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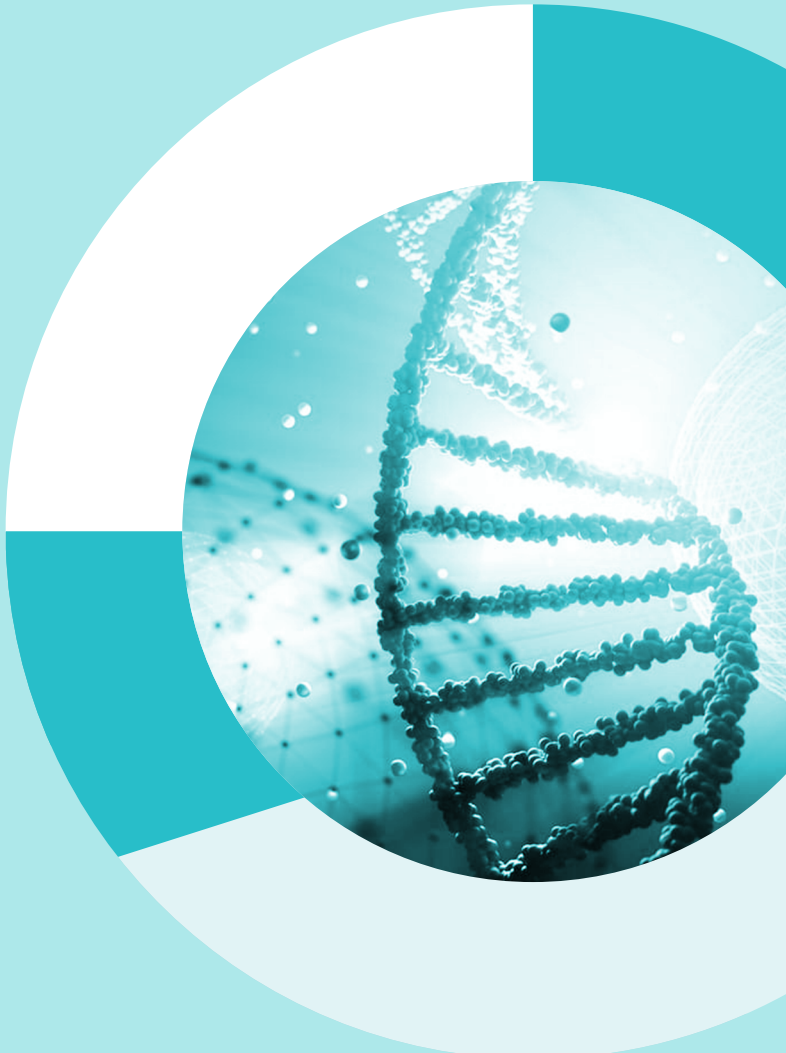

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REVIEW

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Intraoperative Neurophysiological Monitoring in Spine Surgery: Modalities, Interpretation, and Clinical Applications

Nurhak Demir

Department of Neurology, Near East University Hospital, Nicosia, Cyprus

Abstract

Neurological injury remains one of the most serious complications of spine surgery. Intraoperative neurophysiological monitoring (IONM) provides continuous, real-time functional assessment of the spinal cord and nerve roots, and is increasingly integrated into spinal procedures to mitigate the risk of perioperative neurological deficits. The principal IONM modalities used in spine surgery include motor evoked potentials (MEPs), somatosensory evoked potentials (SSEPs), electromyography (EMG), and spinal cord and nerve root mapping. MEPs are highly sensitive to dysfunction of the corticospinal tracts; SSEPs evaluate the integrity of the dorsal column-medial lemniscal pathway; EMG detects nerve root irritation or injury; and mapping techniques facilitate safe surgical navigation, particularly when anatomical landmarks are distorted or obscured. Each modality interrogates distinct neural pathways and is characterized by specific strengths and inherent limitations. When appropriately selected, systematically applied, and accurately interpreted, multimodal IONM enhances intraoperative decision-making, improves surgical safety, and supports maximal safe resection-particularly during high-risk procedures such as spinal deformity correction, intramedullary spinal cord tumor resection, and operations involving the conus medullaris or cauda equina. This review delineates the underlying neurophysiological principles and alarm criteria of these modalities, examines anesthetic considerations that influence signal reliability, and synthesizes current evidence regarding their clinical applications across diverse spinal procedures.

Keywords: Intraoperative neurophysiological monitoring, spinal cord mapping, spine surgery, motor evoked potential, somatosensory evoked potential

INTRODUCTION

Neurological injury remains one of the most devastating complications associated with spinal surgery, often resulting in permanent functional deficits and substantial impairment of quality of life. In this context, intraoperative neurophysiological monitoring (IONM) has become an integral component of contemporary spinal surgical practice. IONM provides continuous, real-time assessment of the functional integrity of the spinal cord, nerve roots, and peripheral neural pathways, thereby furnishing the surgeon with immediate feedback during critical phases of the procedure.[1] Through the integrated use of advanced electrophysiological modalities, IONM facilitates precise neural mapping, early detection of impending neural damage, and timely intervention to mitigate the risk of irreversible neurological injury.[1]

Spinal surgeries most commonly rely on neurophysiological monitoring modalities such as motor evoked potentials (MEPs), somatosensory evoked potentials (SSEPs), and both free-running and triggered electromyography (EMG). Additional techniques-including D-wave recording, bulbocavernosus reflex (BCR) monitoring, and spinal cord and nerve root mapping-are used in spinal procedures to provide further information on neural structures and their functional status.

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ORCID ID of the author: N.D. 0000-0002-7106-2247.

Corresponding author: Nurhak Demir
E-mail: nurhakdemidir@gmail.com
ORCID ID: orcid.org/0000-0002-7106-2247

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Although each modality possesses distinct strengths and inherent limitations, these approaches are complementary and together enable comprehensive multimodal monitoring of spinal cord and nerve root function.

This review discusses the fundamental IONM modalities and their applications in commonly performed spinal surgeries.

Intraoperative Neurophysiological Monitoring Techniques In Spine Surgeries

Neurophysiological Monitoring Modalities:

Transcranial Motor evoked potentials and D-wave recording

Avoiding postoperative paralysis remains a primary objective of spine surgery, and intraoperative monitoring of motor pathways enables early detection of impending motor compromise. MEPs provide real-time assessment of the functional integrity of descending motor pathways and are widely applied across a broad spectrum of spinal procedures.[2,3]

MEPs primarily assess fast-conducting corticospinal tract (CST) fibers that project monosynaptically to anterior horn cells, with subsequent transmission through peripheral nerves to target muscles.[4] MEPs are most commonly elicited via transcranial electrical stimulation using scalp electrodes positioned over the motor cortex (Figure 1).

In awake individuals, single-pulse stimulation of the motor cortex produces a series of descending volleys that can be recorded from the spinal cord (Figure 2). The initial response, termed the D-wave, represents direct activation of corticospinal axons, whereas subsequent I-waves are generated through indirect transsynaptic activation mediated by intracortical interneuronal circuits.[5] In conscious subjects, temporal summation of excitatory postsynaptic potentials generated by D- and I-waves leads to the activation of lower motor neurons (LMNs).

General anesthesia profoundly alters this physiology by suppressing intracortical synaptic transmission, thereby attenuating or abolishing I-waves and reducing LMN excitability.[3,6] As a result, single-pulse stimulation is typically insufficient to elicit reliable muscle MEPs under general anesthesia. To overcome this limitation during IONM, short trains of electrical stimuli are delivered, generating multiple descending volleys whose temporal summation facilitates LMN depolarization and produces reliable myogenic MEP responses.[3]

Myogenic MEPs are recorded from multiple limb muscle groups using surface electrodes, subdermal electrodes, or intramuscular needle electrodes. Because of their high signal-to-noise ratios, signal averaging is not required. In the upper extremities, recordings are commonly obtained from intrinsic hand muscles such as the abductor pollicis brevis, abductor digiti minimi, or first dorsal interosseous. In the lower extremities, commonly monitored muscles include the tibialis anterior and abductor hallucis. The selection of additional muscles spanning different spinal segments is tailored to the surgical level.[3]

In contrast to myogenic MEPs, D-waves provide a direct electrophysiological measure of CST integrity. D-waves are recorded from the spinal cord using epidural or subdural electrodes placed caudal to the surgical field and are elicited by single-pulse transcranial electrical stimulation. Placement of an additional rostral control electrode enables differentiation between global physiological changes and focal spinal cord injury.[7] However, D-wave recording is not feasible below the T10-T11 spinal level because insufficient CST fibers are present to generate a recordable signal.[7]

Somatosensory evoked potentials

SSEPs are among the most commonly used IONM modalities in spine surgery. SSEPs assess the functional integrity of ascending sensory pathways by recording cortical and subcortical responses following electrical stimulation of peripheral nerves.

Peripheral nerve stimulation is delivered through surface or needle electrodes placed distally on the limbs, generating afferent action potentials that ascend via the dorsal column-medial lemniscal pathway to the somatosensory cortex.[8,9] In routine clinical practice, SSEPs are elicited by stimulation of the median or ulnar nerves for the upper extremities and the posterior tibial or common peroneal nerves for the lower extremities (Figure 3). Responses are recorded from multiple sites, including the scalp, cervical spine, and peripheral locations, allowing assessment of neural conduction across different anatomical levels.[8,9]

Because SSEPs are low-amplitude signals embedded within background electroencephalographic activity, signal averaging is required to achieve an adequate signal-to-noise ratio.[10,11] Consequently, acquisition of

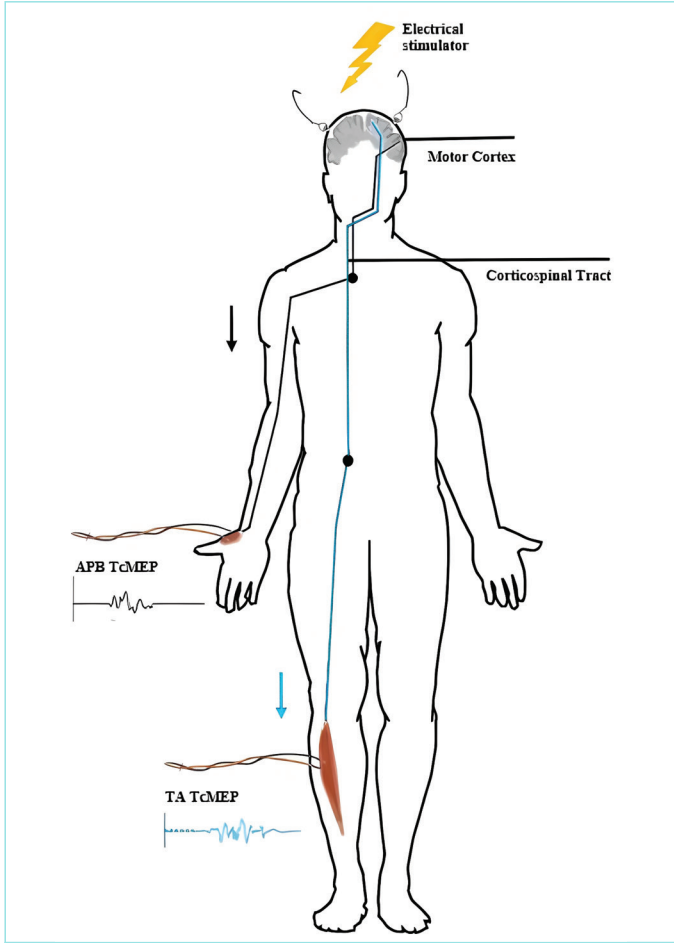

Figure 1. Schematic representation of TcMEP recordings from the upper (black) and lower (blue) extremities.

APB: Abductor pollicis brevis, TA: Tibialis anterior, TcMEP: Transcranial motor evoked potential.

stable SSEP waveforms typically requires repeated stimulation over time, which may delay the detection of acute neurological compromise compared with myogenic MEPs.[11]

Free-running and triggered electromyography

EMG is a fundamental IONM modality for assessing the functional integrity of individual nerve roots during spine surgery. EMG is particularly valuable for detecting neurotonic discharges and reducing the risk of postoperative radiculopathy, particularly during procedures involving pedicle screw placement.[9]

Free-running electromyography: Free-running, or spontaneous, EMG involves recording muscle electrical activity from selected muscle groups innervated by nerve roots at risk during surgery. Appropriate muscle selection should sample the relevant spinal nerve roots, with one representative muscle per nerve root generally being sufficient for effective monitoring.

Common EMG recording sites for cervical nerve roots include the trapezius (C3-4), deltoid (C5-6), biceps brachii (C5-6), brachioradialis (C6), triceps brachii (C6-7), extensor digitorum communis (C7), abductor pollicis brevis (C8-T1), first dorsal interosseous (C8-T1), and abductor digiti minimi (C8-T1). Upper thoracic roots can be monitored by recording from intercostal or paraspinal muscles. For lower thoracic and lumbosacral roots, commonly monitored muscles include the intercostal muscles, paraspinal muscles, rectus abdominis (T7-12), internal oblique (L1-2), adductor longus (L2-3), vastus lateralis (L2-4), tibialis anterior (L4-5), gastrocnemius (S1-S2), abductor hallucis (S1-2), and external anal sphincter (EAS) (S2-4).

Needle electrodes placed in selected muscle groups continuously record muscle fiber activity, thereby enabling real-time monitoring throughout the surgical procedure. EMG monitoring is performed in the absence of neuromuscular blockade. Under adequate general anesthesia and in the absence of nerve irritation, intact nerve roots typically exhibit no spontaneous EMG activity. However, nerve dissection, mechanical or thermal irritation, prolonged traction, or compression of a nerve root during surgery may elicit distinct EMG patterns, some of which are suggestive of axonal injury.[12]

Triggered electromyography: Triggered EMG is a technique that uses electrical stimulation delivered via a handheld probe to evoke and record compound muscle action potentials (CMAPs) from targeted muscles. Intraoperative triggered EMG during spinal surgery is used to identify and map functional motor nerve roots and to differentiate viable neural tissue from non-neural structures during tumor resection or tethered cord release procedures.[13,14]

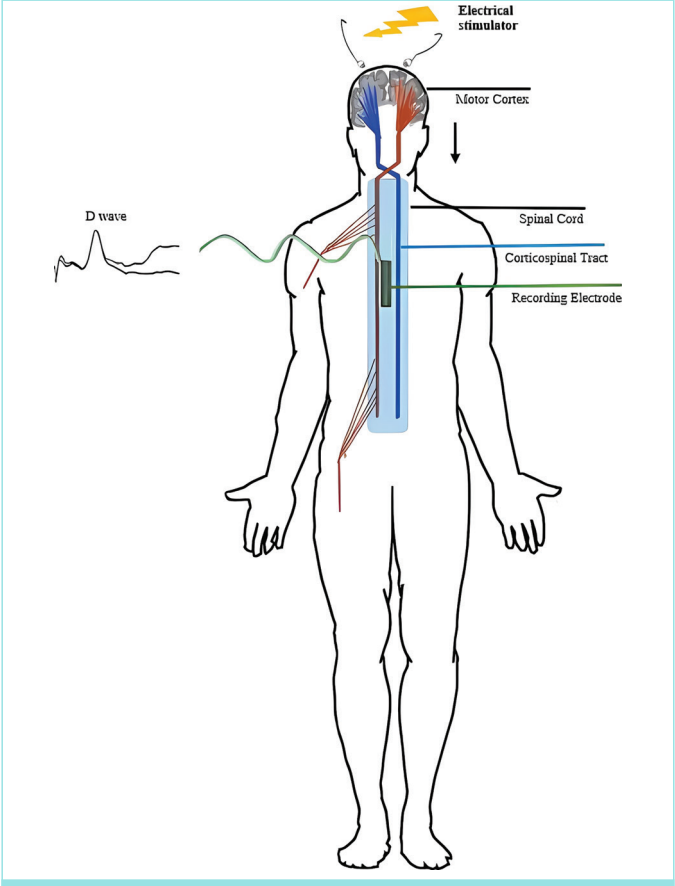

Figure 2. Schematic representation of D-wave recording.

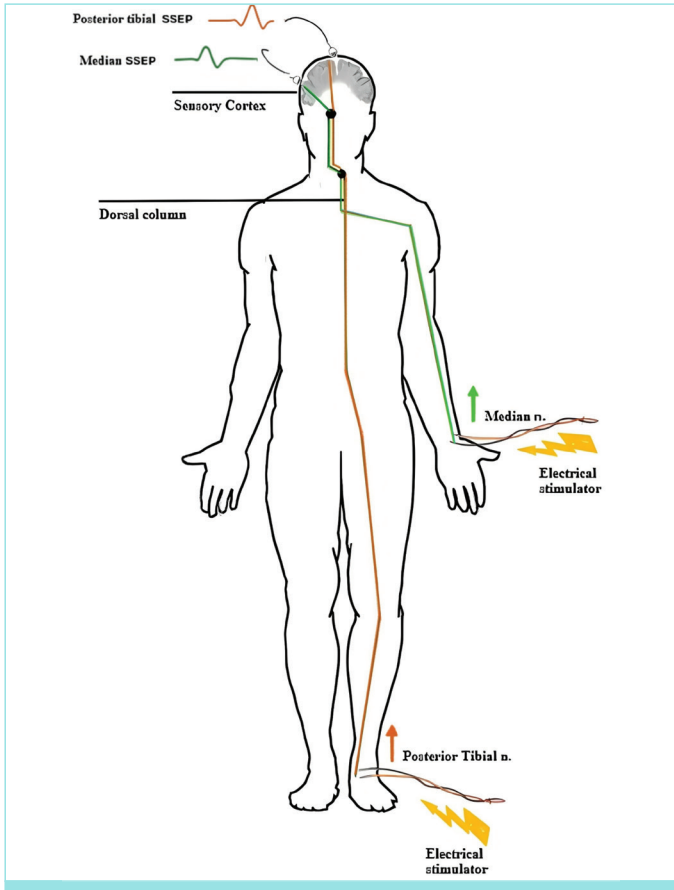

Figure 3. Schematic representation of SSEP recordings from the upper (green) and lower (orange) extremities.

SSEP: Somatosensory evoked potentials.

Triggered EMG is most commonly applied during spinal instrumentation procedures, particularly for assessing pedicle screw placement. In this technique, a handheld monopolar stimulating probe is applied directly to the pedicle holes or implanted screws to evaluate a potential breach of the pedicle wall and the proximity to adjacent nerve roots. When a pedicle screw is correctly positioned, the surrounding cortical bone acts as an electrical insulator, requiring relatively higher stimulation currents to evoke a CMAP response.[15] If the screw breaches the medial pedicle wall, the reduced impedance permits electrical current to reach the adjacent nerve root, thereby eliciting an EMG response at lower stimulation thresholds.[15]

The bulbocavernosus reflex

Intraoperative BCR monitoring is a specialized neurophysiological technique used during spinal surgery involving the conus medullaris and cauda equina to preserve bowel, bladder, and sexual function.[16] BCR monitoring provides real-time assessment of the functional integrity of the sacral sensory and motor nerve roots and the S2-S4 spinal cord segments.

The reflex is elicited by electrical stimulation of the dorsal penile nerve in males or of the dorsal clitoral nerve in females, with recordings obtained from the EAS muscle (Figure 4). Activation of this reflex arc reflects intact afferent sensory input, sacral spinal cord interneuronal processing, and efferent motor output to the EAS.[16]

In males, surface electrodes are placed on the dorsum of the penis for stimulation. In females, the cathode is positioned over the clitoris and the anode is positioned on the adjacent labium. Electromyographic responses are recorded from the EAS using wire or needle electrodes. To improve reliability under general anesthesia, stimulation is typically delivered as short trains of 2-5 pulses or using a double-train paradigm, which enhances temporal summation and increases the likelihood of eliciting a reproducible reflex response.[17]

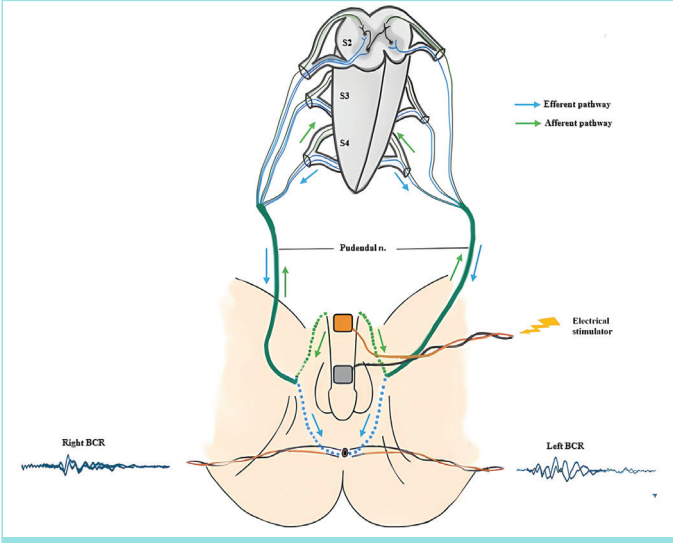

Figure 4. Schematic representation of BCR recording during dorsal penile nerve stimulation in males.

BCR: Bulbocavernous reflex.

Spinal Cord and Nerve Root Mapping Modalities:

Neurophysiological spinal cord and nerve root mapping techniques enable accurate intraoperative identification of critical neuroanatomical structures within the surgical field, thereby reducing the risk of iatrogenic neural injury.

The principal mapping modalities used in spine surgery include dorsal column mapping, CST mapping, motor root mapping, and sensory root mapping. Among these, dorsal column and CST mapping are most commonly applied during intramedullary spinal cord tumor (IMSCT) resection.[13] In contrast, nerve root mapping techniques are primarily used during procedures involving the cauda equina to differentiate functional neural tissue and during instrumented spinal surgeries to assess the osseous integrity of instrumented pedicles.[14]

Dorsal column mapping

Surgical treatment of IMSCTs often involves a posterior midline myelotomy performed through the posterior median sulcus between the right and left dorsal columns.[18] The dorsal columns consist of large-diameter, heavily myelinated sensory fibers responsible for proprioception, vibration sense, and fine touch. Injury to these pathways during myelotomy may result in postoperative numbness, dysesthesias, impaired proprioception, and sensory ataxia.

Because pathological processes may obscure or displace normal surface landmarks, dorsal column mapping has become an essential adjunct for identifying the physiological midline of the spinal cord and delineating the boundaries between the left and right dorsal columns. Accurate localization of the midline enables the surgeon to perform a safer myelotomy while minimizing the risk of posterior column dysfunction.

Several neurophysiological techniques have been described for dorsal column mapping. One approach involves direct electrical stimulation of the exposed dorsal columns with recording of antidromic sensory nerve action potentials from a peripheral nerve.[19] A second technique relies on recording scalp SSEPs and identifying a phase reversal following dorsal column stimulation, which corresponds to the functional midline.[20] A third method employs peripheral nerve stimulation with recording of SSEPs directly from the exposed spinal cord using a multielectrode grid placed perpendicular to the longitudinal axis of the cord.[21] In this configuration, an amplitude gradient across the grid reflects the somatotopic organization of the dorsal columns and allows precise identification of the physiological midline.

Corticospinal tract mapping

Another critical structure at risk during intramedullary tumor resection is the CST. CST mapping is performed using direct electrical stimulation within the resection cavity, typically with a handheld bipolar probe, to assess proximity to descending motor pathways and to guide safe tumor removal.

Stimulation of the CST produces myogenic responses in limb muscles; however, similar muscle responses may also arise from stimulation of the dorsal columns through activation of spinal interneurons, resulting in a centrally mediated reflex resembling the H-reflex.[22] To distinguish CST activation from dorsal column-mediated responses, a double-train stimulation paradigm can be applied.[23]

This technique exploits differences in the recovery times of interneuronal circuits within the spinal gray matter.[23] Interneurons activated by CST fibers exhibit a shorter refractory period than those activated by dorsal column fibers. When two stimulus trains are delivered with an intertrain interval of approximately 60 ms, stimulation near the CST is expected to evoke muscle responses following both the first and second trains. In contrast, stimulation near the dorsal columns typically produces a response only to the first train because dorsal column-related interneurons remain in their refractory period during the second train.

Accordingly, persistence of a muscle response after both stimulus trains indicates close proximity to the CST, whereas absence of a response to the second train suggests stimulation of dorsal column pathways.[23] This differentiation provides valuable real-time information regarding the spatial relationship between the surgical field and critical motor tracts, thereby facilitating maximal tumor resection while preserving neurological function.

Motor and sensory root mapping

Intraoperative motor and sensory root mapping employs direct electrical stimulation to precisely identify individual nerve roots within the surgical field. This technique is particularly valuable in procedures involving the cauda equina, resection of intradural extramedullary tumors (IDEMTs), and revision surgeries, in which normal anatomy may be distorted by tumor infiltration, inflammation, or scar tissue. Accurate root identification facilitates safe dissection, prevents iatrogenic neural injury, and optimizes surgical outcomes.

Motor root mapping is performed by directly stimulating a nerve root with a handheld monopolar or bipolar probe while recording CMAPs from muscles innervated by the corresponding myotome. This technique allows the surgeon to distinguish functional motor roots from non-neural tissue and non-functional roots, thereby preserving motor function during lesion resection.

Electrical stimulation of a sensory nerve root may also evoke a muscle response, which can complicate interpretation. This response does not result from direct motor fiber activation but instead represents a posterior root-muscle reflex (PRMR), analogous to the H-reflex mediated by proximally excited sensory axons near the spinal cord.[24] PRMRs arise from monosynaptic activation of alpha motor neurons in the anterior horn following afferent stimulation of the dorsal root.[24] Compared with CMAPs generated by motor root stimulation, PRMRs are characterized by longer latency, lower amplitude, and a higher stimulation threshold.[25] Despite these distinguishing features, overlap in response characteristics may occur, necessitating additional strategies to reliably differentiate sensory from motor roots.

Reliable differentiation between motor root-evoked CMAPs and sensory root-mediated PRMRs can be achieved using a double-train stimulation paradigm.[25] When two stimulus trains are delivered with an interstimulus interval of 60 ms, stimulation of either motor or sensory roots produces a muscle response following the first stimulus.[25] However, after stimulation of a sensory root, the reflex arc remains within its refractory period, thereby preventing the generation of a second response. In contrast, motor axons recover more rapidly, allowing stimulation of a motor root to elicit a second CMAP in response to the second stimulus.

Warning Criteria for Intraoperative Neurophysiological Monitoring Modalities

Practice guidelines and technical standards have been issued by professional societies such as the American Clinical Neurophysiology Society; however, no universally accepted, procedure-independent alarm criteria exist across all IONM modalities.[2] Interpretation of intraoperative changes therefore requires integration of neurophysiological findings with clinical judgment and the specific surgical context.

Motor evoked potentials and D-waves

Commonly used alarm criteria for MEPs are based on amplitude reduction or complete signal loss, with thresholds ranging from 50% to 80% reported in the literature.[26]

Alternative criteria-such as increased stimulation threshold, waveform simplification, or morphological changes-have also been described;[2] however, these approaches are technically demanding, time-consuming, and less frequently used in routine clinical practice.

As an indicator of irreversible CST injury, D-wave monitoring provides complementary prognostic information to MEPs in spinal cord neuromonitoring, particularly during intramedullary tumor resection.[3,27] Whereas MEPs primarily reflect immediate postoperative motor function, D-wave recordings are more predictive of long-term motor outcomes and functional recovery.

A reduction in D-wave amplitude of more than 50% or its complete disappearance, indicates significant CST injury and is associated with a high-risk of permanent motor deficit during intramedullary tumor surgery. In contrast, depending on surgical risk and tumor characteristics, resection may be continued despite loss of MEPs if the D-wave amplitude remains within 50% of baseline. Such preservation is strongly associated with favorable long-term motor outcomes, even in the presence of significant transient postoperative weakness.[27]

In addition to intramedullary tumor surgery, D-wave recording can be applied in selected cases involving IDEMTs and syringomyelia.[28,29] However, its utility in scoliosis correction and other orthopedic procedures remains uncertain.

Somatosensory evoked potentials

SSEPs are typically monitored continuously throughout surgery to detect changes suggestive of evolving spinal cord or nerve injury. Commonly accepted alarm criteria include a reduction in amplitude greater than 50% and/or a latency prolongation exceeding 10% relative to baseline recordings.[9,10]

Bulbocavernosus reflex

The presence of a stable BCR waveform with preserved amplitude and morphology throughout the procedure indicates intact sacral reflex arc function and sphincter control.[17,30] Conversely, intraoperative attenuation or loss of the BCR may reflect transient or permanent compromise of sacral sensory or motor pathways and has been associated with postoperative bowel, bladder, or sexual dysfunction, particularly when the loss is sustained and not attributable to anesthetic or technical factors.[30]

Free-running and triggered electromyography

Continuous free-running EMG is recorded from multiple muscle groups to detect electromyographic discharges indicative of motor nerve root irritation or mechanical compromise. Normal EMG recordings are electrically silent; however, intraoperative injury to motor roots may evoke high-frequency burst or train activity.

During surgery, mechanical or thermal irritation of a nerve root-such as traction, compression, stretching, cold irrigation, or electrocautery-may elicit spikes, burst discharges, or short-lasting, low-frequency train activity.[9,12,31] Short-lasting, intermittent burst activity is often associated with transient nerve root contact or decompression and generally reflects proximity rather than structural injury, being associated with a low risk of permanent nerve damage.[9,32]

Another form of EMG activity observed during IONM is long-lasting, high-frequency train activity (A-trains), also referred to as neurotonic discharges. This pattern is more commonly associated with prolonged traction or compression and is considered a warning sign of potential nerve injury if not promptly addressed. Accordingly, sustained or repetitive neurotonic discharges suggestive of possible axonal injury should be distinguished from transient irritation-related activity.[31]

In addition, preexisting chronic radiculopathy may produce baseline low-amplitude, low-frequency EMG activity that can confound interpretation; however, this activity is typically present from the onset of monitoring and lacks temporal correlation with surgical manipulation.[32] In some situations, such as sharp dissection of a motor nerve root, no EMG activity may be observed despite nerve injury, thereby creating a false-negative interpretation regarding nerve integrity.[12]

In addition to continuous EMG monitoring to detect spike, burst, or train-type discharges, triggered EMG is useful for evaluating the presence of functional neural tissue, differentiating neural tissue from scar tissue, and mapping motor roots entrapped within a tumor. When electrical stimulation of a suspected motor nerve root with a handheld probe within the surgical field evokes CMAPs in innervated muscles, the location of motor fibers can be inferred based on the stimulation threshold, latency, and amplitude of the CMAPs. Generally, obtaining a CMAP response with a stimulation threshold of 1 mA or less indicates close proximity to functional motor roots that should be preserved.[33]

Triggered EMG is also used to detect pedicle screw breach based on stimulation thresholds. The stimulation threshold reflects the proximity of the screw to adjacent neural structures. Reported warning thresholds indicating a low likelihood of medial pedicle wall breach typically range from 6 to 11 mA.[34] An EMG response elicited at currents below these thresholds raises concern for screw malposition and warrants further evaluation.[35]

However, the spinal level should be taken into account when determining appropriate warning thresholds. Threshold values vary by region and are generally higher in the cervical spine. Moreover, in the presence of osteoporosis, the stimulation threshold required to elicit an EMG response may be reduced because of decreased bone mineral density.

Several additional factors may limit the reliability of triggered EMG. Chronically compressed or injured nerve roots may exhibit elevated stimulation thresholds, potentially resulting in false-negative findings.[14] In addition, current shunting may occur when stimulation is delivered in the presence of conductive fluids, such as saline irrigation, within the surgical field, thereby further increasing the risk of false-negative results.[14]

Anesthetic Consideration During Intraoperative Neurophysiological Monitoring

Any pharmacological agent or physiological parameter that influences axonal conduction, synaptic transmission, or motor neuron excitability may alter evoked potential waveforms during IONM. In general, neural pathways with longer conduction distances and a greater number of synapses are more sensitive to anesthetic effects.[36] Accordingly, SSEPs, MEPs, EMG activity, and reflex-based responses such as the BCR are all affected-albeit to differing degrees-by anesthetic agents in a dose- and mechanism-dependent manner.

Appropriate selection of anesthetic agents and close coordination between them are therefore essential to obtain stable and interpretable IONM recordings. Most anesthetic agents depress synaptic transmission, resulting in reduced waveform amplitudes and prolonged latencies.[37] Although both inhalational and intravenous anesthetics exert these effects, inhalational agents have a more pronounced suppressive impact and are more likely to degrade or abolish IONM signals, particularly at higher concentrations.[38]

Among the various modalities, MEPs are generally more sensitive to anesthetic suppression than SSEPs, reflecting their dependence on intact corticospinal excitability and spinal motor neuron recruitment.[36] In addition, both MEPs and EMG responses are markedly attenuated by neuromuscular blocking agents.[14,36] For this reason, neuromuscular blockade is typically limited to facilitating tracheal intubation and is avoided thereafter in procedures requiring motor pathway or EMG monitoring.

Special anesthetic considerations are required for BCR monitoring. Because the BCR is an oligosynaptic reflex, it is particularly sensitive to anesthetic agents. Neuromuscular blocking agents and inhalational anesthetics can significantly suppress or abolish the reflex and should therefore be avoided.[16]

Consequently, total intravenous anesthesia-most commonly using propofol in combination with a short-acting opioid such as remifentanil-without maintenance neuromuscular blockade is widely regarded as the anesthetic technique of choice for IONM.[36,39] Avoiding large bolus doses and maintaining stable drug infusion rates further enhance signal reliability and reduce abrupt changes in evoked potential recordings.

Interpretation of Intraoperative Neurophysiological Monitoring Signal Changes

IONM is typically performed by an IONM technologist under the supervision of an IONM professional, such as a qualified physician, a neurologist, or a clinical neurophysiologist. The IONM technologist carries out electrode placement, signal acquisition, and artifact management. The technologist plays a critical role in maintaining signal integrity, ensuring accurate documentation of baseline recordings and intraoperative events, and adhering to established monitoring protocols. Professional guidelines emphasize that high-quality data acquisition and timely reporting are essential components of safe IONM practice.

However, interpretation of neurophysiological data, assessment of alarm significance, diagnostic decision-making, and intraoperative clinical judgment remain the responsibility of the supervising physician or clinical neurophysiologist.[2,31]

When a significant change in waveforms is detected by the IONM technologist, non-surgical causes should be excluded before issuing a warning. Technical factors should be verified, including correct placement of stimulating and recording electrodes and proper functioning of the monitoring equipment. Interpretation of intraoperative changes also requires careful consideration of confounding systemic factors, including hypotension, hypothermia, anemia, depth of anesthesia, and bolus administration of anesthetic agents.[38,39] Monitoring SSEPs and MEPs from rostral control recording electrodes is useful for distinguishing global physiological effects from focal spinal cord compromise. Additionally, focal MEP and SSEP changes resulting from surgical manipulation must be differentiated from peripheral nerve dysfunction caused by limb malpositioning, compression, or ischemia. If no correctable cause is identified, attention should be directed toward recent surgical maneuvers-particularly those temporally associated with an intraoperative potential change-with consideration given to reversing or modifying these actions and assessing signal recovery.

Intraoperative Neurophysiological Monitoring Practice in Spinal Surgeries

In current practice, IONM is employed during a wide range of spinal operations, including surgery for degenerative disease, spinal deformity, intramedullary tumors and IDEMTs, and tethered cord syndrome.

The selection of monitoring modalities is influenced by several factors, including the surgical approach, anatomical level, underlying pathology, and surgeon preference. During procedures involving the cervical and thoracic spine, preservation of the integrity of motor and sensory pathways within the spinal cord is critical. In addition to long-tract monitoring, nerve root monitoring remains important in selected cervical and lumbosacral surgeries, particularly those performed below the conus medullaris, where the thecal sac and cauda equina nerve roots are encountered. The most commonly used monitoring modalities in spine surgery include MEPs, SSEPs, and EMG; their techniques, advantages, disadvantages, and typical clinical applications are summarized in Table 1.

Although the clinical value of IONM is increasingly recognized, no universal consensus exists regarding its routine use across all spinal procedures.[1] Numerous studies have demonstrated that multimodal monitoring provides superior sensitivity and predictive value compared with single-modality techniques, particularly in high-risk procedures.[1]

IONM is generally accepted as an effective adjunct for predicting and reducing neurological injury in complex, high-risk spine and spinal cord surgeries, whereas its role in routine, low-risk cervical and lumbar procedures remains controversial because of limited and inconsistent supporting evidence.[40]

Degenerative spine disease

Degenerative disease of the cervical and lumbar spine encompasses a spectrum of conditions, including intervertebral disc degeneration, spondylolisthesis, and spinal canal or foraminal stenosis. Surgical management is primarily directed toward neural decompression and/or stabilization with instrumentation and fusion.

For routine, low-risk degenerative cervical and lumbar procedures-particularly single-level lumbar decompressions or fusions in patients without preoperative neurological deficits-the benefit of routine IONM remains controversial.[41] Current evidence does not consistently support the routine use of IONM in low-risk degenerative spine surgery.

In contrast, in patients with degenerative cervical myelopathy (DCM), in whom chronic compression of the cervical spinal cord is present, IONM may play an important role in reducing the risk of perioperative neurological injury.[42] Surgical treatment of DCM, using anterior, posterior, or combined approaches, aims to decompress the spinal cord, restore cervical alignment, and achieve spinal stability. However, these procedures carry an inherent risk of perioperative spinal cord injury. Neurological compromise during DCM surgery may occur at multiple stages, including induction of anesthesia, patient positioning (particularly prone positioning or excessive cervical extension), decompression maneuvers, instrumentation, deformity correction, or intraoperative hypotension with impaired spinal cord perfusion. Multimodal IONM has therefore emerged as a valuable adjunct in DCM surgery, providing real-time functional assessment of the spinal cord and nerve roots. The combined use of SSEPs, MEPs, and EMG improves diagnostic sensitivity and specificity compared with single-modality monitoring, allowing earlier detection of evolving spinal cord dysfunction and enabling timely corrective interventions, such as adjustment of surgical maneuvers, optimization of blood pressure, revision of instrumentation, or modification of patient positioning.[42]

Spinal deformity surgery

The utility of IONM in spinal deformity surgery is well established and widely accepted.[43] Surgical correction of spinal deformities-particularly in cases involving scoliosis, kyphosis, or complex multiplanar deformities-carries a substantial risk of neurological injury. Such injury may result from direct mechanical trauma to the spinal cord, ischemia secondary to compromised spinal cord perfusion, excessive distraction, compression during corrective maneuvers, or malpositioned instrumentation.[43]

Accordingly, continuous intraoperative assessment of both motor and sensory pathway integrity is critical throughout all phases of deformity correction, including positioning, exposure, deformity manipulation, osteotomy, and instrumentation.

The combined multimodal application of SSEPs and MEPs is now considered the standard of care in most centers performing complex spinal deformity surgery, particularly in pediatric and adult patients undergoing high-risk corrective procedures.[44,45]

Spinal tumor surgery

Although spinal cord tumors are relatively uncommon, their close proximity to critical neural structures-including long ascending and descending tracts, nerve roots, and segmental vascular supply-places patients at substantial risk for perioperative neurological morbidity.

