, 92)	-16.499	<0.001
Laboratory parameters					
WBC count (10^9/L)	11.3 (7.0, 16.3)	11.4 (7.2, 16.3)	11.2 (6.6, 16.2)	-0.348	0.728
RDW (10^9/L)	15.1 (14, 16.65)	14.9 (13.8, 16.3)	15.6 (14.5, 17.4)	-1.825	<0.001
Hemoglobin	9.22±1.94	9.25±1.94	1.19±1.93	3.951	<0.001
Lymphocytes (10^9/L)	0.7 (0.4, 1.2)	0.7 (0.4, 1.1)	0.7 (0.3, 1.3)	-0.467	0.64
Platelet count (10^9/L)	164 (107, 237)	167 (112, 240)	157 (94, 231)	-3.208	<0.001
Creatinine (mEq/L)	1.3 (0.9, 2.1)	1.2 (0.8, 1.9)	1.5 (1.0, 2.4)	-6.504	<0.001
BUN (mg/dl)	3 (1.9, 4.7)	2.8 (1.8, 4.3)	3.6 (2.3, 5.6)	-7.563	<0.001
Albumin (g/L)	27.79±6.46	28.61±6.19	26.77±6.65	8.136	<0.001
PNI	32.70 (27.90, 37.90)	33.25 (28.90, 38.26)	31.02 (25.40, 36.75)	3.285	<0.001
Anion gap (mmol/L)	10 (8, 13)	10 (8, 12)	12 (9, 15)	-13.278	<0.001
Hemoglobin (g/dl)	25 (21, 28.50)	26 (22, 29)	23 (18, 26)	5.451	<0.001
Potassium (mmol/L)	3.91±0.65	3.88±0.62	3.96±0.89	-4.29	<0.001
Bicarbonate	24.56±6.15	26.11±5.52	22.65±6.34	11.441	<0.001
Chloride (mmol/L)	105.97±7.21	105.89±6.62	105.96±7.78	-0.187	0.852
ICU LOS	3.7 (2.0, 7.8)	3.7 (2.0, 7.9)	3.8 (2.1, 7.7)	-0.645	0.519

SOFA: Sequential Organ Failure Assessment; APACHE III: Acute Physiology and Chronic Health Evaluation III score; WBC: White blood cell; RDW: Red cell distribution width; BUN: Blood urea nitrogen; PNI: Prognostic nutritional index; ICU: Intensive care unit; ICU LOS: length of stay in ICU

scores; APACHE-III scores; red cell distribution width; and serum creatinine, blood urea nitrogen, anion gap, and potassium levels compared to the survival group ($p<0.05$). Additionally, the mortality group had significantly lower platelet counts; PNI values; and hemoglobin, albumin, and bicarbonate levels ($p<0.05$). These findings suggest that higher inflammatory and organ dysfunction indicators are closely related to poor prognosis in elderly septic patients. Detailed data are presented in Table 1.

Restricted Cubic Spline Analysis

To assess the relationship between PNI and 28-day all-cause mortality risk, restricted cubic spline (RCS) analysis was used to determine two critical cutoff values for PNI: 29.24 and 47.77. RCS analysis revealed a significant nonlinear relationship between PNI and 28-day mortality risk ($X^2<0.001$, $p<0.001$). As PNI increased from lower values, mortality risk significantly decreased. However, when PNI exceeded 47.77, mortality risk began to rise. Based on these findings, patients were divided into three groups: PNI<29.24 (extreme group), 29.24≤ PNI≤47.77 (moderate group), and PNI>47.77 (extreme group). Survival analysis showed that the cumulative survival curves for the PNI<29.24 and PNI>47.77 groups were nearly identical and significantly lower than that of the moderate PNI group (29.24≤ PNI≤47.77). Therefore, the PNI<29.24 and PNI>47.77 groups were combined into a single extreme PNI group to more clearly illustrate the relationship between PNI and outcome. Detailed analysis results are shown in Figure 2.

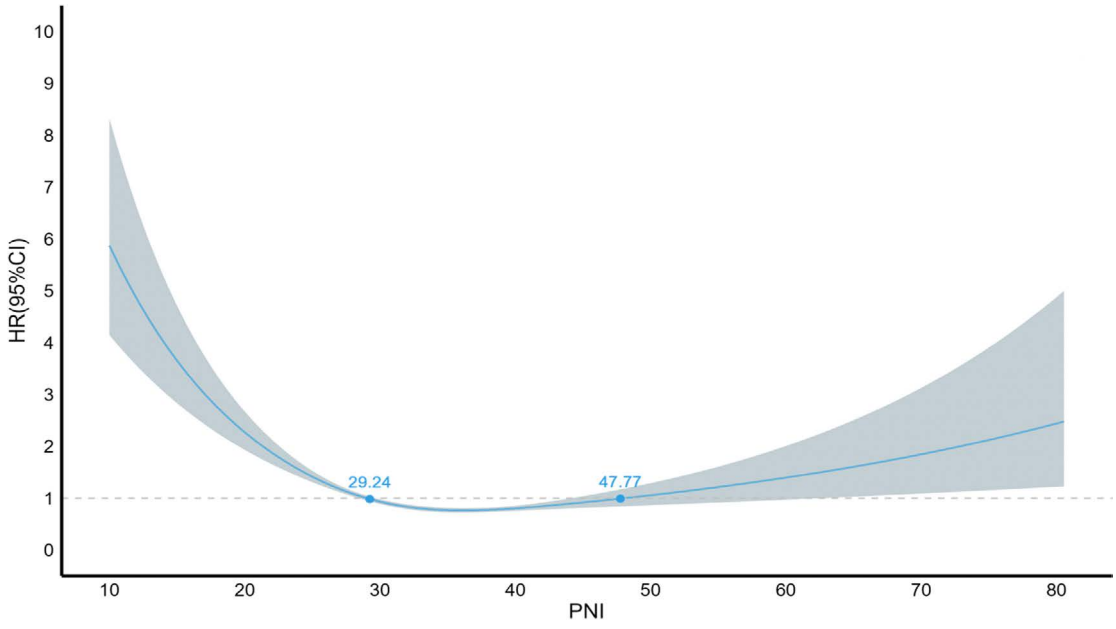

FIGURE 2. PNI Levels and 28-day all-cause mortality risk.
HR: Hazard ratio; CI: Confidence interval; PNI: Prognostic nutritional index

Kaplan-Meier Survival Curves

Kaplan-Meier survival curve analysis demonstrated that patients in the extreme PNI group (PNI<29.24 or PNI>47.77) had significantly lower cumulative survival rates at 28 days compared to those in the moderate PNI group (29.24≤ PNI≤ 47.77) (Log-rank test, p<0.001). This finding further confirmed the importance of PNI in the clinical outcomes of elderly septic patients, indicating that abnormal fluctuations in PNI levels are closely related to higher mortality (Fig. 3).

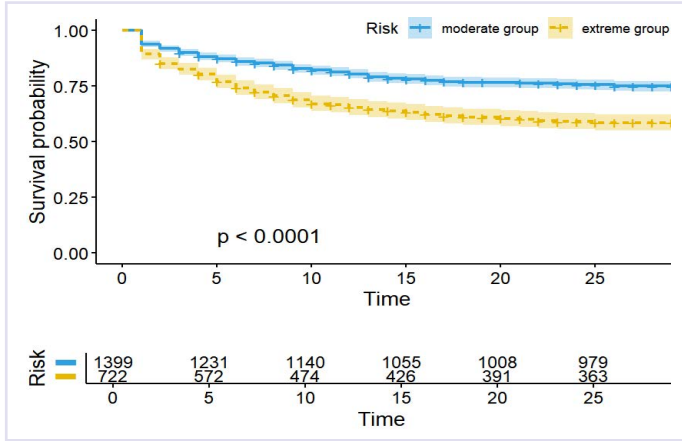

FIGURE 3. 28-day cumulative survival rate curve.

Cox Regression Analysis

The unadjusted Cox regression analysis model disclosed that the mortality risk was significantly higher in the extreme PNI group than in the moderate PNI group (HR=1.864, p<0.001). After adjusting for confounding factors such as age, gender, SOFA score, APACHE-III score, serum albumin, and lymphocyte count, the mortality risk in the extreme PNI group remained significantly elevated (HR=1.349, p=0.004). Additionally, age, serum albumin, red cell distribution width, and lymphocyte count were also associated with mortality risk in the adjusted model (p<0.05). Notably, hemoglobin level shifted from a protective factor to a risk factor after adjustment (HR=1.045, p=0.044), highlighting the importance of adjusting for confounding factors. Detailed results are presented in Table 2.