Depending on their anatomical location, spinal tumors are broadly classified into two groups: extradural and intradural. Extradural spinal tumors, which are most commonly of metastatic origin,

Table 1. MEP, SSEP, and EMG in spine surgery: a comparative summary

	SSEP	MEP	EMG (free-running)	EMG (triggered)
Stimulation	Electrical stimulation of peripheral nerves (e.g., median, ulnar, posterior tibial)	Transcranial electrical stimulation over the motor cortex	None	Direct electrical stimulation with a handheld probe
Recording	Scalp (cortical responses) ± subcortical/spinal recordings	Extremity muscles	Myotome-specific muscles	Myotome-specific muscles
Modality type	Sensory (dorsal column pathways)	Motor (corticospinal tract)	Motor (nerve root irritation)	Motor (nerve root integrity testing)
Pathway monitored	Dorsal column-medial lemniscus pathways	Corticospinal tracts	Motor nerve roots	Motor nerve roots
Monitoring style	Near-continuous (requires signal averaging → delayed detection)	Intermittent (stimulus-evoked)	Continuous (real-time activity)	Intermittent (stimulus-dependent)
Advantages	Continuous monitoring; relatively resistant to anesthetic effects	Direct motor pathway assessment; large signal amplitude (no averaging required)	Real-time detection of nerve root irritation	Localizes and tests specific neural structures (e.g., pedicle screws)
Disadvantages	Small amplitude → requires averaging, delayed detection; does not assess motor pathways	Highly sensitive to anesthesia (especially inhalational agents); not continuous	Monitors only motor nerve roots; limited anatomical localization; may miss silent injury	Not continuous; operator-dependent; threshold variability (false-positive or false-negative results)
Typical clinical use	Monitor functional integrity of dorsal column sensory pathways	Monitor functional integrity of motor tracts (e.g., deformity surgery)	Detect nerve root irritation during instrumentation	Confirm safe pedicle screw placement or root proximity; map neural elements at risk

EMG: Electromyography, MEP: Motor evoked potential, SSEP: Somatosensory evoked potential.

produce neurological deficits secondary to spinal cord or nerve root compression. Intradural tumors arise within the dura and are further subdivided, based on their relationship to the spinal cord parenchyma, into intramedullary and extramedullary categories.

Approximately two-thirds of intradural spinal cord tumors are IDEMTs, which typically include meningiomas and nerve sheath tumors. IDEMTs are usually benign and well circumscribed, allowing total resection in most cases.[46] However, IDEMT surgery can be technically demanding because of limited operative corridors, spinal cord displacement, and close adherence to nerve roots or the pial surface. IONM assists in preserving neurological function while enabling complete tumor removal.[47] SSEPs, MEPs, and D-wave recordings are the IONM modalities most commonly used during IDEMT surgery to reduce the risk of spinal cord injury and improve surgical outcomes.[48]

IMSCTs are rare, accounting for approximately 20-35% of all intradural spinal cord tumors, and most commonly include ependymomas, astrocytomas, and hemangioblastomas.[49] Surgical resection is the standard of care for these lesions, although the extent of resection is strongly influenced by tumor histology, growth pattern, and the presence or absence of a well-defined tumor-cord interface.[49] Because IMSCTs arise within the spinal cord parenchyma, surgical manipulation inherently places the CST, dorsal columns, and segmental gray matter at high-risk of injury from traction, compression, or vascular compromise. In this setting, IONM is of particular importance and has become an integral component of modern IMSCT surgery. Clinical studies have demonstrated that IONM provides critical real-time functional feedback that can guide the extent of resection and facilitate maximal safe tumor removal.[50] Commonly employed modalities include SSEPs, MEPs, D-wave recordings, and free-running and triggered EMG.[27,48] In addition, functional mapping techniques-such as dorsal column mapping and CST mapping-can assist in identifying safe entry zones and minimizing injury to eloquent spinal cord regions.[51]

Conus medullaris and cauda equina surgeries

Surgical procedures involving the conus medullaris and cauda equina-including decompressive surgery, intradural and extradural tumor resection, and tethered cord release-are associated with a substantial risk of neurological injury. Owing to the dense concentration of motor, sensory, and autonomic pathways within this region, even limited intraoperative insult may result in significant postoperative deficits affecting lower extremity motor function, sensation, and bladder, bowel, or sexual function.

During these procedures, the conus medullaris or the individual lumbosacral nerve roots may be compromised by traction, compression, thermal injury (from coagulation), ischemia, or inadvertent transection. The risk is further heightened by distorted anatomy caused by tumors, adhesions, lipomas, prior surgery, or congenital malformations, which may obscure visual identification of neural structures. Consequently, reliance on anatomical landmarks alone may be insufficient to ensure neurological preservation.

Accordingly, a multimodal IONM strategy integrating monitoring and mapping techniques is typically employed in conus medullaris and cauda equina surgery. Commonly used modalities include MEPs, SSEPs, and free-running and triggered EMG.[52] In addition, BCR monitoring provides real-time assessment of sacral reflex arcs and autonomic pathways, particularly those arising from sacral segments S2-S4, which are critical for sphincter function.[52]

Root mapping plays a particularly important role in these surgeries, especially when normal anatomy is obscured. By applying focal electrical stimulation to suspected neural tissue and observing corresponding EMG responses, root mapping enables differentiation between functional nerve roots and non-neural structures such as fibrous tissue, tumor capsule, or lipomatous elements.[52] This technique is especially valuable in tethered cord release and intradural tumor surgery, where

preservation of functional nerve roots is essential to prevent irreversible motor or sphincter deficits.[52,53]

CONCLUSION

IONM has become an integral component of modern spine and spinal cord surgery, providing real-time functional assessment of neural structures at risk during operative intervention. By enabling early detection of evolving neurological compromise, IONM facilitates timely corrective measures that may prevent or mitigate permanent postoperative deficits. The effectiveness of neuromonitoring is closely linked to the appropriate selection, interpretation, and integration of multiple complementary modalities rather than to reliance on a single technique.

MEPs, SSEPs, EMG, reflex monitoring, and mapping techniques each interrogate distinct aspects of neural function and exhibit differing sensitivities to mechanical, ischemic, anesthetic, and physiological perturbations. Multimodal IONM therefore offers a more comprehensive evaluation of spinal cord and nerve root integrity, particularly in high-risk procedures such as deformity correction, IMSCT resection, and surgery involving the conus medullaris and cauda equina.

Despite its widespread adoption, the routine use of IONM in low-risk degenerative spinal procedures remains controversial, reflecting variability in the evidence base, cost considerations, and practice patterns. Importantly, IONM should be regarded as an adjunct to-not a substitute for-meticulous surgical technique, sound anatomical knowledge, and close interdisciplinary communication among the surgical, anesthesia, and neurophysiology teams.

Future advances in quantitative signal analysis, standardized alarm criteria, and integration of emerging technologies may further refine the clinical utility of IONM. Continued prospective studies are needed to better define procedure-specific indications and associated outcome benefits. When appropriately applied and interpreted, IONM remains a powerful tool for enhancing surgical safety and optimizing neurological outcomes in spine surgery.

MAIN POINTS

- Intraoperative neurophysiological monitoring (IONM) has become a cornerstone of contemporary spine surgery by enabling real-time functional assessment of neural structures and facilitating early identification of impending neurological injury.
- Multimodal IONM, rather than reliance on a single modality, provides the most comprehensive assessment and confers the greatest benefit in high-risk procedures.
- The routine application of IONM in low-risk degenerative spine surgery remains a subject of ongoing debate.

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RESEARCH ARTICLE

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Antioxidant Activity and Chemical Composition of *Origanum Majorana, Cedrus Atlantica, and Commiphora Myrrha* Essential Oils: Implications for Antifungal Applications

Merve Nenni

Department of Analytical Chemistry, Çukurova University Faculty of Pharmacy, Adana, Türkiye

Abstract

BACKGROUND/AIMS: Rising antifungal resistance has encouraged the investigation of plant-derived compounds as alternative or adjunctive agents in fungal infections. Essential oils possess bioactive constituents with antioxidant and antifungal properties. This study aimed to evaluate the antioxidant activity of essential oils obtained from *Origanum majorana*, *Cedrus atlantica*, and *Commiphora myrrha* and to characterize their chemical composition using gas chromatography-mass spectrometry (GC-MS), focusing on their potential relevance to antifungal applications.

MATERIALS AND METHODS: Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazine (DPPH) radical scavenging assay, and half-maximal inhibitory concentration (IC_{50}) values were calculated with ascorbic acid (AA) as the reference standard. Chemical profiles of the essential oils were determined by GC-MS analysis. Statistical comparisons were performed using one-way ANOVA, with $p<0.05$ considered statistically significant.

RESULTS: Among the tested essential oils, *C. myrrha* exhibited the strongest antioxidant activity (IC_{50}: 18.167±0.972 mg/mL), followed by *C. atlantica* (IC_{50}: 213.037±74.219 mg/mL) and *O. majorana* (IC_{50}: 333.207±31.954 mg/mL). AA showed substantially higher antioxidant activity (IC_{50}: 0.021±0.0003 mg/mL). GC-MS analysis revealed that *C. myrrha* oil was rich in oxygenated sesquiterpenes, particularly curzerene and furanoeudesma-1,3-diene; *O. majorana* was characterized by terpinen-4-ol and γ-terpinene; and *C. atlantica* was dominated by himachalene derivatives.

CONCLUSION: The findings demonstrate that *C. myrrha* essential oil possesses superior antioxidant capacity among the oils tested, likely attributable to its high content of oxygenated sesquiterpenes. While direct antifungal activity was not assessed, the observed antioxidant properties may support the use of myrrh essential oil as a natural adjuvant in antifungal formulations targeting fungal infections associated with oxidative stress.

Keywords: *Origanum majorana*, *Cedrus atlantica*, *Commiphora myrrha*, essential oil, antioxidant, DPPH

INTRODUCTION

Alternative approaches are of great importance in the treatment of fungal infections, especially due to increasing resistance problems.[1] In this context, the antimicrobial and especially antifungal properties of essential oils obtained from plants offer the potential for the development of new therapeutic strategies.[2] Essential oils have attracted attention with their antibacterial, antifungal, anticancer, and antioxidant effects in various sectors such as food, cosmetics, pharmaceuticals, textiles, and agriculture.[3] These natural compounds, secondary metabolites of plants, function as defense mechanisms against pathogens and exhibit broad-spectrum antifungal activities.[4] With these properties, they constitute a promising therapeutic alternative, especially against pathogenic fungi such as Candida species in humans.[5] In addition, the antioxidant capacity of essential oils may contribute to the treatment process by reducing oxidative stress caused by fungal infections.[6]

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ORCID IDs of the authors: M.N. 0000-0003-3165-1060.

Corresponding author: Merve Nenni
E-mail: mnenni@cu.edu.tr, merve.ecz@gmail.com
ORCID ID: orcid.org/0000-0003-3165-1060

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Compared to other species in the Origanum genus, *Origanum majorana (Sweet Marjoram*) essential oil contains higher concentrations of monoterpene alcohols and hydrocarbons and lower concentrations of phenolic compounds such as carvacrol and thymol. Recent chromatographic studies indicate that the principal component of this oil is frequently terpinen-4-ol. Gamma-terpinene, sabinene, alpha-terpinene, cis-sabinene hydrate, and linalyl acetate are other important phytochemicals. Some chemotypes contain thymol and carvacrol, whereas *Origanum majorana* usually contains terpinen-4-ol and sabinene as its main constituents. These components give the oil its unique smell and make it function better within the body. The antioxidant properties of this essential oil are based on the scavenging ability of its monoterpenes. Terpinen-4-ol and gamma-terpinene specifically reduce reactive oxygen species, thereby preventing lipid peroxidation. The antifungal mechanism of action is highly effective and primarily targets the fungal cell membrane. The lipophilic structure of *Origanum majorana* essential oil penetrates the chitin and glucan layers of the fungal cell wall and integrates into the cell membrane. This makes the membrane less flexible and less permeable. It also stops the production of ergosterol, causing intracellular ions (such as potassium) and essential macromolecules to leak out. This stops energy synthesis in the fungal cell (mitochondrial failure), thereby killing the cell. This method shows a lot of inhibition, especially against Candida species and dermatophytes.[7]

The essential oil of *Cedrus atlantica* exhibits a complex chemical composition, with sesquiterpenes constituting a major component. Recent studies show that the major constituents of the oil are beta-himachalene (30-50%), alpha-himachalene, and gamma-himachalene. The "himachalene" group is responsible for the biological activity of cedar oil. The oil also contains significant amounts of alpha-atlantone (a ketone), delta-cadinene, and cedrol, a sesquiterpene alcohol. Cedrol, a component of the oil, can crystallize, giving it its distinct aroma. The antioxidant properties of cedar essential oil are due to its sesquiterpenes, especially himachalenes, which can donate electrons. However, this effect is usually weaker than that of phenolic oils, such as thyme. Its antifungal mechanism is more narrowly focused. The essential oil of *Cedrus atlantica* has a unique ability to alter the structure of fungal biofilms. Studies show that this oil alters protein organization, facilitating fungal cell adhesion to surfaces and weakening the cell membrane. Studies show that the vapor phase of oil prevents mold, such as Penicillium, from forming spores and developing mycelium by irreversibly damaging the cytoplasmic membrane. The hydrophobic properties of sesquiterpene hydrocarbons interact with fungal membrane lipids, reducing the membrane potential and impeding adenosine triphosphate synthesis.[8]

The essential oil from *Commiphora myrrha* resin differs from other essential oils because it is rich in sesquiterpenes, specifically furano-sesquiterpenes that contain furan rings. Furanoeudesma-1,3-diene, curzeren, and lindestrene are the primary components. There are also other sesquiterpenes, such as beta-elemene and germacrene. The unique chemical composition of myrrh oil, which includes molecules with furan rings, gives it strong therapeutic and antibacterial properties. The antioxidant properties of myrrh essential oil are attributed to furan-ring-containing compounds that mitigate oxidative stress and bind metal ions. It has a complex mechanism for combating fungi. *Commiphora myrrha* essential oil can directly inhibit enzymatic activity in fungal cells. Studies, particularly those focused on Candida albicans and other pathogenic yeasts, have shown that myrrh oil selectively disrupts cell membrane permeability and promotes the coagulation of intracellular components. Furthermore, recent studies indicate that the sesquiterpenes in myrrh oil undermine the defensive mechanisms of fungi and inhibit hyphal (thread-like) development, thus preventing the spread of infection and avoiding secondary fungal infections during the wound healing process.[9,10]

This study evaluated the antioxidant activity of marjoram (*Origanum majorana),* cedarwood (*Cedrus atlantica*), and myrrh *(Commiphora myrrha*) essential oils and characterized their chemical compositions using gas chromatography-mass spectrometry (GC-MS). The results are discussed in the context of their potential relevance to future antifungal applications.

MATERIALS AND METHODS

The current study was performed only using commercial essential oils and *in vitro* experimental techniques. No human subjects or experimental animals were used. Consequently, the ethical committee approval and informed consent were not needed for the present study.

Samples of Chemicals and Essential Oils

2,2-diphenyl-1-picrylhydrazine (DPPH), [ascorbic acid (AA); positive control], and methanol were purchased from Sigma-Aldrich (USA) and were of analytical reagent grade. Commercial essential oils of *Origanum majorana*, *Cedrus atlantica,* and *Commiphora myrrha* were obtained from a pharmacy in Adana, Türkiye, under the brand name Art de Huile. The oils were used as received, according to the product labeling, without additional botanical authentication or chemotype verification.

Antioxidant Activity

Free radical scavenging is measured using 1,1-diphenyl-2-picrylhydrazyl. The decrease in absorbance of the stable free radical DPPH at 517 nm can be used to evaluate the scavenging capacity of natural substances. DPPH is colorless when mixed with a scavenger and purple in its free-radical form. A 0.1 mM DPPH stock solution in methanol was prepared. This foil-wrapped solution is refrigerated to avoid degradation. The essential oil samples were tested for DPPH.[11,12] Twelve MeOH dilutions were performed on essential oil samples. A 96-well plate was filled with 120 µL of diluted essential oil samples and AA. To initiate the reaction, 40 µL of 0.1 mM DPPH in MeOH was added. The absorbance of the reaction mixture at 517 nm was measured with a UV spectrophotometer after 45 minutes at room temperature. The mean was computed from three observations. Measurements were performed in triplicate wells (technical replicates), and mean values were used for calculations. The DPPH radical scavenging activity of samples and standards was calculated from the decrease in absorbance. The DPPH removal % was $[(A_{Control} A_{Sample})/A_{Control}) \times 100]$. The half-maximal inhibitory concentration (IC_{50}) is then calculated. The antioxidant activities of the tested compounds were evaluated by comparing their IC_{50} values, which denote the concentrations necessary to block 50% of free-radical activity. Lower IC_{50} values correspond to higher antioxidant potency. Effective concentration (EC_{50}), antiradical power (ARP), and ascorbic acid equivalent antioxidant capacity (AEAC) were measured.[13-16]

Composition Analysis of the Essential Oil by Gas Chromatography-Mass Spectrometry

The content analysis was conducted utilizing GC-MS.[17] The content analysis was conducted on a Shimadzu GC-MS/QP2010 Ultra equipped with a DB-5MS stationary-phase column (i.d. 0.25 mm; film thickness 0.25 µm). Upon completion of the analysis, intricate chromatograms were deconvoluted using AMDIS. Moreover, retention time adjustment and data matrix creation were performed using SpectConnect software. The correlation analysis was conducted using Microsoft Excel.

Statistical Analysis

For the purpose of expressing all of the data, the mean and standard deviation of the IC_{50} measurements were utilized. Statistical comparisons were performed using one-way analysis of variance (ANOVA). When the overall ANOVA was significant, Dunnett's post-hoc test was applied to compare each essential oil with AA, the reference standard. A p-value <0.05 was considered statistically significant.

RESULTS

As the IC_{50} value decreased, the antioxidant ability of the essential oil samples correspondingly increased. A lower IC_{50} value indicates the presence of antioxidant action. An antioxidant is deemed more potent if it has a low IC_{50}-EC_{50} value and a high ARP-AEAC value. The essential oils were evaluated for their antioxidant efficacy according to the specified criteria. AA (IC_{50}: 0.021±0.0003 mg/mL) is more effective than *Commiphora myrrha* (myrrh) essential oil (IC_{50}: 18.167±0.972 mg/mL), *Cedrus atlantica* (cedarwood) essential oil (IC_{50}: 213.037±74.219 mg/mL), and *Origanum majorana* (marjoram) essential oil (IC_{50}: 333.207±31.954 mg/mL) (Figure 1 and 2).The other antioxidant parameters are displayed in Table 1.

The GC-MS analysis of the essential oils revealed distinctive chemical profiles closely associated with their antioxidant potentials. The essential oil of Origanum majorana (marjoram) was predominantly composed of terpinen-4-ol, γ-terpinene, and cis-thujanol-4, accompanied by smaller proportions of α-terpinene and sabinene. The GC-MS profile of *Commiphora myrrha* (myrrh) revealed a complex mixture rich in sesquiterpenes, with curzerene, furanoeudesma-1,3-diene, lindestrene, and β-elemene as major constituents. The essential oil of *Cedrus atlantica* consisted mainly of himachalenes.

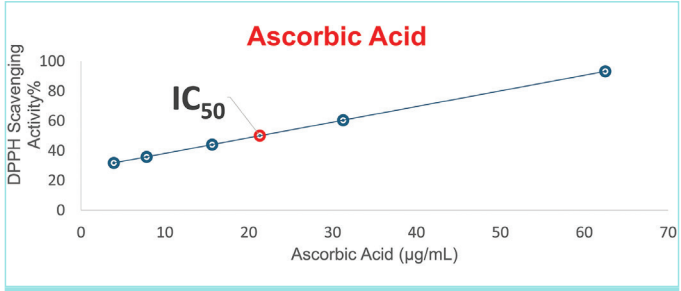

Figure 1. DPPH scavenging activity % of ascorbic acid as control group.

DPPH: 2,2-diphenyl-1-picrylhydrazine, IC_{50}: Half-maximal inhibitory concentration.

DISCUSSION

Essential oils contain bioactive compounds, such as terpenes and phenolics, that exhibit strong antifungal activity by disrupting fungal cell membranes, inhibiting cell wall synthesis, and interfering with mitochondrial function in fungi. They also inhibit fungal biofilm formation, which contributes to infection persistence and drug resistance. Marjoram *(Origanum majorana)* essential oil shows antifungal activity attributed to its content of oxygenated monoterpenes and terpenoids that disrupt fungal cell membranes and reduce ergosterol synthesis, essential for fungal cell viability. Cedarwood (*Cedrus atlantica)* essential oil exhibits antifungal effects mainly due to sesquiterpenes such as cedrol that impair fungal growth and mycelial integrity, with reported activity particularly against dermatophytes such as Trichophyton species, which are common agents of cutaneous fungal infections, including those affecting the hands. Myrrh *(Commiphora myrrha)* essential oil demonstrates antifungal properties through multiple mechanisms, including cell membrane disruption, mitochondrial dysfunction, and inhibition of fungal biofilm development, thereby enhancing its efficacy against persistent fungal infections. Studies report that essential oils have potential as natural antifungal agents for the treatment of hand fungal infections, with advantages including low toxicity, reduced development of resistance, and suitability for topical application. Their efficacy is linked to chemical composition variability dependent on plant source and extraction method.[1,18,19]

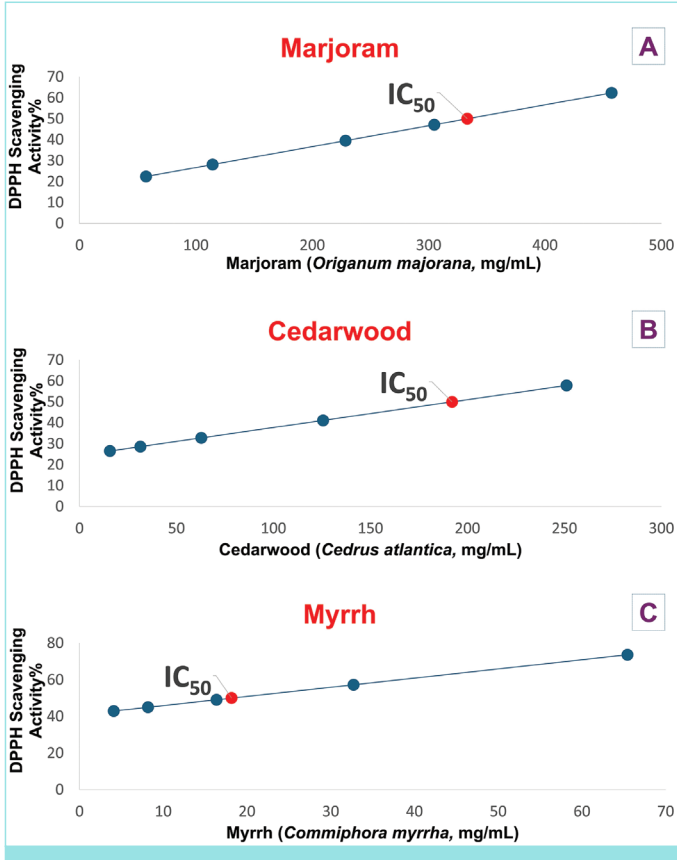

Figure 2. DPPH scavenging activity % of marjoram (A), cedarwood (B) and myrrh (C) essential oils.

DPPH: 2,2-diphenyl-1-picrylhydrazine, IC_{50}: Half-maximal inhibitory concentration.

Table 1. Antioxidant properties of the samples of essential oils

Compounds	IC_{50} (mg/mL)		EC_{50} (mg/mL)		ARP (mL/mg)		AEAC
	Mean ± SD	p-values	Mean ± SD	p-values	Mean ± SD	p-values	
Ascorbic acid	0.021±0.0003	-	0.0005±0.0000069	-	184937.934±2347.026	-	-
Marjoram (*Origanum majorana*) essential oil	333.207±31.954	0.000027	8.450±0.810	0.000027	11.834±1.087	0.0001	6.399
Cedarwood (*Cedrus atlantica*) essential oil	213.037±74.219	0.003	5.403±1.883	0.0038	18.509±8.976	0.0001	10.008
Myrrh (*Commiphora myrrha*) essential oil	18.167±0.972	0.0000027	0.461±0.024	0.000002	217.049±11.980	0.0001	117.363

IC_{50} results are presented as means ± standard deviation from three measurements. Group differences were assessed using one-way ANOVA followed by Dunnett's post-hoc test (ascorbic acid as reference). The values of the essential oil samples with superscripts varied significantly ($p<0.005$).
EC_{50}: Effective concentration, IC_{50}: Half-maximal inhibitory concentration, ARP: Antiradical power, AEAC: Ascorbic acid equivalent antioxidant capacity.

Similar studies have reported potential antioxidant effects of the same essential oils. The DPPH antioxidant activity of *Commiphora myrrha* resin was found to be IC_{50}: 26.86 mg/L, indicating moderate antioxidant activity.[20] For *Cedrus atlantica* essential oil, an IC_{50} value of 0.126 mg/mL and similar values were reported using the DPPH method, indicating moderate-to-high antioxidant capacity.[21] Antioxidant activity testing and health effects of *Origanum majorana* water and ethanol extracts; despite the high IC_{50} value, potential antioxidant effects were reported.[22]

In this study, the antioxidant capacity of AA is markedly superior to that of essential oils. These are natural results of potent antioxidants, such as AA. Among the essential oils tested, myrrh demonstrated the most potent antioxidant effect with IC_{50} values of 18.167±0.972 mg/mL, showing a markedly higher radical scavenging activity compared to marjoram and cedarwood.

The GC-MS analysis confirmed that each essential oil possesses a unique chemical composition, which influences its antioxidant potential. *Commiphora myrrha* oil contained high levels of curzerene and furanoeudesma-1,3-diene, that are strongly associated with antioxidant and cytoprotective effects. Consequently, myrrh demonstrated the most potent antioxidant activity among the tested oils. *Origanum majorana* oil, rich in terpinen-4-ol and γ-terpinene, exhibited moderate activity, reflecting the contribution of oxygenated monoterpenes. In contrast, *Cedrus* atlantica oil, dominated by hydrocarbon sesquiterpenes such as β-himachalene, showed weaker antioxidant activity. These findings highlight the close relationship between chemical composition and biological activity. The presence of oxygenated terpenes, particularly furanosesquiterpenes and alcohol monoterpenes, appears to enhance antioxidant capacity, suggesting that *Commiphora myrrha* essential oil could serve as a promising natural antioxidant source for pharmaceutical or nutraceutical applications.

Study Limitations

This work assessed antioxidant activity using an *in vitro* DPPH assay and did not include direct antifungal testing (e.g., MIC/MFC, inhibition zones, or dermatophyte culture models). In addition, the essential oils were purchased as commercial products from a local market, and no independent botanical authentication, chemotype verification, or batch standardization was performed; therefore, compositional variability may have influenced the measured activity.

CONCLUSION

This study aimed to evaluate the antioxidant potential of three essential oils, *Origanum majorana* (marjoram), *Cedrus atlantica* (cedarwood), and *Commiphora myrrha* (myrrh), and to determine their chemical composition using GC-MS. The antioxidant activities were assessed using the DPPH radical-scavenging method, and the IC_{50} values were compared with those of AA. The results showed that *Commiphora myrrha* exhibited the highest antioxidant activity with IC_{50}: 18.167±0.972 mg/mL among other essential oils, which may be related to its high content of oxygenated sesquiterpenes. No direct antifungal assays were performed; the present findings should be interpreted as supportive evidence for potential formulation research rather than treatment efficacy. Further *in vitro* and *in vivo* studies are required to evaluate antifungal activity, safety, and batch-to-batch variability.

MAIN POINTS

- The study evaluates the antioxidant activities of *Origanum majorana*, *Cedrus atlantica,* and *Commiphora myrrha* essential oils using the 2,2-diphenyl-1-picrylhydrazine radical-scavenging assay.
- *Commiphora myrrha* essential oil exhibited the highest antioxidant capacity (IC_{50}: 18.167±0.972 mg/mL) among the tested oils.
- Gas chromatography-mass spectrometry analysis revealed that the high antioxidant potential of *Commiphora myrrha* is associated with its rich content of oxygenated sesquiterpenes, such as curzerene and furanoeudesma-1,3-diene.
- The findings suggest that *Commiphora myrrha* essential oil could serve as a natural adjuvant in antifungal formulations targeting oxidative stress-related fungal infections.
- This study highlights the potential of essential oils as alternatives for antifungal formulations, particularly as antioxidant adjuncts, and emphasizes the need for direct antifungal efficacy testing.

ETHICS

Ethics Committee Approval: The author declare that the materials and methods used in the current study do not need approval from an ethics committee or special legal permission. The study was not conducted on humans or experimental animals.

Informed Consent: The study did not include human participants or samples. Informed consent was not applicable.

Acknowledgement

During the preparation of this work, the author(s) used Perplexity to generate summaries of research articles relevant to the topic. The summaries were assessed by juxtaposing them with expert-authored summaries in the field. After the precision and pertinence of the generated summaries were verified, they were incorporated into the literature review section of the article. QuillBot was used to edit the language of the article. After carefully reviewing and editing the content as necessary, the author(s) take full responsibility for the publication's content. The integration of AI tools substantially improved the efficiency of the literature review process and the thoroughness of the collected research insights.

DISCLOSURES

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Declaration of Generative AI and AI-assisted Technologies in the Writing Process: During the preparation of this work, the author used ChatGPT to improve "readability" and "language". After using this tool/service, the author(s) reviewed and edited the content as needed and took full responsibility for the content of the publication.

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RESEARCH ARTICLE

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Do Serum Bilirubin and Uric Acid Reflect Oxidative Stress in Vitiligo? A Cross-Sectional Analysis

Tuğcan Yüksek[1], Simge Ünal Işık[2]

[1]Clinic of Dermatology, Kyrenia Dr. Akçiçek Hospital, Kyrenia, Cyprus
[2]Department of Dermatology, Uşak Training and Research Hospital; Uşak University Faculty of Medicine, Uşak, Türkiye

Abstract

BACKGROUND/AIMS: Oxidative stress is central to vitiligo, driven by excess reactive oxygen species and weakened antioxidant defenses. Although bilirubin and uric acid (UA) are important endogenous antioxidants routinely measured, current evidence regarding their systemic relevance to vitiligo is limited.

MATERIALS AND METHODS: To compare serum bilirubin and UA levels between vitiligo patients and healthy controls and to determine whether these biomarkers correlate with clinical characteristics or reflect disease burden. This cross-sectional study included 44 patients with non-segmental vitiligo and 45 healthy controls. Serum total bilirubin (Tbil), direct bilirubin (Dbil), and UA levels were measured. Disease severity was evaluated using the vitiligo extent score (VES) and vitiligo area scoring index (VASI). Disease stability was defined as the absence of new or enlarging lesions for ≥12 months. Correlation and subgroup analyses were performed to assess associations between biomarkers and clinical parameters. Receiver operating characteristic (ROC) analysis was used to evaluate diagnostic performance.

RESULTS: No significant differences were found between vitiligo patients and controls in Tbil (p=0.070), Dbil (p=0.978), or UA (p=0.311). Subgroup analyses showed no associations of bilirubin or UA with disease activity, severity (VES, VASI), duration, or anti-thyroid peroxidase status (all p>0.05). Correlation analyses revealed no significant relationships between these markers and clinical features. ROC analyses demonstrated low diagnostic accuracy.

CONCLUSION: Serum bilirubin and UA levels appear unaltered in vitiligo and show no correlation with clinical features. These findings suggest that oxidative stress may be predominantly localized, highlighting the need for research on skin-specific oxidative pathways and alternative biomarkers.

Keywords: Vitiligo, oxidative stress, bilirubin, uric acid, antioxidants

INTRODUCTION

Vitiligo is a chronic autoimmune depigmenting disorder characterized by the progressive loss of melanocytes, leading to well-defined depigmented macules on the skin. The prevalence of vitiligo ranges from 0.5% to 2% worldwide, and its unpredictable course and visible skin changes often result in a significant psychosocial burden for affected individuals.[1,2] While the precise etiology of vitiligo remains unclear, growing evidence suggests that oxidative stress plays a pivotal role in its pathogenesis. Several genetic, autoimmune, and environmental factors have been implicated in melanocyte destruction; however, oxidative stress is increasingly recognized as a key trigger for disease onset and progression.[3,4]

Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms,

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ORCID IDs of the authors: T.Y. 0000-0003-2612-8080; S.Ü.I. 0000-0002-4226-0463.

Corresponding author: Tuğcan Yüksek
E-mail: tugcanyuksek6@gmail.com
ORCID ID: orcid.org/0000-0003-2612-8080

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leading to lipid peroxidation, DNA damage, mitochondrial dysfunction, and apoptosis of melanocytes. Several studies have demonstrated that melanocytes in vitiligo patients are particularly vulnerable to oxidative damage, primarily due to their high metabolic activity and ROS accumulation during melanogenesis. Previous research has reported elevated serum malondialdehyde (MDA) levels, a marker of lipid peroxidation, and reduced antioxidant enzyme activity, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in vitiligo patients compared to healthy controls.[5,6] Additionally, hydrogen peroxide accumulation has been observed in vitiligo lesions, further supporting the role of oxidative stress in melanocyte degeneration.[7,8] A systematic review by Speeckaert et al.[9] confirmed that oxidative stress pathways are significantly dysregulated in vitiligo patients, highlighting increased lipid peroxidation, disrupted antioxidant enzyme activity, and an overall imbalance in the oxidative stress response.

Recent studies have further demonstrated that oxidative stress correlates with the severity and activity of vitiligo. Mathachan et al.[10] have found that oxidative stress markers were more significantly altered in patients with generalized and active vitiligo, suggesting that oxidative imbalance worsens as the disease progresses. Given this association, assessing oxidative stress in relation to disease severity is crucial for understanding its impact on vitiligo pathogenesis.

To counteract oxidative damage, the body relies on enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants, such as SOD, CAT, GPx, and glutathione reductase, directly neutralize ROS, whereas non-enzymatic antioxidants, including vitamins C and E, glutathione, albumin, ceruloplasmin, bilirubin, and uric acid (UA), act as circulating free radical scavengers. Studies in vitiligo have consistently reported reduced enzymatic antioxidant activity and altered levels of non-enzymatic antioxidants, indicating a defective oxidative defense system.[11]

Among non-enzymatic antioxidants, bilirubin and UA play crucial roles in neutralizing oxidative damage. Bilirubin, a product of heme degradation, is generated through the heme oxygenase-1 (HO-1) pathway, which breaks down heme into biliverdin, carbon monoxide, and free iron. Biliverdin is subsequently reduced to bilirubin by biliverdin reductase, forming a biliverdin-bilirubin redox cycle that provides continuous antioxidant protection.[12] Earlier studies demonstrated that bilirubin has a greater antioxidant capacity than vitamin C and α-tocopherol, particularly in preventing lipid peroxidation in cellular membranes.[13,14]

UA is another endogenous antioxidant that acts as a free radical scavenger, neutralizing hydroxyl radicals, singlet oxygen, and peroxynitrite. Additionally, UA has metal-chelating properties, reducing iron-catalyzed oxidative damage and preventing further ROS generation.[15] Studies have shown that UA contributes significantly to total plasma antioxidant capacity, offering protection against oxidative stress-related conditions, including cardiovascular diseases, neurodegenerative disorders, and autoimmune diseases.[16]

Several studies have investigated the role of bilirubin and UA in inflammatory and autoimmune diseases, such as pemphigus vulgaris (PV), Crohn's disease, and polymyositis/dermatomyositis, in which these markers have generally been found to be lower, suggesting potential antioxidant depletion during oxidative stress.[17-19] However, findings are not entirely consistent across diseases; for example, studies in psoriasis have shown differing trends. Despite the recognized role of oxidative stress in vitiligo, research on bilirubin and UA in this condition is scarce, and their potential involvement remains unclear. Further investigation is needed to determine whether these antioxidants play a role in oxidative imbalance and disease progression in vitiligo.

Based on their well-established antioxidant properties, bilirubin and UA are hypothesized to be potential biomarkers of oxidative imbalance in vitiligo, offering a cost-effective and widely accessible option for clinical monitoring. Therefore, this study aimed to compare serum bilirubin and UA levels between vitiligo patients and healthy controls and to evaluate their association with disease progression.

MATERIALS AND METHODS

This cross-sectional study was conducted between June 2024 and December 2024 and included patients aged 18 to 65 years diagnosed with non-segmental vitiligo and healthy control subjects. Ethical approval for the study was obtained from the Uşak University Non-Interventional Clinical Research Ethics Committee (approval no: 349-349-07, date: 04.04.2024). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

The required sample size was calculated using G*Power (version 3.1.9.7). Using an Independent Samples t-test and assuming a moderate effect size (Cohen's d=0.5, based on prior studies of antioxidant markers in autoimmune skin diseases), a power of 80%, and an alpha of 0.05, the minimum required sample size was 42 per group.

Patients were included if they had a dermatologist-confirmed clinical diagnosis of non-segmental vitiligo. Healthy controls had no history of vitiligo, autoimmune diseases, or chronic inflammatory diseases. Participants were excluded if they had systemic diseases (e.g., diabetes mellitus, cardiovascular disorders, hepatic disorders, or renal disorders), hyperbilirubinemia (e.g., Gilbert's syndrome), hyperuricemia, metabolic disorders that influence oxidative stress, or recent infections or malignancies. Additional exclusions were antioxidant supplementation (e.g., vitamins C/E, N-acetylcysteine), immunosuppressive or UA-lowering drugs (e.g., allopurinol), corticosteroid use, pregnancy/lactation, active smoking (≥10 cigarettes/day), regular alcohol consumption, significant dietary restrictions (e.g., vegan, ketogenic diets), or high-intensity physical activity (>5 hours/week) and chronic stress, due to their potential impacts on oxidative biomarkers.

For each participant, demographic data including age, sex, and body mass index (BMI) were recorded. Vitiligo severity was evaluated using two validated scoring systems: the vitiligo area scoring index (VASI) and the vitiligo extent score (VES). The VASI score was calculated by estimating the body surface area (BSA) involvement in hand units (1 hand ≈1% of total BSA) across body regions, each multiplied by the degree of depigmentation (graded from 0 to 1.0). The VES classification was used to categorize patients based on the extent of BSA affected by vitiligo, grouping them into five severity categories: no vitiligo (0%), mild (1-10%), moderate (11-30%), severe (31-50%), and extensive (>50%). This classification system allows for a structured comparison of disease severity across different patient groups. In addition to severity, disease activity was assessed to determine whether patients had active or stable vitiligo. Disease activity was classified as active if new depigmented lesions had appeared or existing lesions had expanded within the past 12 months, whereas vitiligo was considered stable if no new lesions had developed, and existing depigmented areas remained unchanged for at

least one year.[20] The presence of thyroid autoimmunity was evaluated by measuring anti-thyroid peroxidase (anti-TPO) antibody levels.

Fasting (8-12 hours) venous blood samples were collected from all participants. Serum total bilirubin (Tbil), direct bilirubin (Dbil) and UA levels were measured using an automated enzymatic colorimetric method on a fully automated biochemical analyzer. Blood samples were processed by centrifugation at 3,500 rpm for 10 minutes, and serum was separated and stored at -80 °C until analysis.

Statistical Analysis

Statistical analyses were conducted using PASW 18.0 for Windows. The normality of continuous variables was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. For comparisons between vitiligo patients and the control group, Independent Samples t-tests were used for normally distributed variables, while Mann-Whitney U tests were applied for non-normally distributed data. For comparisons involving multiple groups, one-way ANOVA was used for normally distributed data, while the Kruskal-Wallis test was applied for non-normally distributed data. Correlation analyses between bilirubin, UA, disease severity (VASI), disease duration, and anti-TPO positivity were conducted using Pearson's correlation coefficient for normally distributed variables and Spearman's rank correlation coefficient for non-normally distributed data. As an exploratory analysis, receiver operating characteristic (ROC) curve analysis was conducted to evaluate the potential diagnostic performance of bilirubin and UA in distinguishing vitiligo patients from healthy controls, and area under the curve (AUC) values were calculated. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 89 participants were included in this study, comprising 44 patients with non-segmental vitiligo and 45 healthy controls. No significant differences were found between vitiligo patients and healthy controls regarding age (30.25±8.7 vs. 29.87 ±7.9 years, p=0.838), gender distribution (male: 50% vs. 51.1%, p=0.912), BMI (24.85±4.5 kg/m^2 vs. 24.34±4.2 kg/m^2, p=0.597), weight (68±14 kg vs. 66±13 kg, p=0.483), or height (168.3±9.5 cm vs. 168.4±9.7 cm, p=0.996) (Table 1). The mean VASI score among vitiligo patients was 5.4±2.2 (Table 1). The median VES score among vitiligo patients was 2.5% (range: 0.4-95%). According to the VES classification, most patients were categorized as mild (n=31), followed by moderate (n=7), severe (n=3), and extensive (n=3).

The comparison of serum Tbil, Dbil and UA levels between vitiligo patients and controls revealed that Tbil levels were 0.39±0.20 mg/dL in vitiligo patients and 0.33±0.20 mg/dL in controls, with no significant difference (p=0.070). Similarly, Dbil levels were comparable between vitiligo patients (0.33±0.20 mg/dL) and controls (0.33±0.10 mg/dL, p=0.978), and serum UA levels were also similar between vitiligo patients (4.83±1.23 mg/dL) and controls (5.14±1.22 mg/dL, p=0.311), indicating that bilirubin and UA metabolism do not significantly differ between vitiligo patients and healthy individuals (Table 2).

Further subgroup analyses did not reveal statistically significant differences in serum Tbil, Dbil, or UA levels when patients were stratified by gender, anti-TPO positivity, disease activity (active vs. stable), or vitiligo severity assessed by VES (p>0.05 for all comparisons) (Table 3).

Correlation analysis was performed to determine associations of bilirubin and UA with vitiligo severity (as assessed by the VASI), disease duration, and anti-TPO positivity. Tbil, Dbil, and UA levels did not correlate with VASI (p=0.579, 0.457, 0.693, respectively) or disease duration (p=0.798, 0.552, 0.245, respectively). Similarly, anti-TPO positivity was not associated with bilirubin or UA levels (p=0.692, 0.620, 0.364, respectively) (Table 4).

ROC curve analysis was performed to assess the diagnostic value of bilirubin and UA in distinguishing vitiligo patients from healthy controls. The results showed that Tbil had an AUC of 0.53, indicating poor discriminatory ability, whereas Dbil had an AUC of 0.50, indicating limited diagnostic value. UA had an AUC of 0.54, indicating that it does not effectively differentiate vitiligo patients from controls. The optimal cut-off values for Tbil (0.37 mg/dL), Dbil (0.095 mg/dL), and UA (4.65 mg/dL) exhibited low sensitivity and specificity, making them unsuitable as diagnostic biomarkers for vitiligo (Table 5). The results of ROC analysis are also illustrated in Figure 1.

DISCUSSION

In this study, we evaluated bilirubin and UA-two endogenous antioxidants-to determine their potential role as oxidative stress biomarkers in vitiligo. Although we hypothesized that these markers might be altered due to their ROS-scavenging properties, no significant differences in Tbil, Dbil, or UA levels were observed between vitiligo patients and healthy controls.

Subgroup analyses further showed that these markers did not differ according to disease activity, severity (VES), or anti-TPO status, suggesting that bilirubin and UA are not influenced by disease burden or thyroid autoimmunity. These findings indicate that systemic antioxidant levels do not adequately reflect the oxidative processes underlying vitiligo.

Table 1. Baseline characteristics of vitiligo patienrs and healthy controls

Characteristic	Vitiligo patients (mean ± SD or n%)	Controls (mean ± SD or n%)	p-value
Age (years)	30.25±8.7	29.87±7.9	0.838[a]
Gender (male/female)	22 (50%)/22 (50%)	23 (51.1%)/22 (48.9%)	0.912[b]
BMI (kg/m^2)	24.85±4.5	24.34±4.2	0.597[a]
Weight (kg)	68±14	66±13	0.483[a]
Height (cm)	168.3±9.5	168.4±9.7	0.996[a]
VASI (mean ± SD)	5.4±2.2	N/A	N/A

[a]Independent t-test, [b]Chi-square test.
BMI: Body mass index, VASI: Vitiligo area severity index, SD: Standard deviation.

A plausible explanation is that oxidative stress in vitiligo predominantly operates at a localized, skin-specific level, rather than being systemic. A lternative antioxidant defense mechanisms, such as enhanced local enzymatic activity (CAT, SOD, or GPx), may restrict oxidative stress to affected skin sites, thereby preventing measurable alterations in circulating bilirubin and UA levels.

No correlations were found between bilirubin/UA levels and VASI score, disease duration, or anti-TPO positivity. ROC analyses also demonstrated low diagnostic accuracy (AUC <0.7), reinforcing that these systemic antioxidants are neither sensitive nor specific markers for diagnosing vitiligo or assessing its characteristics.

To date, only one previous study has assessed serum bilirubin and UA levels in vitiligo, reporting significantly lower bilirubin levels in patients while UA remained unchanged.[21] Our study, in contrast, found slightly higher though not statistically significant, Tbil levels, suggesting an alternative oxidative-stress response rather than systemic depletion. This discrepancy may reflect differences in patient selection, disease duration at enrollment, the proportion of active versus stable cases, and methodological variation in bilirubin assay techniques or pre-analytical variables such as fasting status and sample storage. From a biological standpoint, the slightly higher bilirubin levels in our cohort may reflect an early adaptive response mediated by HO-1, a cytoprotective enzyme induced under oxidative stress, which generates bilirubin as a byproduct of heme catabolism. In low-grade or early oxidative states, as may occur in stable vitiligo, HO-1 upregulation could transiently elevate circulating bilirubin before sustained oxidative damage potentially depletes these reserves. Unlike Türkmen and Altunışık,[21] our study further examined Tbil, Dbil, and UA in relation to disease severity (VES and VASI), activity, duration, and anti-TPO positivity-none of which showed significant associations-which reinforces the conclusion that systemic antioxidant status is not a reliable indicator of disease burden in vitiligo. Of note, a recent Mendelian randomization study reported a causal association between genetically elevated bilirubin levels and reduced vitiligo risk, with no significant reverse causality.[22] While this supports the biological relevance of bilirubin in the pathogenesis of vitiligo, the mechanistic relationship between genetically determined bilirubin levels and changes in circulating antioxidants observed in established disease warrants further investigation.

Table 2. Comparison of serum bilirubin and uric acid levels in vitiligo and control groups

Parameter	Vitiligo patients mean ± SD)	Controls (mean ± SD)	p-value*
Total bilirubin (mg/dL)	0.39±0.20	0.33±0.20	0.070
Direct bilirubin (mg/dL)	0.33±0.20	0.33±0.10	0.978
Uric acid (mg/dL)	4.83±1.23	5.14±1.22	0.311

*Independent t-test.
SD: Standard deviation.

Table 3. Subgroup analysis of serum bilirubin and uric acid levels

Variable	Category	n	Tbil (mg/dL) Median (min-max)	Statistical analysis (Tbil)	Dbil (mg/dL) Median (min-max)	Statistical analysis (Dbil)	UA (mg/dL) median (min-max)	Statistical analysis (UA)
Anti-TPO	Negative	23	0.6 (0.36-2.23)	p=0.638*	0.29 (0.08-1.46)	p=0.629*	5.6 (2.6-7.6)	p=0.546*
	Positive	21	0.69 (0.37-1.4)		0.31 (0.17-0.6)		4.8 (3.0-7.9)	
Disease activity	Active	34	0.62 (0.36-2.23)	p=0.922*	0.3 (0.08-1.46)	p=0.575*	5.2 (2.6-7.9)	p=0.713*
	Stable	10	0.625 (0.36-1.19)		0.29 (0.18-0.41)		5.1 (3.1-6.9)	
Gender	Female	22	0.57 (0.36-1.71)	p=0.192*	0.29 (0.08-0.65)	p=0.626*	5.1 (3.0-7.4)	p=0.469*
	Male	22	0.69 (0.37-2.23])		0.3 (0.17-1.46)		5.2 (2.6-7.9)	
	Mild	35	0.6 (0.36-2.23)		0.3 (0.08-1.46)		5.25 (2.6-7.9)	
VES	Moderate	3	0.65 (0.37-0.85)	p=0.843**	0.34 (0.18-0.37)	p=0.882**	4.25 (3.5-5.0)	p=0.617**
	Severe	1	N/A		N/A		N/A	
	Extensive	5	0.75 (0.38-1.12)		0.29 (0.19-0.41)		6.3 (3.9-6.9)	

*Mann-Whitney U test, **Kruskal-Wallis test.
Anti-TPO: Anti-thyroid peroxidase, VES: Vitiligo extent score, Tbil: Total bilirubin, Dbil: Direct bilirubin, UA: Uric acid.

Table 4. Correlation analysis of bilirubin, uric acid, and clinical parameters

Biomarker	Correlation with	Spearman r	p-value
Tbil	VASI	-0.101	0.579
Dbil	VASI	-0.136	0.457
UA	VASI	-0.06	0.693
Tbil	Disease duration	-0.042	0.798
Dbil	Disease duration	-0.108	0.552
UA	Disease duration	-0.198	0.245
Tbil	Anti-TPO	0.06	0.692
Dbil	Anti-TPO	0.08	0.620
UA	Anti-TPO	-0.14	0.364

Tbil: Total bilirubin, Dbil: Direct bilirubin, UA: Uric acid, VASI: Vitiligo area severity index, Anti-TPO: Anti-thyroid peroxidase.

Unlike our findings, previous studies in other autoimmune and inflammatory diseases have reported significant alterations in bilirubin and UA levels. Chen et al.[19] found that patients with polymyositis and dermatomyositis (PM/DM) had significantly lower serum bilirubin and UA levels, with oxidative stress identified as a major factor in these diseases. Similarly, Li et al.[17] reported that PV patients exhibited lower total, direct, and indirect bilirubin, along with lower UA levels, with disease severity negatively correlated with bilirubin. In Crohn's disease, Su et al.[18] demonstrated that bilirubin and UA levels were significantly lower in patients, correlating with disease progression and oxidative stress severity. These studies support the idea that oxidative stress leads to systemic depletion of antioxidants in inflammatory diseases, contrasting with our vitiligo findings where bilirubin and UA remained unchanged.

A key explanation for these differences lies in the nature of the oxidative process: systemic diseases such as PV, Crohn's disease, and PM/DM involve widespread chronic inflammation that depletes circulating antioxidant reserves, while vitiligo's oxidative stress appears predominantly confined to melanocyte-rich skin areas. However, systemic depletion of bilirubin and UA has been observed in some localized inflammatory skin diseases. A previous study in 2020 has shown that rosacea patients exhibited significantly reduced bilirubin and UA levels, suggesting that oxidative stress can lead to systemic depletion even in disorders with primarily localized pathology.[23] On the other hand, Rahimi et al.[24] have shown that bilirubin levels were significantly higher in melasma patients and positively correlated with disease extent. Since melasma involves functional but oxidatively stressed melanocytes, elevated bilirubin may reflect an HO-1-mediated adaptive antioxidant response in structurally intact cells. In vitiligo, by contrast, melanocytes are progressively destroyed, potentially rendering a compensatory bilirubin response insufficient or transient. Our finding of no correlation of bilirubin or UA levels with disease duration further suggests that systemic antioxidant levels do not track disease progression. Future studies should directly assess oxidative stress markers within lesional skin to better characterize the spatial and temporal dynamics of the oxidative imbalance in vitiligo.

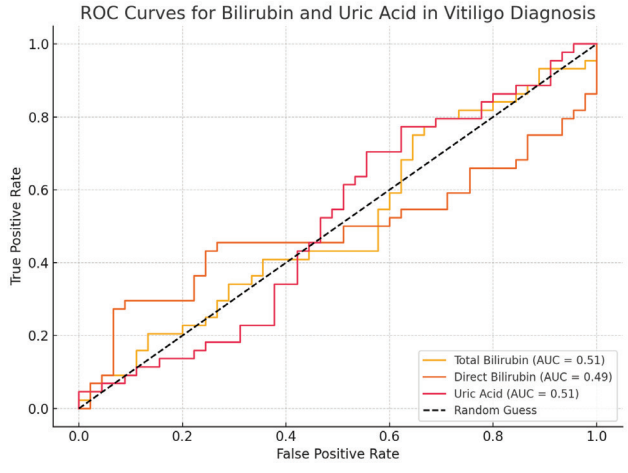

Figure 1. ROC curves assessing the diagnostic performance of serum bilirubin and uric acid in vitiligo.

ROC: Receiver operating characteristic, AUC: Area under the curve.

Table 5. ROC curve analysis for diagnostic performance of bilirubin and uric acid

Biomarker	AUC	95% CI	p-value
TBil	0.53	0.44-0.62	0.307
Dbil	0.50	0.41-0.59	0.857
UA	0.54	0.45-0.63	0.285

AUC: Area under the curve, CI: Confidence interval, Tbil: Total bilirubin, Dbil: Direct bilirubin, UA: Uric acid, ROC: Receiver operating characteristic.

Studies in other inflammatory skin diseases have yielded variable results regarding systemic antioxidant profiles.[25] In psoriasis, Solak et al.[26] reported elevated UA levels suggesting a compensatory antioxidant response, while Nemati et al.[27] found no significant difference in bilirubin or UA despite elevated MDA and SOD. Severin et al.[28] similarly observed elevated bilirubin and UA in psoriasis patients, yet total antioxidant capacity remained unchanged. While a psoriatic arthritis study reported significantly lower indirect bilirubin, inversely correlating with CRP.[29] In lichen planus, Barikbin et al.[30] found slightly lower bilirubin and UA levels that did not reach statistical significance, though vitamin C levels were markedly reduced, indicating selective antioxidant depletion. As summarized in Table 6, these heterogeneous findings suggest that bilirubin and UA are inconsistent indicators of oxidative stress, and that our vitiligo results align most closely with conditions characterized by localized oxidative stress and preserved systemic antioxidant status.

Study Limitations

Several limitations of this study should be considered. First, its cross-sectional design limits the ability to assess temporal changes in, or causal relationships between, oxidative biomarkers and disease dynamics. Second, although we implemented strict exclusion criteria, unmeasured variables such as subclinical stress, unreported supplement use, or genetic predispositions may still have influenced systemic antioxidant levels. Third, subgroup comparisons by VES category (Table 3) are exploratory, as certain severity tiers-particularly the severe (n=1) and extensive (n=5) subgroups-have very small sample sizes; therefore, interpretations of null findings for these subgroups should be made with caution. Furthermore, only systemic markers were assessed; the lack of lesional oxidative stress markers and additional antioxidants (e.g., MDA, SOD) limits mechanistic interpretation. Future studies should incorporate longitudinal designs, larger sample sizes, and tissue-level sampling to better understand the role of bilirubin and UA in vitiligo.

CONCLUSION

This study showed that serum bilirubin and UA levels did not differ between vitiligo patients and controls, nor did they correlate with disease activity, severity, duration, or thyroid autoimmunity. These findings suggest that systemic antioxidant levels remain stable and do not reflect disease burden in this cohort. The slightly higher bilirubin levels observed in patients may represent a limited compensatory response rather than a meaningful oxidative stress marker. Overall, oxidative stress in vitiligo may be predominantly localized to the skin, highlighting the need for future studies focusing on lesional oxidative markers rather than serum antioxidants.

Table 6. Comparative overview of serum bilirubin and uric acid levels in vitiligo and other autoimmune or inflammatory diseases

Disease	Study (author, year)	Bilirubin findings	Uric acid findings	Summary conclusion
Vitiligo	Türkmen and Altunışık,[21]	↓ Bilirubin	No change	Possible systemic depletion.
Pemphigus vulgaris	Li et al.,[17]	↓ Total, direct, indirect	↓ Uric acid	Reduced antioxidants with severity.
PM/DM	Chen et al.,[19]	↓ Bilirubin	↓ Uric acid	Systemic oxidative imbalance.
Crohn's disease	Su et al.,[18]	↓ Bilirubin	↓ Uric acid	Depletion linked to disease activity.
Rosacea	Turkmen[23]	↓ Bilirubin	↓ Uric acid	Systemic effect despite local disease.
Melasma	Rahimi et al.,[24]	↑ Bilirubin	Not assessed	Compensatory antioxidant response.
Psoriasis	Solak et al.,[26]	Not assessed	↑ Uric acid	Possible compensation.
Psoriasis	Nemati et al.,[27]	No change	No change	Stable systemic antioxidants.
Psoriasis	Severin et al.,[28]	↑ Bilirubin	↑ Uric acid	Elevated antioxidants; total capacity normal.
Psoriatic arthritis	Wang et al.,[29]	↓ Indirect Bilirubin	Not assessed	IBIL inversely correlated with CRP.
Lichen planus	Barikbin et al.,[30]	No change	No change	Slightly lower levels; not statistically significant.
Vitiligo (current study)	Yüksek and Ünal Işık	No change	No change	Suggests localized oxidative stress.

↓: Decreased, ↑: Increased, PM/DM: Polymyositis/dermatomyositis, IBIL: Indirect bilirubin, CRP: C-reactive protein.

MAIN POINTS

- Although oxidative stress is considered central in vitiligo pathogenesis, systemic antioxidant markers such as bilirubin and uric acid may remain unchanged.
- These biomarkers were also not associated with disease severity, activity, duration, or thyroid autoimmunity.
- The findings suggest that oxidative stress in vitiligo may predominantly occur at the cutaneous rather than the systemic level.
- Future research should focus on lesional oxidative stress pathways and skin-specific biomarkers rather than circulating antioxidants.

ETHICS

Ethics Committee Approval: Ethical approval for the study was obtained from the Uşak University Non-Interventional Clinical Research Ethics Committee (approval no: 349-349-07, date: 04.04.2024).

Informed Consent: Written consent was obtained from the patient participating in this study.

Footnotes

Authorship Contributions

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

DISCLOSURES

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RESEARCH ARTICLE

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Predictors of Clinically Significant Urinary Leakage Requiring Ureteral Stenting after Percutaneous Nephrolithotomy

Ferhat Çoban[1], Hüseyin Kutlu[2]

[1]Department of Urology, Adıyaman University Faculty of Medicine, Adıyaman, Türkiye
[2]Department of Biostatistics and Medical Informatics, Adıyaman University Faculty of Medicine, Adıyaman, Türkiye

Abstract

BACKGROUND/AIMS: Urinary leakage after percutaneous nephrolithotomy (PNL) is a clinically relevant complication that may prolong hospitalization and necessitate additional interventions such as ureteral stenting. However, reliable predictors of clinically significant leakage remain insufficiently defined.

MATERIALS AND METHODS: This retrospective study included 305 patients who underwent PNL. The primary outcome was urinary leakage requiring double-J stent placement, defined as persistent drainage >24 hours after nephrostomy tube removal. Patients were divided into leakage (n=46) and non-leakage (n=259) groups. Clinical, radiological, and operative variables were analyzed. Univariate and multivariable analyses were performed using Firth penalized logistic regression. Model performance was evaluated using receiver operating characteristic analysis and calibration testing.

RESULTS: Urinary leakage occurred in 15.1% of patients. On multivariable analysis, multiple stones [odds ratio (OR)=55.66, p<0.001] and longer operative time (OR=1.017 per minute, p<0.001) were identified as independent risk factors; nephrostomy tube placement was strongly protective (OR=0.059, p<0.001). Lower calyceal stone involvement also remained significant (OR=0.255, p=0.044). The model demonstrated acceptable discrimination (area under the curve=0.798, 95% confidence interval: 0.729-0.865) and good calibration (Hosmer-Lemeshow p=0.754).

CONCLUSION: Clinically significant urinary leakage after PNL is primarily associated with stone complexity and operative factors. Multiple stones and prolonged operative time increase the risk, whereas nephrostomy tube placement has a protective effect. The proposed model may assist in perioperative risk stratification and clinical assessment.

Keywords: Percutaneous nephrolithotomy, urinary leakage, double-J stent, nephrostomy tube, stone multiplicity

INTRODUCTION

Percutaneous nephrolithotomy (PNL) is currently one of the most effective and widely utilized surgical techniques for the management of renal stones, particularly those larger than 2 cm or with complex configurations.[1,2] Despite its high stone-free rates, PNL is inherently invasive and associated with a spectrum of complications. While bleeding and infectious events are more commonly emphasized, urinary leakage from the nephrostomy tract represents a frequently encountered yet relatively underrecognized clinical issue.[3,4]

Postoperative urinary leakage may lead to several adverse outcomes, including prolonged hospitalization, the need for additional

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ORCID IDs of the authors: F.Ç. 0000-0003-1248-1940; H.K. 0000-0003-0091-9984.

Corresponding author: Ferhat Çoban
E-mail: coban_ferhat@hotmail.com
ORCID ID: orcid.org/0000-0003-1248-1940

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interventions such as double-J (DJ) stent placement, impaired patient comfort, and an increased risk of infection. The reported incidence of this complication varies depending on the definition used, with most studies reporting rates of approximately 5-15%.[4-6] These findings suggest that post-PNL urinary leakage is a clinically relevant complication that may significantly affect patient management.

Previous studies investigating factors associated with urinary leakage after PNL have primarily focused on stone burden, degree of hydronephrosis, number of access tracts, operative time, and renal parenchymal thickness. However, the existing literature remains heterogeneous, with inconsistent findings across studies. In particular, it is still unclear which specific aspects of stone characteristics-such as volume, location, complexity, or multiplicity-play the most decisive role. Moreover, parameters like stone number and calyceal involvement have often not been sufficiently detailed or have been evaluated without considering their interactions with other variables.[4,7]

In this context, identifying reliable clinical predictors of urinary leakage after PNL is of considerable importance for optimizing perioperative management and developing preventive strategies in high-risk patients.

The aim of the present study was to determine the independent preoperative and intraoperative predictors of clinically significant urinary leakage requiring DJ stent placement following PNL. Furthermore, beyond identifying statistical associations, this study sought to translate these findings into a clinically applicable risk estimation approach. By integrating readily available perioperative variables into a predictive framework, we aimed to facilitate preoperative risk stratification and support individualized decision-making regarding postoperative drainage strategies.

MATERIALS AND METHODS

Study Design and Population

This retrospective cohort study initially screened 328 consecutive patients who underwent PNL at our institution between 2020 and 2025. Patients who underwent PNL and had complete clinical, laboratory, and imaging data, as well as clearly documented postoperative drainage status and urinary leakage outcomes, were included in the study. Patients with incomplete clinical data (n=12), concomitant major surgical procedures (n=2), urinary diversion (n=1), active malignancy (n=1), or early reoperation due to major complications (n=6) were excluded. Pregnant patient (n=1) was also excluded. After applying these inclusion and exclusion criteria, 305 patients were included in the final analysis. The study was approved by the Adıyaman University Non-Interventional Clinical Research Ethics Committee (approval no: 2026/3-1, date: 14.04.2026). Due to the retrospective nature of the study, informed consent was not required. Patients were categorized into the leakage group (n=46, 15.1%) and the non-leakage group (n=259, 84.9%).

Data Collection and Surgical Technique

Patient demographics (age, gender), stone-related factors (history of stone passage and surgery, laterality, staghorn status, stone number and location, stone dimensions, Hounsfield unit, surface area, volume), anatomical parameters (skin-to-stone distance, hydronephrosis grade), and operative details (operation time, access tract number, hospital stay, nephrostomy tube placement and duration, stone-free status) were collected from medical records and non-contrast computed tomography scans.

All PNL procedures were performed under general anesthesia in the prone position. Percutaneous renal access was obtained under fluoroscopic guidance, followed by tract dilation using amplatz dilators. Stone fragmentation was accomplished using pneumatic lithotripsy. The decision regarding nephrostomy tube placement (20-22 Fr) was made at the surgeon's discretion based on intraoperative findings. The primary outcome was urinary leakage requiring DJ stent placement, defined as persistent urinary drainage from the nephrostomy tract lasting more than 24 hours after nephrostomy tube removal. No quantitative threshold for the amount of leakage was used. The need for DJ stent insertion was determined by the treating urologist's clinical judgment. In all 46 patients who developed urinary leakage and required DJ stent placement, the leakage resolved completely following stent insertion.

Statistical Analysis

Continuous variables were assessed for normality using the Shapiro-Wilk test. Normally distributed variables were compared using an Independent Samples t-test (mean ± standard deviation), while non-normally distributed variables were compared using the Mann-Whitney U test (median, interquartile range). Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate.

Univariate logistic regression analyses were performed for each variable. Variables with $p<0.25$, as well as those considered clinically relevant, were included as candidates for multivariable analysis. Multicollinearity was assessed using variance inflation factors (VIFs). Due to the potential for sparse-data bias and quasi-complete separation, multivariable analysis was also performed using Firth penalized logistic regression to provide bias-reduced estimates. Model discrimination was evaluated using the area under the receiver operating characteristic curve (AUROC) with 95% confidence intervals obtained via 1000-iteration bootstrap resampling. The final multivariable model included four variables. The optimal cut-off value was determined using Youden's J index. Model calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test. All analyses were conducted using Python version 3.10, NumPy version 1.26.4, and PyTorch version 2.5.1 with CUDA 12.1 support, together with the SciPy, scikit-learn, and statsmodels libraries. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

A total of 305 patients were included. Urinary leakage occurred in 46 participants (15.1%). The demographic and clinical characteristics are presented in Table 1. Median age was comparable between groups (39.0 vs. 42.0 years, p=0.524). No significant difference between gender was observed (60.9% vs. 60.6% male, p=1.000).

Compared with the non-leakage group, patients with urinary leakage had significantly higher rates of multiple stones, involvement of the upper, middle, and lower calyces, prolonged operative time, and residual stones. In contrast, nephrostomy tube placement was significantly less frequent in the leakage group (Table 1).

Univariate Logistic Regression

Univariate analysis identified 12 variables with p<0.25 as candidates for multivariate modeling (Table 2). The strongest univariate predictors were multiple stones [odds ratio (OR)=9.312], lower calyx stone (OR=3.391), residual stones (OR=3.057), upper calyx stone (OR=2.836), and multiple access tracts (OR=2.819). Nephrostomy tube placement (OR=0.240) and non-staghorn status (OR=0.281) were protective.

Multivariate Logistic Regression

All candidate variables showed acceptable multicollinearity (VIF <10; range: 1.05-5.63). Given the potential for sparse-data bias and quasi-complete separation, the final multivariable analysis was performed using Firth penalized logistic regression (Table 3). Multiple stones remained the strongest independent predictors of urinary leakage (OR=55.66, p<0.001). Each additional minute of operation time was associated with a 1.7% increase in the odds of leakage (OR=1.017, p=0.001). Nephrostomy tube placement remained strongly protective (OR=0.059, p=0.001). Lower calyceal stone involvement remained statistically significant after penalization (OR=0.255, p=0.044). The direction and magnitude of associations were consistent with those observed in conventional logistic regression analysis, supporting the robustness of the findings.

Model Performance

The Hosmer-Lemeshow test was not statistically significant (χ^2=5.032, p=0.754), indicating adequate calibration. The AUC was 0.798 (bootstrap 95% CI: 0.729-0.865). Using Youden's J index (0.444), the optimal cut-off of 0.185 yielded 65.2% sensitivity, 79.2% specificity, 35.7% positive predictive value, 92.8% negative predictive value, and 77.0% accuracy (Table 4, Figures 1-3).

Effect Sizes

Operation time demonstrated a medium effect size (Cohen's d=0.649); all other continuous variables exhibited negligible effects. Among categorical variables, multiple stones (Cramér's V=0.238), staghorn stone (V=0.194), and residual stones (V=0.181) had the largest, though still small, effect sizes.

Descriptive Subgroup Comparisons

Leakage rates were consistent across genders (females 15.0% vs. males 15.1%). Staghorn patients had higher leakage (32.6% vs. 12.0%), as did those with multiple access (30.8% vs. 13.6%), no nephrostomy tube (40.0% vs. 13.8%), residual stones (28.6% vs. 11.6%), and severe hydronephrosis (20.4% vs. 9.2% for moderate). In subgroups with adequate sample sizes, the trends were consistent with those in the main analysis, particularly for multiple stones and operation time.

Table 1. Comparison of baseline and operative characteristics between urinary leakage and non-leakage groups

Variable	No leakage (n=259)	Leakage (n=46)	p-value
Continuous variables			
Age (years)[a]	42.0 (29.0-54.0)	39.0 (28.3-53.0)	0.524
Stone height (mm)[a]	19.2 (16.1-24.1)	19.0 (15.1-24.9)	0.786
Stone width (mm)[a]	23.0 (18.7-28.8)	25.9 (19.4-33.9)	0.078
Stone length (mm)[a]	25.7 (21.2-33.4)	28.1 (21.3-36.4)	0.435
HU[a]	1111.5 (881.2-1308.5)	1146.6 (851.8-1333.3)	0.844
SSD (cm)[b]	7.86±2.21	7.68±2.03	0.603
Stone area (mm^2)[a]	453.7 (320.0-709.0)	581.7 (308.1-951.2)	0.227
Stone volume (mm^3)[a]	5749.9 (3438-11266)	7502.4 (3322-15186)	0.418
Op. time (min)[a]	110.0 (95.0-125.0)	122.5 (101.3-150.0)	**0.001**
Hospital stay (h)[a]	115.0 (80.0-150.0)	120.0 (94.3-162.0)	0.268
NT time (h)[a]	64.0 (40.0-72.0)	64.5 (44.3-71.5)	0.567
Categorical variables			
Male gender	157 (60.6)	28 (60.9)	1.000
Hx stone passage	49 (18.9)	13 (28.3)	0.211
Hx stone surgery	51 (19.7)	10 (21.7)	0.905
Left side	127 (49.0)	24 (52.2)	0.816
Non-staghorn	228 (88.0)	31 (67.4)	**0.001**
Multiple stones	157 (60.6)	43 (93.5)	**<0.001**
Pelvis stone	239 (92.3)	42 (91.3)	0.769
Upper calyx stone	41 (15.8)	16 (34.8)	**0.005**
Middle calyx stone	90 (34.7)	27 (58.7)	**0.004**
Lower calyx stone	161 (62.2)	39 (84.8)	**0.005**
Multiple access	18 (6.9)	8 (17.4)	**0.039**
Nephrostomy tube	250 (96.5)	40 (87.0)	**0.015**
Residual stones	45 (17.4)	18 (39.1)	**0.002**

Continuous variables were compared using Mann-Whitney U or Independent Samples t-test as appropriate, whereas categorical variables were compared using chi-square or Fisher's exact test. [a]Median (IQR); [b]Mean ± SD. Bold p-values indicate statistical significance (p<0.05).
NT: Nephrostomy tube, HU: Hounsfield unit, SSD: Skin-to-stone distance, Hx: History.

Table 2. Univariate logistic regression: variables with p<0.25

Variable	OR	95% CI	p-value
Staghorn (no)	0.281	0.137-0.578	**<0.001**
Multiple stones	9.312	2.814-30.814	**<0.001**
Upper calyx	2.836	1.419-5.667	**0.003**
Middle calyx	2.668	1.407-5.062	**0.003**
Lower calyx	3.391	1.460-7.878	**0.005**
Operation time	1.017	1.008-1.026	**<0.001**
Multiple access	2.819	1.146-6.935	**0.024**
Nephrostomy tube	0.240	0.081-0.711	**0.010**
Residual stones	3.057	1.559-5.997	**0.001**
Hydronephrosis	1.564	0.978-2.501	0.062
Stone width	1.027	0.996-1.058	0.085
Hx stone passage	1.688	0.827-3.445	0.150

Variables with p<0.25 were considered candidate variables for multivariable analysis. Bold p-values indicate significance (p<0.05).
OR: Odds ratio, CI: Confidence interval, Hx: History.

Table 3. Firth penalized multivariate logistic regression model

Variable	B	SE	OR	95% CI	p-value
Multiple stones	4.019	0.982	**55.66**	8.12-381.72	**<0.001**
Operation time (min)	0.016	0.005	**1.017**	1.007-1.027	**<0.001**
Nephrostomy tube	-2.825	0.811	**0.059**	0.012-0.296	**<0.001**
Lower calyx stone	-1.367	0.677	**0.255**	0.068-0.961	**0.044**

Pseudo R^2=0.201; AIC=216.6; LLR p<0.001. Firth penalized logistic regression was applied to address potential sparse-data bias and quasi-complete separation, providing bias-reduced estimates. Bold values indicate statistical significance (p<0.05).
B: Coefficient, SE: Standard error, OR: Odds ratio, CI: Confidence interval, AIC: Akaike information criterion.

Table 4. Model performance metrics

Metric	Value
AUC (95% CI)	0.798 (0.729-0.865)
Hosmer-Lemeshow χ^2 (p)	5.032 (0.754)
Youden's J index	0.444
Optimal cut-off	0.185
Sensitivity	65.2%
Specificity	79.2%
PPV	35.7%
NPV	92.8%
Accuracy	77.0%
McFadden R^2	0.201
AIC	216.6

AUC: Area under the curve, CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value, AIC: Akaike information criterion.

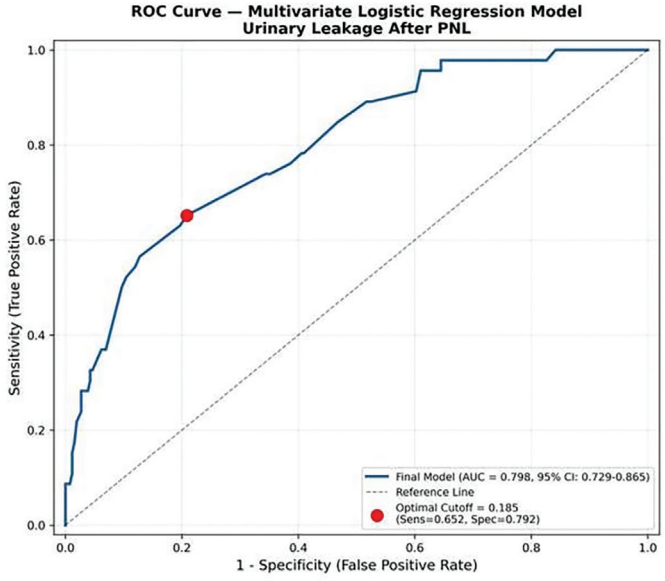

Figure 1. ROC curve of the final multivariate logistic regression model. AUC=0.798 (95% CI: 0.729-0.865). Red dot indicates the optimal cut-off (Youden's J =0.444).

ROC: Receiver operating characteristic, AUC: Area under the curve, CI: Confidence interval, PNL: Percutaneous nephrolithotomy.

DISCUSSION

In this study, the main predictors associated with clinically significant urinary leakage requiring DJ stent placement after PNL were identified. The most notable finding was that the presence of multiple stones emerged as the strongest independent determinant of urinary leakage. In addition, prolonged operative time significantly increased the risk of leakage, whereas nephrostomy tube placement demonstrated a clear protective effect. Furthermore, the presence of lower calyceal stones remained an independent factor in the multivariable analysis. These findings indicate that both factors related to stone complexity and intraoperative parameters play a critical role in the development of postoperative urinary leakage. Moreover, the model developed in this study demonstrated acceptable discrimination and calibration,

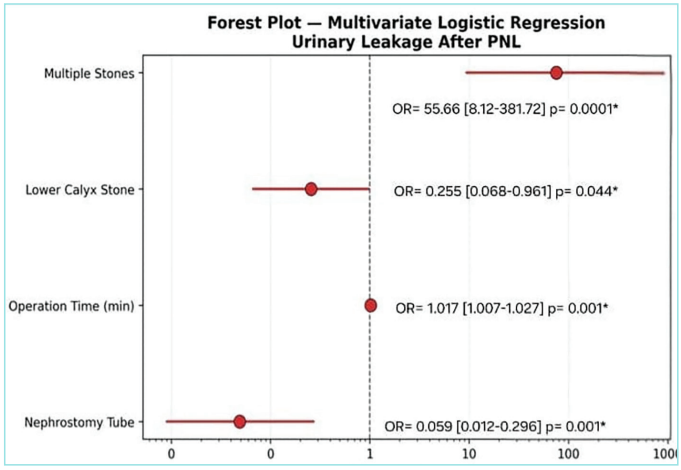

Figure 2. Forest plot of the final multivariate logistic regression model. Error bars represent 95% confidence intervals. Logarithmic scale; dashed line indicates OR=1 (null effect). *Statistically significant (p<0.05).

PNL: Percutaneous nephrolithotomy, OR: Odds ratio.

supporting its potential utility in perioperative risk assessment in clinical practice.

When the literature on urinary leakage after PNL is examined, factors frequently reported to be associated include stone complexity, degree of hydronephrosis, parenchymal thickness, presence of multiple access tracts, and residual stones. In particular, it has been reported that increased stone complexity and multiple access tracts may exacerbate renal parenchymal injury, delay tract healing, and consequently increase the risk of leakage. Similarly, several studies have demonstrated that the degree of hydronephrosis and parenchymal thickness are associated with the duration of urinary leakage. However, the existing literature is highly heterogeneous, and the predictors identified vary across studies, with no unified risk model currently established. This suggests that urinary leakage after PNL is a multifactorial complication and that both stone-related characteristics and intraoperative parameters should be evaluated together.[4,7-10]

The findings of the present study are generally consistent with those reported in the literature; however, several important differences should be emphasized. The presence of multiple stones was the strongest independent predictor of urinary leakage. Although previous studies have demonstrated an association between stone burden or complexity and leakage, data demonstrating such a pronounced effect size specifically for stone multiplicity remain limited. This suggests that the presence of multiple stones may not only increase overall stone burden but may also be associated with the involvement of multiple calyces, a broader dissection area, and a greater need for intrarenal manipulation.[10-15] However, the markedly asymmetric distribution of multiple stones between groups may also have contributed to inflation of the magnitude of the estimated OR despite the use of Firth penalization. Therefore, the reported effect size for stone multiplicity should be interpreted cautiously.