DISCUSSION

The clinical manifestations of sepsis are often atypical in elderly patients and easily overlooked, leading to diagnostic and treatment delays, which in turn expedite further clinical deterioration. Therefore, early diagnosis and prognostic assessment are crucial for improving clinical outcomes in elderly septic patients. To our knowledge, this study is the first to systematically explore the relationship between PNI and 28-day all-cause mortality in elderly septic patients, filling a critical knowledge gap. Our findings suggest that PNI can serve as an indepen-

Table 2. Cox regression analysis model

	Model 1			Model 2		
	HR	95% CI	p	HR	95% CI	p
PNI						
Moderate group	Reference			Reference		
Extreme group	1.799	1.460–2.218	<0.001	1.349	1.099–1.656	0.004
Age	1.005	0.992–1.018	0.476			
Albumin	0.946	0.930–0.963	<0.001	0.978	0.955–1.002	0.067
RDW	1.145	1.103–1.1.187	<0.001	1.060	1.014–1.108	0.010
Lymphocyte	1.085	0.973–1.210	0.143			
WBC	0.992	0.979–1.005	0.226			
Hemoglobin	0.918	0.869–0.969	0.002	1.034	0.974–1.097	0.274
Platelets	0.999	0.999–1.000	0.262			
BUN	1.075	1.039–1.113	<0.001	0.969	0.918–1.022	0.249
Creatinine	1.129	1.056–1.208	<0.001	0.940	0.840–1.052	0.284
Potassium	1.307	1.120–1.526	<0.001	1.097	0.934–1.288	0.260
Weight	1.002	0.997–1.007	0.381			
SOFA	1.290	1.232–1.351	<0.001	1.173	1.116–1.233	<0.001
Gender	0.885	0.719–1.089	0.248			
Total_ICU_stay	1.002	0.988–1.017	0.738			
APACHE-III	1.031	1.027–1.035	<0.001	1.018	1.013–1.022	<0.001
Anion gap	1.164	1.145–1.183	<0.001	1.091	1.065–1.117	<0.001
Bicarbonate	0.897	0.880–0.914	<0.001	0.956	0.936–0.978	<0.001
Chloride	0.987	0.972–1.002	0.086			

HR: Hazard ratio; CI: Confidence interval; PNI: Prognostic nutritional index; RDW: Red cell distribution width; WBC: White blood cell; BUN: Blood urea nitrogen; SOFA: Sequential Organ Failure Assessment; ICU: Intensive care unit; APACHE III: Acute Physiology and Chronic Health Evaluation III score

dent indicator for prognostic risk stratification in elderly septic patients. Its calculation is relatively simple and easily performed, thereby facilitating its clinical application.

Our study identified both high (PNI>47.77) and low (PNI<29.24) PNI levels as independent risk factors for 28-day all-cause mortality. This result indicates that PNI can effectively predict the short-term prognosis of elderly septic patients. Its prognostic value is likely closely related to the pathophysiology of sepsis. Specifically, PNI integrates albumin and peripheral blood lymphocyte count, simultaneously reflecting nutritional status and immune function, thereby providing a comprehensive assessment of the pathodynamics of sepsis.

Albumin is the primary negative acute-phase serum protein, with multiple functions in inflammatory responses that include antioxidant and anticoagulant activities and maintenance of plasma colloid osmotic pressure. Hypoalbuminemia typically indicates hyperinflammation and malnutrition, which are closely related to poor prognosis in septic patients [15, 16]. As a regulatory adaptation, lymphocytes secrete anti-inflammatory cytokines (e.g., interleukin-10, IL-10) to suppress excessive inflammatory responses. However, hyperinflammation frequently leads to lymphopenia and consequent immune suppression [16].

Although CRP and PCT are currently the most widely used biomarkers for monitoring sepsis, their sensitivity and specificity vary widely between numerous studies [17]. Some reports suggest that PCT and CRP may be more helpful in ruling out rather than ruling in sepsis [18]. Therefore, the use of PNI to comprehensively assess nutritional and immune status is worth consideration. Additionally, compared to the Geriatric Nutritional Risk Index (GNRI) [19], PNI places greater emphasis on immune function assessment, which is significant for the prognosis of septic patients. GNRI can reflect nutritional status, but its

assessment of immune function is insufficient, and its component serum creatinine changes slowly, impeding the rapid identification of disease progression.

Multiple recent studies have explored the prognostic value of PNI in numerous diseases. For example, Wu et al. [20] demonstrated that PNI is an independent prognostic factor for 30-day all-cause mortality in septic patients, consistent with the findings of this study. However, our study further revealed the nonlinear relationship between PNI and 28-day all-cause mortality, suggesting that the prognostic value of PNI in elderly septic patients may be more complex. Moreover, Li et al. [21] found that PNI is independently associated with the presence and severity of neonatal sepsis, indicating that PNI may have universal prognostic significance across age groups of septic patients. Elderly septic patients have more comorbidities and immunodeficiencies than their neonatal counterparts. Therefore, the prognostic value of PNI may be more clinically significant in elderly patients. Our RCS analysis found a nonlinear relationship between PNI and 28-day all-cause mortality, with both high and low PNI levels being risk factors for death. We hypothesize that this may be related to the complex pathophysiology of sepsis: high PNI levels may indicate excessive inflammation, while low PNI levels may reflect malnutrition and immune suppression. This dual relationship suggests that in clinical practice, PNI should not be simply regarded as a single prognostic indicator but rather assessed in combination with individual patient conditions (e.g., nutritional status and severity of inflammation).

Limitations of this study must be acknowledged. First, its retrospective study design may have introduced selection and information biases. Although we used RCS analysis and Cox regression models to minimize the impact of confounding factors, potential biases cannot be completely excluded. Second, the MIMIC database designates all patients aged over 90 years as 90 years old, which may have degraded the precision of age-related analyses. Additionally, the lack of long-term follow-up data precluded the assessment of the impact of PNI on long-term clinical outcomes. Finally, this study did not address all variables that may affect outcomes, such as treatment plans and comorbidities, which may have impacted the study results. Future research should prioritize: (1) directly comparing PNI's prognostic performance against established scores (e.g., APACHE III, SOFA, POSMI) using the same cohort, as demonstrated in Reference 15, to validate its clinical utility; (2) adopting multicenter prospective designs to minimize retrospective biases; (3) integrating PNI with dynamic biomarkers (e.g., cytokines, DAMPs) to enhance prognostic accuracy; (4) developing comprehensive models that synergize inflammatory, nutritional, and immune indicators; and (5) evaluating PNI's long-term prognostic value to optimize therapeutic strategies for elderly septic patients.

CONCLUSION

To our knowledge, our MIMIC-based study is the first investigation of the relationship between PNI and 28-day all-cause mortality in elderly septic patients. The results suggest that PNI may serve as an independent indicator for prognostic risk stratification in elderly septic patients. The calculation of PNI is relatively simple and easily performed, facilitating its clinical application. However, given the limitations of this investigation, future prospective studies are needed to further validate the prognostic value of PNI.

Informed Consent: The establishment of this database was approved by the Massachusetts Institute of Technology (Cambridge, MA, USA) and Beth Israel Deaconess Medical Center (Boston, MA, USA), and informed consents were exempted due to all patients' data were anonymized before the data were obtained.

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

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Original Article UROLOGY

Aggravation of lower urinary tract symptoms in patients with benign prostatic hyperplasia after COVID-19

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ABSTRACT

OBJECTIVE: To evaluate the effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on prostate volume, prostate-specific antigen (PSA) values, International Prostate Symptom Score (IPSS), maximum urinary flow rate (Q_{max}) and postvoid residual urine volume (PVR) of patients with BPH.

METHODS: After retrospectively review the hospital database, the study included 104 patients aged 40-75 years, who were detected to have SARS-CoV-2 according to the reverse transcription-polymerase chain reaction (RT-PCR) test of combined oro-nasopharyngeal swab samples between March 2020 and December 2020 who were being followed for BPH and had undergone prostate volume, PSA, IPSS, Q_{max} and PVR measurements within one year prior to their COVID-19 diagnosis. The prostate volume, PSA, IPSS, Q_{max} and PVR parameters were compared before and after COVID-19.