Similarly, prolonged operative time was identified as an independent risk factor, consistent with the literature, likely reflecting increased tissue trauma and sustained intrarenal pressure. The clear protective

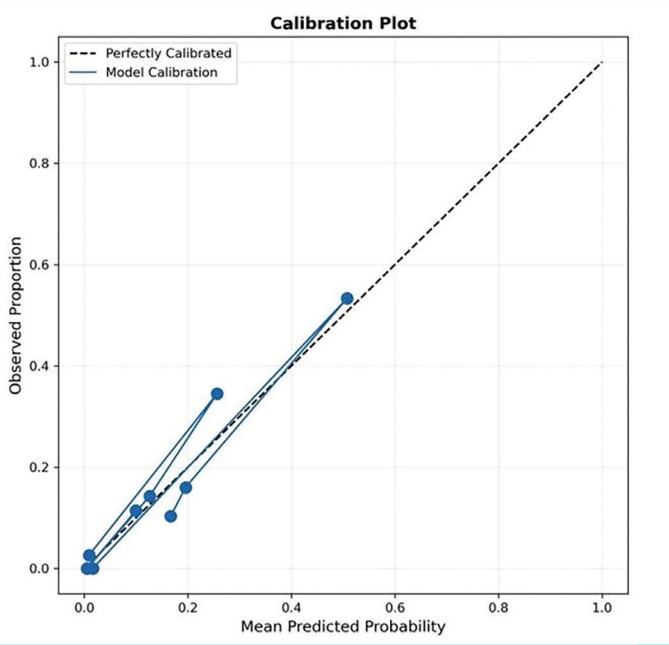

Figure 3. Calibration plot comparing predicted probabilities with observed proportions. Dashed diagonal represents perfect calibration.

effect of nephrostomy tube placement is noteworthy. While this finding is consistent with some previous reports, the role of nephrostomy placement remains controversial in the literature.[16-19] Therefore, it may be reasonable to reconsider nephrostomy use based on careful patient selection.

Interestingly, lower calyceal stone involvement showed a reversal in direction between the univariate and multivariable analyses. While lower calyx stones appeared to be associated with an increased risk of leakage in univariate analysis, the adjusted multivariable model demonstrated an inverse association after accounting for other covariates. This finding may reflect a suppression effect related to the strong co-occurrence between lower calyx involvement and the presence of multiple stones, where the presence of multiple stones was the dominant predictor in the final model. Therefore, the observed association for lower calyx stones should be interpreted cautiously and not necessarily as evidence of an isolated protective effect.

The development of urinary leakage after PNL is a multifactorial process, as emphasized in the literature, arising from the interplay among renal parenchymal injury, disruption of collecting-system integrity, and alterations in intrarenal pressure dynamics. Previous studies have reported that, particularly in cases with large stone burden and complex stone configurations, the need for manipulation of multiple calyces and prolonged intrarenal instrumentation may delay tract healing and increase the risk of leakage.[20]

In addition, increased irrigation pressure and prolonged operative time may contribute to microperforations within the collecting system and to the development of parenchymal edema, thereby facilitating postoperative urinary extravasation along the tract. Indeed, some studies have demonstrated that in cases with reduced parenchymal thickness and significant hydronephrosis, tract closure may be delayed and the duration of leakage prolonged.[10,21,22] Within this context, the identification in our study of the presence of multiple stones and of prolonged operative time as independent risk factors appears consistent with the current pathophysiological framework.

The clinical implications of these findings are particularly noteworthy. The preoperative identification of factors such as multiple stones and an anticipated prolonged operative time may enable the surgeon to plan the drainage strategy. In this context, individualized consideration of nephrostomy tube placement or early DJ stent use for selected high-risk patients may be reasonable.

Furthermore, the acceptable discriminative performance of the developed model suggests that patient-specific risk prediction may be feasible through the combined evaluation of these parameters. This approach may assist perioperative risk stratification and support more individualized clinical assessment in selected patients.

Another important strength of our study is that it not only identifies independent risk factors but also presents a predictive model in which these factors are evaluated collectively. The model's AUC of 0.79 indicates a clinically meaningful level of discrimination, while satisfactory calibration further supports its reliability. However, the model's moderate explanatory power suggests that urinary leakage after PNL is not fully predictable and that unmeasured factors may contribute to its development. Therefore, prospective external validation is required before broader clinical implementation. This underscores the need for future studies to incorporate more detailed anatomical and intraoperative parameters to further improve predictive performance.

Study Limitations

This study has several limitations. First, due to its retrospective design, it is subject to selection bias and therefore limits the ability to establish causal relationships. Second, although the study includes a relatively large patient cohort, its single-center nature may restrict the generalizability of the findings. Third, some potentially important anatomical and physiological parameters-such as renal parenchymal thickness along the access tract and intrarenal pressure measurements-were not available and, therefore, could not be included in the analysis. Fourth, validated stone complexity scoring systems, such as CROES, S.T.O.N.E., Guy's, and S-ReSC, were not incorporated into the present analysis; therefore, a comparative evaluation against established nephrolithometric systems could not be performed. In addition, decisions regarding nephrostomy tube placement and DJ stent insertion were based on the intraoperative judgment of the surgeon, which may have introduced variability into clinical practice. Moreover, the observed protective association of nephrostomy tube placement may have been influenced by indication (protopathic) bias, since intraoperative decisions regarding drainage were likely guided by case complexity, bleeding severity, and anticipated postoperative complications. Therefore, the reported effect estimate for nephrostomy tube placement should not be interpreted as causal. Although Firth penalized regression analysis was used to mitigate the effects of rare events and separation bias, residual confounding cannot be entirely excluded. Finally, while the developed model demonstrated acceptable discrimination and calibration, it has not undergone external validation; therefore, its clinical applicability should be supported by prospective, multicenter studies.

CONCLUSION

Clinically significant urinary leakage requiring DJ stent placement after PNL is a multifactorial complication influenced by stone-related and intraoperative factors. In this study, the presence of multiple stones and a prolonged operative time were independently associated with urinary leakage, whereas nephrostomy tube placement was associated with a lower risk of leakage. The proposed predictive model showed acceptable discrimination and calibration, suggesting its potential utility in perioperative risk assessment. Identification of high-risk patients may assist in perioperative risk assessment and postoperative monitoring. Further prospective, multicenter studies are warranted to validate these findings and enhance their applicability in clinical practice.

MAIN POINTS

- Clinically significant urinary leakage requiring ureteral stenting occurred in 15.1% of patients after percutaneous nephrolithotomy.
- Stone multiplicity was the strongest independent predictor of postoperative urinary leakage.
- Prolonged operative time was significantly associated with an increased risk of leakage, likely reflecting cumulative intrarenal trauma and pressure.
- Nephrostomy tube placement demonstrated a strong protective effect against clinically significant leakage.
- The proposed predictive model showed acceptable discrimination (area under the curve: 0.798) and may support individualized perioperative drainage strategies.

ETHICS

Ethics Committee Approval: The study was approved by the Adıyaman University Non-Interventional Clinical Research Ethics Committee (approval no: 2026/3-1, date: 14.04.2026).

Informed Consent: Due to the retrospective nature of the study, informed consent was not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices: F.Ç., Concept: F.Ç., Design: H.K., Data Collection and/or Processing: F.Ç., H.K., Analysis and/or Interpretation: H.K., Literature Search: F.Ç., H.K., Writing: F.Ç., H.K.

DISCLOSURES

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

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RESEARCH ARTICLE

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Bridging Clinical Severity and Cognitive Flexibility: A Mediation Analysis of Emotion Regulation in Affective Disorders

Nefise Demir, Zuhal Koç Apaydın, Dilanur Cinkavuk, Melisa Ataseven, Rıfat Tarhan

Department of Psychiatry, Karabük University Faculty of Medicine, Karabük, Türkiye

Abstract

BACKGROUND/AIMS: Depression and anxiety are conditions that often accompany each other, and multiple factors have been implicated in their development. The present research investigates the interplay between emotion regulation (ER) deficits, cognitive flexibility, and the manifestation of neurotic symptoms.

MATERIALS AND METHODS: Patients meeting diagnostic and statistical manual of mental disorders, fifth edition diagnostic criteria for anxiety disorders (n=79) and depression (n=72), as well as a healthy control group (n=82). All groups completed a socio-demographic data form, the difficulties in ER scale-16, and the cognitive flexibility inventory (CFI). In addition, the patient groups completed the hamilton depression rating scale and the hamilton anxiety rating scale. Descriptive, mediation, and regression analyses were conducted using SPSS.

RESULTS: Scores on the CFI control subscale ($p<0.001$) and the CFI total score ($p=0.002$) differed significantly across groups. Post-hoc analyses indicated superior performance in the control group compared with both clinical groups across all measures, with no statistically significant difference between the anxiety and depression groups. Scores on the difficulties in ER scale also differed significantly among groups ($p<0.001$), with the control group showing lower scores than both clinical groups. Moreover, no significant difference was observed between the anxiety and depression groups. As cognitive flexibility increased, capacity for ER and levels of anxiety and depression decreased. Mediation analyses showed that ER ability mediated the association between cognitive flexibility and depressive symptoms, but did not mediate the association between cognitive flexibility and anxiety.

CONCLUSION: Cultivating ER strategies could mitigate the adverse effects of cognitive rigidity on depressive symptoms. Interventions aimed at improving cognitive flexibility may be considered a therapeutic option for anxiety by promoting greater internal control over anxiety symptoms. These findings are important for understanding how cognitive flexibility and ER contribute to depression and anxiety. In psychotherapy programs, these characteristics should be assessed and taken into account.

Keywords: Depression, anxiety, cognitive flexibility, emotion regulation, affective disorders

INTRODUCTION

Depression and anxiety are conditions that often co-occur, impair functioning, and may become life-threatening. Individuals with anxiety disorders tend to exhibit widespread responses to situations perceived as threatening, along with a range of biopsychosocial alterations.[1] They typically emerge early in life and, with their increasing prevalence, pose a substantial threat to quality of life.[2] The global population prevalence of anxiety disorders is estimated to be approximately 4%, and the number of affected individuals has increased by more than 55% over the past two decades.[3] When anxiety comes to be perceived

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ORCID IDs of the authors: N.D. 0000-0001-6683-9085; Z.K.A. 0000-0003-3893-5314; D.C. 0009-0008-5048-5284; M.A. 0009-0009-7466-2684; R.T. 0009-0005-8781-0560.

Corresponding author: Nefise Demir
E-mail: nefisedemir@karabuk.edu.tr
ORCID ID: orcid.org/0000-0001-6683-9085

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as a threat, it may trigger a fight-or-flight response, manifested as psychophysiological reactions such as dizziness, increased heart rate, and sweating,[4] and these symptoms may contribute to the development of various physical conditions, including cardiovascular diseases, ophthalmologic and otologic disorders, dermatologic conditions, and genitourinary diseases.[5] Depression, likewise, poses a substantial public health challenge globally, standing out as one of the foremost causes of disability attributed to mental health conditions.[6] Symptoms of depression include unhappiness, decreased interest, feelings of guilt, sleep disturbances, changes in appetite, inability to enjoy life, and difficulty concentrating.[7] The lifetime risk of developing depression is estimated to be 15-18%. In recent years, depression has shown the most rapid increase among adolescents and young adults and has risen across all sex, racial/ethnic, income, and educational groups.[8] For these reasons, a better understanding of depression and anxiety, as well as of the factors influencing their treatment, is of considerable importance.

Depression and anxiety disorders can result from several factors. One such factor frequently associated with these disorders is difficulty in emotion regulation (ER).[9] ER refers to the conscious or automatic processes through which individuals manage their emotions, including when and how they experience and express them.[10] ER skills are widely considered essential for modifying negative emotional experiences in everyday life.[11] Variations in individuals' ER abilities have been suggested as factors that influence the onset and progression of depression and anxiety disorders. Impairments in these skills correlate with the symptom burden of depression and anxiety and have consequently been targeted in clinical interventions.[9] Beyond intra-individual factors, maladaptive strategies in interpersonal ER, such as excessive reassurance-seeking and co-rumination, have been consistently linked to the severity of both depressive and anxiety-related symptomatology.[12] It has been suggested that psychiatric disorders, including depression and anxiety, may, in part, result from failures to effectively select and implement ER strategies.[11] Conversely, adaptive ER strategies have been found to contribute to enhanced resilience against depression and anxiety.[13]

Similarly, the literature has shown that cognitive flexibility is another variable that may play a central role across multiple domains of psychopathology.[14] Cognitive flexibility represents the mental capacity for shifting and modifying one's patterns of thought in accordance with changing conditions in the environment. This construct reflects the capacity to perceive various possibilities within a given context, to remain open to changing circumstances, and to formulate new, more adaptive ways of thinking.[14] In individuals with depression, cognitive control has been reported to increase, whereas cognitive flexibility has been shown to decrease.[15] Conversely, mood state has been shown to influence cognitive flexibility in patients with major depressive disorder.[16] Similarly, anxiety, depending on its duration and intensity, may also lead to impairments in cognitive flexibility.[17] Processes commonly observed in depression, such as rumination and difficulty appropriately shifting attention between tasks, are likewise associated with reduced cognitive flexibility.[18] Moreover, cognitive flexibility has been shown to moderate the association between trait anxiety and depression, attenuating the impact of trait anxiety on depressive symptoms.[19]

In patients with anxiety disorders and depression who fail to achieve full clinical remission, psychological factors contributing to chronicity remain an important focus of investigation. Although the relationship between cognitive flexibility and ER, including their potential mediating roles in depression and anxiety, remains incompletely understood, an association between ER ability and cognitive flexibility has been demonstrated.[20] Earlier research indicates that greater cognitive flexibility is associated with fewer difficulties with ER and less severe depressive symptoms.[21] Both ER strategies and cognitive flexibility have emerged as significant correlates of depression and anxiety symptomatology.[22] In addition, significant associations have been reported among neuroticism, depression, ER, and cognitive flexibility.[23]

Mood and anxiety disorders vary widely in their causes and underlying biological mechanisms, making it challenging to identify markers that could be targeted in future treatments. Therefore, there is a substantial need to investigate risk factors and broader determinants of mental health. In this regard, the current study was designed to examine the relationships among the severity of anxiety and depression, ER, and perceived cognitive flexibility in patients diagnosed with anxiety disorders and depression. As a secondary aim, contingent upon identifying such associations, we sought to evaluate potential mediating factors that may influence clinical severity. Based on the current literature, we tested the following hypotheses:

- (H1) Patients diagnosed with depression and anxiety disorders exhibit significantly lower cognitive flexibility and ER compared to healthy controls.
- (H2) Perceived cognitive flexibility and ER are negatively associated with the severity of anxiety and depressive symptoms.
- (H3) ER mediates the relationship between perceived cognitive flexibility and depressive and anxiety symptom severity.

MATERIALS AND METHODS

This cross-sectional study included 151 patients and 82 healthy controls, recruited from among adults aged 18-75 years who presented to the Outpatient Psychiatry Clinic of Karabük Training and Research Hospital. Patients who met the diagnostic and statistical manual of mental disorders, fifth edition (DSM-5) diagnostic criteria for anxiety disorders or depression, met the research eligibility criteria, and provided written informed consent were enrolled. The inclusion criteria were: absence of psychiatric comorbidities (including intellectual disability, learning disorder, autism, psychosis, or bipolar disorder), absence of chronic medical illness (such as autoimmune disease, allergic disorders, hypertension, diabetes mellitus, etc.), absence of alcohol or substance use disorder, age over 18 years, and ability to read and write in Turkish.

Experienced psychiatrists conducted all diagnostic evaluations and excluded psychiatric comorbidities using DSM-5 criteria during standard clinical interviews. Clinicians administered the hamilton depression rating scale (HAM-D) and the hamilton anxiety rating scale (HAM-A), while participants completed self-report questionnaires individually, under researcher supervision. For patients diagnosed with anxiety disorders or depression who consented to participate and met the study criteria, a socio-demographic data form was completed, and the following measures were administered: the HAM-D, the HAM-A, the difficulties in ER scale (DERS)-short form, and the cognitive flexibility inventory (CFI).

Formal ethical approval was granted by the Karabük University Non-Interventional Research Ethics Committee (approval no: 2025/2422, date: 28.07.2025).

Measures

Socio-demographic data form: All participants completed a form designed by the authors to collect socio-demographic and clinical information. The data form recorded demographic variables, including gender, relationship status, and academic background; occupational status; comorbid medical or psychiatric conditions; substance use patterns (e.g., smoking and alcohol consumption); prior psychiatric interventions; and any forensic or suicidal history.

Hamilton depression rating scale: This scale was introduced by Hamilton in 1960 to evaluate depression severity. While the original version comprised 17 items, extended versions consisting of 21 and 24 items were later developed.[24] The Turkish adaptation and psychometric evaluation were carried out by Akdemir et al.[25]

Hamilton anxiety rating scale: The HAM-A was developed by Hamilton to assess the severity and distribution of anxiety symptoms and to measure changes in symptom intensity over time. It consists of 14 items assessing both psychic and somatic symptoms. Each item is rated by the interviewer.[26] The Turkish validity and reliability evaluation was performed by Yazıcı et al.[27]

Difficulties in emotion regulation scale-16: Originally introduced by Bjureberg et al.[28] in 2016, consists of 16 items rated on a 5-point Likert-type scale. The scale encompasses five distinct subdimensions, namely clarity, goals, impulse, strategies, and non-acceptance, each capturing a different facet of ER difficulty. Total scores range accordingly, with higher scores indicating greater overall difficulty in ER.[28] The measurement invariance and structural integrity of the tool in a Turkish-speaking cohorts were examined and established by Yiğit and Guzey Yiğit.[29]

The cognitive flexibility inventory: Originally developed by Dennis and Vander Wal[30] in 2010, consists of 20 items designed to evaluate cognitive flexibility. Responses are provided on a Likert-type scale ranging from 1 to 5, with six items (2, 4, 7, 9, 11, and 17) requiring reverse scoring. The inventory produces three distinct scores reflecting overall cognitive flexibility, perceived control, and the ability to generate alternatives; higher scores correspond to greater cognitive flexibility.[30] The Turkish adaptation was carried out by Gülüm and Dağ.[31]

Statistical Analysis

All statistical procedures were carried out using IBM SPSS Statistics version 30.0, while mediation analyses were performed with the PROCESS Macro (v4.2) developed by Andrew F. Hayes. Normality of continuous variables was assessed using both visual inspection methods (including histograms and Q-Q plots) and formal statistical tests. Continuous variables deviating from normality were expressed as median (minimum-maximum), while categorical variables were reported as counts and percentages (n, %). Differences in continuous variables among the three groups were examined using the Kruskal-Wallis test. For variables with significant overall group differences, pairwise comparisons were conducted using Dunn-Bonferroni-adjusted post-hoc tests, and the adjusted p-values were reported. Categorical variables were compared across study cohorts using Pearson's chi-square test; however, when expected cell counts were low, the Fisher-Freeman-Halton exact test was performed using Monte Carlo simulation. Associations between variables were examined using Spearman's rank-order correlation coefficient (rho). In correlation analyses, missing data were handled using the pairwise method. The mediating role of the DERS in the association between total CFI scores and HAM-A and HAM-D scores was tested using PROCESS Model 4, controlling for age, total years of education, and sex. Indirect effects were evaluated based on 95% bootstrap confidence intervals (CIs) generated from 5,000 resamples; an indirect effect was regarded as statistically meaningful when the corresponding CI did not encompass zero. The alpha level for determining statistical significance was set at 0.05 (two-tailed).

RESULTS

Statistical analyses indicated that the anxiety (n=79), depression (n=72), and control (n=82) groups were comparable in terms of age and sex distributions; however, educational level differed significantly across groups ($p<0.001$). A notable disparity in prior psychiatric hospitalizations was evident: the prevalence reached 12.5% among depressed individuals, compared with 2.5% among those with anxiety and 0% among controls ($p<0.001$). The prevalence of known medical illnesses was significantly lower in the control group (anxiety: 41.8%, depression: 34.7%, control: 9.8%; $p<0.001$). Relevant group results are outlined in Table 1.

Regarding scale scores, group differences reached statistical significance for CFI-control scores ($p<0.001$) and CFI-total scores ($p=0.002$). Follow-up comparisons indicated that the control group scored notably higher than both clinical groups on these measures, with no statistically significant difference between the anxiety and depression groups. DERS scores also differed significantly across groups ($p<0.001$), with the control group showing lower scores than both clinical groups, and no significant difference was observed between the anxiety and depression groups. Relevant group results are outlined in Table 2.

To assess the associations among clinical variables, Spearman correlation analysis was performed. As years of education increased, the ability to generate alternative ways of thinking increased ($r=0.136$). Higher levels of cognitive flexibility (including both subdimensions) were associated with lower levels of ER difficulties, anxiety, and depression. In contrast, deficits in ER were directly correlated with both anxiety and depression scores ($r=0.367$ and $r=0.382$, respectively). The correlation results for the study variables are presented in Table 3.

Indirect effects were evaluated using SPSS PROCESS Macro v4.2, Model 4. Two separate mediation models were constructed in which the total CFI score served as the independent variable (X), the DERS score as the mediator (M), and the HAM-D and HAM-A scores as the outcome variables (Y). All models were adjusted for age, total years of education, and sex. Coefficients are presented as unstandardized B values with corresponding 95% CIs. The indirect effect ($a \times b$) was estimated using 5,000 bootstrap samples; mediation was determined to be significant when the bootstrap CI did not include zero. In PROCESS Model 4 mediation analyses, controlling for covariates (age, total years of education, and sex), the effect of total CFI score on DERS was significant in both models [path a: B=-0.5963, 95% CI (-0.7556, -0.4371), $p<0.001$].

In the model with HAM-D as the outcome, DERS positively predicted HAM-D after controlling for total CFI score [path b: B= 0.1350, 95% CI (0.0550, 0.2150), $p=0.0011$], whereas the direct effect of total CFI score on HAM-D was not significant (c': B=-0.0153, $p=0.741$). In contrast, the indirect effect was significant [$a \times b$ =-0.0805; boot CI (-0.1501, -0.0306)], indicating that the association between total CFI score and HAM-D was mediated by DERS. In the model with HAM-A as the outcome, the effect

Table 1. Socio-demographic and clinical characteristics of anxiety, depression, and health control

Variables		Anxiety (n=79)	Depression (n=72)	Control (n=82)	p	Intergroup
Median (min-max)						
Age		47 (18-71)	44 (18-70)	40 (18-60)	0.094[a]	
Gender	Female Male	52 27	42 30	53 29	0.595	
Duration of education (years)		8 (5-18)	12 (2-10)	12 (2-22)	0.636[a]	
		n (%)	n (%)	n (%)		
Education level	Primary school and below high school associate's degree and above	42 (53.2) 15 (19.0) 22 (27.8)	33 (45.8) 15 (20.8) 24 (33.3)	9 (11.0) 26 (31.7) 47 (57.3)	**<0.001**[c]	**Primary school and below** Anxiety (53.2%)[a] Depression (45.8)%[a] Control (11.0%)[b] **High school** Anxiety (19.0%)[a] Depression (%20.8%)[a] Control (31.7%)[a] **Associate' degree and above** Anxiety (27.8%)[a] Depression (33.3%)[a] Control (57.3%)[b]
Marriage status	Married Single Widow Divorced Separated	46 (58.2) 24 (30.4) 5 (6.3) 4 (5.1) 0 (0.0)	45 (62.5) 19 (26.3) 3 (4.2) 3 (4.2) 2 (2.8)	64 (78.0) 13 (15.9) 1 (1.2) 3 (3.7) 1 (1.2)	0.126	
Working status	Employed Unemployed	28 (35.4) 51 (64.6)	31 (43.1) 41 (56.9)	43 (52.4) 39 (47.6)	0.093	
Psychiatric hospitalization	No Yes	77 (97.5) 2 (2.5)	63 (87.5) 9 (12.5)	82 (100) 0 (0.0)	**<0.001**	Anxiety (2.5%)[a] Depression (12.5%)[b] Control (0%)[a]
History of suicide	No Yes	77 (97.5) 2 (2.5)	68 (94.4) 4 (5.6)	81 (98.8) 1 (1.2)	0.263	
Physical illness	No Yes	46 (58.2) 33 (41.8)	47 (65.3) 25 (34.7)	74 (90.2) 8 (9.8)	**<0.001**[c]	Anxiety (41.8%)[a] Depression (34.7%)[a] Control (9.8%)[b]
Smoking	No Yes	46 (58.2) 33 (41.8)	39 (54.2) 33 (45.8)	55 (67.1) 27 (32.9)	0.242	
Alcohol use	No Yes	67 (84.8) 12 (15.2)	61 (84.7) 11 (15.3)	76 (92.7) 6 (7.3)	0.217	

[a]Kruskal-Wallis test, [b]Dunn-Bonferroni test, [c]Chi-square test.
Because continuous variables did not show a normal distribution, group comparisons were performed using the Kruskal-Wallis test; for variables with significant overall group differences, pairwise comparisons were evaluated using Dunn's post-hoc test with Bonferroni correction. Categorical variables were evaluated applying the chi-square test; when expected cell counts were low, the Fisher-Freeman-Halton exact test (Monte Carlo method) was used. Adjusted p-values were interpreted according to the Bonferroni correction.
min-max: Minumum-maximum.

Table 2. Comparison of cognitive flexibility and emotion regulation scores among anxiety, depression, and healthy control

Variables	Anxiety (n=79)	Depression (n=72)	Control (n=82)	p	Intergroup
Median (min-max)					
CFI-control	21 (7-34)	21 (11-59)	28 (8-35)	**<0.001**[a]	[b]Anxiety < control **p<0.001** Depression < control **p<0.001** Anxiety < depression p=0.864
CFI-alternative	52 (26-65)	51 (24-65)	52 (17-65)	0.614[a]	
CFI-total	73 (9-95)	72 (42-99)	79 (45-100)	**0.002**[a]	[b]Anxiety < control **p=0.001** Depression < control **p=0.003** Anxiety < depression p=0.866
DERS-16	38 (16-79)	41.50 (16-79)	24 (16-80)	**<0.001**[a]	[b]Anxiety < control **p<0.001** Depression < control **p<0.001** Anxiety < depression p=0.618

[a]Kruskal-Wallis test, [b]Dunn-Bonferroni test.
Because continuous variables did not show a normal distribution, group comparisons were performed using the Kruskal-Wallis test; for variables with significant overall group differences, pairwise comparisons were evaluated using Dunn's post-hoc test with Bonferroni correction. Adjusted p-values were interpreted according to the Bonferroni correction.
DERS: Difficulties in emotion regulation scale, CFI: Cognitive flexibility inventory, min-max: Minumum-maximum.

Table 3. Results of the spearman correlation analysis (correlation coefficients)

	1	2	3	4	5	6	7	8
1. CFI-control	1.000							
2. CFI-alternative	0.266**	1.000						
3. CFI- total	0.667**	0.831**	1.000					
4. DERS-16	-0.729**	-0.276**	-0.539**	1.000				
5. HAM-A	-0.293**	-0.222**	-0.344**	0.367**	1.000			
6. HAM-D	-0.375**	-0.228**	-0.332**	0.382**	0.414**	1.000		
7. Age	0.083	0.045	0.088	-0.158*	-0.020	-0.094	1.000	
8. Duration of education	0.020	0.136*	0.084	0.040	-0.045	-0.122	-0.429**	1.000

*p<0.05, **p<0.001. Values are presented as Spearman's rho (two-tailed) correlation coefficients. Sample sizes varied by variable: for CFI control, CFI alternatives, CFI total, DERS, age, and years of education, n=233; for correlations including HAM-A and HAM-D, n=151. Missing data was handled using the pairwise method.
CFI: Cognitive flexibility inventory, DERS-16: Difficulties in emotion regulation scale-16, HAM-A: Hamilton anxiety rating scale, HAM-D: Hamilton depression rating scale.

of DERS on HAM-A was not significant (path b: B=-0.0266, p=0.644); the mediating effect was not supported [a × b =0.0158; boot CI (-0.1794, 0.1004)]. In this model, the direct effect of total CFI score on HAM-A remained significant [c': B= -0.3469, 95% CI (-0.4762, -0.2176), p<0.001], suggesting that the association between total CFI score and HAM-A was explained by a direct effect independent of DERS. Table 4 presents the mediating role of ER in the association of total CFI scores with HAM-D and HAM-A.

DISCUSSION

In this study, the relationships among ER, cognitive flexibility, and symptoms of depression and anxiety were examined in clinically diagnosed patient groups and a healthy control group. Both clinical groups, relative to the control group, exhibited reduced internal control (CFI-control), lower cognitive flexibility (CFI-total), and poorer ER abilities; however, no significant differences were observed between the anxiety and depression groups in these domains. Increases in cognitive flexibility and ER capacity were found to be associated with lower anxiety and depression scores. Mediation analyses indicated that the relationship between cognitive flexibility and depressive symptoms may have been mediated by ER ability, whereas the association between cognitive flexibility and anxiety appeared to be driven by a direct effect independent of ER.

Cognitive flexibility, defined as the ability to shift mentally between tasks in response to environmental demands, is thought to facilitate thinking and problem-solving and enhances resilience to adverse life events.[32] These findings suggest that cognitive flexibility may not merely be an accompanying characteristic, but also an important regulatory mechanism shaping the co-occurrence of anxiety and depression. Indeed, cognitive flexibility may reduce the risk that trait anxiety culminates in depression, thereby limiting the extent to which anxiety acts as a risk factor for depressive symptoms.[19] Corroborating this view, diminished cognitive flexibility has been linked to greater depressive symptomatology in those with social anxiety.[33] Previous studies have similarly reported that higher anxiety and depression levels are related to lower levels of cognitive flexibility.[34] In line with the literature, our findings showed that the control group had a higher internal locus of control and greater cognitive flexibility than both clinical groups. No significant difference in cognitive flexibility was found between the anxiety and depression groups. Reduced cognitive flexibility may contribute to the deepening of depressive symptoms by allowing negative automatic thoughts to become more persistent, maintaining threat-focused interpretations, and diminishing the effectiveness of cognitive restructuring. The cognitive rigidity observed in the clinical groups may therefore increase vulnerability to the onset of these disorders or contribute to their chronicity. For this reason, future studies

Table 4. The mediating role of emotion regulation in the association of total CFI scores with HAM-D and HAM-A: PROCESS Model 4 mediation analyses (covariates: age, years of education, and sex)

Section	Road/model	Effect (B)	SE	t	p	95% lower CI	95% upper CI
Model summary	M model: DERS	R=0.5892	R^2=0.3471	F (4,146) =19.4046	<0.001		
Model summary	Y model: HAM-D	R=0.3887	R^2=0.1511	F (5,145) =5.1624	0.0002		
Roads	a: CFI total → DERS	-0.5963	0.0806	-7.4021	<0.001	-0.7556	-0.4371
Roads	b: DERS → HAM-D (X control)	0.1350	0.0405	3.3342	0.0011	0.0550	0.2150
Roads	c': CFI total → HAM-D (M control)	-0.0153	0.0462	-0.3316	0.741	-0.1067	0.0760
Indirect effect	a×b: CFI-total → DERS → HAM-D	-0.0805	Boot SE=0.0301		Boot strap	Boot LLCI=-0.1501	Boot ULCI=-0.0306
Section	**Road/model**	**Effect (B)**	**SE**	**t**	**p**	**95% lower CI**	**95% upper CI**
Model summary	M model: DERS	R=0.5892	R^2=0.3471	F (4,146) =19.4046	<.001		
Model summary	Y model: HAM-A	R=0.4651	R^2=0.2163	F (5,145) =8.0056	<.001		
Roads	a: CFI-total → DERS	-0.5963	0.0806	-7.4021	<.001	-0.7556	-0.4371
Roads	b: DERS → HAM-A (X control)	-0.0266	0.0573	-0.4637	0.644	-0.1398	0.0867
Roads	c': CFI-total → HAM-A (M control)	-0.3469	0.0654	-5.3026	<.001	-0.4762	-0.2176
Indirect effect	a × b: CFI-total → DERS → HAM-A	0.0158	Boot SE=0.0834		Boot strap	Boot LLCI=-0.1794	Boot ULCI=0.1004

DERS: Difficulties in emotion regulation scale, HAM-D: Hamilton depression rating scale, HAM-A: Hamilton anxiety rating scale, CFI: Cognitive flexibility inventory, CI: Confidence interval, SE: Standard error.

should examine the impact of cognitive flexibility on illness course and outcomes (e.g., symptom severity, functioning, relapse, and treatment response) using longitudinal designs. Furthermore, given that cognitive flexibility may support the development of alternative coping and cognitive reappraisal strategies that mitigate the impact of adverse experiences, it could serve as a protective resource against mental disorders and be considered a target variable in psychoeducation and psychotherapy programs.

One of the strategies consistently associated with enhanced resilience against depression and anxiety is the presence of effective ER skills.[13] The adaptive deployment of ER strategies has been found to correspond with lower depressive symptom severity at all assessment time points.[35] In groups receiving ER-based interventions, psychiatric symptoms improved while reliance upon maladaptive ER strategies decreased.[36] Viewed through this lens, effective ER may be a relevant factor supporting clinical recovery. Consistent with this, ER ability was lower in the clinical groups than in the control group in the current study, and no meaningful difference emerged between the anxiety and depression groups. Given the substantial symptomatic overlap between depression and anxiety disorders, particularly with regard to cognitive functioning and emotional dysregulation, the failure to detect a meaningful difference between the two clinical groups is perhaps unsurprising. Moreover, shared dispositional characteristics such as neuroticism, which has been associated with both disorders,[23] may further account for the observed DER. The results of this study underline the relevance of ER among individuals with anxiety and depression; nevertheless, these findings warrant further confirmation through longitudinal research conducted with larger and more representative samples. Screening for ER difficulties, developing appropriate psychotherapy programs, and incorporating ER skills training into treatment may therefore be beneficial. Given the well-established comorbidity between major depressive disorder and anxiety disorders, skills-based interventions targeting ER may provide benefits for both conditions.

Extant research suggests that cognitive flexibility and ER strategies are pivotal correlates of affective health, potentially influencing the clinical profiles of anxiety and depression.[22] Furthermore, evidence suggests a mediating intersection between these two domains, in which impairments in executive cognitive control may inherently undermine an individual's capacity for effective emotional modulation.[20] Previous research examining the effects of cognitive flexibility on the two key components of ER-strategy use and flexibility- has shown that cognitive flexibility predicts the flexibility component of ER.[37] In addition, EEG findings suggest that ER and cognitive processes share overlapping neural circuits.[38] Consistent with the literature, our study observed an association between ER and cognitive flexibility. Our findings suggest that the association between total cognitive flexibility and depressive symptom severity is largely mediated by ER, presenting a pattern consistent with full mediation in depression. Clinically, this finding suggests that cognitive flexibility may influence depressive symptom severity less by directly reducing symptoms and more by altering the individual's cognitive appraisal and processing style, and related regulatory processes. Accordingly, interventions aimed at enhancing cognitive flexibility in depression might yield better outcomes when they also target cognitive processes linked to ER.

In contrast, a different pattern emerged for anxiety symptoms. Relationships between cognitive flexibility and anxiety symptoms did not appear to be explained by ER, but rather by a more direct relationship. Prior work has highlighted that cognitive flexibility may operate as an important moderating factor within the relationship between ER and anxiety symptoms.[39] In anxiety, processes such as threat perception, intolerance of uncertainty, attentional bias, and avoidance tend to play a more prominent role; therefore, cognitive flexibility may influence symptom severity through a mechanism independent of the cognitive components assessed by measures of ER. These findings suggest that the relationships of cognitive flexibility with depression with anxiety do not operate through the same mechanism. In depression, the mediating role of cognitive processes, as represented by ER, appears more prominent in the association between symptom severity and

cognitive flexibility; by contrast, in anxiety disorders, the effect of cognitive flexibility appears to follow a more independent and direct pathway. This distinction could also have implications for treatment planning: whereas ER may be particularly critical alongside efforts to enhance cognitive flexibility in the treatment of depressive symptoms, approaches aimed at strengthening cognitive flexibility alone may make a more direct contribution in the treatment of anxiety symptoms.

Evaluating cognitive flexibility with self-report tools rather than performance-based assessments directly affects the interpretation of our findings. This choice reflects the study's primary aim. Rather than measuring neurocognitive processing speed or executive functions in a laboratory setting, the study primarily examined patients' subjective assessments of their adaptive cognitive abilities in daily life. Such self-report measures offer insight into how individuals perceive their ability to cope with difficulties, a core component of cognitive-behavioral models for depression and anxiety. However, the observed relationships primarily reflect perceived cognitive flexibility rather than objective neurocognitive capacity. Therefore, our results indicate that subjective cognitive rigidity is associated with symptom severity rather than with definitive neurocognitive deficits. Reliance on subjective evaluation remains a limitation, highlighting the need for future research incorporating objective neuropsychological batteries.

Finally, these findings should be interpreted within the limitations of a cross-sectional design. Although mediation models may suggest possible mechanisms linking variables, longitudinal and/or experimental studies are required to draw causal inferences. Future research should further investigate which cognitive components mediate the effects of cognitive flexibility on depression and anxiety. In addition, examining the role of other potential mediators such as rumination, intolerance of uncertainty, avoidance, attentional bias, and cognitive reappraisal may contribute to the development of more targeted interventions.

Study Limitations

This study has several limitations. First, the cross-sectional design, the relatively small sample size, and the single-center setting limit the generalizability of the findings. Second, psychiatric diagnoses and the exclusion of comorbidities were based on clinical psychiatric evaluations rather than structured diagnostic interviews such as the SCID-5. Therefore, diagnostic heterogeneity cannot be entirely excluded. Similarly, the lack of detailed categorization within the clinical groups, specifically the absence of subclassification for anxiety subtypes and depressive specifiers (e.g., melancholic or atypical features), may obscure more nuanced relationships between the variables. Third, cognitive flexibility was assessed only through a self-report instrument rather than performance-based executive function measures (such as the wisconsin card sorting test, trail making test part B, or stroop test). The self-report instrument may primarily reflect subjective cognitive appraisal rather than objective neuropsychological capacity. Because patients were receiving psychotropic medications at different treatment stages, the potential impact of these medications on cognitive and emotional processing could not be separately evaluated; thus, caution is warranted when interpreting findings related to executive functioning. Future longitudinal studies that employ structured clinical interviews and objective neuropsychological batteries and include larger samples are required to draw causal inferences.

CONCLUSION

The current findings suggest that cognitive flexibility and ER may exert direct and/or mediating effects on anxiety and depression. From a preventive standpoint, interventions targeting improvement in ER and cognitive flexibility may contribute to reducing the likelihood of onset of depressive and anxiety disorders, while also alleviating the severity of existing symptoms. The findings highlight the potential value of personalized interventions that prioritize an individual's cognitive flexibility and ER capacity in the management of depression and anxiety. I ncorporating techniques designed to enhance cognitive adaptability within traditional psychotherapeutic frameworks may significantly optimize therapeutic outcomes for patients with low cognitive flexibility. Beyond clinical treatment, however, these skills should be viewed as core components of primary prevention. Implementing psychoeducational programs within educational and occupational settings, aimed at bolstering these faculties in at-risk populations before the onset of clinical symptoms, could alleviate the long-term burden on public mental health services. Future longitudinal studies are needed to evaluate the effectiveness of such intervention programs. Investigating the underlying neurobiological processes may help identify biological pathways that clarify the shared roles of ER and cognitive flexibility in anxiety and depression, which may, in turn, provide guidance for future pharmacological treatment approaches.