RESULTS: The prostate volume, PSA, IPSS, Q_{max} and PVR values were compared pre-COVID-19 and post-COVID-19. No statistically significant changes were found in prostate volume and PSA levels ($p>0.05$), while a statistically significant difference was observed in IPSS, Q_{max} and PVR values, independent of the severity of the disease ($p<0.001$).

CONCLUSION: COVID-19 appears to have a negative impact on IPSS, Q_{max}, and PVR in BPH patients in the short term.

Keywords: Benign prostatic hyperplasia; COVID-19; lower urinary tract symptoms; SARS-CoV-2; urinary tract.

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Coronavirus disease (COVID-19) first began in Wuhan, China, and affected the whole world and caused the death of hundreds of thousands of people. The first case of COVID-19 is thought to have occurred in consequence of a zoonosis from a livestock market, but subsequent contamination was seen through human-to-human droplets, and symptomatic individuals have become the most important agents in the spread of the disease [1]. The COVID-19 pandemic is one of the worst disasters in human history, having economic impacts on most countries, restricting people's freedom, and posing an increasingly pressing problem on a global scale [2]. In that time according to the World Health Organization (WHO) report, there were approximately 152 million verified cases and three million deaths [3]. Owing to this very important public health problem, most countries have closed their borders, and the disease has been tried to be kept under control with vaccines [4].

COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, usually

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presents with respiratory symptoms and can progress with a wide range of forms from asymptomatic disease to severe pneumonia and multi-organ failure [5]. It is known that the virus is more likely to bring about acute respiratory distress syndrome (ARDS) and multiple organ failure, especially in the elderly population and people with comorbidities (chronic lung disease, diabetes, hypertension, etc.), compared to the normal and healthy population [6]. SARS-CoV-2 has been shown to use the angiotensin-converting enzyme 2 (ACE-2) receptor and the transmembrane serine protease TMPRSS2 in its pathophysiology [7]. It has been reported that COVID-19 is serious and fatal in men [8], which has been associated with the androgen-mediated regulation of ACE-2 receptors and the TMPRSS2 enzyme group [9].

Benign prostatic hyperplasia (BPH) is a common health problem in elderly men and negatively affects their quality of life [10]. The two most important etiological factors implicated in the development of BPH are aging and androgens [11]. There are also studies showing that prostate tissue expresses ACE-2 and TMPRSS2 [12, 13]. Therefore, considering that both BPH and COVID-19 have a more negative effect on the elderly and male population and androgens play an important role in both processes, it makes sense that the elderly male population with BPH is at greater risk. In the light of this idea, we aimed to investigate whether the prostate volume, prostate-specific antigen (PSA) value, International Prostate Symptom Score (IPSS), maximum urinary flow rate (Q_{max}) and postvoid residual urine volume (PVR) of patients with BPH were affected by COVID-19. For this purpose, in this study, these parameters between pre- and post-disease periods have been evaluated.

Highlight key points

- COVID-19 infection may exacerbate lower urinary tract symptoms (LUTS) in patients with benign prostatic hyperplasia (BPH).
- The urogenital effects of COVID-19, which are often overlooked, should be considered by clinicians.
- COVID-19 was associated with worsening IPSS, decreased Q_{max}, and increased PVR, indicating impaired urinary function after infection.

MATERIALS AND METHODS

Study Population and Design

We retrospectively screened the database of our tertiary care institution and identified 236 male patients aged 40–75 years who were verified to have COVID-19 by a positive reverse transcription-polymerase chain reaction (RT-PCR) test of combined oro-nasopharyngeal swab samples between March and December 2020 while being followed up with a diagnosis of BPH. Eighty-three patients were excluded from the study due to a history of previous transurethral or pelvic surgery or pelvic radiotherapy, a PSA value above 4 ng/ml, a history of anticholinergic drug use, active urinary system infection, signs of prostatitis, bladder stone, urethral stricture, severe neurological disease (cerebrovascular disease, Parkinson's disease, etc.) or neurogenic bladder. In addition, 47 patients who did not have prostate volume, PSA, uroflowmetry, and IPSS records within the last year before COVID-19 and those for whom medical records could not be obtained were excluded from the study. Lastly, of the screened patients, two that received intensive care were excluded due to the insufficient number of cases to form a severely symptomatic group.

This study was conducted in accordance with the Declaration of Helsinki. Ethics committee approval was obtained on 20.04.2021 with the number 02-2021/05 from Karamanoğlu Mehmetbey University Faculty of Medicine.

Detailed medical histories of all patients in the study were questioned. The patients' age, smoking, body mass index, accompanying comorbid diseases, and the applied COVID-19 treatment were questioned in detail. A total of 104 patients who met the study criteria were mildly symptomatic outpatients and moderately symptomatic patients receiving oxygen therapy in the hospital without the need for intensive care. After confirming recovery from COVID-19 based on RT-PCR negativity in two consecutive tests, the patients were invited to a urological follow-up. During this evaluation, the patients were asked to complete the IPSS questionnaire, their hemogram, C-reactive protein (CRP), kidney function tests (urea and creatinine) and prostate volume, PSA values were measured, and urine analysis was performed. In addition, uroflowmetry and PVR measurement were performed. Patients with active urinary tract infection and prostatitis in the follow-up examination were evaluated again after their appropriate treatment. All patients were compared in point of the prostate volume, PSA, IPSS, Q_{max} and PVR values before and after COVID-19.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences, version 22.0 (SPSS,

Chicago, IL, USA). It was determined whether the data were normally distributed using Kolmogorov-Smirnov and Shapiro-Wilk tests. Student's t-test and the Mann-Whitney U test were used for continuous data with and without a normal distribution, respectively, while the chi-square test was used for the comparison of categorical variables. The paired t-test or Wilcoxon test was used in dependent groups to compare the parameters before and after COVID-19. $P<0.05$ was considered statistically significant.

RESULTS

The demographic data of the patients, their comorbidities, and the distribution of treatments applied for COVID-19 are shown in Table 1. Table 2 presents the comparison of the prostate volume, PSA, IPSS, Q_{max}, and PVR parameters before and after COVID-19. When the prostate volume and PSA values before and after COVID-19 were compared, no statistically significant difference was found ($p>0.05$). In the IPSS evaluation, a statistically significant increase was observed compared to the pre-disease values ($p<0.001$). In addition, there was a statistically significant decrease in the Q_{max} values and a statistically significant increase in the PVR values compared to their pre-disease evaluation ($p<0.001$).

Table 1. Distribution of demographic data

Characteristics	n=104 Mean±SD
Age (years)	55.98±0.65
BMI (kg/m²)	28.36±0.38
	%
Smoker	37.5
Hospitalized	21
Comorbidities	
None	46.1
Hypertension	29.8
Diabetes	25
Cardiovascular disease	8.6
Hyperlipidemia	4.9
Respiratory diseases	2.8
BPH medications	
Alpha-blockers	79.8
5-ARI±Alpha-blockers	6.7
Lifestyle modifications	20.2
	Mean±SD
Time from the last negative RT-PCR of naso-oropharyngeal swab to the post-COVID-19 urological examination, (day)	93.12±31.40

SD: Standard deviation; BMI: Body mass index; BPH: Benign prostatic hyperplasia; RT-PCR: Reverse transcription-polymerase chain reaction

Table 2. Comparison of the prostate volume, PSA, IPSS, Q_{max} and PVR parameters before and after COVID-19

Parameters	Before COVID-19	After COVID-19	p
Prostate volume	50.19±14.70	50.73±14.93	0.794[a]
PSA (ng/ml)	1.24±0.82	1.22±0.85	0.561[a]
IPSS	10.69±6.25	15.24±6.58	<0.001[b]
Q_{max} (ml/sec)	17.20±3.79	14.29±4.80	<0.001[b]
PVR (ml)	34.33±32,93	77.31±46.15	<0.001[a]

The results are shown as mean±standard deviation. a: Wilcoxon test: b: Paired t-test. PSA: Prostate-specific antigen; IPSS: International prostate symptom score; Q_{max}: Maximum urinary flow rate; PVR: Postvoid residual urine volume

DISCUSSION

Respiratory system symptoms are common in COVID-19, and the most common clinical presentation is fever, cough and dyspnea. Among severe cases, there is a substantial incidence of ARDS development as a result of cytokine storm caused by COVID-19 [5]. Although the most clinical presentation of COVID-19 is respiratory symptoms, recent studies have shown other organs and systems could be involved [14]. Zou et al. [15] created a risk map by determining the level of ACE2 receptor expression of various tissues and cells to determine which organs are more vulnerable to COVID-19. According to this mapping, it was determined that the cardiovascular, renal and gastrointestinal systems, especially the respiratory system, were at high risk due to the highest expression of ACE2. Varying levels of ACE2 and TMPRSS2 expression in human tissues and organs are considered to be the reason for the wide range of the forms of COVID-19 and variations in the clinical presentation of the disease [16].