MAIN POINTS

- Patients with depression and anxiety exhibit significant deficits in cognitive flexibility and emotion regulation (ER) compared to healthy individuals.
- ER significantly mediates the effect of cognitive flexibility on depressive symptoms, but not on anxiety.
- Higher cognitive flexibility directly reduces anxiety levels by promoting greater internal control of symptoms.
- Psychotherapeutic and preventive interventions should specifically target cognitive adaptability and ER to improve clinical outcomes.

ETHICS

Ethics Committee Approval: Ethical approval for the study was obtained from the Karabük University Non-Interventional Research Ethics Committee (approval no: 2025/2422, date: 28.07.2025) and institutional permission was granted from the mentioned research center.

Informed Consent: Written informed consent was obtained from all participants included in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.D., Z.K.A., D.C., M.A., R.T., Concept: N.D., Z.K.A., Design: N.D., Z.K.A., Data Collection and/or Processing: N.D., Z.K.A., D.C., M.A., R.T., Analysis and/or Interpretation: N.D., Z.K.A., D.C., M.A., R.T., Literature Search: N.D., Z.K.A., D.C., M.A., R.T., Writing: N.D., Z.K.A., D.C., M.A., R.T.

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RESEARCH ARTICLE

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Mapping Time Perception Research in Cognitive Aging and Early Cognitive Decline: A Bibliometric Analysis

Özlem Önder

Department of Neurology, Near East University Faculty of Medicine, Nicosia, Cyprus

Abstract

BACKGROUND/AIMS: Time perception is a core cognitive function that relies on distributed neurocognitive systems and may be sensitive to early changes across the cognitive aging continuum, including subjective cognitive decline (SCD) and mild cognitive impairment (MCI). Despite growing interest, the knowledge structure and thematic organization of research linking time perception to age-related cognitive vulnerability have not been quantitatively mapped. This study aimed to characterize publication trends, disciplinary distribution, geographic contributions, and conceptual themes in the literature on time perception in cognitive aging, SCD, and MCI using bibliometric methods.

MATERIALS AND METHODS: A bibliometric analysis was conducted using the Web of Science Core Collection. A targeted query combined time-perception constructs in the title field with constructs related to aging, SCD, MCI, and dementia in the topic field. Records were exported in plain-text format and descriptively analyzed with respect to annual production, document types, countries, institutions, and research areas. The thematic structure of the field was examined using a frequency-based analysis of title-derived terms.

RESULTS: The final dataset included 64 records published between 1984 and 2025. Publication output remained limited until the mid-2000s and then expanded markedly after 2015, with peak activity concentrated in the last decade. Research output showed a geographically uneven distribution, led by the United States (n=17), France (n=10), China (n=7), Italy (n=6), and Canada and the Netherlands (n=5 each). Research areas were highly interdisciplinary, with the highest representation in neurosciences/neurology and psychology (n=25 each), followed by geriatrics/gerontology (n=11) and psychiatry (n=8). Frequency-based analysis of title-derived terms showed that the literature is centered on core timing concepts, such as time perception and time estimation, with additional emphasis on Alzheimer's disease, memory, and age-related constructs.

CONCLUSION: Research on time perception in cognitive aging and early cognitive decline has expanded rapidly over the last decade and is primarily anchored in neuropsychology and cognitive neuroscience. Thematic mapping suggests that timing paradigms form the conceptual core of the field, whereas clinically framed work on early cognitive decline remains comparatively limited. These findings highlight the need for further methodologically standardized and empirically oriented research in preclinical and prodromal cognitive states.

Keywords: Time perception, interval timing, cognitive aging, cognitive decline, bibliometric analysis

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ORCID ID of the author: Ö.Ö. 0000-0002-7133-9808.

Corresponding author: Özlem Önder
E-mail: ozlem.onder@neu.edu.tr
ORCID ID: orcid.org/0000-0002-7133-9808

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INTRODUCTION

Time perception-the ability to estimate, reproduce, and compare temporal intervals-represents a fundamental component of human cognition that underpins goal-directed behavior, prospective memory, decision-making, and adaptive interaction with the environment.[1] Rather than reflecting a unitary faculty, temporal processing emerges from distributed neural systems involving fronto-striatal and fronto-parietal networks, cerebellar contributions, and neuromodulatory mechanisms.[2] As a result, temporal judgments are intrinsically linked to attentional allocation, working memory maintenance, and executive control. Alterations in these cognitive systems due to aging or neurodegenerative processes are therefore expected to manifest as measurable changes in interval timing and subjective temporal experience.[1-3]

Cognitive aging encompasses a heterogeneous continuum ranging from normative age-related changes to prodromal and syndromic forms of cognitive impairment. Within this framework, subjective cognitive decline (SCD) is commonly conceptualized as a preclinical state characterized by self-perceived cognitive worsening in the absence of clear objective deficits, whereas mild cognitive impairment (MCI) denotes objectively measurable cognitive impairment with relative preservation of daily functioning and an increased risk of progression to dementia.[4,5]

Because time perception tasks impose simultaneous demands on attention, working memory, and monitoring processes, they have been proposed as potentially sensitive behavioral probes of early neurocognitive vulnerability.[3] A growing empirical literature has applied time estimation, reproduction, bisection, and judgment paradigms to older adults and clinical populations along the Alzheimer's disease continuum.[6] Nevertheless, findings remain heterogeneous, reflecting substantial variability in experimental paradigms, temporal scales, analytic metrics, and clinical characterization.[7]

Despite this expanding literature, the intellectual and thematic structure of research at the intersection of time perception, cognitive aging, and early cognitive decline has not been systematically mapped. Studies are dispersed across multiple disciplines, often progressing in parallel rather than through integrative conceptual frameworks, complicating the identification of dominant research themes and underexplored areas. In such contexts, bibliometric science-mapping approaches offer a valuable methodological framework by quantitatively characterizing publication trends, citation structures, and conceptual networks within a defined research domain.[8,9]

Accordingly, this study conducted a bibliometric analysis of publications addressing time perception in cognitive aging and early cognitive decline, using data indexed in the Web of Science (WoS) Core Collection and identified through a targeted search strategy that yielded 64 records. The objectives are to characterize (i) temporal trends in publication output, (ii) the disciplinary and source distribution of the literature, and (iii) the thematic structure of the field, using a frequency-based analysis of title-derived terms.

MATERIALS AND METHODS

Literature Source

This bibliometric study was conducted using the WoS Core Collection (Clarivate Analytics), selected because of its standardized indexing structure, citation-tracking capability, and wide use in bibliometric and science-mapping research. Only records indexed in the WoS Core Collection were considered. The exact database search was performed on February 2, 2026.

Search Strategy

A targeted search strategy was developed to identify publications addressing time perception in the context of cognitive aging and early cognitive decline. To improve conceptual specificity and reduce retrieval of unrelated basic timing literature, time-perception-related terms were restricted to the title (TI) field, whereas aging- and cognition-related constructs were searched in the topic (TS) field, which includes the title, abstract, and keywords indexed in WoS. No restriction on publication year was applied, and only English-language records were included. Records were exported in plain-text format with cited references. The final search query was as follows:

TI =("time perception" or "interval timing" or "duration perception" or "time estimation" or "time reproduction" or "time bisection" or "temporal judgment") and TS =("cognitive aging" or "older adults" or elderly or geriatric* or "mild cognitive impairment" or MCI or "subjective cognitive decline" or SCD or dementia or Alzheimer*)

This title-restricted approach was chosen to maximize specificity and yield a more focused dataset. As a trade-off, this strategy may have reduced sensitivity by excluding potentially relevant studies whose time-perception-related concepts appeared only in abstracts or keywords.

Eligibility Criteria

Eligible records included publications relevant to time perception or interval timing in older adults or in the context of early cognitive decline, including SCD and MCI. No additional post-hoc manual relevance screening beyond the predefined query was applied, consistent with a reproducible bibliometric approach. Because WoS document-type labels may overlap within the same record, document-type classifications were interpreted descriptively rather than as mutually exclusive categories. This is particularly relevant for records indexed simultaneously, for example, as "article; proceedings paper" or "article; early access".

Data Extraction and Preprocessing

All retrieved records were exported from WoS in plain-text format, including full bibliographic information and cited references. Extracted variables included publication year, document type, journal title, authors, affiliations, countries/regions, WoS research areas, titles, and citation counts. For title-based thematic analyses, titles were normalized by lowercasing and harmonizing common lexical variants. Duplicate term occurrences within the same record were removed to avoid artificial inflation of frequencies.

Ethical Consideration

This study is a bibliometric analysis based exclusively on publicly available bibliographic records. It did not involve human participants, patient-level data, identifiable personal information, or animal experiments. Therefore, ethics committee approval and informed consent were not required.

Statistical Analysis

Descriptive statistics were used to summarize annual publication output, document types, countries, institutions, and WoS research

areas. Citation impact was reported using total citation counts and, where appropriate, measures of central tendency.

To characterize the thematic structure of the field, a frequency-based analysis of title-derived terms was performed. Unigrams and bigrams were extracted from publication titles following standard text preprocessing, including lowercasing, punctuation removal, and stop-word filtering. Term frequencies were summarized descriptively, and the most frequent title-derived terms were visualized to provide an overview of the dominant concepts represented in the dataset.

Analyses and visualizations were conducted using WoS export files, reproducible computational scripts, and established bibliometric tools, including R, bibliometrix, and VOSviewer.[8,9] Graphical outputs included annual publication and citation trends, research- area and productivity distributions, and frequency-based visualizations of title-derived terms. All figures were generated at publication-ready resolution. In addition, a title-term co-occurrence network was generated to provide a complementary visualization of thematic relationships among frequently occurring terms. In this network, node size reflected term occurrence frequency, edge thickness reflected co-occurrence strength, and node color indicated mean publication year.

RESULTS

Study Selection and Dataset Characteristics

A total of 64 records were retrieved from the WoS Core Collection, using a predefined query that combined time-perception terms in the title field and cognitive aging/SCD/MCI-related terms in the topic field. The retrieved literature spanned 1984-2025, indicating a long but relatively sparse historical record, with a marked intensification in recent years. The dataset accumulated 1,707 citations across all documents, with a median citation count of 10.5 per record, suggesting that the field is supported by a small number of highly cited contributions alongside many modestly cited publications.

Annual Publication and Citation Trends

The annual publication profile indicates a prolonged period of limited scientific output from the mid-1980s through the early 2000s, during which annual output rarely exceeded a single paper. Publication activity increased gradually beginning around 2008 and rose more markedly after 2015; the highest annual outputs were concentrated between 2018 and 2024, reflecting a recent intensification of scholarly attention to the topic. Citation dynamics closely tracked this expansion but followed a steeper trajectory: annual citation counts remained minimal until the mid-2000s, increased steadily thereafter, and accelerated substantially after 2015, reaching their highest levels between 2019 and 2023 (Figure 1). The decline observed in the most recent year is best interpreted as a citation-lag effect, because newly published articles have had limited time to accrue citations.

Document Type Distribution

The literature was dominated by articles. A smaller number of records were also indexed as meeting papers, review articles, or early access items. Because WoS document-type labels may overlap within the same record, document-type categories were interpreted descriptively rather than as mutually exclusive counts.

Source Journals

Publications were distributed across 53 journals, indicating a broad disciplinary spread rather than concentration in a single outlet. The most frequent source titles were the Journal of the International Neuropsychological Society and Brain and Cognition (3 records each), followed by Neurobiology of Aging, Frontiers in Aging Neuroscience, Memory, Scientific Reports, Heliyon, Epidemiology and Infection, and International Journal of Aging & Human Development (2 records each) (Figure 2).

Geographic Distribution of Publications

Research output showed a geographically uneven distribution, with major contributions from North America, Western Europe, and parts of Asia. The United States contributed the largest share (17/64 records; 26.6%), followed by France (10/64; 15.6%), China (7/64; 10.9%), Italy (6/64; 9.4%), and Canada and the Netherlands (5/64; 7.8% each). Additional contributions were received from South Korea, Japan, England, Germany, Spain, Taiwan, Türkiye, Australia, and Portugal (Figure 3).

Institutional Contributions

Institution-level analyses indicated that a small number of organizations contributed multiple records, while most institutions contributed only one or two. Overall, the distribution suggested a dispersed research field rather than a strong dominance by a single center (Figure 4).

Research Area Classification

WoS research area assignments demonstrated a multidisciplinary profile. The most represented categories were neurosciences/neurology (n=25) and psychology (n=25), followed by geriatrics/gerontology (n=11) and psychiatry (n=8). Smaller, but notable, contributions were observed in science & technology—other topics (n=6) and behavioral sciences (n=4), as well as several additional categories, including engineering; public, environmental & occupational health; pharmacology & pharmacy; physiology; rehabilitation; biochemistry & molecular biology; and biophysics. Because WoS research area assignments may overlap across records, these categories should be interpreted as descriptive rather than mutually exclusive (Figure 5).

Title-Derived Term Frequency Analysis and Thematic Structure

To characterize the thematic structure of the field, a frequency-based analysis of title-derived terms was performed across the 64 records. The most common terms included “perception” (n=32), “time perception” (n=31), “time estimation” (n=25), and “estimation” (n=25). Additional terms related to Alzheimer’s disease, memory, and age-related concepts. These findings suggest that the literature is organized primarily around core timing paradigms, with further extensions toward aging-related cognitive constructs and neurodegenerative disease contexts (Figure 6). A complementary title-term co-occurrence network further illustrates the relational structure among core timing concepts and their links to aging- and disease-related terms, supporting the interpretation that the field is organized around shared temporal-processing paradigms (Figure 7).

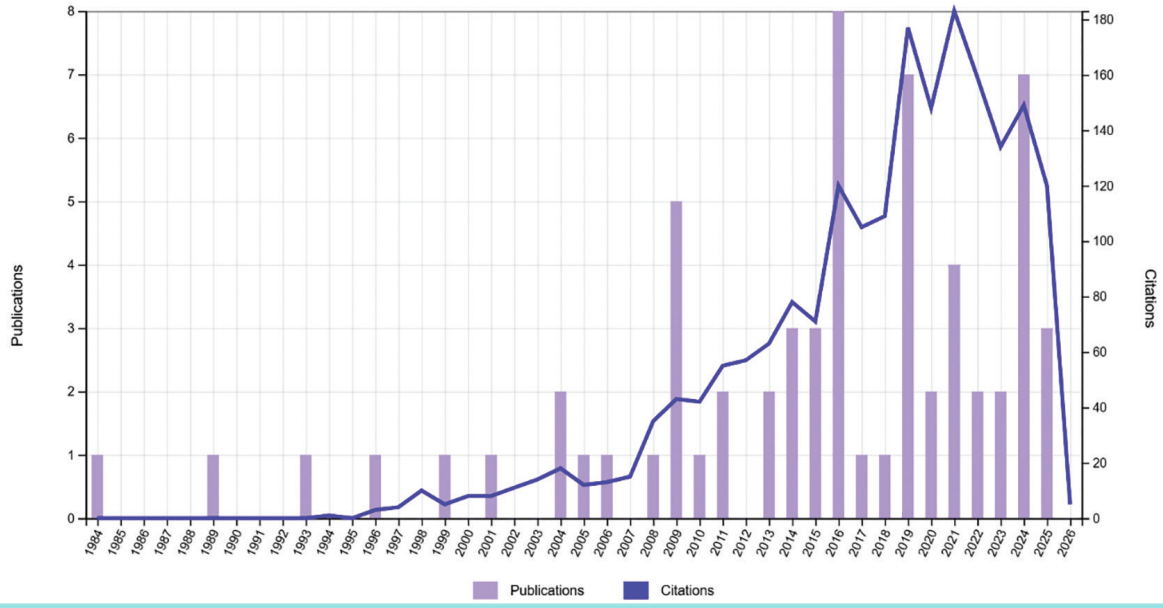

Figure 1. Annual trends in scientific output and citation activity related to time perception in cognitive aging and early cognitive decline. Bars represent the number of publications per year, while the line indicates the total number of citations received annually. The figure illustrates the prolonged period of low publication activity followed by a marked increase after 2015, along with a parallel acceleration in citation counts.

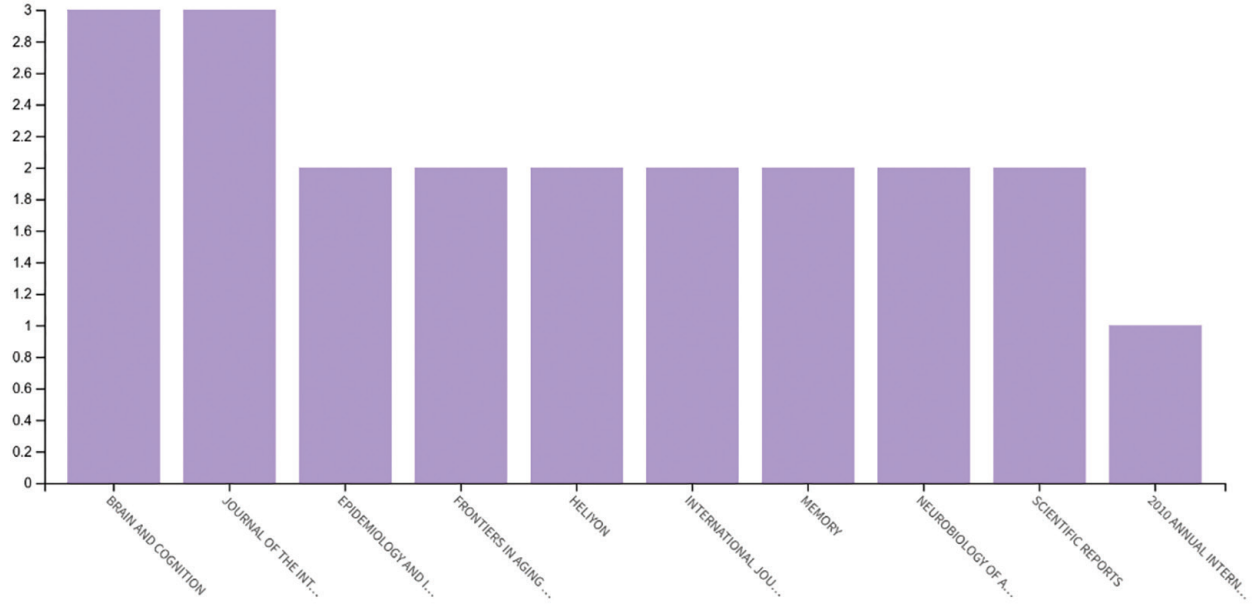

Figure 2. Distribution of source journals contributing to publications on time perception in cognitive aging and early cognitive decline. The figure displays the journals with the highest number of publications, highlighting the multidisciplinary dissemination of research across neuropsychology, neuroscience, aging, and general science journals.

DISCUSSION

This bibliometric analysis mapped the evolution, thematic focus, and disciplinary profile of research examining time perception in cognitive aging and early cognitive decline, including SCD and MCI. Overall, the findings indicate a field that has clearly expanded over the last decade, remains strongly interdisciplinary, and is still more closely aligned with experimental paradigms than with clinically oriented research on early cognitive decline.

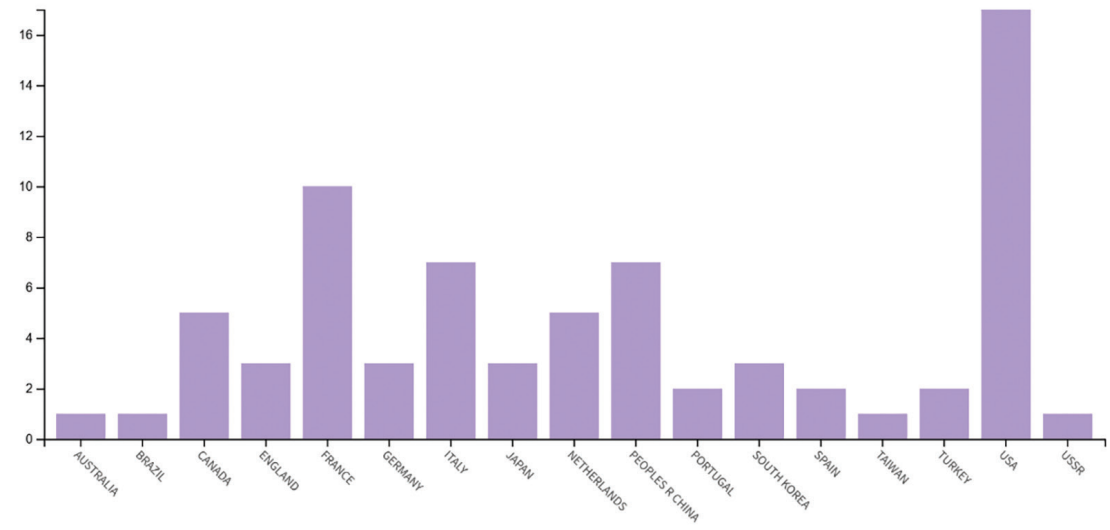

Figure 3. Country-level productivity based on author affiliations. Counts reflect the number of publications assigned to each country from the address/affiliation field; multi-country papers contribute to each represented country according to WoS affiliation parsing.

WoS: Web of Science.

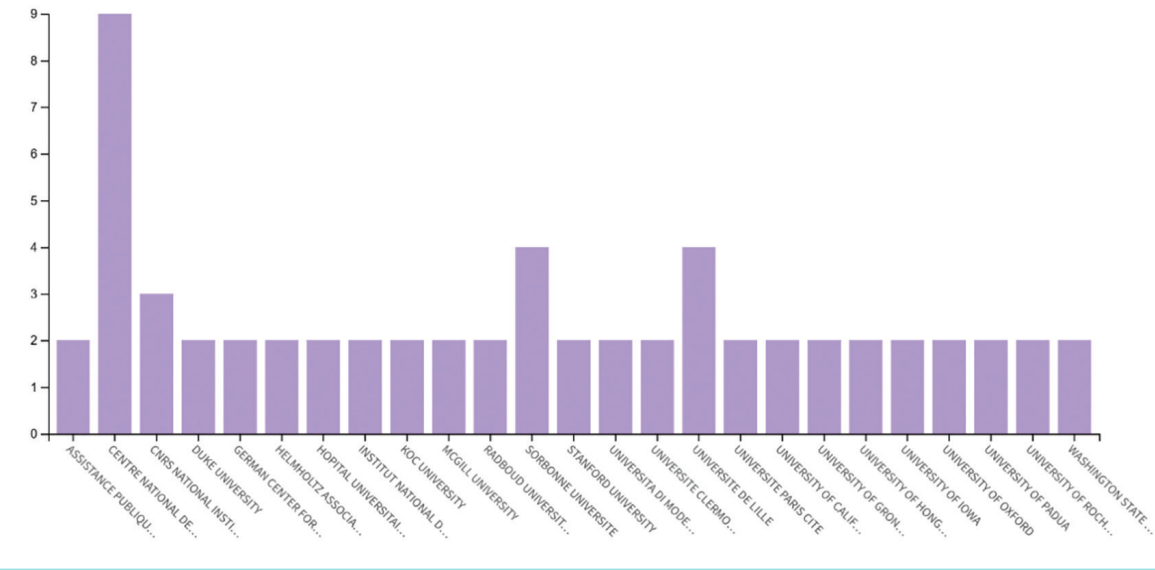

Figure 4. Institutional contributions derived from author affiliations. The figure displays the most productive institutions; values represent the number of records associated with each institution as indexed in WoS.

WoS: Web of Science.

One of the main observations of this study is the delayed integration of time perception research into aging- and dementia-related contexts. Although temporal processing has long been studied in cognitive neuroscience and experimental psychology, its more explicit application to cognitive aging and early cognitive decline appears to have gained momentum more recently.[1-3,7] This pattern may reflect the longstanding emphasis of aging research on memory, language, and executive functions as primary cognitive domains, with temporal processing receiving comparatively less clinical attention.[10]

The disciplinary distribution of the literature further supports this interpretation. The strong representation of neurosciences/neurology and psychology suggests that the field is anchored primarily in cognitive and neurobiological approaches, whereas the relative underrepresentation of geriatrics/gerontology and psychiatry indicates that translation into routine clinical and geriatric settings remains limited. Taken together, these findings suggest that time perception has been studied more often as a methodological or experimental construct than as a clinically integrated domain of investigation.

Figure 5. Treemap visualization of the Web of Science research areas represented in the retrieved literature on time perception in cognitive aging and early cognitive decline. Each rectangle corresponds to a research area assigned by Web of Science, and rectangle size is proportional to the number of records associated with that area. Because Web of Science research area assignments may overlap across records, categories should be interpreted descriptively rather than as mutually exclusive.

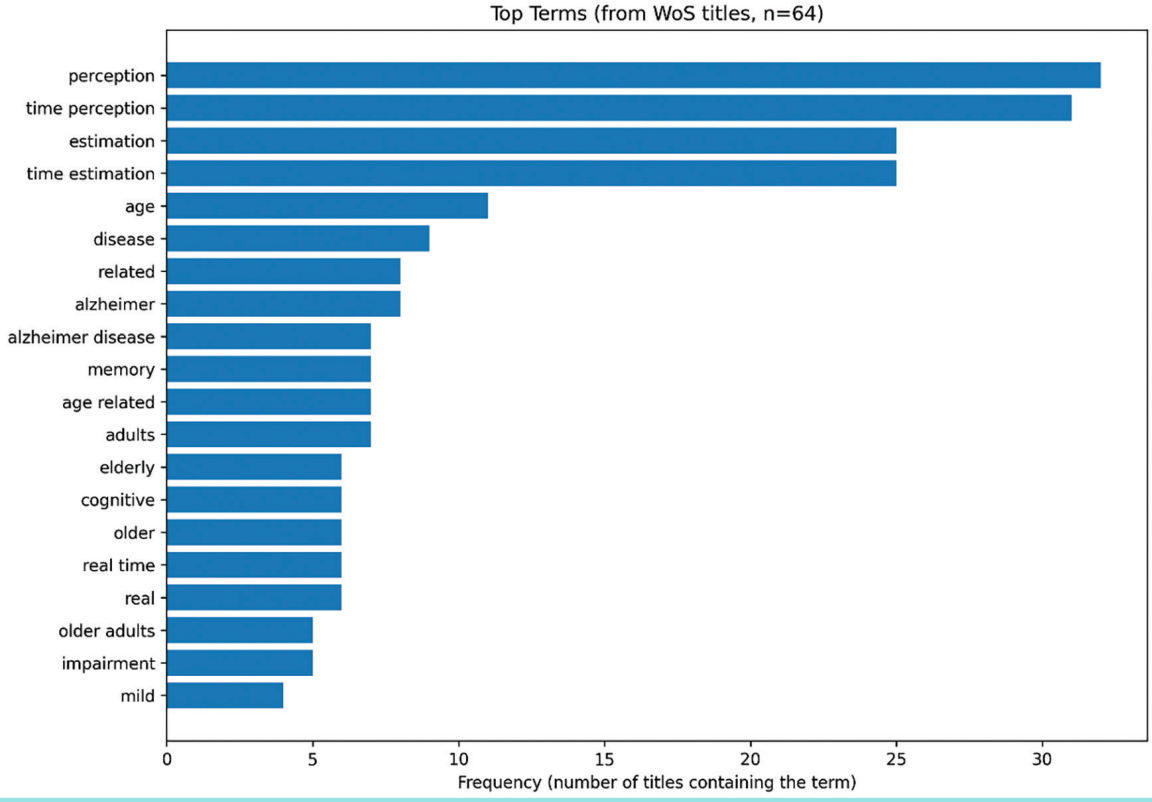

Figure 6. Most frequent title-derived terms in the dataset. Bars indicate the number of records in which each term appears, providing a frequency-based overview of the dominant concepts represented in the literature.

WoS: Web of Science.

The frequency-based analysis of title-derived terms provides additional insight into the thematic structure of the field. The prominence of terms such as time perception, time estimation, and related timing constructs indicates that the literature is organized largely around shared experimental paradigms.[1-3] By contrast, clinically framed concepts such as SCD, MCI, and Alzheimer-related terminology were less prominent at the title level.[4-6] This pattern suggests that early cognitive decline has often served as the clinical context in the literature rather than as its primary conceptual driver.

These findings should be interpreted cautiously. The present study is bibliometric and therefore describes patterns of publication, indexing, and thematic emphasis rather than the diagnostic or clinical utility of temporal processing measures. Nevertheless, the observed trends identify a potentially important area for future empirical research. Because temporal processing tasks engage attention, working memory, and executive control, and rely on neural systems relevant to neurodegenerative disease, they may warrant further investigation in clinically characterized aging populations.[10-12]

Another notable finding is the methodological heterogeneity of the field. Even within central timing paradigms, studies vary substantially in task design, temporal intervals, outcome definitions, and analytic strategies.[1-3,7] While such diversity may support exploratory work, it also limits comparability across studies and complicates synthesis of findings across populations. More harmonized task selection and outcome reporting may therefore improve interpretability in future studies.[5,6,10]

Study Limitations

Several limitations should be acknowledged. First, the analysis was restricted to the WoS Core Collection and may therefore have missed relevant studies indexed elsewhere. Second, the thematic analysis was based on title-derived terms, which capture dominant themes but may not fully reflect concepts emphasized in abstracts or full texts. Third, the relatively small dataset requires cautious interpretation of prominence at the country, institution, and theme levels. Finally, bibliometric methods describe the structure of the literature but do not assess the methodological quality or validity of individual studies.

Overall, the present findings indicate increasing scientific interest in time perception in research on cognitive aging and early cognitive decline. Future work, particularly in SCD and early MCI populations, may help clarify whether temporal processing paradigms have broader relevance in clinically oriented cognitive aging research.

CONCLUSION

This bibliometric analysis provides an overview of the scientific literature linking time perception with cognitive aging and early cognitive decline, including SCD and MCI. The findings show that despite the long-standing experimental tradition of temporal processing research, its integration into aging- and dementia-oriented frameworks has mainly accelerated over the past decade.

The literature is characterized by strong interdisciplinary foundations in neuroscience and psychology, alongside a more limited clinical

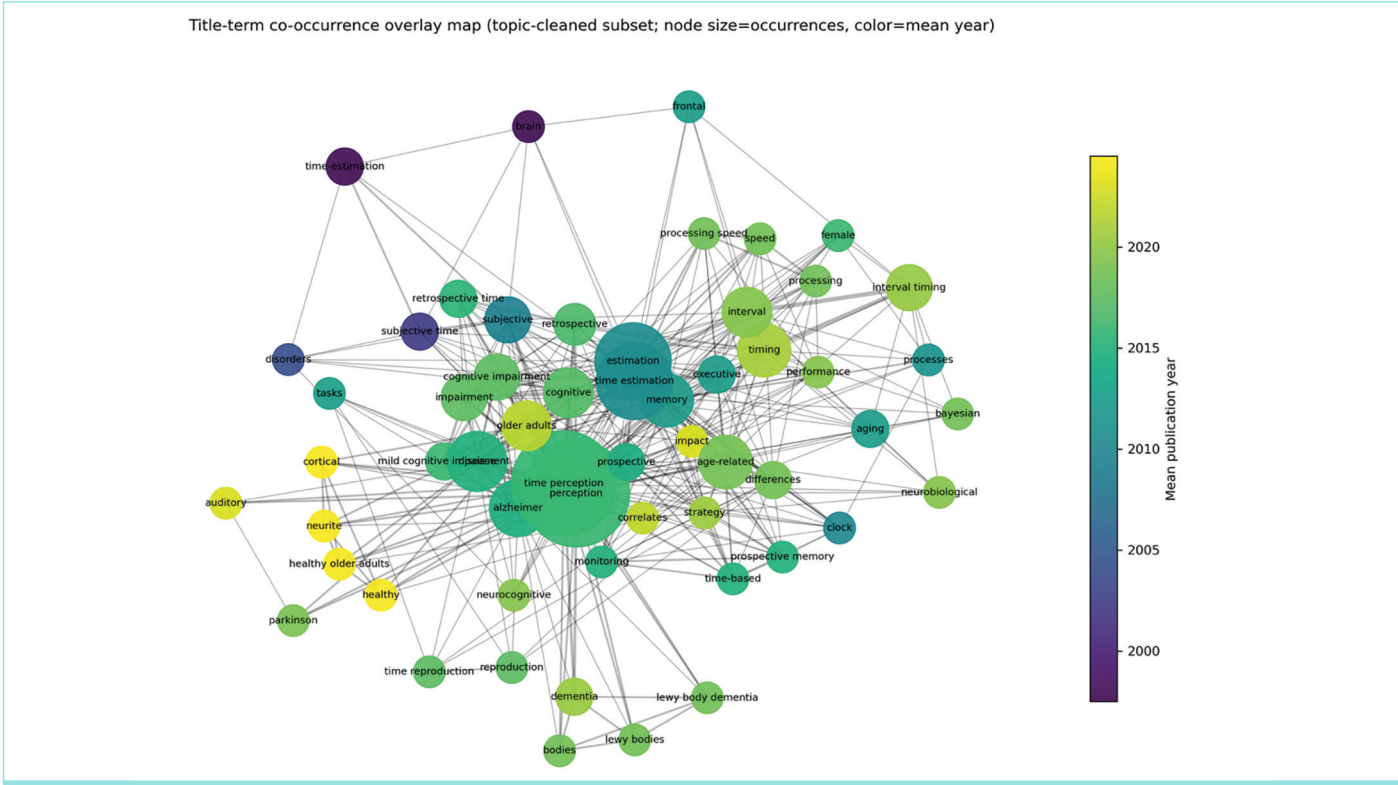

Figure 7. Title-term co-occurrence overlay network for the retrieved literature on time perception in cognitive aging and early cognitive decline. Node size reflects term occurrence frequency, line thickness indicates co-occurrence strength, and node color represents the mean publication year. The figure provides a complementary network-based visualization of thematic relationships among the most prominent title-derived terms.

orientation. Thematic analysis suggests that timing paradigms remain the conceptual core of the field, whereas clinically framed early cognitive decline constructs appear less prominent. This pattern indicates that time perception has been used more often as an experimental framework than as a clinically integrated domain.

Overall, the observed publication trends and thematic structure highlight an opportunity for future research to further connect experimental and clinical approaches. Greater methodological standardization and more systematic study of temporal processing in preclinical and prodromal cognitive states may help clarify its relevance in early cognitive decline research.

MAIN POINTS

- Research on time perception in cognitive aging has expanded markedly over the last decade following a prolonged period of limited output.
- The literature is interdisciplinary but remains centered primarily on timing paradigms rather than clinically framed early cognitive decline constructs.
- Future studies using more standardized temporal processing measures may clarify the relevance of this field to early cognitive decline research.

ETHICS

Ethics Committee Approval: Not applicable. This bibliometric study used publicly available bibliographic data and did not involve human participants, identifiable personal data, or experimental procedures.

Informed Consent: Not applicable. No human participants or identifiable individual-level data were included in this study.

DISCLOSURES

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Declaration of Generative AI and AI-assisted Technologies in the Writing Process: During the preparation of this work, the author(s) used ChatGPT to improve “readability” and “language”. After using this tool/service, the author(s) reviewed and edited the content as needed and took full responsibility for the content of the publication.

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RESEARCH ARTICLE

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Seroprevalence Trends of HBV, HCV, and HIV Before and During the COVID-19 Pandemic: Five-Year Retrospective Data from a Tertiary Hospital in Northern Cyprus

Behich Koyutourk[1], Melis Kalaycı[1], Ertan Direnç[2], Kaya Süer[2]

[1]Department of Medical Microbiology and Clinical Microbiology, Near East University Faculty of Medicine, Nicosia, Cyprus
[2]Department of Infectious Diseases and Clinical Microbiology, Near East University Faculty of Medicine, Nicosia, Cyprus

Abstract

BACKGROUND/AIMS: To assess five-year seroreactivity trends of hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) in Northern Cyprus, with emphasis on the coronavirus disease 2019 (COVID-19) and post-pandemic periods.

MATERIALS AND METHODS: This retrospective cross-sectional study evaluated 278,016 serological test results for hepatitis B surface antigen (HBsAg), anti-HCV, and anti-HIV between 2020 and 2024. All tests were carried out using enzyme-linked immunosorbent assay technology on the ARCHITECT Plus ci4100 system (Abbott Laboratories, Chicago, IL, USA). The annual rate of seroreactivity was calculated and compared across years and sex groups. The results were also categorised based on signal-to-cut-off (S/CO) ratios for HCV and HIV and on IU/mL for HBsAg.

RESULTS: Overall seroreactivity rates were 1.2% for HBsAg, 0.6% for anti-HCV, and 0.2% for anti-HIV. HBV seroreactivity declined significantly from 1.5% in 2020 to 1.0% in 2023-2024 (p=0.001). HCV prevalence remained below 1%, increasing non-significantly from 0.5% to 0.7% (p=0.192); most positive samples fell within the low S/CO category. HIV prevalence remained stable at 0.2% across the study period (p=0.043).

CONCLUSION: HBV seroreactivity exhibited a declining trend over the years; HCV levels remained low but exhibited a rising trend; HIV levels remained stable and low. The results indicate that Northern Cyprus remains a low-endemic region and highlight the importance of sustained surveillance and prevention in the post-COVID-19 era. The results need to be interpreted cautiously, as they may not entirely indicate the levels of infection.

Keywords: HIV, hepatitis B virus, hepatitis C virus, COVID-19, SARS-CoV-2

INTRODUCTION

Hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) remain major global public health challenges. According to estimates, 296 million people were living with chronic HBV, 58 million with chronic HCV, and 39 million with HIV as of 2022.[1] These infections substantially contribute to global morbidity and mortality through complications such as liver cirrhosis, hepatocellular carcinoma, and acquired immunodeficiency syndrome.[2,3]

In Europe and the Eastern Mediterranean, HBV and HCV transmission is particularly associated with people who inject drugs, migrants, and individuals with multiple sexual partners.[4] Although HIV prevalence in these regions remains relatively low compared to global averages, rising rates among men who have sex with men (MSM) and young adults are noteworthy.[5] Despite advances in prevention and treatment, important challenges persist. HBV continues to circulate in populations with low vaccination coverage, while HCV remains largely underdiagnosed due to limited awareness and access to testing.[6,7]

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ORCID IDs of the authors: B.K. 0009-0009-8278-9394; M.K. 0000-0002-5779-5532; E.D. 0000-0002-3311-9388; K.S. 0000-0002-2565-3425.

Corresponding author: Behich Koyutourk
E-mail: behic.koyuturk@neu.edu.tr
ORCID ID: orcid.org/0009-0009-8278-9394

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On the other hand, the coronavirus disease 2019 (COVID-19) pandemic (2020-2022) further disrupted healthcare systems worldwide, interrupting routine screening, surveillance, and public health programs.[8,9] Lockdowns, travel restrictions, and the diversion of health resources led to marked declines in serological tests like HIV, HBV, and HCV.[9,10] In the World Health Organization (WHO) European Region, community-based testing fell by up to 80% in early 2020, disproportionately affecting key populations such as people who inject drugs, MSM, migrants, and sex workers.[9-12] These disruptions delayed diagnosis and treatment, thereby increasing the risk of ongoing transmission. According to that, several studies across Europe, Latin America, and the Middle East have documented significant reductions in testing volumes alongside paradoxical increases in positivity rates, reflecting a shift toward testing primarily symptomatic or high-risk individuals.[4,8,9]

In Northern Cyprus, previous research has consistently indicated low endemicity for HBV, HCV, and HIV.[13,14] However, updated data describing recent temporal trends of these infections in this setting remain limited. Therefore, this study aimed to evaluate five-year trends in HBV, HCV, and HIV seroreactivity at a tertiary care hospital during and after the COVID-19 pandemic. In this study, we aimed to address this gap and provide updated information on trends in the seroprevalence of HBV, HCV, and HIV during the COVID-19 pandemic and the post-pandemic period.

MATERIALS AND METHODS

Ethical Approval and Patient Consent

This study was approved by the Near East University Scientific Research Ethics Committee (approval number: 2025/138, date: 28.11.2025). Due to the retrospective nature of the study and the use of anonymised laboratory data, the requirement for informed patient consent was waived by the Ethics Committee. All procedures were conducted in accordance with the ethical standards of the institutional and national research committees and with the Declaration of Helsinki.

Study Design and Setting

This cross-sectional study analysed 278,016 retrospectively collected serological test results for HBV, HCV, and HIV from a tertiary care hospital in Northern Cyprus, Nicosia, covering the period from January 2020 to December 2024. All serological analyses were performed using the ARCHITECT Plus ci4100 system (Abbott Laboratories, Chicago, IL, USA). According to the manufacturer's data, the assays demonstrate high analytical performance, with reported sensitivity and specificity values exceeding 99% for hepatitis B surface antigen (HBsAg), anti-HCV, and anti-HIV detection. These tests were primarily conducted as part of routine clinical screening, including preoperative assessments and infectious disease screening protocols. Duplicate test results from the same individual within the same year were excluded when identifiable. Records with incomplete or missing key data were also excluded from subgroup analyses where applicable.

Data from the hospital's electronic laboratory information system were provided as de-identified serology results. These results included information on year of testing, test type (HBsAg, anti-HCV, anti-HIV), test result [signal-to-cut-off (S/CO) ratio of ≥1 was considered a positive result, while a S/CO ratio of <1 was considered a negative result], and demographic information that may be available (sex and age). Although geographical origin data were available in the hospital system, they were not included in the analysis due to variability in data recording and the need for extensive standardisation. Confirmatory testing (e.g., nucleic acid amplification tests or immunoblot assays) was not consistently available; therefore, the results reflect screening test reactivity rather than confirmed infection.

Furthermore, to refine the interpretation of positive results and reduce potential false-positive misclassification, HBsAg-positive samples were reclassified into three analytic strata: 0.20-9.90 IU/mL, 10.0-20.0 IU/mL, and ≥20.0 IU/mL.[15] For HIV, positive cases were classified as strongly positive when the S/CO ratio exceeded 400.[16] For HCV, positive samples were characterised into three categories based on their S/CO values: low reactivity (1-5), moderate reactivity (5-10), and high reactivity (>10).[17] The high S/CO category was often reported separately because low-level reactivity may indicate false-positive results. By including S/CO distributions, we aimed to limit our overestimation of prevalence and to provide a more reliable interpretation of the true infection burden in the population studied.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics, version 18 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to summarise demographic characteristics and calculate annual seropositivity rates for HBsAg, anti-HCV, and anti-HIV from 2020 to 2024. Categorical variables were presented as frequencies and percentages. Comparisons across years and demographic subgroups (sex) were conducted using cross-tabulations, and the statistical significance of group differences was assessed using chi-square tests. A p-value of <0.05 was considered statistically significant.

RESULTS

From 2020 to 2024, 278,016 individuals underwent screening for HBV, HCV, and HIV at a tertiary hospital in Northern Cyprus (Table 1). The overall seroreactivity rates were 1.2% for HBV (HBsAg), 0.6% for HCV (anti-HCV), and 0.2% for HIV (anti-HIV). HBV positivity was identified in 1,081 of 92,339 individuals (1.2%), with males exhibiting significantly higher rates than females (1.4% versus 0.9%, $p<0.001$). Anti-HCV antibodies were detected in 554 of 92,946 individuals (0.6%), with a slight but non-significant increase over the study period, from 0.5% in 2020 to 0.7% in 2024 ($p=0.192$). Seroreactivity for HCV was comparable between the sexes (0.6% in males versus 0.5% in females, $p=0.081$). HIV seropositivity was observed in 181 of 92,731 individuals (0.2%); the highest prevalence was in 2021 (0.3%), and subsequent annual rates ranged from 0.1% to 0.2%. HIV positivity was significantly higher in males than in females (69.6% vs. 30.4%, $p=0.001$). Detailed prevalence data, including sex-specific and annual distributions, are presented in Table 2.

Figure 1 illustrates the annual trends in HBV, HCV, and HIV seroreactivity between 2020 and 2024. The highest rate was reported for HBV during the study period, but it declined sharply from 1.5% in 2020 to 1.0% in 2023-2024, demonstrating a consistent downward trend. On the other hand, the prevalence of HCV remained below 1% but showed a consistent upward trend, rising from 0.5% in 2020 to 0.7% in 2024. The prevalence of HIV was the lowest of the three, remaining low and stable, ranging between 0.1% and 0.3%, with a rise in 2021 followed by a decline to 0.1% in 2024 (Figure 1). Overall, these results indicate a prolonged downward trend in seropositivity for HBV, a gentle but

Table 1. Overall seroprevalence of HBV, HCV, and HIV in Northern Cyprus (2020-2024), including male and female distributions with corresponding p-values

Marker	Total tested (n)	Positive (n)	Seroprevalence (%)	Male positive (n, %)	Female positive (n, %)	p-value* (male vs. female)
HBsAg	92,339	1,081	1.2	740 (1.4%)	341 (0.9%)	<0.001
Anti-HCV	92,946	554	0.6	338 (0.6%)	216 (0.5%)	0.081
Anti-HIV	92,731	181	0.2	126 (0.3%)	55 (0.1%)	0.001

*Chi-square test (Pearson) was used to compare male vs. female seropositivity. All positive cases are reported with signal-to-cut-off values ≥1, indicating reactivity above the negative threshold.
HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, HBsAg: Hepatitis B surface antigen.

Table 2. Annual distribution of HBV, HCV, and HIV seroreactivity in Northern Cyprus, 2020-2024

Year	HBV tested (n)	HBV positive (n, %)	HCV tested (n)	HCV positive (n, %)	HIV tested (n)	HIV positive (n, %)
2020	12,134	179 (1.5%)	13,097	67 (0.5%)	13,034	24 (0.2%)
2021	16,442	232 (1.4%)	16,343	101 (0.6%)	16,290	42 (0.3%)
2022	17,693	221 (1.2%)	17,600	94 (0.5%)	17,539	34 (0.2%)
2023	20,966	205 (1.0%)	20,851	121 (0.6%)	20,827	48 (0.2%)
2024	25,104	244 (1.0%)	25,055	171 (0.7%)	25,041	33 (0.1%)
Total	92,339	1,081 (1.2%)	92,946	554 (0.6%)	92,731	181 (0.2%)
p-value	p=0.001		p=0.192		p=0.043	

Data are presented as number of individuals tested and number (percentage) of reactive cases. Seroreactivity is defined as ELISA screening positivity (S/CO ≥1). p-values represent comparisons across years.
HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, ELISA: Enzyme-linked immunosorbent assay, S/CO: Signal-to-cut-off.

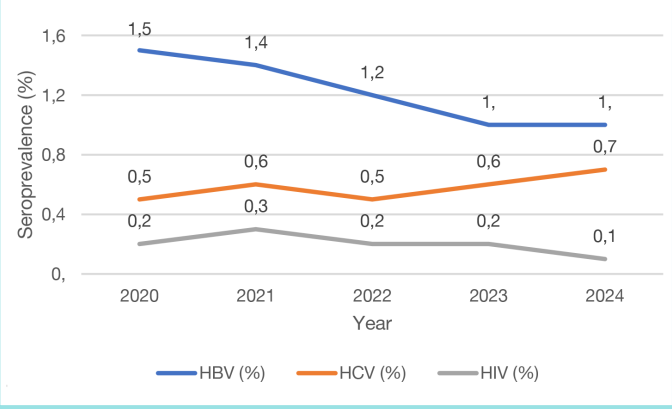

Figure 1. Annual seroprevalence of HBV (HBsAg), HCV (anti-HCV), and HIV (anti-HIV) in Northern Cyprus between 2020 and 2024. Rates are expressed as percentages of tested individuals.

HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, HBsAg: Hepatitis B surface antigen.

consistent upward drift in HCV prevalence, and very low HIV prevalence during the five-year surveillance period (Table 2).

In our cohort, all positive results were considered to be those with an S/CO value greater than 1.[15,16] To better characterise the strength of seropositivity and minimise potential false-positive interpretations, all positive cases were further stratified by quantitative values. The distribution of results across these categories is presented in Table 3. Based on that, low-to-moderate antigenemia likely represents carrier or early infection, while ≥20 IU/mL indicates active infection for HBsAg in 277 patients between 2020 and 2024. Additionally, among the 554 HCV-positive individuals, the majority (72.6%) showed low reactivity in the 1-5 S/CO range, 9.2% were in the intermediate 5-10 S/CO range, and 18.2% were strongly positive (S/CO >10). Moreover, among the 181 HIV-positive results detected between 2020 and 2024, only 26.6% of cases demonstrated strong reactivity with S/CO values above 400, consistent with a high likelihood of true HIV infection.

Additionally, an age-stratified analysis of seroreactive cases showed a consistent pattern across HBV, HCV, and HIV. Age distribution data for seroreactive cases are presented in Table 4. The majority of cases were observed in the 19-30 age group (HBV: 47.1%, HCV: 44.0%, HIV: 49.2%), followed by the 31-45 age group (HBV: 30.0%, HCV: 25.1%, HIV: 32.8%). Lower proportions of cases were observed among older age groups, whereas cases among individuals under 18 years of age remained rare across all infections.

The sex distribution of HBV, HCV, and HIV seroreactivity between 2020 and 2024 showed that males had higher HBV seroreactivity (1.4%) than females (0.9%), indicating a notable sex difference (Table 1). HCV seroreactivity was similar between sexes, with rates of 0.6% in males and 0.5% in females. In contrast, HIV seroreactivity demonstrated a more pronounced disparity, with rates approximately three times higher in males (0.3%) than in females (0.1%). Overall, seroreactivity for HBV and HIV was higher among males, whereas the slight difference observed for HCV was not statistically significant (p=0.081).

DISCUSSION

This study provides the first post-pandemic assessment of HBV, HCV, and HIV seroreactivity trends in Northern Cyprus. The main finding is that Cyprus remains a region of low endemicity for all three infections, with HBV prevalence showing a marked decline, HCV showing a modest but non-significant upward trend, and HIV remaining low and stable. These findings support our hypothesis that the overall endemicity profile

Table 3. Distribution of reactive HBV (HBsAg), HCV, and HIV results according to S/CO and IU/mL categories

Test	Category (S/CO or IU/mL)	Number of patients (n)	Percentage (%)	Interpretation
HBsAg	0.20-9.90 IU/mL	687	63.6%	Low-level reactivity
	10.0-20.0 IU/mL	117	10.8%	Moderate reactivity
	≥20.0 IU/mL	277	25.6%	High-level reactivity
	Total	**1081**	**100%**	
Anti-HCV	1.0-5.0 S/CO	402	72.6%	Low-level reactivity
	5.0-10.0 S/CO	51	9.2%	Moderate reactivity
	≥10.0 S/CO	101	18.2%	High-level reactivity
	Total	**554**	**100%**	
Anti-HIV Ag/Ab	1.0-400 S/CO	134	74.0%	Low-level reactivity
	≥400 S/CO	47	26.0%	High-level reactivity
	Total	**181**	**100%**	

Positive results were defined as samples with an S/CO value ≥1. For anti-HCV, S/CO ranges of 1-5, 5-10, and >10 are presented to illustrate the distribution of reactivity levels. For anti-HIV, samples with S/CO >400 are shown separately to indicate strong reactivity. However, all reactive HIV results require confirmatory testing regardless of S/CO values. These categorizations are provided for descriptive purposes only and do not replace confirmatory diagnostic testing.
HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, S/CO: Signal-to-cut-off, HBsAg: Hepatitis B surface antigen.

Table 4. Age distribution of seroreactive cases by infection type

Age group	HBV n (%)	HCV n (%)	HIV n (%)
0-18	14 (1.3%)	11 (2.0%)	3 (1.6%)
19-30	509 (47.1%)	244 (44.0%)	90 (49.2%)
31-45	324 (30.0%)	139 (25.1%)	60 (32.8%)
46-60	117 (10.8%)	81 (14.6%)	17 (9.8%)
>60	117 (10.8%)	79 (14.3%)	11 (6.6%)
Total	**1081 (100%)**	**554 (100%)**	**181 (100%)**

HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus.

would remain low, while also revealing pandemic-related complexities in transmission dynamics.

HBV showed the highest overall prevalence (1.2%), but demonstrated a significant decline during the study period, from 1.5% in 2020 to 1.0% in 2023-2024. This downward trend aligns with the long-term success of vaccination and public awareness campaigns, which have been central to hepatitis B control in Cyprus as reported in previous studies.[4,18-20] Nevertheless, the persistence of higher positivity among males (1.4% vs. 0.9%) indicates that behavioural and occupational risk factors, as well as potential gaps in vaccination coverage, continue to shape transmission.[21] The overall prevalence remains below the 2% threshold defining low endemicity, reaffirming Cyprus as a country where HBV is effectively controlled.[22,23]

On the other hand, HCV prevalence remained under 1% throughout the study period, with a slight but consistent upward trend (0.5% to 0.7%). Although not statistically significant, this increase may reflect changes in risk behaviours and healthcare access during the COVID-19 pandemic, such as reduced harm reduction services, delayed treatment, and altered testing patterns.[24,25] The lack of significant gender differences suggests that exposure risks are broadly shared across sexes.[26] Compared with neighbouring countries such as Türkiye and Greece, HCV prevalence (<1%) from Northern Cyprus is consistent with the Greek results (0.9%) and lower than Türkiye, where population estimates ranged from 0.8 - 1.5%.[27-29] These findings suggest that Cyprus is more consistent with Southern European epidemiology than in higher-prevalence regions in the Middle East and North Africa, where the prevalence of HCV is higher than 3-4%.[30] However, the persistence of even low-level increases emphasises the need for continuous surveillance and harm reduction initiatives, particularly among people who inject drugs.[31,32]

Conversely, HIV prevalence was lowest overall, at 0.2%, and remained relatively stable throughout the five-year period, with a peak of 0.3% in 2021 and a decline to 0.1% in 2024. While these rates are comparable with small European nations such as Malta and Slovenia (≤0.3%, ECDC 2023), the marked male predominance (≈70%) is consistent with global epidemiology, especially among MSM and highlights the need for targeted interventions, including community-based testing, behavioural education, and stigma reduction.[31,32] Importantly, the comparative stability of HIV levels during the COVID-19 pandemic is compared to reports from countries such as Italy and Spain, where temporary decreases in levels of testing led to underreporting.[33] This would suggest that Cyprus's smaller health system may have been better placed to be more resilient in maintaining continuity of essential HIV services.[34,35]

Furthermore, the predominance of seroreactive cases among young adults suggests an increased risk of exposure in this age group, likely related to behavioural and social factors, whereas the low proportion of cases in younger individuals may reflect lower exposure and/or vaccination effects, particularly for HBV. The COVID-19 pandemic is a particularly critical contextual factor when interpreting these results. Reductions in routine testing and delayed care were widely reported in Europe and the Eastern Mediterranean, potentially leading to underdiagnosis of chronic viral infections.[8,36,37] In Cyprus, while some of the observed fluctuations may reflect deferred testing or shifts in the tested population, the overall stability of HIV and the decline in HBV indicate relative resilience of the healthcare infrastructure.[35]

Nevertheless, an important limitation of this study relates to the reliance on enzyme-linked immunosorbent assay-based screening assays without confirmatory testing. While we stratified S/CO values to differentiate weak from strong reactivity, false positives cannot be excluded, particularly for borderline results.[15-17] In regards to HBsAg,

positive samples were reassigned among three analytic strata: 0.20-9.90 IU/mL (low reactivity), 10.0-20.0 IU/mL (moderate reactivity), and ≥20.0 IU/mL (strong reactivity).[15] This was done to minimise ambiguity in the interpretation of borderline results, though confirmatory assays, including neutralisation assays or HBV deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) assays, would be necessary for unambiguous classification. For HIV, we addressed this by reporting cases with S/CO >400 separately as highly likely true positives; 69.9% of positives with lower S/CO may require confirmatory testing, such as HIV ribonucleic acid (RNA) PCR. According to international guidelines (CDC and WHO), all reactive HIV screening results require confirmatory testing, regardless of S/CO values. Therefore, the findings of this study should be interpreted as screening reactivity rather than as confirmed infection. However, such confirmatory data were unavailable in this retrospective dataset. A similar approach was applied to HCV, where classification into low, moderate, and high reactivity categories aimed to refine interpretation.

Study Limitations

This study has several limitations. First, its retrospective single-centre design may limit the generalizability of the findings to the broader population of Northern Cyprus. Second, serological data were obtained from hospital-based testing and may not fully reflect community-level prevalence. Third, confirmatory molecular testing (e.g., HBV DNA, HCV RNA, HIV RNA) was not available for all positive cases, which may have led to misclassification, particularly in low (S/CO) results.[15] Finally, fluctuations in healthcare access and testing behaviour during the COVID-19 pandemic may have influenced annual testing volumes and observed prevalence trends.

Cyprus remains a low-endemic setting for HBV, HCV, and HIV. The prevalence of HBV is declining, that of HCV is slightly increasing, and HIV prevalence is low, with a notable male predominance. These findings reinforce the value of vaccination, harm reduction, and gender-sensitive interventions, while also stressing the importance of resilient screening systems that can withstand disruptions such as those caused by the COVID-19 pandemic.[38-40] Strengthening surveillance and tailoring prevention strategies to high-risk groups remain essential priorities for sustaining control of blood-borne infections in the post-pandemic era.[41]

CONCLUSION

The integrated analysis of HIV, HBV, and HCV seroprevalence in Northern Cyprus highlights important trends in disease prevalence. It suggests that while public health initiatives have contributed to reductions in HBV and HIV rates, there is a need for enhanced focus on HCV prevention and gender-targeted interventions. As these diseases continue to pose challenges to public health, prevention, education, and treatment strategies must evolve to meet the changing needs of the population. This study provides a five-year overview of seroreactivity trends during and after the COVID-19 pandemic. However, it is limited by its single-centre scope, lack of detailed patient risk-factor data, and absence of confirmatory or nucleic acid amplification testing follow-up. Such follow-up could have provided a more comprehensive understanding of the true infection burden and diagnostic accuracy. The rebound in testing volumes and sustained high positivity rates underscore the need for adaptable, resilient public health systems that can preserve essential surveillance during crises.

MAIN POINTS

- The current research provides a five-year (2020-2024) overview of the patterns of seroreactivity to hepatitis B virus, hepatitis C virus (HCV), and human immunodeficiency virus (HIV) in Northern Cyprus during the coronavirus disease 2019 pandemic.
- HBV showed the highest seroreactivity rate but demonstrated a consistent decline over the study period, supporting the long-term impact of vaccination and prevention programs.
- The rate of HCV seroreactivity remained below 1%, but showed a slight increase, indicating the need for continuous monitoring and preventive measures.
- Seroreactivity to HIV stays low and steady during the study period despite the clear male predominance among the positive results.
- Continuous screening campaigns and a strong public health surveillance system are vital for controlling blood-borne diseases.

ETHICS

Ethics Committee Approval: This study was approved by the Near East University Scientific Research Ethics Committee (approval number: 2025/138, date: 28.11.2025).

Informed Consent: Due to the retrospective nature of the study and the use of anonymised laboratory data, the requirement for informed patient consent was waived by the Ethics Committee.

Footnotes

Authorship Contributions

Concept: B.K., M.K., K.S., Design: B.K., M.K., K.S., Data Collection and/or Processing: B.K., M.K., E.D., K.S., Analysis and/or Interpretation: B.K., M.K., E.D., K.S., Literature Search: B.K., M.K., E.D., Writing: B.K., M.K., E.D.

DISCLOSURES

Conflict of Interest: One author of this article, Kaya Süer, is a member of the Editorial Board of the Cyprus Journal of Medical Sciences. However, he was not involved in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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Evaluation of the C-Reactive Protein to HDL Cholesterol Ratio as a Marker of Cardiovascular and Renal Risk Among Type 2 Diabetic Patients

Irena Kostovska, Svetlana Cekovska, Katerina Tosheska Trajkovska, Sonja Topuzovska

Department of Institute of Medical and Experimental Biochemistry, Ss. Cyril and Methodius University in Skopje Faculty of Medicine, Skopje, North Macedonia

Abstract

BACKGROUND/AIMS: Type 2 diabetes (T2D) is a metabolic disorder commonly accompanied by persistent low-grade inflammation, accelerated development of atherosclerosis, and gradual decline in renal function. The ratio of C-reactive protein (CRP) to high-density lipoprotein-cholesterol (HDL-C) has recently been proposed as a composite biomarker reflecting inflammatory activity and anti-atherogenic capacity. The present study aimed to investigate the relationship between the CRP/HDL-C ratio and the presence of coronary artery disease (CAD) as well as the stages of chronic kidney disease (CKD) in individuals with T2D.

MATERIALS AND METHODS: A total of 148 individuals with T2D were enrolled in this cross-sectional study: 56 patients without CAD and 92 patients with CAD confirmed by angiography; additionally, 44 healthy controls were included. Renal function and CKD staging were determined from the estimated glomerular filtration rate (eGFR) calculated using the CKD-epidemiology collaboration equation. Clinical characteristics and a broad range of biochemical, inflammatory, lipid, and renal markers were evaluated, and the CRP/HDL-C ratio was derived for each participant. Statistical analysis included intergroup comparisons, Spearman correlation testing, and receiver operating characteristic (ROC) curve analysis.

RESULTS: T2D patients, particularly those with CAD, had significantly higher CRP levels, CRP/HDL-C ratios, blood pressure, glucose levels, abnormal lipid levels, and lower eGFR than controls ($p<0.001$). Progressive stages of CKD were associated with worsening renal function, elevated CRP levels, poor glycemic control, and dyslipidemia. The CRP/HDL-C ratio correlated positively with age, body mass index, fasting blood glucose, glycated hemoglobin, total cholesterol, triacylglycerols, low-density lipoprotein cholesterol, and blood pressure, and correlated negatively with eGFR and serum creatinine. ROC analysis demonstrated that the CRP/HDL-C ratio had good discriminatory ability for the presence of CAD (area under the curve 0.82; 95% confidence interval: 0.740-0.890), with an optimal cut-off value of 2.2, sensitivity of 78%, and specificity of 75%.

CONCLUSION: The CRP/HDL-C ratio is significantly associated with cardiovascular and renal complications in T2D. An elevated ratio reflects increased inflammatory burden, dyslipidemia, and renal impairment, supporting its potential role as a simple and accessible biomarker for cardio-renal risk assessment in patients with T2D.

Keywords: CRP/HDL-C ratio, diabetic nephropathy, chronic kidney disease, coronary artery disease

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ORCID IDs of the authors: I.K. 0000-0003-0971-6710; S.C. 0000-0001-7152-1725; K.T.T. 0000-0002-7636-4631; S.T. 0000-0001-5020-1861.

Corresponding author: Irena Kostovska
E-mail: irena.kostovska@medf.ukim.edu.mk
ORCID ID: orcid.org/0000-0003-0971-6710

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INTRODUCTION

Type 2 diabetes (T2D) is a multifactorial metabolic condition defined not only by sustained hyperglycemia but also by ongoing low-grade systemic inflammation and enhanced atherogenesis. Individuals with T2D have a substantially increased risk of coronary artery disease (CAD) and chronic kidney disease (CKD), which are leading causes of cardiovascular morbidity and mortality worldwide.[1] The coexistence of coronary and renal complications reflects shared pathophysiological mechanisms, including endothelial dysfunction, oxidative stress, lipid abnormalities, and systemic inflammation. C-reactive protein (CRP) is a widely recognized biomarker of systemic inflammation and has been repeatedly linked to adverse cardiovascular outcomes as well as the progression of diabetic kidney disease. Elevated CRP levels promote endothelial dysfunction, increase vascular permeability, and contribute to atheromatous plaque instability.[2] However, inflammation in T2D is not solely defined by elevated pro-inflammatory markers; it is also characterized by qualitative and quantitative alterations in protective lipoproteins. High-density lipoprotein-cholesterol (HDL-C) is known to have anti-inflammatory and antioxidant properties and contributes to endothelial protection and vascular homeostasis. In the diabetic milieu, HDL undergoes structural and functional modifications, losing its anti-atherogenic properties. Reduced HDL concentrations, together with increased inflammatory burden, create a pro-atherogenic environment that accelerates both vascular and renal injury.[3] The CRP to HDL-cholesterol ratio (CRP/HDL-C ratio) integrates these opposing biological processes into a single inflammatory index, reflecting the balance between systemic inflammation and anti-atherogenic capacity. Emerging evidence suggests that combined inflammatory-lipid indices may have superior predictive value compared to isolated biomarkers.[4] However, data on the association between the CRP/HDL-C ratio and CAD and CKD stages in T2D remain limited. In addition to systemic inflammation, vascular-specific inflammatory pathways contribute to diabetic complications. The interplay between systemic inflammatory markers, lipid dysfunction, vascular inflammation, and renal impairment represents a critical yet insufficiently explored area in diabetic patients.[5] Therefore, this study was designed to examine the relationship between the CRP/HDL-C ratio and the presence and extent of CAD and the severity across CKD stages, and to evaluate its potential role as a simple inflammatory index reflecting combined cardio-renal risk in patients with T2D.

We hypothesized that a higher CRP/HDL-C ratio would be significantly associated with both CAD and progressive renal dysfunction, thereby supporting its potential utility as an accessible, clinically relevant biomarker in routine clinical practice.

MATERIALS AND METHODS

Study Design and Participants

This cross-sectional study enrolled 192 individuals, including 148 patients with T2D and 44 apparently healthy participants who served as a control group (50% male, 50% female). The diagnosis of T2D was established in accordance with the criteria of the American Diabetes Association.[6] All individuals with T2D had a documented disease duration and received regular clinical follow-up. Patients with T2D were stratified into two groups: those without documented CAD (n=56, 65% male, 35% female) and those with CAD confirmed by coronary angiography (n=92, 61% male, 39% female). Patients with diabetic nephropathy were further categorized according to estimated glomerular filtration rate (eGFR) and staged for CKD using the CKD-epidemiology collaboration (CKD-EPI) equation as follows: stage II (n=47), stage IIIa (n=40), stage IIIb (n=54), and stage IV (n=7). The control group consisted of apparently healthy individuals with no history of diabetes, cardiovascular disease, or CKD. The control participants were not individually age-matched to the T2D groups. Inclusion criteria: age ≥18 years, diagnosed T2D, available complete biochemical and clinical data, for the CAD group: angiographically confirmed CAD. Exclusion criteria: acute inflammatory or infectious disease, malignancy, autoimmune disorders, severe hepatic dysfunction, acute coronary syndrome within the previous 3 months. For all participants, the following data were collected: age, sex, body weight, height, body mass index (BMI), glycemic control parameters, duration of diabetes, and smoking status. Blood pressure was assessed using standardized measurement procedures following a 10-minute resting period.

Laboratory Investigations

Venous blood samples were obtained following a 12-hour overnight fast. Serum concentrations of the following parameters were measured using standardized, automated photometric methods: fasting glucose, total cholesterol, triacylglycerols, HDL-C, low-density lipoprotein cholesterol (LDL-C), blood urea, and creatinine. CRP levels were determined using an immunoturbidimetric assay. Renal function was assessed using eGFR calculated from the CKD-EPI creatinine equation. CKD stages were defined according to Kidney Disease: Improving Global Outcomes guidelines based on eGFR values. The CRP/HDL-C ratio was computed using the formula: CRP/HDL-C ratio = CRP (mg/L) divided by HDL-C (mg/dL). Prior to analysis, all values were standardized to ensure uniformity across measurements. For unit conversion of HDL-C from mmol/L to mg/dL, a conversion factor of 38.67 was applied (i.e., mmol/L ×38.67).

Ethical Considerations

The study protocol was reviewed and approved by Ss. Cyril and Methodius University in Skopje Faculty of Medicine Ethics Committee for Human Research (approval no: 03-5602/11, date: 16.12.2022). All participants provided written informed consent prior to enrollment. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and its subsequent amendments.

Statistical Analysis

Statistical analysis was performed using MedCalc for Windows, version 23.1.7 (MedCalc Software, Ostend, Belgium). The normality of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed variables are presented as mean ± standard deviation, while non-normally distributed variables were analyzed using non-parametric methods. For comparisons between two groups, Student's t-test was applied to normally distributed variables, and the Mann-Whitney U test to non-normally distributed variables. For comparisons among more than two groups, one-way analysis of variance with appropriate post-hoc analysis was used for normally distributed variables, whereas the Kruskal-Wallis test was applied for non-normally distributed variables. Categorical variables were compared using the chi-square test. Correlations between continuous variables were assessed using Spearman's correlation analysis because several variables did not meet normality assumptions. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminatory ability of the

CRP/HDL-C ratio for CAD. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Comparison of Clinical and Laboratory Characteristics in T2D Patients, with and without CAD, and in Healthy Controls

Patients with T2D differed significantly from healthy controls in several clinical and laboratory parameters, including glycemic control, blood pressure, lipid profile, inflammatory markers, and renal function. Overall comparisons among the three study groups revealed significant differences in CRP, the CRP/HDL-C ratio, eGFR, serum creatinine, fasting blood glucose, and lipid parameters (Table 1). Patients with CAD generally exhibited less favorable metabolic, inflammatory, and renal profiles than those without CAD; however, the overall p-values presented in Table 1 reflect comparisons across all three groups.

Comparison of Clinical and Laboratory Characteristics in T2D Patients Stratified by CKD Stage and Healthy Controls

The data demonstrate that advancing CKD in patients with T2D is associated with worsening renal impairment, poorer glycemic control, worsening dyslipidemia, and heightened systemic inflammation. Serum creatinine, blood urea, eGFR, glycated hemoglobin (HbA1c), triacylglycerols, and CRP were strongly associated with CKD stage progression and may serve as useful indicators of disease severity. In contrast, BMI, duration of diabetes, and LDL cholesterol did not differ significantly across CKD stages, suggesting a weaker association with renal disease progression in this study population. Table 2 presents the results.

Table 1. Comparison of clinical and laboratory characteristics in T2D patients, with and without CAD, and in healthy controls

Clinical and laboratory characteristics	T2D patients without CAD n=56	T2D patients with CAD n=92	Healthy controls n=44	p-value
Age (years)	58.2±6.005	54.6±6.4	40.5±8.13	<0.001
Male/female (%)	65/35	61/39	50/50	/
BMI (kg/m^2)	28.93±0.67	27.8±0.74	26.5±0.68	0.029
Duration of T2D (years)	6.78±2.62	7.1±2.75	/	0.324
Smoking	68%	62%	54%	0.367
Alcohol	41%	37%	28%	0.325
Blood glucose (mmol/L)	7.46±1.6	7.61±1.45	4.73±0.8	<0.001
HbA1c (%)	7.45±1.45	6.46±1.5	4.36±0.5	<0.001
SBP (mm/Hg)	155.5±2.23	157.04±2.14	125±1.54	<0.001
DBP (mm/Hg)	87.7±0.95	91.93±1.06	87.7±0.95	0.0179
CRP (mg/L)	111.86±9.08	135.25±10.05	2.25±0.13	<0.001
Total cholesterol (mmol/L)	6.93±1.38	7.14±1.36	4.93±0.08	<0.001
Triacylglycerols (mmol/L)	5.02±2.2	5.08±3.02	4.08±1.23	<0.001
HDL-C (mg/dL)	41.04±19.4	27.6±19.5	67.3±11.3	<0.001
LDL-C (mmol/L)	4.7±1.54	4.54±2.14	3.3±0.08	<0.001
Blood urea (mmol/L)	7.83±2.78	8.4±3.1	4.4±2.1	<0.001
Serum creatinine (mmol/L)	128.5±5.2	135.5±3.07	66.7±2.08	<0.001
CRP/HDL-C ratio	2.1±1.7	2.34±0.34	0.009±0.003	<0.001
eGFR (mL/min per 1.73 m^2)	79.4±11.2	50.05±13.7	132.6±22.3	<0.001

Results are expressed as mean ± standard deviation.
T2D: Type 2 diabetes, HbA1c: Glycated hemoglobin, BMI: Body mass index, eGFR: Estimated glomerular filtration rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, CRP: C-reactive protein, HDL-C: High-density lipoproteins-cholesterol, LDL-C: Low-density lipoproteins-cholesterol, CAD: Coronary artery disease.

Table 2. Comparison of clinical and laboratory characteristics in T2D patients stratified by CKD stage and healthy controls

	Stage II n=47	Stage IIIa n=40	Stage IIIb n=54	Stage IV n=7	Healthy subjects n=44	p-value
Age (years)	52.3±5.6	56.3±6.7	59.5±5.05	58.8±4.1	40.5±8.1	<0.01
BMI (kg/m^2)	28.4±4.2	29.1±6.6	29.5±5.1	32.4±5.04	22.3±2.4	0.39
Duration of T2D (years)	6.5±2.8	5.7±1.9	6.7±1.6	8.8±1.5	/	0.56
Blood glucose (mmol/L)	6.4±0.6	7.5±0.9	7.5±1.2	7.8±0.5	4.7±0.4	<0.001
HbA1c (%)	6.3±1.2	6.1±1.3	6.7±1.6	8.8±1.5	4.36±0.5	<0.001
Total cholesterol (mmol/L)	6.8±0.9	6.9±0.9	7.01±0.9	7.5±1.1	4.9±0.6	<0.001
Blood urea (mmol/L)	7.7±1.6	7.8±2.1	9.1±1.6	9.7±1.3	4.3±1.3	<0.001
Serum creatinine (mmol/L)	99.3±12.6	130.1±19.2	152.2±20.1	207.7±26.8	66.7±13.8	<0.001

Table 2. Continued

	Stage II n=47	Stage IIIa n=40	Stage IIIb n=54	Stage IV n=7	Healthy subjects n=44	p-value
Triacylglycerols (mmol/L)	4.72±1.2	5.28±1.02	5.08±1.3	5.6±1.5	5.4±2.1	0.15
HDL-C (mg/dL)	54.9±17.8	46.2±10.05	12.1±69.9	12.5±30.9	67.3±11.3	<0.001
LDL-C (mmol/L)	4.7±1.54	4.4±1.14	4.3±0.8	4.2±1.1	4.5±1.7	0.123
CRP (mg/L)	54.7±12.7	66.5±21.4	89.8±15.4	115.2±24.4	2.25±0.13	<0.001
CRP/HDL-C ratio	0.99±1.04	1.43±2.12	0.8±0.22	2.71±0.78	0.13±0.01	<0.001

Results are expressed as mean ± SD.
T2D: Type 2 diabetes, HbA1c: Glycated hemoglobin, BMI: Body mass index, eGFR: Estimated glomerular filtration rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, CRP: C-reactive protein, HDL-C: High-density lipoproteins-cholesterol, LDL-C: Low-density lipoproteins-cholesterol, CKD: Chronic kidney disease, SD: Standard deviation.

Correlation of the CRP/HDL-C Ratio with Clinical and Laboratory Parameters in Study Subjects

Spearman correlation analysis demonstrated that the CRP/HDL-C ratio was positively correlated with several metabolic and cardiovascular risk factors. Specifically, higher CRP/HDL-C ratios were moderately correlated with age (ρ=0.54), BMI (ρ=0.34), total cholesterol (ρ=0.51), triglycerides (ρ=0.54), LDL-C (ρ=0.42), and blood pressure (systolic blood pressure: ρ=0.55; diastolic blood pressure: ρ=0.51). Strong positive correlations were observed with fasting blood glucose (ρ=0.70) and HbA1c (ρ=0.71), indicating that poorer glycemic control was associated with higher CRP/HDL-C ratios. Conversely, the CRP/HDL-C ratio was negatively correlated with blood urea (ρ=-0.60), serum creatinine (ρ=-0.68), and eGFR (ρ=-0.78), suggesting an association with declining renal function. The duration of T2D showed only a weak correlation (ρ=0.02), indicating that the CRP/HDL-C ratio may reflect the current metabolic and inflammatory status rather than disease duration. These findings support the potential value of the CRP/HDL-C ratio as an integrative marker linking metabolic dysregulation, cardiovascular risk, and renal impairment in patients with T2D. Table 3 presents the results.

Table 3. Correlation of the CRP/HDL-C ratio with clinical and laboratory parameters in study subjects

Clinical and laboratory parameters	CRP/HDL-C ratio
	Spearman ρ
Age (years)	0.54
Duration of T2D (years)	0.02
BMI (kg/m²)	0.34
Glucose (mmol/L)	0.7
HbA1c (%)	0.71
Total cholesterol (mmol/L)	0.51
Triacylglycerols (mmol/L)	0.54
LDL-C (mmol/L)	0.42
Blood urea (mmol/L)	-0.6
Serum creatinine (µmol/L)	-0.68
SBP (mm/Hg)	0.55
DBP (mm/Hg)	0.51
eGFR (mL/min per 1.73 m²)	-0.78

HbA1c: Glycated hemoglobin, BMI: Body mass index, eGFR: Estimated glomerular filtration rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HDL-C: High-density lipoproteins-cholesterol, LDL-C: Low-density lipoproteins-cholesterol. CRP: C-reactive protein.

Diagnostic Performance of the CRP/HDL-C Ratio for Discrimination of CAD

ROC curve analysis demonstrated that the CRP/HDL-C ratio had good discriminatory ability for detecting CAD. The area under the curve was 0.82 (95% confidence interval: 0.740-0.890; p<0.0001), with an optimal cut-off value of <2.2, yielding a sensitivity of 78% and a specificity of 75%. The positive and negative predictive values were 87% and 70%, respectively. These findings suggest that the CRP/HDL-C ratio may be a useful and accessible biomarker for identifying T2D patients with CAD. However, because ROC analysis was performed within the same cohort without external validation, these results should be considered exploratory and should be confirmed in independent populations (Table 4).