Most studies in the literature have focused on the effects of COVID-19 on many organs and systems, whereas relatively fewer studies have been performed with the urogenital system. Various studies have shown that COVID-19 has effects on the male genital system, reporting that it impairs semen parameters through Ser-

toli and Leydig cells, alters sex hormones through follicle-stimulating hormone, luteinizing hormone, and testosterone levels, and leads to scrotal discomfort and orchitis symptoms [17–19]. In addition, a study reported that 0.32% of ACE 2 and 18.65% of TMPRSS22 were expressed in human prostate epithelial cells [20]. It is also known that the SARS-CoV-2 virus requires the coexistence of both ACE2 and TMPRSS2 in the host cell for its attachment to, penetration into, and replication of a cell [7]. Therefore, it is not unreasonable to consider that the urinary system, which has high expression levels for both ACE2 and TMPRSS2, will be affected by a virus that uses both of these factors in its pathogenesis.

BPH is a common health problem in aging men, affecting approximately 210 million men worldwide. Although 50–75% of men aged 50–80 years have histological findings of BPH, half of these cases also become symptomatic [21]. Although the pathophysiology of BPH is not yet clarified, the two etiological factors that are most implicated in its pathophysiology are aging and androgens [11]. It has been shown that approximately half of existing patients with COVID-19 are over 50 years, and men are more affected by the disease and have a higher mortality rate than women [22]. It is believed that androgens play a role in men being more affected by COVID-19 by aggregating the effects of the virus [9]. Considering the prevalence of BPH in elderly men and its possible etiologies, we aimed to investigate this situation, assuming that COVID-19 would have significant effects on BPH, an elderly male disease. In this cohort evaluating the effect of COVID-19 on BPH, the prostate volume, PSA, IPSS, Q_{max} and PVR parameters were compared before and after the disease. While there was no statistically significant difference in the post-disease prostate volume and PSA values compared to the pre-disease period, the disease was found to have negative effects on the IPSS, Q_{max} and PVR parameters independent of the intensity of the disease.

In a study evaluating the effect of LUTS in predicting the prognosis of COVID-19, it was shown that lower urinary tract symptoms (LUTS) could guide clinicians in predicting the prognosis of COVID-19, and patients with severe LUTS had a longer hospital stay, more frequent intensive care requirement, and a higher mortality rate compared to the mild group [23]. From an opposite perspective, when studies examining the effects of COVID-19 on the urinary system are reviewed, Kashi et al. [24] reported worsening in new-onset LUTS and pre-existing LUTS in patients with COVID-19 and stated that storage symptoms were generally at the forefront. In a study conducted by Kaya et al. [25], 27 female and 19 male patients were evaluated during active COVID-19 infection using IPSS and the Urinary Symptom Profile (USP) scoring system, and it was observed that LUTS became prominent in the early period of the disease. However, that study had the limitations of the questioning of the patients being performed during the active disease, subjective scoring based on the IPSS questionnaire, and the low number of male patients. In comparison, the advantages of our study can be considered a sound comparative evaluation of pre- and post-disease parameters, adequate sample size, evaluation performed after confirming recovery from COVID-19 rather than during active disease that could provide misleading results, and inclusion of objective parameters such as Q_{max} and PVR to support our findings.

SARS-CoV-2 is isolated from nasal, nasopharyngeal and lower respiratory secretions and transmitted by these secretions. Although the virus genome has been rarely detected in other biological fluids such as urine, the general consensus is that it cannot be isolated from urine [26]. In addition, in a study investigating whether SARS-CoV-2 was detected in expressed prostatic secretions (EPS), none of the patients had positive COVID-19 RNA in EPS [27]. Despite the fact that both ACE-2 receptor and TMPRSS2 protein expression has been shown in urinary system, it remains unclear which mechanism a virus that cannot be isolated from urine or prostatic secretion cause LUTS. One of our hypotheses is that the virus reaches the urinary system through the hematogenous route via viremia. Additionally, in a study in the literature, increased levels of interleukin-6 were assessed in the blood of patients with COVID-19 and the resulting immune response was shown to trigger the procoagulant process and cause multi-organ failure, which can be another hypothesis explaining how the urinary system can be affected by this immune response [28]. In a case report that might be related to this pro coagulant condition, it was stated that an elderly patient with BPH who had severe COVID-19 developed prostatic infarction, and the authors recommended that urologists should be alert to this phenomenon [29].

Our study also has limitations. First, it was designed retrospectively, and there was no control group. Second, viral RNA in urine samples was not investigated to prove the invasion of the urinary system by SARS-CoV-2. Third and lastly, the data obtained do not reflect long-term results due to the short duration of follow-up.

CONCLUSION

Based on the results obtained from our study, it was shown that COVID-19 had a negative effect on IPSS, Q_{max}, and PVR values and caused worsening of lower urinary tract symptoms. Although it is known that COVID-19 disease has effects on many organs and systems, we emphasize that its effects on the urogenital system, which may be overlooked, should also be taken into consideration. The data obtained from our study, in which we predict that COVID 19 causes worsening of LUTS, needs to be supported by larger-scale prospective studies.

Ethics Committee Approval: The Karamanoglu Mehmetbey University Faculty of Medicine Ethics Committee granted approval for this study (date: 20.04.2021, number: 02-2021/05).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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Original Article GYNECOLOGY

Management of adnexal torsion: A 5-year experience from a tertiary center

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ABSTRACT

OBJECTIVE: We aimed to examine the clinical characteristics, surgical findings, histopathological results, laboratory parameters of histopathologically confirmed ovarian torsion cases.

METHODS: This was a retrospective study that analyzed 96 surgically proven cases of ovarian torsion treated at a tertiary care referral hospital between 2018 and 2024. The study reviewed demographic characteristics, clinical features, surgical details, laboratory findings, and histopathological results of patients through the hospital's electronic medical records. In addition, a comparative analysis was conducted to evaluate differences between patients undergoing laparoscopic and laparotomy surgical approaches.

RESULTS: Ninety-six surgically confirmed ovarian torsions with a median age 27 years were included. The main presenting symptoms were pelvic pain (93.8%) and abdominal pain (40.6%). Laparoscopy was performed in 61 (63.5%) patients, while laparotomy was performed in 35 (36.5%) patients. Surgical approaches included salpingo-oophorectomy with cystectomy (12.1%), salpingo-oophorectomy alone (40.6%), adnexectomy (41.7%), and hysterectomy with bilateral salpingo-oophorectomy (5.2%). Ovarian size ranged from 2-30 cm (mean 7.0 cm) with right-sided involvement in 60.4%. Hemorrhagic infarction was the most common histopathologic finding, followed by simple cysts, with only one case of borderline serous cystadenoma.

CONCLUSION: Adnexal torsion is a rare emergency requiring a high index of clinical suspicion due to nonspecific symptoms. Surgical intervention is the definitive approach, with laparoscopy preferred for faster recovery and superior outcomes.

Keywords: Ovary; ovarian torsion; laparoscopic surgery.

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Adnexal torsion is a medical condition where the ovary or tuba uterina partially or completely rotates around its vascular pedicles, leading to a decrease in blood flow and potential damage to the adnexal structures [1]. It is responsible for 3% of gynecologic patients presenting to the emergency room with acute abdominal pain [2]. Adnexal torsion can be caused by adnexal cysts, neoplasia, pregnancy, or other factors [3]. It is most common during the reproductive period and more often occurs on the right side than on the left [4, 5]. The diagnosis of adnexal torsion can be challenging due to nonspecific clinical findings, but it typically presents as acute, sharp unilateral abdominal pain accompanied by nausea and vomiting. A mass may be felt during a pelvic examination, and peritoneal symptoms such as pelvic tenderness may be observed [6]. However, the clinical presentation may resemble other causes of acute abdomen, so it is important to consider the differential diagnosis, including

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non-torsioned pelvic cysts, masses or tumors, pelvic inflammatory disease, ruptured ovarian cysts, ectopic pregnancies, appendicitis, diverticulitis, and urolithiasis.