Table 4. Diagnostic performance of the CRP/HDL-C Ratio for discrimination of CAD

The area under the ROC curve (AUC)	0.82
95% confidence interval (95% CI)	0.740-0.890
Significance level p (area=0.5)	<0.0001
Youden index J	0.53
Optimal cut-off	<2.2
Sensitivity %	78
Specificity %	75
NPV-negative predictive value %	70
PPV-positive predictive value %	87

CRP/HDL-C: C-reactive protein/high-density lipoprotein cholesterol, CAD: Coronary artery disease, ROC: Receiver operating characteristic.

DISCUSSION

The study indicates a significant association of the CRP/HDL ratio with cardiovascular and renal complications in patients with T2D. Patients with T2D, particularly those with angiographically confirmed CAD, exhibited significantly higher CRP levels and CRP/HDL-C ratios, impaired renal function, poorer glycemic control, and more pronounced lipid abnormalities compared with patients with T2D without CAD and healthy controls. These findings support the hypothesis that the imbalance between systemic inflammation and protective HDL-C-mediated anti-atherogenic activity contributes to the progression of both macrovascular and renal complications in T2D.[7] Inflammation is a key mechanism in the development of both atherosclerosis and diabetic nephropathy. CRP is a widely recognized marker of inflammation that has been linked to endothelial dysfunction, oxidative stress, instability of atherosclerotic plaques,

and vascular damage. Conversely, HDL-C exerts anti-inflammatory, antioxidant, and endothelial-protective effects. In patients with diabetes, HDL particles undergo structural and functional modifications, leading to reduced anti-atherogenic capacity. The CRP/HDL-C ratio therefore reflects the combined influence of increased inflammatory burden and impaired lipid-mediated vascular protection, making it a potentially valuable integrative biomarker of cardio-renal risk.[4] In this study, the overall comparison demonstrated that CRP/HDL-C ratios differed significantly among the three study groups, with the highest values observed in patients with CAD. ROC analysis demonstrated that the CRP/HDL-C ratio had good discriminatory ability to identify CAD in patients with T2D. However, because the ROC analysis was performed within the same cohort and lacked external or internal validation, these findings should be interpreted cautiously. Future studies using independent cohorts and appropriate validation methods are required to confirm the clinical utility of this marker. Similar observations have been reported in previous studies, which have shown that combined inflammatory-lipid indices may predict cardiovascular events more accurately than isolated inflammatory or lipid parameters.[4,8,9] The present study also demonstrated a significant association between the CRP/HDL-C ratio and renal dysfunction. Patients with more advanced stages of CKD exhibited higher CRP concentrations, worsening renal function parameters, and altered lipid profiles. Although the CRP/HDL-C ratio did not increase completely linearly across all CKD stages, the highest values were observed in advanced renal impairment, particularly stage IV CKD, suggesting that progressive renal dysfunction is accompanied by enhanced systemic inflammation and reduced anti-atherogenic protection. These results align with earlier research showing that persistent inflammation plays a major role in the development and progression of diabetic kidney disease as well as cardiovascular complications.[9,10] Correlation analysis further supported the relationship between the CRP/HDL-C ratio and metabolic, cardiovascular, and renal abnormalities. Higher CRP/HDL-C ratios were positively correlated with age, BMI, blood pressure, fasting blood glucose, HbA1c, total cholesterol, triglycerides, and LDL-C levels, indicating that an increased inflammatory burden is closely associated with poorer metabolic control and a less favorable cardiovascular risk profile. In addition, the CRP/HDL-C ratio showed significant correlations with renal function parameters, including serum creatinine and eGFR, suggesting an association with renal dysfunction in patients with T2D. The weak correlation with diabetes duration indicates that the CRP/HDL-C ratio may reflect the current inflammatory and metabolic status rather than cumulative disease duration. The clinical implications of these findings are important. The CRP/HDL-C ratio is inexpensive, widely available, and easily calculated from routine laboratory parameters. Therefore, it may be used as a practical marker for early identification of T2D patients at increased risk of cardiovascular and renal complications. Incorporating this ratio into routine clinical assessment could improve risk stratification and facilitate earlier preventive interventions that target inflammation and dyslipidemia and provide cardio-renal protection.[11,12]

Study Limitations

Several limitations of the present study should be considered. First, its cross-sectional design precludes causal inference. Second, the absence of an a priori sample size calculation represents another limitation of this study. Although significant associations were identified, the sample size, particularly within advanced CKD stages, may have limited the statistical power. Future studies should include prospective sample size estimation and larger populations to validate these findings. Third, because this was a single-center study, the generalizability of the findings may be limited. Furthermore, multivariable regression analyses were not performed, thereby restricting the ability to determine independent predictive associations. An additional limitation is the age difference between the control group and the T2D mellitus (T2DM) groups. Although the control participants were healthy individuals without diabetes or cardiovascular disease, they were younger than the diabetic participants. Because age is an important determinant of inflammation, renal function, lipid metabolism, and cardiovascular risk, residual confounding by age cannot be excluded. Future studies with age-matched controls or analyses adjusted for age and other relevant confounders are needed to confirm these findings. Another limitation is that multiple comparisons were performed among several clinical and biochemical variables without formal adjustment for multiple testing. Therefore, the possibility of a type I error cannot be completely excluded, and the findings should be considered exploratory and should be confirmed in future studies using appropriate correction methods. Prospective, multicenter studies with larger cohorts are needed to validate the prognostic significance of the CRP/HDL-C ratio. In addition, studies incorporating multivariable regression analyses are warranted to determine whether the observed associations are independent of established cardiovascular and renal risk factors. Further research should evaluate whether interventions targeting systemic inflammation and improving HDL-C functionality can reduce cardio-renal risk in patients with T2D.

CONCLUSION

The CRP/HDL-C ratio is associated with cardiovascular and renal complications in patients with T2D. Its good discriminatory performance for CAD suggests potential utility as an accessible marker for cardio-renal risk assessment; however, further studies with external validation and adjusted analyses are required before clinical implementation.

MAIN POINTS

- The C-reactive protein/high-density lipoprotein-cholesterol (CRP/HDL-C) ratio was significantly higher in patients with T2D mellitus (T2DM) than in healthy controls and was highest in patients with coronary artery disease (CAD) and advanced chronic kidney disease (CKD).
- Higher CRP/HDL-C ratios were associated with poorer glycemic control, dyslipidemia, elevated blood pressure, and declining renal function.
- The CRP/HDL-C ratio demonstrated good discriminatory ability for identifying CAD, with an area under the curve of 0.82 (95% confidence interval: 0.740-0.890), a sensitivity of 78%, and a specificity of 75%.
- Progressive CKD stages in patients with T2DM were associated with increased systemic inflammation, worsening renal function, and adverse lipid profiles.
- The CRP/HDL-C ratio is a simple, inexpensive, and readily available biomarker that may help assess combined cardiovascular and renal risk in patients with T2DM.

ETHICS

Ethics Committee Approval: The study protocol was reviewed and approved by Ss. Cyril and Methodius University in Skopje Faculty of Medicine Ethics Committee for Human Research (approval no: 03-5602/11, date: 16.12.2022).

Informed Consent: All participants provided written informed consent prior to enrollment.

Footnotes

Authorship Contributions

Surgical and Medical Practices: I.K., Concept: I.K., Design: I.K., Data Collection and/or Processing: I.K., K.T.T., Analysis and/or Interpretation: S.C., S.T., Literature Search: I.K., Writing: I.K.

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RESEARCH ARTICLE

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Blood Urea Nitrogen to Albumin Ratio in Acute Pancreatitis: A Potential Supportive Marker for Early Triage between Ward and Intensive Care

Mehmet Erdem[1], Tuğba Raika Kıran[1], Sevgi Yumrutepe[2], Feyza İnceoğlu[3]

[1]Department of Medical Biochemistry, Malatya Turgut Özal University Faculty of Medicine, Malatya, Türkiye
[2]Department of Emergency Medicine, Malatya Turgut Özal University Training and Research Hospital, Malatya, Türkiye
[3]Department of Biostatistics and Medical Informatics, Malatya Turgut Özal University Faculty of Medicine, Malatya, Türkiye

Abstract

BACKGROUND/AIMS: This study aimed to evaluate the prognostic significance of the blood urea nitrogen (BUN) to albumin ratio (BAR) in patients with acute pancreatitis (AP) by comparing the clinical, biochemical, and hematological profiles of patients directly admitted to the ward and the intensive care unit (ICU). Unlike previous studies that primarily evaluated BAR in critically ill populations or focused on mortality outcomes, the present study investigates its potential role in the early clinical assessment of AP by comparing ward and ICU patients.

MATERIALS AND METHODS: This study was a retrospective, single-center analysis of the medical records of patients with AP admitted to the ward or the ICU. Biochemical and hematological data, including BAR values, were obtained from initial laboratory measurements at hospital admission. Patients were stratified into two groups based on their initial admission location; comparisons between groups were performed using appropriate statistical tests to assess the relationship between BAR levels and illness severity.

RESULTS: Among the 111 patients included (59 ward and 52 ICU), BAR levels were found to be significantly higher in those requiring ICU-level care (p=0.001). Receiver operating characteristic (ROC) analysis demonstrated that BAR exhibited good discriminatory performance in distinguishing patients admitted to the ICU from those admitted to the ward (area under the curve =0.836), supporting its potential role as a marker for early risk stratification.

CONCLUSION: BAR is a simple, cost-effective biomarker that may support early risk stratification in patients with AP. Elevated BAR levels at admission may help identify patients who require closer monitoring or ICU-level care. The favorable discriminative performance observed in ROC analysis suggests that BAR may complement, rather than replace, clinical assessment in early decision-making. However, prospective multicenter studies are needed to validate these findings and establish optimal cut-off values.

Keywords: Acute pancreatitis, blood urea nitrogen to albumin ratio, intensive care, risk stratification, ward

INTRODUCTION

Acute pancreatitis (AP) is characterized by an inflammatory process initiated by the intra-pancreatic activation of digestive enzymes, leading to local tissue injury and systemic inflammatory responses.[1] This leads to autodigestion, inflammation, edema, and even necrosis of the pancreas.[2] The clinical presentation of AP varies considerably, ranging from mild, self-limiting forms to severe disease associated with systemic complications and organ failure.[3] AP is one of the leading causes of gastrointestinal-related hospital admissions worldwide, with reported

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ORCID IDs of the authors: M.E. 0000-0002-9100-2479; T.R.K. 0000-0002-3724-0249; S.Y. 0000-0003-4051-6696; F.İ. 0000-0003-1453-0937.

Corresponding author: Mehmet Erdem
E-mail: mehmet.erdem@ozal.edu.tr
ORCID ID: orcid.org/0000-0002-9100-2479

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incidence rates ranging from approximately 13 to 45 cases per 100,000 individuals.[3-5] A nationwide Turkish study (n~2144) similarly reported biliary causes as the leading etiology (~67%), followed by idiopathic causes (12%), hyperlipidemic causes (6%), and alcoholic causes (4.2%). In this cohort, 73% of cases were mild, while only 2.6% progressed to severe AP.[6] Recognizing patients at higher risk for unfavorable outcomes at an early stage remains challenging, given that clinical status may worsen rapidly despite an initially mild presentation.[2,3] Despite extensive investigation of various laboratory parameters such as C-reactive protein (CRP), procalcitonin, blood urea nitrogen (BUN), and lactate, their applicability in early clinical decision-making remains constrained due to their limited specificity and susceptibility to confounding by systemic factors.[7]

BUN reflects renal perfusion, catabolic status, and systemic inflammation; elevated BUN on admission has been associated with increased mortality in AP.[8] Similarly, serum albumin is a negative acute-phase reactant and an indicator of nutritional and inflammatory status, with hypoalbuminemia linked to worse outcomes in critically ill patients, including those with AP.[9] Recent studies have proposed the BUN to albumin ratio (BAR) as a simple, cost-effective, and clinically accessible prognostic marker in sepsis, severe coronavirus disease 2019 (COVID-19), and acute exacerbations of chronic obstructive pulmonary disease. Because BAR incorporates both inflammatory and metabolic components, it may better reflect systemic stress responses than either BUN or albumin alone.[10-12] In recent years, growing evidence has supported the prognostic significance of BAR in patients with AP. These studies have reported that elevated BAR levels are associated with increased disease severity and higher short- and long-term mortality rates in AP. Therefore, BAR has been proposed as a simple and readily available biomarker that may help clinicians identify high-risk patients at an early stage.[13-15] Given that intensive care unit (ICU) admission in AP is frequently associated with persistent organ failure and markedly elevated mortality, there is a need for pragmatic biomarkers that can discriminate risk at the time of hospital presentation.[16]

This study investigated the relationship between BAR levels and disease severity in AP by comparing patients managed in the ward to those requiring ICU admission at initial presentation. Unlike previous studies that focused primarily on mortality or on critically ill patients, the present study examines the potential utility of BAR in early triage to determine whether it provides supportive information for initial clinical decision-making about the appropriate level of care.

MATERIALS AND METHODS

Study Plan and Participants

This retrospective, single-center, observational study was conducted at Malatya Turgut Özal University Training and Research Hospital, Malatya, Türkiye. All adult patients who presented to the emergency department with abdominal pain suggestive of AP during the study period were evaluated retrospectively. Patients were allocated to the ward or the ICU based on their clinical status at presentation. Patients who were hemodynamically stable, had no evidence of organ dysfunction, and did not require close monitoring or advanced supportive care were admitted to the ward. In contrast, patients with hemodynamic instability, respiratory compromise requiring support, acute kidney injury or oliguria, altered mental status, or evidence of significant organ dysfunction were admitted to the ICU. Admission decisions were made by experienced clinicians based on an integrated assessment of clinical findings and laboratory results, in accordance with routine institutional practice. A total of 111 patients admitted between October 1, 2024, and March 31, 2025 (59 to the ward and 52 to the ICU) were included in the analysis, and all relevant biochemical markers were retrieved from the electronic medical records. Comorbid conditions such as hypertension, diabetes mellitus, coronary artery disease, and chronic kidney disease were documented. Individuals with incomplete clinical or laboratory data were excluded from further evaluation.

Ethical approval for the study was granted by the Malatya Turgut Özal University Health Sciences Scientific Research Ethics Committee (approval no: 2025/208, date: 30.07.2025). The study protocol adhered to the principles outlined in the Declaration of Helsinki and relevant institutional and national guidelines. Patient confidentiality and data protection were strictly maintained throughout the study.

Definition

During the study period, adult patients presenting with clinical suspicion of AP were screened. The diagnosis was established by meeting at least two of the three criteria defined by the Revised Atlanta classification:

(1) Characteristic abdominal pain,

(2) Serum amylase/lipase ≥3× the upper limit of normal,

(3) Imaging findings consistent with AP.[17]

The study population consisted of patients aged 18 years or older who met the revised Atlanta criteria for AP and for whom comprehensive clinical and laboratory data were available at initial presentation. Patients were excluded from the analysis if any admission-time variable was missing or could not be assessed. Individuals with chronic pancreatitis, pancreatic malignancy, or a history of pancreatic surgery were not eligible. Pregnant patients and those under 18 years of age were also excluded. Cases representing recurrent episodes during the study period were omitted, with only the initial (index) admission considered. Patients whose diagnosis could not be reliably supported by imaging or biochemical findings were similarly excluded. In addition, individuals who left the hospital against medical advice, those transferred to another healthcare facility, and patients hospitalized primarily for another condition in which AP was a secondary diagnosis were not included in the study. In this cohort, biliary etiology accounted for the majority of AP cases, consistent with epidemiological patterns reported in Türkiye. One patient had hereditary spherocytosis as an underlying hematological condition, and another had a history of chronic alcohol consumption without a diagnosis of chronic pancreatitis.

Patients were allocated to the ward or the ICU based on their clinical status at presentation. Patients who were hemodynamically stable, without evidence of organ dysfunction, and who did not require close monitoring or advanced supportive care were admitted to the ward. In contrast, patients with hemodynamic instability, respiratory compromise requiring support, acute kidney injury or oliguria, altered mental status, or evidence of significant organ dysfunction were admitted to the ICU. Admission decisions were made by experienced clinicians based on an integrated assessment of clinical findings and laboratory results, in accordance with routine institutional practice.

Data Sources and Collection

Clinical characteristics, demographic data, laboratory results, and disease severity information were obtained from the hospital's electronic health record database. All analyses were based on laboratory measurements obtained from the initial blood samples collected at hospital presentation for both ward and ICU patients.

Serum biochemical parameters, including urea, amylase, lipase, glucose, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin (TBil), direct bilirubin (DBil), total protein, albumin, calcium (Ca^{2+}), sodium (Na^{+}), potassium (K^{+}), and CRP, were measured using an automated biochemistry analyzer (Abbott Architect c16000, Illinois, United States of America).

Hematological parameters, including white blood cell (WBC) count, platelet count, hemoglobin, hematocrit, neutrophil, lymphocyte, and monocyte counts, were analyzed using an automated hematology analyzer (Sysmex XN-10, Sysmex Corporation, Kobe, Japan).

Statistical Analysis

Statistical analyses were carried out using SPSS software (version 28.0; IBM Corp., Armonk, NY, USA) and R (version 4.3.1). Continuous variables were summarized as medians interquartile range, while categorical variables were presented as counts and percentages. The distributions of variables were evaluated using the Kolmogorov-Smirnov test. As the data did not follow a normal distribution, group comparisons were performed using the Mann-Whitney U test. The discriminative ability of urea, BUN, and BAR for predicting ICU admission was assessed using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) with 95% confidence intervals (CIs), optimal cut-off values based on the Youden index, and sensitivity and specificity with their corresponding 95% CIs were calculated. Differences between AUCs were examined using the DeLong test. To adjust for the potential confounding effect of age while accounting for the non-normal distribution of the data, a rank-based analysis of covariance (rank-based ANCOVA) was performed. The dependent variables (urea, BUN, and BAR) and age were rank-transformed; admission group (ward vs. ICU) was included as the fixed factor, with age entered as the covariate. A two-sided p-value of <0.05 was considered statistically significant.

RESULTS

Gender Characteristics and Age Comparison Between Ward and ICU Groups

A total of 111 patients were included, of whom 59 (53.2%) were admitted to the ward and 52 (46.8%) were admitted to the ICU. Sex distribution did not differ significantly between the ward and ICU groups (p=0.277) (Table 1). However, patients admitted to the ICU were significantly older than those admitted to the ward [77.00 (63.75-80.00) vs. 62.00 (53.50-73.50), p=0.001]. These findings indicate that older age was more common among patients who required ICU care than among those managed on the ward, suggesting a possible association between advanced age and the need for higher-level clinical support (Table 2).

Comparison of Clinical Parameters between Ward and ICU Patients

Serum urea, creatinine, TBil, DBil, CRP, and neutrophil levels were significantly higher in ICU patients than in those admitted to the ward ($p<0.05$). In contrast, total protein, albumin, Ca^{2+}, and lymphocyte levels were significantly higher among ward patients ($p<0.05$). No significant differences were observed between the groups in terms of amylase, lipase, glucose, AST, ALT, ALP, GGT, Na^{+}, K^{+}, WBC, platelet, hemoglobin, hematocrit, and monocyte levels ($p>0.05$) (Table 3).

Evaluation of BUN and BAR Levels

Median BUN levels were significantly higher in ICU patients than in ward patients [21.59 (16.93-29.21) vs. 14.02 (11.17-18.22), p=0.001]. Similarly, BAR values were significantly elevated in the ICU group [6.35 (4.55-8.14) vs. 3.54 (2.83- 4.75), p=0.001] (Table 4).

Receiver Operating Characteristic Analysis

ROC curve analysis was performed for urea, BUN, and the BAR, as these parameters differed significantly between ward and ICU patients ($p<0.05$). BAR demonstrated the highest discriminative performance, with an AUC of 0.836, compared with 0.788 for both urea and BUN. Pairwise comparisons of ROC curves, performed using the DeLong test, demonstrated that the diagnostic performance of BAR was significantly superior to both urea (Z=2.393, p=0.023) and BUN (Z=2.393, p=0.023). These findings support the superior discriminative performance of BAR compared with individual parameters for identifying patients requiring ICU (Table 5, Figure 1).

Age-Adjusted Comparison of Urea, BUN, and BAR Between Ward and ICU Groups

After adjustment for age using rank-based ANCOVA, urea, BUN, and BAR levels remained significantly higher in ICU patients than in ward patients ($p<0.001$), indicating that these differences were not solely explained by age (Table 6).

DISCUSSION

This study demonstrated that BAR levels were significantly higher in patients requiring ICU admission than in those managed in the ward (p=0.001). ROC analysis demonstrated that BAR had superior discriminative performance compared with urea and BUN, suggesting its potential utility in early risk stratification.

Table 2. Age comparison between ward and ICU groups

Variable	Group	Median (IQR)	U	p
Age	Ward	62.00 (53.50-73.50)	966.500	**0.001**
	ICU	77.00 (63.75-80.00)		

ICU: Intensive care unit, IQR: Interquartile range, U: Mann-Whitney U test, $p<0.05$: indicates statistical significance (bold value).

Table 1. Gender characteristics of the ward and ICU groups

Variable	Group	Ward (n, %)	ICU (n, %)	Total (n, %)	χ^2	p
Gender	Male	18 (30.51%)	21 (40.38%)	39 (35.14%)	1.183	0.277
	Female	41 (69.49%)	31 (59.62%)	72 (64.86%)		

ICU: Intensive care unit, n: Frequency, %: Percentage.

Table 3. Comparison of clinical parameters between ward and ICU patients

Variable	Group	Median (IQR)	U	p
Urea (mg/dL)	Ward	30.00 (23.90-39.00)	649.000	**0.001**
	ICU	46.20 (36.22-62.50)		
Amylase (U/L)	Ward	1322.00 (698.50-2403.50)	1416.500	0.487
	ICU	1179.50 (582.25-2109.50)		
Lipase (U/L)	Ward	842.00 (588.50-1000.00)	1430.500	0.531
	ICU	761.50 (457.50-1000.00)		
Glucose (mg/dL)	Ward	137.00 (110.50-164.50)	1515.000	0.911
	ICU	134.00 (107.25-176.25)		
Creatinine (mg/dL)	Ward	0.73 (0.61-0.90)	704.500	**0.001**
	ICU	1.08 (0.76-1.41)		
AST (U/L)	Ward	106.00 (38.00-338.50)	1391.500	0.400
	ICU	95.00 (41.50-200.00)		
ALT (U/L)	Ward	104.00 (42.50-269.00)	1426.500	0.525
	ICU	103.50 (32.00-220.25)		
ALP (U/L)	Ward	136.00 (97.00-194.00)	1516.500	0.918
	ICU	126.50 (93.75-224.00)		
GGT (U/L)	Ward	93.00 (55.00-255.00)	1473.500	0.721
	ICU	102.00 (58.75-192.75)		
TBil (mg/dL)	Ward	1.10 (0.60-2.10)	1152.000	**0.024**
	ICU	1.75 (0.75-3.50)		
DBil (mg/dL)	Ward	0.56 (0.20-1.20)	1182.500	**0.037**
	ICU	0.89 (0.30-2.00)		
Protein (g/L)	Ward	7.60 (7.10-8.00)	744.000	**0.001**
	ICU	6.80 (6.07-7.40)		
Albumin (g/dL)	Ward	4.00 (3.65-4.25)	833.500	**0.001**
	ICU	3.55 (3.08-3.92)		
Ca^{2+} (mg/dL)	Ward	9.30 (8.85-9.65)	1006.000	**0.002**
	ICU	9.05 (8.28-9.33)		
Na^{+} (mmol/L)	Ward	138.00 (137.00-140.00)	1220.000	0.062
	ICU	137.00 (134.00-139.25)		
K^{+} (mmol/L)	Ward	4.00 (3.75-4.40)	1518.000	0.925
	ICU	4.00 (3.74-4.40)		
CRP (mg/dL)	Ward	0.90 (0.30-1.65)	933.500	**0.001**
	ICU	2.35 (1.08-4.05)		

Table 3. Continued

Variable	Group	Median (IQR)	U	p
Leukocyte ($10^3/\mu L$)	Ward	10.30 (8.20-14.05)	1249.000	0.092
	ICU	13.20 (9.38-15.65)		
Platelet ($10^3/\mu L$)	Ward	260.00 (215.00-320.50)	1252.000	0.096
	ICU	240.00 (190.50-296.75)		
Hemoglobin (g/dL)	Ward	14.20 (13.25-15.10)	1344.500	0.263
	ICU	13.60 (12.73-15.05)		
Hematocrit (%)	Ward	41.70 (39.55-45.55)	1250.000	0.093
	ICU	40.50 (36.50-44.25)		
Neutrophil ($10^3/\mu L$)	Ward	7.60 (5.95-10.10)	1142.000	**0.021**
	ICU	9.65 (6.80-13.22)		
Lymphocyte ($10^3/\mu L$)	Ward	1.50 (1.10-1.80)	1074.000	**0.006**
	ICU	1.10 (0.57-2.00)		
Monocyte ($10^3/\mu L$)	Ward	0.60 (0.47-0.80)	1506.000	0.868
	ICU	0.64 (0.40-1.00)		

ICU: Intensive care unit, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, GGT: Gamma-glutamyl transferase, TBil: Total bilirubin, DBil: Direct bilirubin, Ca^{2+}: Calcium, Na^{+}: Sodium, K^{+}: Potassium, CRP: C-reactive protein, IQR: Interquartile range, U: Mann-Whitney U test, $p<0.05$: indicates statistical significance (bold values).

Table 4. Comparison of BUN and BAR between ward and ICU patients

Variable	Group	Median (IQR)	U	p
BUN	Ward	14.02 (11.17-18.22)	649.000	**0.001**
	ICU	21.59 (16.93-29.21)		
BAR	Ward	3.54 (2.83-4.75)	503.500	**0.001**
	ICU	6.35 (4.55-8.14)		

ICU: Intensive care unit, BUN: Blood urea nitrogen, BAR: Blood urea nitrogen to albumin ratio, IQR: Interquartile range, U: Mann-Whitney U test, $p<0.05$: indicates statistical significance (bold values).

Timely recognition of patients at risk for severe disease remains a major challenge in AP, as clinical deterioration may occur rapidly despite an initially mild presentation.[7] Given that decisions regarding the appropriate level of care are often made at the time of emergency department presentation, reliable biomarkers that can support early triage are of particular clinical importance.[7,18] BAR is a composite parameter derived from BUN and serum albumin levels, integrating information related to renal perfusion, intravascular volume status, and systemic inflammatory burden.[19] Elevated BUN levels have been associated with hypovolemia and impaired renal perfusion, and are strongly linked to worse outcomes in AP. Notably, early increases in BUN have been shown to predict mortality.[8,20] Similarly, hypoalbuminemia reflects both systemic inflammation and poor nutritional status, and has been associated with persistent organ failure and increased disease severity in AP.[9] By integrating these parameters, BAR may better systemic stress response than either marker alone. In line with this, previous studies have proposed BAR as a practical and accessible biomarker for predicting adverse outcomes in various critical conditions, including sepsis, COVID-19, and acute exacerbations of chronic obstructive pulmonary disease.[10-12]

Table 5. ROC analysis results

Variable	AUC (95% CI)	p	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)
Urea	0.788 (0.704-0.873)	0.001	34.500	0.808 (0.675-0.904)	0.644 (0.509-0.764)
BUN	0.788 (0.704-0.873)	0.001	16.121	0.808 (0.675-0.904)	0.644 (0.509-0.764)
BAR	0.836 (0.762-0.910)	0.001	5.243	0.692 (0.549-0.813)	0.864 (0.750-0.940)

ROC: Receiver operating characteristic, BUN: Blood urea nitrogen, BAR: Blood urea nitrogen to albumin ratio, AUC: Area under the curve, CI: Confidence interval, $p<0.05$: indicates statistical significance.

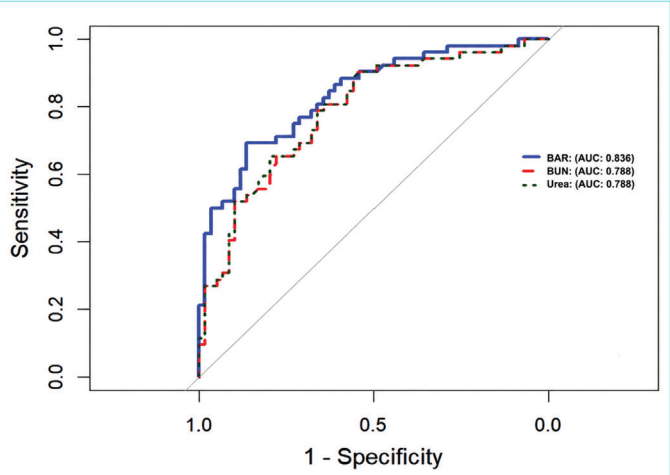

Figure 1. ROC curves for the study variables. The gray diagonal line represents the reference line corresponding to an AUC of 0.5 (no discriminatory ability).

ROC: Receiver operating characteristic, AUC: Area under the curve, BUN: Blood urea nitrogen, BAR: Blood urea nitrogen to albumin ratio.

Table 6. Age-adjusted comparison of urea, BUN, and BAR between ward and ICU groups using rank-based ANCOVA

Variables		Urea	BUN	BAR
Median (IQR)		Median (IQR)	Median (IQR)	
Ward		30.00 (23.90-39.00)	14.02 (11.17-18.22)	3.54 (2.83-4.75)
ICU		46.20 (36.22-62.50)	21.59 (16.93-29.21)	6.35 (4.55-8.14)
Groups	F	21.631	21.631	38.484
	p	0.001	0.001	0.001
Age	F	29.575	29.575	43.433
	p	0.001	0.001	0.001

ICU: Intensive care unit, BUN: Blood urea nitrogen, BAR: Blood urea nitrogen to albumin ratio, IQR: Interquartile range, p<0.05: indicates statistical significance. Because BUN is a fixed linear transformation of urea (BUN = urea/2.14), identical F statistics for these variables are mathematically expected.

The role of BAR as a prognostic indicator in AP has attracted considerable research interest in recent years. Findings from retrospective cohort analyses consistently show that increased BAR values are independently associated with higher mortality in both short- and long-term follow-up.[14,19] For example, Huang et al.[14] reported that higher BAR values were significantly associated with increased 90-day mortality in patients with AP. Similarly, a large database study using the MIMIC-IV dataset demonstrated that BAR was significantly associated with both short- and long-term mortality in patients with AP and that it had superior prognostic performance compared with several individual laboratory parameters.[4] In ICU-based cohorts, the predictive performance of BAR has also been compared with traditional severity scoring systems. Wang et al.[15] reported that BAR demonstrated a high predictive value for mortality in ICU patients with AP, with AUC values ranging between 0.71 and 0.76. Similarly, Cai et al.[13] showed that BAR had strong predictive ability for both 28-day and 90-day mortality in patients with AP, with AUC values approaching 0.80. These findings highlight the potential clinical utility of BAR as a simple laboratory-based index capable of providing prognostic information comparable to more complex scoring systems.[13] Another important aspect of the current literature is the comparison of BAR with established clinical scoring tools. For instance, Efgan et al.[21] compared BAR with the Bedside Index for Severity in Acute Pancreatitis score and demonstrated that BAR had comparable diagnostic accuracy for identifying severe cases of pancreatitis in the emergency department. Because the calculation of BAR requires only two routinely measured laboratory parameters, it may represent a practical alternative or complementary tool to multivariable scoring systems that require multiple clinical inputs.[21] However, most of these studies have focused on mortality prediction or on critically ill patient populations.

The findings of this study provide additional insight into the existing literature by focusing specifically on the early triage phase of AP management. While many previous studies have primarily evaluated BAR as a predictor of mortality or adverse outcomes in ICU populations, our study directly compared patients admitted to the ward with those admitted to the ICU at the time of initial hospital presentation. This approach allows the evaluation of BAR for early clinical decision-making about the appropriate level of care, a critical step in managing patients presenting with AP. Consistent with previous reports, our results showed that BAR values were significantly higher in patients requiring ICU admission than in those managed in the ward. Additionally, ROC analysis demonstrated that BAR showed good discriminative ability for identifying patients requiring ICU admission, with an AUC value of 0.836. This diagnostic performance appears to be comparable to that reported in previous studies evaluating BAR in AP, in which AUC values have generally ranged from approximately 0.67 to 0.80.[13-15] This finding suggests that BAR may serve as a supportive marker for early risk stratification in patients presenting with AP. Compared with individual laboratory parameters such as urea and BUN, BAR demonstrated slightly better diagnostic performance, which supports the concept that composite biomarkers may better reflect the complex pathophysiological processes involved in AP. The higher AUC value of BAR compared with those of BUN or urea alone may be explained by the fact that this composite parameter reflects not only changes in renal function but also systemic inflammation and nutritional status. During acute inflammatory processes, serum albumin levels decrease as a negative acute-phase reactant, whereas elevated BUN levels may reflect hypovolemia and impaired renal perfusion. Therefore, BAR may more comprehensively represent the underlying pathophysiological processes in AP by integrating these two mechanisms and thus potentially better reflect disease severity than either parameter alone. These findings support the hypothesis that BAR may reflect the overall physiological stress associated with more severe forms of AP. In our study, patients admitted to the ICU were older than those managed in the ward, which is consistent with the established association between advanced age and more severe disease in AP. Although ICU patients were older, rank-based ANCOVA demonstrated that urea, BUN, and BAR levels remained significantly higher after adjustment for age (p<0.001). Furthermore, the persistence of significant group differences after age adjustment using rank-based ANCOVA suggests that the observed association between BAR and ICU admission is unlikely to be explained solely by differences in patient age and may instead reflect underlying disease severity. Therefore, evaluating BAR at the time of initial presentation could provide clinically meaningful insight into which patients may require enhanced monitoring or higher levels of supportive care. Although BAR demonstrated a slightly higher AUC compared with BUN and urea, pairwise comparisons using the DeLong test confirmed its superior discriminative performance.

From a clinical perspective, BAR represents a simple and readily available biomarker that may assist in early risk stratification in patients with AP. As BUN and albumin are routinely measured in emergency settings, BAR can be easily calculated without additional cost or time, and may help identify patients requiring closer monitoring or intensive care.

Overall, these findings support the potential clinical utility of BAR in early triage. However, larger prospective multi-center studies are needed to validate these results, establish optimal cut-off values, and confirm the generalizability of the ROC-derived thresholds identified in this study.

Study Limitations

Several factors should be considered when interpreting the present findings. The retrospective design and single-center setting may limit generalizability and increase susceptibility to bias, while the relatively small sample size, particularly among ICU patients, may have reduced statistical power. Furthermore, BAR was assessed only at admission, and the absence of serial measurements limits the evaluation of its dynamic relationship with disease progression. In addition, ICU admission was used as a surrogate marker of disease severity and may have been influenced by clinical judgment and institutional practices. Finally, residual confounding cannot be excluded because factors such as comorbidities and underlying renal function may have affected BUN and BAR levels.

CONCLUSION

BAR may serve as a simple, accessible biomarker to support early risk stratification in patients with AP. Elevated BAR levels at presentation may provide complementary information to support the identification of patients who may require closer monitoring or ICU-level care, particularly when the initial disposition decision is uncertain. However, BAR should be considered an adjunct to, rather than a replacement for, comprehensive clinical assessment. Prospective multicenter studies are needed to validate its potential role in early triage.

MAIN POINTS

- Blood urea nitrogen to albumin ratio (BAR) is significantly higher in acute pancreatitis patients requiring intensive care unit (ICU) admission at initial presentation than in those managed on the ward.
- BAR demonstrates good discriminative performance in identifying ICU admission at presentation, performing slightly better than blood urea nitrogen and urea.
- The association between BAR and ICU admission at initial presentation remained significant after adjusting for age using a rank-based ANCOVA.
- BAR can be easily calculated from routine laboratory parameters obtained at admission, making it a practical tool in the emergency setting.
- BAR may provide supportive information for early risk stratification and help identify patients who may require ICU-level care at initial clinical evaluation.

ETHICS

Ethics Committee Approval: The requisite approval was obtained from the Malatya Turgut Özal University Health Sciences Scientific Research Ethics Committee (approval no: 2025/208, date: 30.07.2025). All procedures were carried out in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki. In addition, the privacy and data of all patients were respected and protected.

Informed Consent: Because of the retrospective design of the study and the use of anonymized data obtained from existing medical records, the requirement for obtaining written informed consent was waived by the ethics committee. Patient confidentiality and data protection were strictly maintained throughout the study.

Footnotes

Authorship Contributions

Concept: M.E., T.R.K., Design: M.E., T.R.K., S.Y., Data Collection and/or Processing: M.E., S.Y., Analysis and/or Interpretation: M.E., F.İ., Literature Search: M.E., T.R.K., Writing: M.E., T.R.K., S.Y., F.İ.

DISCLOSURES

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RESEARCH ARTICLE

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Systemic Inflammatory Response Index and Systemic Immune-Inflammation Index in Parkinson's Disease

Aysu Yetiş, Selcen Duran

Department of Neurology, Kırşehir Ahi Evran University Faculty of Medicine, Kırşehir, Türkiye

Abstract

BACKGROUND/AIMS: Peripheral immune activity may contribute to Parkinson's disease (PD), but the clinical usefulness of blood cell-derived composite indices is not established. We compared the systemic immune-inflammation index (SII) and systemic inflammatory response index (SIRI) between patients with PD and healthy controls (HCs) and explored their relationships with disease stage, duration, and motor severity.

MATERIALS AND METHODS: Data from 76 patients with PD and 44 HCs were evaluated in a cross-sectional case-control study. Disease severity was characterized using the Hoehn-Yahr (HY) stage and the motor section of the Unified PD rating scale (UPDRS). SII and SIRI were calculated from complete blood count parameters, and analyses were performed using non-parametric statistical methods.

RESULTS: Patients with PD had higher SIRI and monocyte values and lower lymphocyte counts than HCs (p=0.008, p=0.047, and p=0.029, respectively). The groups were comparable with respect to neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and SII. No inflammatory index showed a significant relationship with HY stage, disease duration, or UPDRS motor score.

CONCLUSION: An increased SIRI value may represent a peripheral inflammatory feature of PD. Nevertheless, the absence of group differences in NLR, PLR, and SII and the lack of associations with clinical severity indicate that this finding should not be interpreted as a marker of disease progression. Multicenter longitudinal research with adjustment for potential confounders is warranted.

Keywords: Parkinson's disease, systemic inflammation, SIRI, SII, motor severity

INTRODUCTION

Parkinson's disease (PD) is clinically characterized by a broad spectrum of motor and non-motor manifestations. Degeneration of dopaminergic neurons in the substantia nigra is a central feature of PD, but does not fully explain the biological processes underlying the disorder. Immune dysregulation and inflammation have therefore received increasing attention as possible contributors to its development and progression.[1-5]

Peripheral immune signals can influence the central nervous system and may aggravate neuronal injury in neurodegenerative conditions. In PD, activated microglia can sustain an inflammatory environment within the substantia nigra.[6] These cells release mediators such as interleukin-1beta and tumour necrosis factor alpha together with reactive oxygen species. Such products may damage dopaminergic neurons, weaken blood-brain barrier integrity, and facilitate the entry of circulating immune cells into neural tissue.[7] Immunohistochemical findings also support a pro-inflammatory phenotype of glial cells, particularly microglia, during neurodegeneration.[8]

Routine complete blood counts provide several low-cost measures that may capture different aspects of systemic immune activity. Among them, the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), and systemic inflammatory response index (SIRI) combine circulating cell populations into summary indicators. These measures have been investigated across inflammatory and neurological disorders, although their diagnostic and prognostic relevance in PD remains uncertain.[9] Evidence from

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ORCID IDs of the authors: A.Y. 0000-0003-2139-0848; S.D. 0000-0001-7553-2034.