Adnexal torsion diagnosis has no specific laboratory test, but an elevated white blood cell count is observed in 20–56% of patients. Ultrasonography is the most preferred imaging method for patients suspected of having adnexal torsion [7]. Imaging findings of adnexal torsion on ultrasonography can vary depending on the duration and degree of torsion, as well as whether the fallopian tube is twisted or not. If there is a strong clinical suspicion of adnexal torsion, surgical evaluation of the adnexa is recommended, even if Doppler ultrasonography findings do not support it. Computed tomography is not superior to ultrasonography, and Magnetic Resonance Imaging (MRI) can be used as an advanced diagnostic method in some centers.

Laparoscopy is the recommended surgical approach for definitive diagnosis and treatment of adnexal torsion [8]. However, if the surgeon is not experienced in laparoscopic surgery, a laparotomic approach is an option. The main goal of treatment, particularly in adolescents and young patients, is to restore the function of the torsioned adnexal organs by detorsion and to preserve the ovarian reserve.

This study aimed to examine the clinical, intraoperative findings, pathological results, and symptoms, laboratory parameters of histopathologically confirmed ovarian torsion cases admitted to a tertiary care center over a five-year period.

MATERIALS AND METHODS

This study employed a retrospective analysis of surgically confirmed cases of ovarian torsion at a tertiary care referral hospital from January 2018 to January 2024. The inclusion criteria comprised patients presenting with pelvic pain and a diagnosis of adnexal torsion verified intraoperatively. Patients with a diagnosis of adnexal torsion that lacked surgical confirmation, as well as those with incomplete clinical data, were excluded from the analysis.

Data were extracted from the hospital's electronic medical records, providing a comprehensive review of demographic characteristics, clinical features, laboratory findings, histopathological reports, and surgical documentation. The surgical reports detailed the surgical approach employed, the operative procedure performed, the size of the mass or affected ovary, and the laterality of the torsion.

Highlight key points

- Pelvic pain (93.8%) and abdominal pain (40.6%) were the most frequent presenting symptoms in surgically confirmed ovarian torsion cases.
- Laparoscopy was the preferred surgical approach, performed in 63.5% of patients, compared to laparotomy (36.5%).
- Hemorrhagic infarction was the most common histopathologic finding in torsioned ovaries.
- Ovarian torsion occurred more commonly on the right side (60.4%), with sizes ranging from 2 to 30 cm (mean: 7.0 cm).

Additionally, a comparative analysis was conducted to evaluate differences between patients undergoing laparoscopic and laparotomy surgical approaches. This comparative analysis included various factors, such as preoperative symptoms and signs, demographic data, surgical details, laboratory findings, and histopathological results. The study has received approval from the Research Ethics Committee and is in agreement with the tenets of the Declaration of Helsinki (Number, date: 010.99/47, 28.02.2024).

Statistical Analysis

Data were recorded in an Excel sheet and further coded for analysis. Continuous variables were expressed as mean±standard deviation, median (interquartile range, IQR), and categorical variables as numbers (n) and percentages (%). The distribution of the variables was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests to determine normality. For quantitative independent data exhibiting a normal distribution, the independent samples t-test was employed for analysis. Conversely, the Mann-Whitney U test was utilized for quantitative independent data that did not conform to a normal distribution. The Chi-square test was applied to analyze qualitative independent data, while Fisher's exact test was used when the assumptions of the Chi-square test were not met. Data analysis was conducted using SPSS version 27.0 (Armonk, New York: IBM Corp) software.

RESULTS

Ninety-six cases of surgically confirmed ovarian torsion treated between 2018 and 2024 were retrospectively analyzed. The median age of the patients was 27.0 years (range: 9–67 years). Four patients (4.1%) were pregnant. The mean gravida was 1.21±1.73 and the mean parity was 0.93±1.36. The mean leucocyte count was 8.77±3.42 10^3/μl (4.7–25.0).

Table 1. Comparison of demographic and surgical data in adnexal torsion between laparoscopy and laparotomy cases (n=96)

	Laparoscopy (n=61)		Laparotomy (n=35)		p
	Mean±SD	Median	Mean±SD	Median	
Age	31.7±12.2	31.0	25.2±7.7	24.0	**0.005**^{t}
Gravidity	1.62±1.93	1.00	0.49±0.95	0.00	**0.000**^{m}
Parity	1.21±1.52	1.00	0.43±0.85	0.00	**0.001**^{m}
Laterality (%)					0.711$^{\chi^2}$
Right	59.0		62.9		
Left	41.0		37.1		
Size of mass/ovary (cm)	9.0±4.6	8.0	6.6±3.1	6.0	**0.002**^{m}
Type of surgery (%)					
USO	62.3		5.7		**0.000**$^{\chi^2}$
Detorsion alone	16.4		82.9		**0.000**$^{\chi^2}$
Detorsion with cystectomy	13.1		11.4		0.810$^{\chi^2}$
TAH+BSO	8.2		0.0		0.155$^{\chi^2}$

t: Independent sample t test; m: Mann-Whitney U test; χ^2: Chi-square test (Fischer test); USO: Unilateral salpingo-oophorectomy; TAH: Total abdominal hysterectomy; BSO: Silateral salpingo-oophorectomy.

The torsion had been formed in 60.4% of the right ovary and 39.6% of the left. The most common histopathological finding in the torsioned ovaries was a hemorrhagic infarct, followed by a simple cyst (27.1%), mucinous cystadenoma, serous cystadenoma, and dermoid cyst. Only one serous cystadenoma was of borderline malignant potential.

Patients undergoing laparoscopy had a mean age of 31.7±12.2 years, while those undergoing laparotomy were 25.2±7.7 years old (p=0.005). The laparoscopic group had higher gravidity (1.62±1.93) compared to the laparotomy group (0.49±0.95) (p<0.001). there was a significant difference in parity, with a mean parity of 1.21±1.52 in the laparoscopy group and 0.43±0.85 in the laparotomy group (p=0.001). The distribution of laterality was similar between the two groups, with right-sided involvement in 59.0% (n=36) of laparoscopic cases and 62.9% (n=22) of laparotomy cases (p=0.711), and left-sided involvement at 41.0% (n=25) for laparoscopy and 37.1% (n=13) for laparotomy. The mean size of the torsioned ovary was significantly larger in the laparoscopy group (9.0±4.6 cm) compared to the laparotomy group (6.6±3.1 cm) (p=0.002). In the laparoscopic group, 62.3% underwent unilateral salpingo-oophorectomy (USO), while only 5.7% in the laparotomy group had this procedure (p<0.001). Detorsion was performed in 16.4% of laparoscopic cases compared to 82.9% in the laparotomy group (p<0.001). The rate of detorsion with cystectomy was similar in both groups, with 13.1% in laparoscopy and 11.4% in laparotomy (p=0.810). Total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH+BSO) was performed in 8.2% of the laparoscopic group and none in the laparotomy group (p=0.155) (Table 1).

Abdominal pain was reported by 45.9% of patients in the laparoscopic group (n=28) and 31.4% in the laparotomy group (n=11) (p=0.165). There were no significant differences in pelvic pain between surgical approaches, with 91.8% (n=56) of laparoscopy patients and 97.1% (n=34) of laparotomy patients reporting pelvic pain (p=0.298). In the laparoscopy group, 77.0% of patients (n=47) showed rebound tenderness compared to 68.6% in the laparotomy group (n=24) (p=0.362). Additionally, 62.3% of patients in the laparoscopy group (n=38) did not exhibit abdominal defense, while 80.0% in the laparotomy group (n=28) did (p=0.072). Nausea and/or vomiting were reported in 60.7% of laparoscopy cases (n=37) and 62.9% of laparotomy cases (n=22), with no significant difference between the groups (p=0.831). Fever was also assessed, showing that 85.2% of laparoscopic patients had temperatures below 38.3°C (n=52), while 94.3% of laparotomy patients (n=33) had temperatures below 38.3°C. Fever exceeding 38.3°C was observed in

Table 2. Comparison of clinical features in adnexal torsion between laparoscopy and laparotomy cases (n=96)

	Laparoscopy (n=61) %	Laparotomy (n=35) %	p
Abdominal pain			0.165$^{x^2}$
(-)	54.1	68.6	
(+)	45.9	31.4	
Pelvic pain			0.298$^{x^2}$
(-)	8.2	2.9	
(+)	91.8	97.1	
Rebound			0.362$^{x^2}$
(-)	23.0	31.4	
(+)	77.0	68.6	
Defense			0.072$^{x^2}$
(-)	62.3	80.0	
(+)	37.7	20.0	
Nausea and/or vomiting			0.831$^{x^2}$
(-)	39.3	37.1	
(+)	60.7	62.9	
Fever (>38.3°C)			0.181$^{x^2}$
<38.3	85.2	94.3	
>38.3	14.8	5.7	

14.8% of laparoscopic cases (n=9) and 5.7% of laparotomy cases (n=2), resulting in a p-value of 0.181, indicating no significant difference (Table 2).

The study found a significant difference in leukocyte counts between the laparoscopic and laparotomy groups. The laparoscopic group had a mean leukocyte count of 12.2±3.6 10^3/µl (median: 11.8), while the laparotomy group had 10.9±4.3 10^3/µl (median: 10.7) (p=0.050). The platelet-to-lymphocyte ratio (PLR) was higher in the laparoscopic group with a mean of 319.5±1045.7 (median: 180.3) compared to 172.6±92.5 (median: 164.4) in the laparotomy group (p=0.050). Thrombocyte counts were also higher in the laparoscopic group with a mean of 272.4±64.5 (median: 274.0) compared to 249.9±85.0 (median: 251.0) in the laparotomy group (p=0.050). No significant differences were observed in other hematological parameters, including neutrophils, eosinophils, lymphocytes, monocytes, mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), and C-reactive protein (CRP) (Table 3).

DISCUSSION

The study found that most women diagnosed with ovarian torsion were of reproductive age, ranging from 9 to 67 years, with a median age of 27 years. These results are consistent with previous studies conducted by Gupta et al. [9], Tsafrir et al. [10], and Shwyiat et al. [11], who reported median ages of 29±12 and 24.0 years (range: 14–40 years), respectively.

Prior research suggested that ovarian torsion during pregnancy has a prevalence of 10% to 25% [12]. However, in our study, only 4.1% of patients were pregnant, indicating a lower rate than previously reported.

Adnexal masses greater than 5 cm in size are known to be a risk factor for ovarian torsion [13]. Similar to reports by Houry and Abbott [14] and Shwyiat et al. [11], our data reinforces that adnexal masses within the 2–30 cm range can undergo torsion, suggesting that larger adnexal masses are at risk for ovarian torsion.

Our study confirmed earlier research that right-sided ovarian torsion is more common than left-sided torsion [15]. This tendency is due to the greater anatomical mobility of the cecum and ileum on the right, as opposed to the relatively immobile sigmoid colon on the left [16].

Diagnosing ovarian torsion preoperatively can be challenging due to its nonspecific clinical presentation. The primary symptom is abdominal or pelvic pain [17]. Additionally, 49–85% of patients experience nausea or vomiting, and 16–52% have peritoneal irritation [18]. Our study's findings are consistent with previous research, with pelvic pain being the most common presenting symptom, followed by nausea and vomiting. When a young woman presents with an ovarian mass and experiences acute onset abdominal pain, and gastrointestinal symptoms, it is crucial to consider the possibility of adnexal torsion. Leukocytosis may be present in only a minority of cases (16% to 63%) [19]. Our study, consistent with previous research [20], did not commonly observe leukocytosis.

Historically, laparotomy was the predominant surgical approach for managing ovarian torsion. However, laparoscopy has increasingly become the preferred method due to its superior diagnostic and therapeutic efficacy [21]. Consistent with previous research, our findings indicate that laparoscopy was utilized more frequently, with 61 patients (63.5%) undergoing this procedure compared to 35 patients (36.5%) who received laparotomy. Notably, the proportion of torsion cases treated laparoscopically in our study aligns closely with the findings reported

Table 3. Comparison of hematological parameters in adnexal torsion between laparoscopy and laparotomy cases (n=96)

	Laparoscopy (n=61)		Laparotomy (n=35)		p
	Mean±SD	Median	Mean±SD	Median	
Leukocyte (10^3/µl)	12.2±3.6	11.8	10.9±4.3	10.7	**0.050**[m]
Neutrophil	9.0±2.9	8.6	8.3±4.2	7.4	0.149[m]
Eosinophil	0.1±0.1	0.0	0.1±0.1	0.0	0.587[m]
Lymphocyte	1.8±1.0	1.6	1.8±1.0	1.7	0.787[m]
Trombosit (10^3/µl)	272.4±64.5	274.0	249.9±85.0	251.0	**0.050**[m]
Monocyte	0.7±0.4	0.6	0.6±0.3	0.5	0.314[m]
MPV	9.6±1.4	9.8	9.7±1.3	10.0	0.731[t]
NLR	9.6±22.7	6.1	6.3±4.9	4.2	0.308[m]
PLR (x10^3)	319.5±1045.7	180.3	172.6±92.5	164.4	**0.050**[m]
CRP (mg/L)	26.0±50.6	3.0	16.0±43.2	3.0	0.170[m]

t: Independent sample t test; m: Mann-Whitney U test; NLR: Neutrophil to lymphocyte ratio; PLR: Platelet to lymphocyte ratio; CRP: C-reactive protein; MPV: Mean platelet volume.

by Cohen et al. [22], further solidifying the widespread adoption of laparoscopy in ovarian torsion management.

In our research, we observed twelve patients who underwent cystectomy with detorsion. The ovary is known to become friable due to edema and congestion, which can lead to a theoretical risk of bleeding. To minimize this risk, it has been suggested to wait for 2–3 weeks before performing elective cystectomy, allowing for the resolution of edema and congestion. However, our study found that performing cystectomy after detorsion did not result in increased bleeding or any postoperative complications. Therefore, it is suggested to perform ovarian cystectomy concurrently with detorsion, based on the observed lack of increased intraoperative bleeding.

The study revealed that the most frequent histology type of ovarian masses was a simple ovarian cyst, followed by mucinous cystadenoma. This is in contrast to previous studies which reported dermoid cysts or hemorrhagic cysts as the most common histology [23]. Notably, only one case of borderline malignancy was identified, aligning with the low reported incidence (1–1.8%) [24, 25]. However, in situations where malignancy is suspected, such as in postmenopausal women, oophorectomy should be performed. It is essential to perform histopathological examination on the specimen obtained from oophorectomy to definitely exclude the possibility of malignancy.

Our study had several constraints, including its retrospective nature, single-center design, and a limited sample size of patients with a rare disease. Consequently, the findings may not be widely generalizable to other health care settings or patient populations with different demographic characteristics and clinical practices. Despite these limitations, the study presents several strengths. The comprehensive analysis of surgically confirmed adnexal torsion cases allows for a robust evaluation of clinical features and outcomes associated with laparoscopic versus laparotomy approaches. By utilizing detailed surgical and histopathological data, this research provides valuable insights into the management of adnexal torsion and highlights specific factors that may influence surgical decision-making. Additionally, the study contributes to a growing body of literature on this under-researched area, offering a foundation for future investigations. Future research should focus on multicenter, prospective studies that can validate these findings across diverse populations and clinical settings. Additionally, incorporating advanced imaging techniques and biomarkers into the assessment process may enhance diagnostic accuracy and guide treatment decisions.

Conclusion

Detecting ovarian torsion can be a challenging task that necessitates astute clinical abilities and a heightened level of suspicion. If ovarian torsion is suspected, surgical intervention constitutes the definitive diagnostic and therapeutic modality. Laparoscopic surgery is the preferred approach over laparotomy.

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REVIEW MICROBIOLOGY

Global epidemiology of HTLV: Under-reported and under-studied regions

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ABSTRACT

To examine the global epidemiology of human T-lymphotropic viruses (HTLVs), with a focus on under-reported and under-studied regions such as Turkiye, and to highlight public health challenges, including insufficient surveillance and lack of awareness. A comprehensive review of published literature and epidemiological data was conducted to identify trends, prevalence rates, and gaps in surveillance. Key sources included peer-reviewed journals and global health reports. HTLV-1 was identified as highly endemic in regions such as Japan, the Caribbean, South America, and sub-Saharan Africa. Limited data were available for Turkiye and adjacent regions, with estimated prevalence rates below 0.1%. Insufficient screening, stigma, and resource constraints were noted as major barriers to improved surveillance and prevention. HTLVs remain a neglected public health issue with significant implications for healthcare systems. Targeted research, expanded surveillance, and public health interventions are urgently needed, particularly in under-studied regions like Turkiye and Southeast Asia. Human T-lymphotropic viruses (HTLVs) are globally distributed retroviruses with significant health implications, particularly in endemic regions. Despite the known association of HTLV-1 with adult T-cell leukemia/lymphoma (ATLL) and HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP), comprehensive epidemiological data are lacking for several regions. This review examines the global epidemiology of HTLV, with a particular focus on under-reported and under-studied areas, including Turkiye. Highlighting the public health challenges posed by insufficient surveillance, lack of routine screening, and limited awareness, this review emphasizes the urgent need for global efforts to address this neglected public health issue.

Keywords: Epidemiology; Human T-lymphotropic virus; HTLV; prevention; surveillance; transmission.

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Human T-lymphotropic viruses (HTLVs), belonging to the Deltaretrovirus genus, were the first human retroviruses discovered, with HTLV-1 identified in 1980 [1]. HTLV-1 is associated with severe diseases, including ATLL and HAM/TSP, while HTLV-2, though less pathogenic, has been implicated in certain neurological conditions [2, 3]. Despite an estimated global burden of 5–10 million cases, true prevalence remains uncertain due to under-diagnosis, lack of surveillance, and absence of routine screening in blood banks and antenatal settings [4, 5]. This review provides an updated analysis of the epidemiology of HTLV, with a focus on high-prevalence regions, under-studied areas (including Turkiye), and the barriers to accurate reporting.

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HTLV BIOLOGY AND TRANSMISSION

Biological Structure and Classification

Human T-lymphotropic viruses (HTLVs) belong to the genus Deltaretrovirus in the family Retroviridae. Like other retroviruses, HTLV carries its genetic material as single-stranded RNA, which is reverse-transcribed into DNA upon infection of the host cell. HTLV-1 and HTLV-2 are the two primary types infecting humans, with HTLV-1 being more pathogenic. HTLV-1 is associated with severe diseases such as adult T-cell leukemia/lymphoma (ATLL) and HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP), while HTLV-2 has a weaker association with disease and is predominantly linked to neurological conditions [1, 2].

The viral genome of HTLV encodes structural proteins (Gag, Pol, and Env), regulatory proteins (Tax and Rex), and accessory proteins (p12, p13, p30, and HBZ). Tax and HBZ play key roles in viral replication, cellular transformation, and the development of HTLV-associated diseases. Tax is particularly important for driving the proliferation of infected T-cells, which may contribute to oncogenesis in ATLL [3, 4].

Host Interaction and Pathogenesis

HTLV-1 primarily infects CD4+ T-cells, while HTLV-2 targets CD8+ T-cells. Once integrated into the host genome, the virus persists in a latent state, with clonal proliferation of infected cells driving viral spread rather than active replication. This mode of propagation minimizes immune detection, allowing the virus to persist lifelong in the host [5].

The immunopathogenesis of HTLV-1-associated diseases involves sustained immune system stimulation and inflammatory processes. In HAM/TSP, immune-mediated destruction of spinal cord neurons leads to progressive neurological deficits. In ATLL, tax-driven genetic instability and dysregulated signaling pathways result in malignant transformation [6].

Modes of Transmission

HTLV is transmitted through three primary routes, each associated with specific risk factors:

1. **Mother-to-Child Transmission (MTCT):**

   Vertical transmission occurs predominantly through breastfeeding. Infected mothers transfer the virus to their infants via milk, particularly when breastfeeding is prolonged beyond six months. The rate of transmission ranges from 15% to 25% in endemic areas [7]. Reducing breastfeeding duration or substituting with formula milk significantly lowers transmission rates, as evidenced by Japan's antenatal screening programs [8].

2. **Sexual transmission:**

   HTLV-1 is transmitted via unprotected sexual contact, with male-to-female transmission being more efficient than female-to-male. Transmission risk increases with the presence of co-infections such as sexually transmitted infections (STIs), which compromise mucosal barriers and enhance viral entry [9]. Studies suggest that HTLV-1 is more prevalent in older women, possibly due to cumulative risk from lifelong sexual exposure [10].

3. **Parenteral transmission:**

   Bloodborne transmission occurs through exposure to infected blood or bodily fluids, including:

   - Blood Transfusions: Before the implementation of blood donor screening programs, HTLV transmission through blood transfusions was common, with seroconversion rates reaching 40% in some studies [11]. Current screening has significantly reduced this route of transmission in many countries.
   - Intravenous Drug Use: Sharing needles among intravenous drug users is a major transmission route in some populations, particularly for HTLV-2 [12].
   - Medical Procedures: Unsafe medical practices, such as reusing needles or unsterile surgical equipment, contribute to transmission healthcare-limited environments.

Highlight key points

- HTLV, particularly HTLV-1, is linked to serious health issues like adult T-cell leukemia and HTLV-associated myelopathy, with 5-10 million cases worldwide
- Epidemiologic data on HTLV in underreported regions like Turkiye is insufficient, creating major public health challenges.
- Improving HTLV surveillance, screening, and diagnostic protocols is crucial to better understand the virus's spread and impact.
- In Turkiye, targeted studies on high-risk groups like migrants and refugees are essential to understand HTLV spread and create effective interventions.

Geographic and Social Factors Influencing Transmission

Transmission dynamics vary globally based on cultural practices, healthcare infrastructure, and public health policies. For example:

- In endemic regions such as Japan, the Caribbean, and parts of South America, breastfeeding practices and sexual behaviors influence MTCT and sexual transmission rates.
- In Africa, traditional healing practices and the high prevalence of STIs contribute to increased sexual and parenteral transmission.
- In Turkiye and adjacent regions, migration and refugee movement may facilitate the spread of HTLV among underserved and at-risk populations [13].

Barriers to understanding transmission

1. **Asymptomatic carriers:**
   Most HTLV-infected individuals remain asymptomatic, making it challenging to detect and study transmission patterns. It is estimated that only 2–5% of infected individuals will develop severe diseases such as ATLL or HAM/TSP during their lifetime [14].
2. **Diagnostic gaps:**
   Many regions lack reliable screening programs for blood donors and pregnant women, leading to underdetection of transmission routes and prevalence.
3. **Stigma and awareness:**
   Misconceptions about HTLV transmission, often conflated with HIV, deter individuals from seeking testing and disclosing risk behaviors.

Prevention of Transmission

Efforts to reduce HTLV transmission focus on targeted interventions:

1. **Screening:**
   - Routine blood donor screening has significantly reduced HTLV transmission through blood transfusions in high-income countries. Expanding these programs to low- and middle-income countries (LMICs) is crucial.
   - Antenatal screening for pregnant women, coupled with counseling on breastfeeding alternatives, has proven effective in preventing MTCT [8].
2. **Education and awareness:**
   Public health campaigns promoting safer sexual practices, access to needle exchange initiatives, and alternatives to prolonged breastfeeding can mitigate transmission risks.
3. **Regulation of medical practices:**
   Enforcing strict sterilization protocols and discouraging unsafe injections can reduce parenteral transmission, particularly in LMICs.

GLOBAL DISTRIBUTION

The distribution of HTLV exhibits significant geographical variability, with well-documented endemicity in some regions and a lack of reliable data in others.

High-prevalence Regions

1. **Japan:** Japan has one of the most comprehensive HTLV-1 surveillance systems globally. The prevalence of HTLV-1 is approximately 0.3–1% in the general population, with higher rates in southern regions such as Kyushu and Okinawa [9]. Implementation of a national antenatal screening program in 1987 has reduced vertical transmission rates to below 0.2% [10].
2. **Caribbean and South America:** The Caribbean region is a hotspot for HTLV-1, with prevalence rates ranging from 3–6% in countries like Jamaica, Trinidad, and Haiti [11, 12]. In South America, Brazil has been extensively studied, with general population prevalence rates of 0.4–1.8% and much higher rates in at-risk groups such as intravenous drug users and sex workers [13, 14].
3. **Sub-Saharan Africa:** Sub-Saharan Africa is considered the origin of HTLV, with high prevalence in regions such as Gabon and southern Cameroon, where rates exceed 10% in some communities [15]. HTLV-2 is also found, though predominantly in Pygmy populations [16].

Turkiye and surrounding regions

Turkiye, strategically located between Europe, Asia, and the Middle East, has sparse data on HTLV prevalence. Limited studies among blood donors and high-risk populations have reported prevalence rates of less than 0.1% [17, 18]. However, the true burden in the general population remains unclear due to the lack of routine screening and epidemiological studies. Turkiye's close proximity to regions with higher HTLV prevalence, such as the Middle East and North Africa (MENA), raises concerns about potential under-detection [19]. This warrants further investigation, particularly in vulnerable groups such as immigrants, refugees, and rural populations.

Under-reported and Under-studied Regions

1. **Southeast Asia:** While HTLV is known to circulate in Southeast Asia, particularly Papua New Guinea, limited data exist for populous countries such as Indonesia, Vietnam, and the Philippines. Sporadic studies report prevalence rates of less than 1% [20].
2. **South Asia:** India, with its vast population, has minimal data on HTLV prevalence. A few studies in blood donors and antenatal populations report seroprevalence rates ranging from 0.02% to 0.08%, suggesting the virus is under-detected [21, 22].
3. **Middle East and North Africa (MENA):** The MENA region is vastly under-studied, with isolated reports from Iran and Egypt indicating prevalence rates below 1%. These findings likely underestimate the true burden due to limited screening infrastructure [23, 24].
4. **Central Asia and Eastern Europe:** Epidemiological data from Central Asia and Eastern Europe are almost non-existent. The lack of research in these regions highlights a critical gap in global HTLV surveillance [25].
5. **Pacific Islands:** Data from Melanesia and Polynesia suggest possible endemicity, though systematic studies remain scarce. HTLV transmission in these isolated populations warrants further investigation [26].

CHALLENGES IN SURVEILLANCE

HTLV surveillance is hindered by several factors:

1. **Awareness and stigma:** Misconceptions about HTLV, including its conflation with HIV due to overlapping transmission routes, contribute to stigma and under-reporting [27].
2. **Limited screening programs:** Most countries, including Turkiye, lack routine HTLV screening in blood banks and antenatal care, resulting in missed diagnoses [28].
3. **Resource constraints:** In LMICs, the high cost of testing and limited healthcare infrastructure impede widespread screening [29].
4. **Diagnostic challenges:** Current diagnostic tools, such as serological assays, are not always affordable or accessible in healthcare-limited environments [22, 30].

PUBLIC HEALTH IMPLICATIONS

HTLV-associated diseases have long-term implications for healthcare systems, particularly in endemic regions. For example:

- **Economic burden:** Chronic conditions such as HAM/TSP require lifelong care, imposing financial strain on individuals and healthcare systems [28, 31].
- **Mother-to-child transmission:** Vertical transmission remains a significant route of infection, particularly in regions without antenatal screening programs [8, 32].
- **At-risk populations:** High-risk groups, including intravenous drug users, sex workers, and indigenous communities, require targeted interventions to reduce transmission [27, 33].

FUTURE DIRECTIONS

The current gaps in the epidemiological understanding and management of HTLV highlight the urgent need for a multifaceted and globally coordinated response. Below are key areas for future research, policy development, and intervention:

1. **Global Surveillance Programs**

   A comprehensive global HTLV surveillance framework is essential. This could be modeled after existing HIV surveillance systems and should aim to:

   - Include HTLV testing in routine screening for blood donors, pregnant women, and high-risk populations.
   - Standardize diagnostic protocols to facilitate comparison of data across regions.
   - Collect longitudinal data to better understand disease progression and transmission dynamics.

2. **Regional focus in under-reported areas**

   Special attention must be given to regions like Turkiye, Central Asia, Southeast Asia, and the Middle East. For Turkiye, studies should focus on:

   - Prevalence in urban versus rural areas.
   - HTLV in immigrant and refugee populations, given the region's role as a migration hub.
   - Risk factors unique to Turkiye, such as cultural practices and healthcare access.

3. **Cost-effective diagnostic tools**

   Future efforts should prioritize:

   - Development of point-of-care testing kits for rapid and affordable HTLV detection.
   - Integration of HTLV testing into multiplex platforms.

4. **Mother-to-child transmission prevention**
   Lessons from Japan's successful antenatal screening program should be adapted for other regions.
5. **Vaccine development and therapeutics**
   A vaccine for HTLV remains elusive; targeted research is critical.
6. **Awareness campaigns and education**
   Public awareness and healthcare provider education must be enhanced.

CONCLUSION

Human T-lymphotropic viruses (HTLVs) remain a significant yet under-recognized public health challenge. Despite the virus being identified over four decades ago, there are substantial gaps in our understanding of its epidemiology, transmission dynamics, and associated disease burden. Millions of individuals globally are infected with HTLV, yet the virus is often overlooked in public health initiatives, especially in under-studied regions such as Turkiye, Southeast Asia, Central Asia, and the Middle East. This neglect has resulted in limited screening, poor awareness, and inadequate resources for prevention, diagnosis, and management.

The association of HTLV-1 with debilitating and life-threatening conditions like adult T-cell leukemia/lymphoma (ATLL) and HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) underscores the urgent need for action. These diseases impose substantial socioeconomic and healthcare burdens, particularly in endemic regions where healthcare systems are already strained. Furthermore, the chronic nature of HTLV-associated diseases requires lifelong care, emphasizing the importance of early detection and prevention.

Key Insights

The epidemiology of HTLV is marked by significant geographic variability. High-prevalence regions such as Japan, the Caribbean, South America, and parts of Sub-Saharan Africa have implemented effective strategies, including antenatal screening and blood donor testing, that have demonstrated the feasibility of reducing HTLV transmission. However, many regions, including Turkiye, lack the infrastructure and policies to address the virus. For Turkiye, in particular, its role as a bridge between high-prevalence and low-prevalence regions highlights the potential for HTLV to spread through vulnerable populations such as immigrants and refugees. Targeted research and public health efforts in Turkiye could serve as a model for addressing HTLV in other under-studied regions.

The biology of HTLV also poses unique challenges to its detection and control. The virus's ability to persist in a latent state, its lifelong carriage, and its low progression rate to symptomatic disease complicate efforts to identify and manage infected individuals. This has contributed to a persistent gap in surveillance data, especially in low- and middle-income countries (LMICs). The lack of reliable and affordable diagnostic tools exacerbates this issue, making routine screening infeasible healthcare-limited environments.

Future Priorities

1. **Expanding surveillance:** Strengthening surveillance systems is critical to map the true global burden of HTLV. Integrating HTLV testing into existing HIV/STD frameworks, especially in regions where HTLV remains endemic and poorly studied, can provide an efficient way to enhance detection rates.
2. **Improving access to diagnostics:** Developing and deploying affordable, point-of-care diagnostic tools will enable wider screening in LMICs and resource-limited regions. This will be essential for identifying asymptomatic carriers and preventing mother-to-child transmission.
3. **Prevention strategies:** The success of Japan's antenatal screening program serves as a model for other countries. Expanding such programs globally, especially in regions where HTLV remains endemic and poorly studied, will be a crucial step in reducing vertical transmission rates.
4. **Awareness and Education:** Educating healthcare providers and the public about HTLV's transmission, associated diseases, and prevention measures will be key to reducing stigma and promoting testing and early intervention.

A Call for Global Action

Addressing the global burden of HTLV requires coordinated international efforts, led by public health organizations such as the World Health Organization (WHO), national governments, and research institutions. These efforts must prioritize funding for HTLV research, particularly in the areas of vaccine development, therapeutic interventions, and the identification of biomarkers for

disease progression. Furthermore, fostering collaborations between endemic and non-endemic countries can facilitate the exchange of knowledge and resources to tackle HTLV more effectively.

HTLV also presents an opportunity to integrate neglected tropical diseases (NTDs) into broader health frameworks. The virus disproportionately affects marginalized populations, including those in low-resource settings, which aligns with the global NTD agenda. By including HTLV in these frameworks, international health initiatives can promote equity in addressing infectious diseases.

Final Remarks

HTLV is not just a biological or medical issue—it is a public health and social challenge. The virus's long-term effects on individuals and healthcare systems, coupled with its potential for silent spread, make it a pressing issue that demands attention. With concerted efforts to improve surveillance, prevention, and treatment, the global burden of HTLV can be mitigated. Addressing HTLV effectively will not only reduce the suffering of millions but also provide a model for managing other neglected infectious diseases. This is a challenge that the global health community cannot afford to ignore.

By prioritizing HTLV as a public health concern, we can pave the way for better outcomes for affected populations and a more equitable approach to addressing infectious diseases worldwide. The time to act is now.

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