Corresponding author: Aysu Yetiş
E-mail: draysuyetis@hotmail.com
ORCID ID: orcid.org/0000-0003-2139-0848

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both experimental models and clinical samples has not yet clarified how closely peripheral immune changes reflect the neurodegenerative process.[10]

Accordingly, this study compared peripheral inflammatory indices between patients with PD and healthy controls (HCs) and examined whether these indices varied with Hoehn-Yahr (HY) stage, disease duration, or motor symptom severity.

MATERIALS AND METHODS

Study Design

A cross-sectional case-control design was used. Patients with idiopathic PD who had been monitored for at least six months in the neurology outpatient clinic of Kırşehir Ahi Evran University Training and Research Hospital were enrolled between 2024 and 2025. Ethical approval was obtained from the Kırşehir Ahi Evran University Local Research Ethics Committee (approval no: 2024-09/64, date: 30.04.2024), and written informed consent was obtained from all participants. A neurologist established a diagnosis of idiopathic PD according to the United Kingdom PD Society Brain Bank clinical diagnostic criteria. The HCs group comprised volunteers assessed during the same period who had neither PD nor any other neurodegenerative condition and met the exclusion criteria applied to the patient group.

Demographic and clinical data, including age, sex, education, comorbidities, and medication use, were collected from medical records and participant assessments. Both groups excluded individuals with rheumatologic, hematologic, oncologic, endocrine, infectious, or other inflammatory disorders; users of anti-inflammatory drugs, antidepressants, or corticosteroids; and those with another degenerative neurological disease, including Alzheimer's disease. The HY scale was used to stage PD, whereas motor impairment was quantified with the motor section of the Unified PD Rating Scale (UPDRS). Each UPDRS motor examination was completed in the OFF-medication state. Following a 12-hour fast, routine blood samples were used to obtain neutrophil, lymphocyte, monocyte, and platelet counts, together with 25-hydroxyvitamin D, folate, and vitamin B12 levels. SII was calculated as the product of neutrophils and platelets divided by lymphocytes. SIRI was obtained by multiplying neutrophils by monocytes and dividing by lymphocytes. Comorbidities and medication exposure were considered during the eligibility assessment, but no multivariable adjustment was undertaken.

Statistical Analysis

Analyses were conducted in SPSS 25.0 (IBM Corp., Armonk, NY, USA). Categorical data are presented as numbers and percentages. Continuous variables are described using mean ± standard deviation and median, as appropriate. Distributional assumptions were examined with the Kolmogorov-Smirnov test. As the relevant variables were non-normally distributed, two-group comparisons used the Mann-Whitney U test, while comparisons across more than two groups used the Kruskal-Wallis test. Associations between continuous measures were assessed with Spearman's rho. A two-sided p-value below 0.05 was considered statistically significant. Because adjusted models were not constructed, possible confounding was considered when interpreting the results.

RESULTS

The analysis included 76 patients with PD and 44 HCs. Women accounted for 42 of the patients (55.3%). Disease duration was most commonly 1-5 years (n=33, 43.4%). Based on body mass index (BMI), 46 patients (60.5%) were overweight, 25 (32.9%) were obese, and 5(6.6%) had a normal BMI. HY stage 2 was the most frequent category (n=33, 43.4%), followed by HY stage 1.5 (n=28, 36.8%). Five patients (6.6%) were at stage 1; five (6.6%) were at stage 2.5; three (3.9%) were at stage 3; one (1.3%) was at stage 4; and one (1.3%) was at stage 0. The UPDRS motor score averaged 20.6±11.7, with a median of 18. The corresponding mini-mental test values were 25.2±2.2 and 25. Table 1 provides the clinical and sociodemographic profile of the PD group.

Comparison of hematological measures showed that the PD group had higher monocyte counts and SIRI values than the HCs (p=0.047 and p=0.008, respectively). Conversely, lymphocyte counts were lower among patients with PD (p=0.029). None of the remaining variables listed in Table 2 differed significantly between the groups (all p>0.05).

As shown in Table 3, HY stage was not significantly correlated with NLR, PLR, SII, or SIRI (all p>0.05). The coefficients were small and centered near zero, providing no evidence that these circulating inflammatory indices changed in parallel with stage-defined disease severity.

Table 1. Clinical and socio-demographic profile of the patients

	Number (n)	Percent (%)
BMI		
Normal	5	6.60
Overweight	46	60.50
Obese	25	32.90
Education		
Illiterate	8	10.50
Elementary school	44	57.90
Middle school	6	7.90
High school	16	21.10
University	2	2.60
Duration of illness		
Less than 1 year	13	17.10
1-5 years	33	43.40
5-10 years	17	22.40
Over 10 years	13	17.10
DM	18	23.70
HT	40	52.60
HPL	28	36.80
CAD	20	26.30
Thyroid disease	9	12
HY		
0	1	1.3
1	5	6.60
1.5	28	36.80
2	33	43.40
2.5	5	6.60
3	3	3.90
4	1	1.30

BMI: Body mass index, HT: Hypertension, DM: Diabetes mellitus, HPL: Hyperlipidemia, CAD: Coronary artery disease, HY: Hoehn-Yahr.

When patients were categorized by disease duration (<1 year, 1-5 years, 5-10 years, and >10 years), NLR, PLR, SII, and SIRI did not differ significantly across categories (all p>0.05; Table 4). Although group means varied numerically, no consistent duration-related pattern was evident.

UPDRS motor scores were unrelated to NLR, PLR, SII, and SIRI (all p>0.05; Table 5). BMI, however, showed weak positive correlations with PLR (r=0.253, p<0.05) and SII (r=0.284, p<0.05), as reported in Table 6.

Table 2. Comparison of blood parameters between HCs and PD groups

	PD (n=76)	HC (n=44)	Total (n=120)	p†
	n (%)	n (%)	n (%)	
Gender				
Female	42 (55.30)	24 (54.50)	66 (55)	0.939
Male	34 (44.70)	20 (45.50)	54 (45)	
	PD (n=76)	HC (n=44)	Total (n=120)	p‡
	Mean ± SD (Med)	Mean ± SD (Med)	Mean ± SD (Med)	
UPDRS	20.60±11.70 (18)			
MMT	25.20±2.20 (25)			
Vitamin B12	314.30±110.20 (293.50)	347.80±118.80 (336)	326.60±114.10 (303)	0.108
Folate	7.63±3.30 (7)	7.73±2.90 (7)	7.67±3.10 (7)	0.625
25-hydroxy vitamin D	17.4±8.0 (16)	17±6.6 (16)	17.30±7.50 (16)	0.943
Neutrophil	4.58±1.20 (4.50)	4.52±1.30 (4.70)	4.56±1.20 (4.60)	0.987
Lymphocyte	2.04±0.70 (1.82)	2.30±0.70 (2.20)	2.14±0.70 (1.96)	**0.029***
Platelet	261.70±83.50 (245)	272.80±51.60 (272.50)	265.80±73.40 (258.50)	0.139
Monocyte	0.72±0.90 (0.56)	0.52±0.10 (0.51)	0.65±0.79 (0.54)	**0.047***
NLR	2.49±1.20 (2.22)	2.06±0.60 (2.06)	2.33±1 (2.13)	0.063
PLR	137.30±47.60 (132)	122.80±38.10 (120.50)	131.90±44.70 (127.50)	0.156
SII	637.80±315.40 (571.50)	555.90±166.20 (566.50)	607.80±272.50 (567.50)	0.428
SIRI	1.64±1.60 (1.20)	1.05±0.30 (1.01)	1.42±1.30 (1.12)	**0.008***

†Chi-square test; ‡Mann-Whitney U test. *p<0.05.
PD: Parkinson's disease, HC: Healthy control, UPDRS: Unified Parkinson's disease rating scale, MMT: Mini-mental test, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic immune-inflammation index, SIRI: Systemic inflammatory response index, SD: Standard deviation.

Table 3. Correlations between Hoehn-Yahr stage and inflammatory parameters

	HY	
	r	p
NLR	0.075	0.521
PLR	0.158	0.172
SII	0.070	0.547
SIRI	-0.005	0.966

p<0.05; Spearman's rho.
HY: Hoehn-Yahr; NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic immune-inflammation index, SIRI: Systemic inflammatory response index.

Table 4. Differences between disease duration and inflammation parameters

	Less than 1 year (n=13)	1-5 years (n=33)	5-10 years (n=17)	Over 10 years (n=13)	p
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
NLR	2.01±0.90 (1.71)	2.72±1.50 (2.44)	2.48±0.60 (2.59)	1.85±2.90 (0.96)	0.095
PLR	113.90±44.60 (106)	146.30±54.80 (132)	143.50±34.20 (157)	129.75±40.89 (130)	0.087
SII	510.60±194.30 (464)	700.90±360.50 (651)	618.70±225.10 (588)	629.80±375.30 (548)	0.329
SIRI	1.85±2.97 (0.96)	1.79±1.40 (1.27)	1.38±0.60 (1.30)	1.38±0.80 (1.20)	0.261

Kruskal-Wallis test.
NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic immune-inflammation index, SIRI: Systemic inflammatory response index, SD: Standard deviation.

Table 5. Correlations between UPDRS scores and inflammatory parameters

	UPDRS	
	r	p
NLR	0.027	0.818
PLR	0.149	0.198
SII	0.098	0.402
SIRI	0.056	0.630

Spearman's rho.
UPDRS: Unified Parkinson's disease rating scale, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic immune-inflammation index, SIRI: Systemic inflammatory response index.

Table 6. Correlations between BMI and inflammatory parameters

	BMI	
	r	p
NLR	0.172	0.138
PLR	0.253*	0.028
SII	0.284*	0.013
SIRI	0.125	0.282

*p<0.05; Spearman's rho.
BMI: Body mass index, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic immune-inflammation index, SIRI: Systemic inflammatory response index.

DISCUSSION

This study compared several blood-cell-derived inflammatory measures in PD and HCs. The principal observation was a higher SIRI value in the PD group, accompanied by increased monocytes and reduced lymphocytes. By contrast, NLR, PLR, and SII did not distinguish the groups. Moreover, none of the indices was related to HY stage, disease duration, or UPDRS motor performance. Thus, SIRI may capture a peripheral immune alteration associated with PD, but the present data do not support its use as an indicator of clinical advancement.

Inflammatory activity in PD may involve bidirectional interaction between peripheral immunity and the central nervous system. Infectious agents, including influenza A, HSV-1, EBV, varicella-zoster virus, Japanese encephalitis virus, human immunodeficiency virus, and *Helicobacter pylori*, have been discussed in relation to parkinsonian mechanisms.[11] Molecular mimicry between viral proteins and alpha-synuclein (α-Syn) may promote immune responses within nigrostriatal pathways and facilitate α-Syn aggregation. α-Syn itself can also affect the recruitment of neutrophils and monocytes after infection, linking systemic immune activation with neuroinflammation. Although persistent inflammation is unlikely to initiate every case of PD, it may accelerate neuronal injury once neurodegeneration is underway.[12,13] Positron emission tomography findings in early PD further suggest that greater microglial activation accompanies lower dopaminergic terminal density and worse motor function.[14]

SII integrates neutrophil, platelet, and lymphocyte counts and summarizes the balance between inflammatory activation and immune competence. Studies in PD have produced conflicting results. Zhao et al.[15] associated higher SII with greater odds of PD, whereas Li et al.[10] linked elevated SII to poorer motor performance. Conversely, a study from Türkiye detected neither a group difference in SII nor a convincing association with clinical severity.[16] Our findings align with the latter report: SII was similar in PD and HCs and showed no relationship with HY stage or UPDRS motor score. Variation in disease distribution, participant comorbidities, medication exposure, and lifestyle characteristics may account for these discrepancies.

SIRI, which combines neutrophil, monocyte, and lymphocyte counts, was originally proposed by Qi et al.[17] as an indicator of systemic inflammatory burden. Its relevance to PD has been examined less often than that of SII. Wang et al.[18] observed no association of either SII or SIRI with PD risk, and Alagoz et al.[16] reported similar SIRI values in PD and HCs. In the current cohort, however, SIRI was elevated in PD. This result likely reflects the simultaneous increase in monocytes and a decrease in lymphocytes, both of which directly influence the index. SIRI may, therefore, respond to a cellular pattern not captured to the same extent by SII.

The elevated SIRI value was not associated with HY stage, disease duration, or UPDRS motor score. NLR, PLR, and SII showed a similar lack of clinical association. One interpretation is that these indices represent a general peripheral inflammatory state rather than the extent of neurological impairment. Their values may also fluctuate and need not mirror ongoing changes within the central nervous system. In addition, the concentration of this sample during early and moderate HY stages narrowed the range of disease severity and may have weakened the detectable correlations.

BMI was weakly correlated with PLR and SII. Because adipose tissue can sustain chronic, low-grade inflammatory signaling, body composition may influence cell-count-derived indices independently of PD. Age, diabetes mellitus, hypertension, hyperlipidemia, coronary artery disease, metabolic status, and medication use may exert similar effects. Future analyses should therefore account for these variables when estimating the independent relationship between PD and systemic inflammatory measures.

Study Limitations

Several issues limit the interpretation. Recruitment from a single center and the modest sample size reduce external validity. The cross-sectional case-control design also prevents the evaluation of temporal sequence and does not permit causal conclusions regarding inflammation and PD progression. Although eligibility criteria excluded recognized inflammatory conditions and selected medications, residual confounding by age, BMI, cardiometabolic disease, medication exposure, lifestyle, or clinically silent inflammation remains possible because adjusted analyses were not performed. Patients with advanced PD were uncommon, which further limited evaluation across the full severity spectrum. Even so, simultaneous assessment of SII and SIRI provides comparative information on two composite indices. Larger multicenter cohorts, repeated measurements, central and peripheral inflammatory markers, and multivariable modeling are needed to determine their clinical value.

CONCLUSION

Patients with PD displayed a distinct pattern of higher SIRI and monocyte values together with lower lymphocyte counts relative to HCs. NLR, PLR, and SII did not differ between groups, and none of the examined indices were related to HY stage, disease duration, or UPDRS motor score. These

results suggest altered peripheral immune activity but do not establish peripheral immune activity as a marker of PD severity or progression. Longitudinal studies that include broader disease stages and control for relevant confounders are required.

MAIN POINTS

- Compared with healthy controls (HCs), patients with Parkinson's disease (PD) had higher systemic inflammatory response index (SIRI) and monocyte values but lower lymphocyte counts.
- Neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and systemic immune-inflammation index were similar in the PD and HCs.
- Hoehn-Yahr stage, disease duration, and unified Parkinson's disease rating scale motor scores were not related to the inflammatory indices.
- SIRI may reflect peripheral immune alterations in PD, although its clinical and prognostic value requires confirmation in longitudinal studies.

ETHICS

Ethics Committee Approval: Ethical approval was obtained from the Kırşehir Ahi Evran University Local Research Ethics Committee (approval no: 2024-09/64, date: 30.04.2024).

Informed Consent: Written informed consent was obtained from all participants.

Footnotes

Authorship Contributions

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

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RESEARCH ARTICLE

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Adjunctive Multimodal Therapy Incorporating Iontophoresis-Assisted Growth Factor Delivery for Hair Loss: A Real-World Retrospective Study

Ümit Akpınar[1], Ekrem Civaş[1], Yıldız Hayran[2], Neslihan Fişek İzci[3]

[1]Private Dermatology Practice, Ankara, Türkiye
[2]Clinic of Dermatology, Ankara Bilkent City Hospital, Ankara, Türkiye
[3]Department of Dermatology, Bahçeşehir University Faculty of Medicine, İstanbul, Türkiye

Abstract

BACKGROUND/AIMS: Adjunctive multimodal therapies incorporating iontophoresis-assisted growth factor delivery have emerged as potential non-invasive treatment options for hair loss; however, real-world clinical evidence remains limited. This study evaluated the feasibility, tolerability, patient-reported hair shedding, symptom response, and treatment satisfaction associated with this treatment approach in patients with androgenetic alopecia (AGA), telogen effluvium (TE), and mixed AGA + TE.

MATERIALS AND METHODS: This single-center, retrospective, uncontrolled, real-world observational study included 46 patients who had completed a standardized four-session adjunctive multimodal treatment protocol incorporating iontophoresis-assisted growth factor delivery alongside standard-of-care management; they were selected from 78 consecutively screened individuals. Outcomes included patient-reported daily hair shedding, symptom response, procedural tolerability, and treatment satisfaction.

RESULTS: Daily hair shedding decreased significantly after treatment ($p<0.001$). After Bonferroni correction for the three diagnostic subgroup comparisons (adjusted $\alpha=0.0167$), the reduction remained statistically significant in TE ($p=0.002$), whereas AGA ($p=0.030$) and mixed AGA + TE ($p=0.025$) did not meet the corrected significance threshold. No patients remained in the ≥200 hairs/day shedding categories after treatment. Patients with TE showed greater symptom improvement than those with isolated AGA ($p=0.016$). Reduction in hair shedding was positively correlated with treatment satisfaction ($r=0.48$, $p=0.004$). No severe adverse events were documented in the medical records.

CONCLUSION: Adjunctive multimodal therapy incorporating iontophoresis-assisted growth factor delivery was associated with reduced patient-reported hair shedding and high treatment satisfaction. However, because of the retrospective, uncontrolled design, the concomitant standard-of-care therapies, and the multimodal nature of the intervention, these findings should be considered hypothesis-generating. Prospective, randomized, sham-controlled studies incorporating objective outcome measures and longer follow-up are warranted.

Keywords: Androgenetic alopecia, telogen effluvium, iontophoresis, growth factor therapy, transdermal drug delivery

INTRODUCTION

Hair loss encompasses a heterogeneous group of disorders characterized by alterations in the hair cycle and follicular dynamics. Beyond its biological dimension, hair loss is frequently associated with substantial psychosocial burden, given the central role of hair in self-perception, identity, and social interaction. Among the various causes of hair loss, androgenetic alopecia (AGA) and telogen effluvium (TE) represent the most prevalent entities in clinical practice.[1]

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ORCID IDs of the authors: Ü.A. 0000-0002-5816-760X; E.C. 0000-0001-7976-1125; Y.H. 0000-0003-1942-7285; N.F.İ. 0000-0002-9829-4013.

Corresponding author: Ümit Akpınar
E-mail: drumitakpinar@gmail.com
ORCID ID: orcid.org/0000-0002-5816-760X

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TE is characterized by a premature transition of hair follicles from the anagen (growth) phase to the telogen (resting) phase, resulting in diffuse shedding of club hairs. It is widely recognized as the most common cause of diffuse hair loss. In most cases, diagnosis is established through detailed history taking and physical examination. TE is typically a self-limited condition and lacks a standardized therapeutic approach. When an underlying trigger, such as hormonal imbalance, micronutrient deficiency (e.g., iron, zinc, or vitamin D deficiency), or metabolic disease, is identified, correction of the underlying cause generally leads to spontaneous hair regrowth.[2]

AGA, in contrast, is a non-scarring and progressive miniaturization of hair follicles occurring in genetically predisposed individuals, usually following a characteristic pattern distribution in both men and women.[3] The diagnosis is primarily clinical. Currently, topical minoxidil is Food and Drug Administration (FDA)-approved for AGA, while oral finasteride is FDA-approved for male AGA. In addition, several low-level light therapy devices have received FDA clearance for the treatment of AGA. However, numerous alternative or adjunctive approaches, including platelet-rich plasma, mesotherapy, exosomes, and growth factor-based therapies, are increasingly utilized in clinical practice. The broad spectrum of available interventions, combined with the lack of methodological standardization across studies, further complicates therapeutic decision-making.[4]

Iontophoresis is a technique that applies a low-intensity electrical current to enhance the transdermal delivery of active compounds. Experimental evidence suggests that iontophoresis using gel-based formulations can improve both *in vitro* and *in vivo* drug distribution into hair follicles. Given the cosmetic and psychological impact of alopecia, iontophoresis has been increasingly explored within dermatocosmetic applications. Topical therapies are generally preferred to systemic treatments to minimize systemic adverse effects. Nevertheless, conventional topical drug delivery is limited by the skin barrier, which may restrict drug penetration and reduce local delivery efficiency.[5] To overcome these limitations, technology-assisted delivery systems such as iontophoresis have been proposed.

The role of hair follicles as reservoirs in percutaneous drug delivery has been extensively reviewed.[6-9] Increasing attention has been directed toward follicular drug depots as a strategy for localized therapy, particularly for conditions such as acne, certain non melanoma skin cancers (e.g., Bowen's disease), and alopecia.

Among emerging approaches, the use of preformed growth factors delivered via iontophoresis has gained interest as an innovative modality. This patented technique is proposed to stimulate hair follicle activity through combined mechanisms, including the application of exogenous growth factors, microdermal scalp stimulation, pressure wave effects, and iontophoretic enhancement of transfollicular delivery.[10]

MATERIALS AND METHODS

Study Design

This was a single-center, retrospective, uncontrolled, pre-post observational cohort study evaluating an adjunctive multimodal therapy incorporating iontophoresis-assisted growth factor delivery for hair loss. This retrospective study included patients who underwent treatment between January 1, 2025, and January 1, 2026. Owing to its retrospective and uncontrolled design, this study was intended to evaluate real-world feasibility, tolerability, and patient-reported outcomes rather than to establish a causal treatment effect. Accordingly, the findings should be interpreted as hypothesis-generating rather than confirmatory evidence of treatment efficacy.

Patient Selection

Medical records of 78 consecutive patients who underwent adjunctive multimodal therapy incorporating iontophoresis-assisted growth factor delivery during the study period were retrospectively reviewed.

Patients were eligible if they completed the planned four-session treatment protocol and had adequate baseline and post-treatment clinical documentation.

Patients were excluded if they did not complete the planned four-session treatment protocol or had missing or incompatible diagnostic information. Of the 78 patients screened, 18 did not complete the planned four-session treatment protocol, 14 had missing diagnostic information, and 2 had diagnoses incompatible with the study eligibility criteria (frontal fibrosing alopecia and trichotillomania). Two patients had both missing diagnostic information and incomplete treatment; therefore, the exclusion categories were not mutually exclusive. Overall, 32 unique patients were excluded and 46 were included in the final analysis.

Diagnostic Assessment

All patients underwent a standardized dermatologic evaluation, including clinical examination and trichoscopy, performed by a dermatologist before treatment initiation.

The diagnosis of AGA was established based on characteristic clinical findings together with trichoscopic evidence of follicular miniaturization.

The diagnosis of TE was established using clinical history, hair-pull test findings, trichoscopic examination, laboratory investigations when clinically indicated, and assessment of potential triggering factors, including nutritional deficiencies, systemic diseases, medications, and recent physiological or psychological stressors.

Patients presenting with clinical features of both disorders were classified as having mixed AGA and TE.

Device and Procedure

Skin Patting® (aPS, Faenza, Ravenna, Italy) is a patented device designed to stimulate hair follicle activity through a multimodal approach combining controlled microdermal stimulation, radial pressure wave application, and iontophoresis-assisted transdermal delivery.

No anesthesia was required during the procedure.

The treatment protocol began with controlled microdermal stimulation of the affected scalp areas using a needle length of 0.25 mm. Microperforations were applied in longitudinal, vertical, and diagonal directions, with eight passes in each direction or until mild erythema was achieved, which was considered the procedural endpoint. This controlled stimulation was intended to activate the dermal repair cascade, including increased vascularization, increased fibroblast proliferation, and enhanced collagen and elastin synthesis.

The device simultaneously generated radial pressure waves directed toward the scalp. This mechanical action was designed to support microcirculation, stimulate cellular metabolism, and facilitate uptake of active compounds.

Subsequently, iontophoresis was applied to enhance transdermal penetration of the topical formulation. This electrical stimulation was intended to promote transfollicular delivery by increasing skin permeability and supporting the absorption of bioactive ingredients. Iontophoresis and LED exposure were delivered using the device's standardized preset protocol, according to the manufacturer's specifications.

At the end of each session, the scalp was exposed to red LED light to provide additional biostimulatory support. Each treatment session lasted approximately 20-25 minutes.

Treatment sessions were performed at 3-week intervals, resulting in a total treatment duration of approximately 9 weeks. Clinical outcomes were evaluated immediately after completion of the fourth treatment session.

Composition of the Topical Gel

The topical gel used in this study consisted of bioactive peptides, carrier agents, and stabilizing components formulated to support follicular activity and facilitate transdermal delivery.

Bioactive Components

The formulation included the following peptides: octapeptide-2, a growth factor-like peptide involved in cellular signaling and follicular activation; copper tripeptide-1 (Glycyl-L-Histidyl-L-Lysine-Copper), an angiogenic and reparative peptide associated with dermal papilla activation, vascular support, and extracellular matrix remodeling; sh-oligopeptide-1, an epidermal growth factor analogue promoting cellular proliferation; sh-oligopeptide-2, an insulin-like growth factor-1 (IGF-1) analogue implicated in follicular growth and anagen phase support; and sh-polypeptide-1 and sh-polypeptide-3, recombinant growth factor analogues associated with cellular metabolism and microenvironment modulation.

Carriers and Penetration Modulators

Hydroxyethylcellulose served as the gel matrix to ensure uniform distribution and controlled release. Glycerin functions as a humectant, enhancing stratum corneum hydration and facilitating penetration. Hydrogenated lecithin acted as a lipid-based carrier to improve membrane compatibility and enhance transdermal transport. Glycine soja (soybean) oil provided lipophilic support with antioxidant properties, while sodium oleate functioned as an emulsifying and penetration-enhancing agent.

Stabilizing Components

Phenoxyethanol and imidazolidinyl urea were included as antimicrobial preservatives, and disodium EDTA served as a chelating agent to enhance formulation stability.

The procedure was used as an adjunct to standard-of-care management (e.g., topical minoxidil for AGA and etiologic management for TE), reflecting routine clinical practice.

Patients with AGA were routinely prescribed topical minoxidil unless contraindicated. Patients with documented iron or vitamin D deficiency received appropriate replacement therapy according to routine clinical practice. Therefore, the observed outcomes reflect the combined effect of standard-of-care management and the adjunctive multimodal intervention, and should not be interpreted as the isolated effect of iontophoresis-assisted growth factor delivery.

Outcome Assessment

No predefined long-term follow-up protocol was available due to the retrospective design of the study. Outcome measures included patient-reported daily hair-shedding categories, accompanying scalp symptoms, procedural pain, and overall treatment satisfaction. Hair shedding was assessed by asking patients to estimate the number of hairs shed per day during routine clinical evaluation. Based on predefined investigator-assigned categories, responses were classified as <100, 100-200, 200-300, or >300 hairs/day. No validated questionnaire or standardized hair-counting method was used. Because this was a retrospective real-world study, outcome assessments reflected routine clinical practice rather than a standardized research protocol. Procedural pain was assessed at the end of the fourth treatment session using a 10-point visual analogue scale (VAS), where 0 indicated no pain and 10 indicated the worst imaginable pain. Overall treatment satisfaction was evaluated after completion of the treatment protocol using a 5-point Likert scale (1= very dissatisfied; 5= very satisfied). Adverse events were actively assessed and documented at each treatment session.

This study was approved by the Bahçeşehir University Non-Interventional Clinical Research Ethics Committee (approval number: 2026-01/06, date: 07.01.2026). The study was conducted in accordance with the principles of the Declaration of Helsinki. The evaluated treatments had been performed as part of routine clinical practice. Ethics approval was obtained prior to the retrospective review of the medical records and data analysis. Written informed consent was obtained from all patients prior to treatment. As this was a retrospective review of routinely collected clinical data, no additional consent was required for data analysis.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows (IBM Corp., Armonk, NY, USA; version 23.0). Categorical variables were expressed as frequencies and percentages. Differences between independent categorical variables were evaluated using the chi-square test or Fisher's exact test, as appropriate. Because of the small cell counts, the comparison of symptom response categories between diagnostic groups was performed using Fisher's exact test. The distribution of continuous variables was assessed using histograms and the Kolmogorov-Smirnov test. Normally distributed variables were presented as mean ± standard deviation, whereas non-normally distributed variables were expressed as median interquartile range (IQR). Comparisons of independent continuous variables were performed using the Student's t-test or the Mann-Whitney U test, as appropriate. Changes in daily hair shedding before and after treatment were analyzed using the Wilcoxon signed-rank test. Of the 46 patients, 45 had baseline shedding data and 44 had post-treatment data; therefore, only 44 patients had both measurements recorded and were included in the paired Wilcoxon signed-rank analysis. For correlation analyses, the reduction in hair-shedding was calculated as the ordinal difference between baseline and post-treatment hair-shedding categories (baseline category - post-

treatment category), with larger positive values indicating greater improvement. Correlation analyses were performed using Spearman's correlation coefficient. To account for multiple comparisons across the three diagnostic subgroup analyses (TE, AGA, and combined AGA + TE), a Bonferroni correction was applied, yielding an adjusted significance threshold of α=0.0167 (0.05/3). For all other analyses, a two-tailed p-value <0.05 was considered statistically significant. Due to the retrospective design, some variables were incompletely documented; denominators are therefore reported where applicable.

RESULTS

Patient Characteristics

During the study period, the medical records of 78 consecutive patients who underwent adjunctive multimodal therapy incorporating iontophoresis-assisted growth factor delivery were screened for eligibility. 18 patients did not complete the planned four-session treatment protocol; 14 had missing diagnostic information; and 2 had diagnoses that were incompatible with the study eligibility criteria (frontal fibrosing alopecia and trichotillomania). Two patients had missing diagnostic information and incomplete treatment. Thus, 32 unique patients were excluded and 46 were included in the final analysis. A flow diagram illustrating patient screening, reasons for exclusion, and inclusion in the study is presented in Figure 1.

The baseline characteristics of the study population are summarized in Table 1. A total of 46 patients were included in the analysis. The mean age was 33.3±9.9 years, and the mean body mass index (BMI) was 23.6±4.3 kg/m^2. Diagnostic information was available for 45 patients.

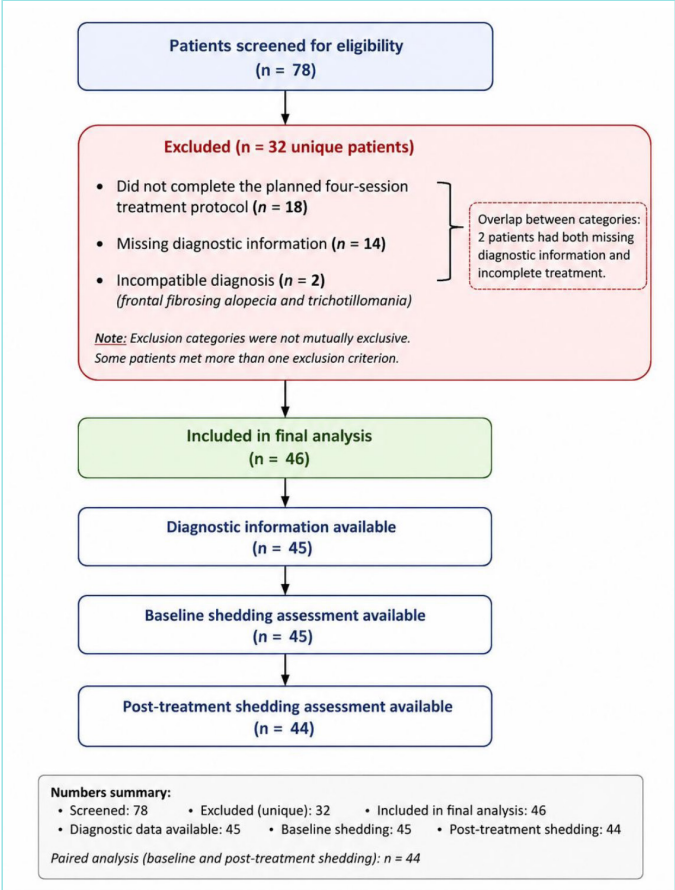

Figure 1. Flow diagram of patient selection and study inclusion.

Changes in Daily Hair Shedding

Baseline shedding data were available for 45 patients and post-treatment data for 44 patients. Paired baseline and post-treatment measurements, required for the Wilcoxon signed-rank test, were available for 44 patients. Missing baseline or post-treatment shedding data reflected incomplete documentation in routine clinical records, rather than protocol-defined exclusions.

Changes in patient-reported daily hair shedding before and after treatment are summarized in Table 2. Among the 44 patients with paired measurements, patient-reported daily hair shedding decreased significantly following treatment (Wilcoxon signed-rank test, Z=-4.318, p<0.001, r=0.65), indicating a large effect size. After applying the Bonferroni correction for the three diagnostic subgroup comparisons (adjusted α=0.0167), the decrease in hair shedding remained statistically significant in the TE subgroup (n=15, Z=-3.169, r=0.82, p=0.002), whereas the decreases observed in the AGA (n=19, Z=-2.165, r=0.50, p=0.030) and combined AGA + TE (n=9, Z=-2.236, r=0.75, p=0.025) subgroups did not meet the Bonferroni-adjusted significance threshold, although both were statistically significant at the unadjusted α=0.05

Table 1. Baseline characteristics of the study population

Characteristic	Value
Overall sample (n=46)	
Age (years), mean ± SD	33.3±9.9
Female sex, n (%)	25 (54.3)
Male sex, n (%)	21 (45.7)
Height (cm), mean ± SD	170.6±10.6
Weight (kg), mean ± SD	72.9±18.7
Body mass index (kg/m^2), mean ± SD	23.6±4.3
Diagnosis (n=45), n (%)*	
Androgenetic alopecia (AGA)	20 (44.4)
Telogen effluvium (TE)	15 (33.3)
AGA + TE	10 (22.2)
Family history of hair loss, n (%)	37 (80.4)
Current smoker, n (%)	11 (23.9)
Alcohol consumption, n (%)	10 (21.7)
Presence of systemic disease, n (%)	12 (26.1)
Systemic medication use, n (%)	20 (43.5)
Daily hair shedding before treatment (n=45), n (%)	
<100 hairs/day	15 (33.3)
100-200 hairs/day	23 (51.1)
200-300 hairs/day	4 (8.9)
>300 hairs/day	3 (6.7)
Presence of scalp symptoms (n=46), n (%)	
Any scalp symptom	15 (32.6)
Pain	4 (8.7)
Itching	11 (23.9)

*Diagnostic information was available for 45 patients; percentages for diagnostic categories were calculated using the number of patients with available diagnostic data.
SD: Standard deviation.

Table 2. Distribution of patient-reported daily hair shedding categories before and after treatment according to diagnosis

Hair shedding category	Baseline	After 4th session
Overall (n=44), p<0.001		
<100 hairs/day	15 (34.1)	36 (81.8)
100-200 hairs/day	22 (50.0)	8 (18.2)
200-300 hairs/day	4 (9.1)	0
>300 hairs/day	3 (6.8)	0
Androgenetic alopecia (AGA) (n=19), p=0.030		
<100 hairs/day	11 (57.9)	17 (89.5)
100-200 hairs/day	5 (26.3)	2 (10.5)
200-300 hairs/day	3 (15.8)	0
>300 hairs/day	0	0
Telogen effluvium (TE) (n=15), p=0.002		
<100 hairs/day	1 (6.7)	11 (73.3)
100-200 hairs/day	10 (66.7)	4 (26.7)
200-300 hairs/day	1 (6.7)	0
>300 hairs/day	3 (20.0)	0
AGA + TE (n=9), p=0.025		
<100 hairs/day	3 (33.3)	8 (88.9)
100-200 hairs/day	6 (66.7)	1 (11.1)
200-300 hairs/day	0	0
>300 hairs/day	0	0

Values are presented as n (%). Percentages were calculated using the number of patients with paired baseline and post-treatment assessments in each group. Hair shedding before and after treatment was compared using the Wilcoxon signed-rank test. For the three diagnostic subgroup comparisons, a Bonferroni correction was applied, with an adjusted significance threshold of α=0.0167; reported p-values are unadjusted.

level. Patients without documented diagnostic classification (n=1) were excluded only from the diagnosis-based subgroup analyses and were included in the overall paired analysis. Notably, no patients remained in the ≥200 hairs/day categories after treatment.

These findings indicate a consistent reduction in daily hair shedding reported by patients following the adjunctive multimodal treatment protocol in this real-world clinical cohort.

Symptom Response

Baseline scalp symptoms, symptom response after treatment, procedural pain, treatment satisfaction, and adverse events are summarized in Table 3.

Symptom response was analyzed separately from patient-reported hair shedding, to distinguish subjective symptom improvement from changes in shedding. Patients with TE (n=8) demonstrated significantly greater symptom improvement compared with patients with isolated AGA (n=7) (Fisher's exact test, p=0.016).

No statistically significant associations were observed between symptom response and age, sex, BMI, smoking status, alcohol consumption, systemic disease, medication use, family history of hair loss, or baseline shedding category (all p>0.05).

Table 3. Patient-reported outcomes following adjunctive multimodal therapy

Outcome	Result
Baseline scalp symptoms (n=46), n (%)	
Any scalp symptom	15 (32.6)
Pain	4 (8.7)
Itching	11 (23.9)
Symptom response among symptomatic patients (n=15), n (%)	
No improvement	3 (20.0)
Partial improvement	10 (66.7)
Complete improvement	2 (13.3)
Procedural pain at the end of the fourth session (VAS, 0-10; n=46), median (IQR)	5 (2-7)
Treatment satisfaction (5-point Likert scale) (n=46), n (%)	
1 (very dissatisfied)	3 (6.5)
2	1 (2.2)
3	8 (17.4)
4	22 (47.8)
5 (very satisfied)	12 (26.1)
Adverse events	
Severe adverse events	None documented

Values are presented as n (%) unless otherwise stated. Pain was assessed using a 10-point visual analogue scale (VAS). Treatment satisfaction was assessed using a 5-point Likert scale. Symptom response was evaluated only in patients reporting baseline scalp symptoms (n=15). No severe adverse events were documented in the medical records. IQR: Interquartile range.

Pain During Treatment

Procedural pain and adverse events are summarized in Table 3. Procedural pain was assessed in 46 patients at the end of the fourth treatment session. The median VAS pain score was 5 (IQR, 2-7). No severe adverse events were documented in the medical records.

Patient Satisfaction

Treatment satisfaction scores are summarized in Table 3.

A moderate positive correlation was observed between the reduction in daily hair shedding and patient satisfaction (Spearman's correlation test, n=44, r=0.48, p=0.004), indicating that greater perceived clinical improvement was associated with higher satisfaction levels.

Age showed a weak negative correlation with satisfaction (r=-0.25), which did not reach statistical significance (p=0.098; Spearman's correlation test, n=46). Satisfaction was not significantly associated with sex, BMI, smoking status, alcohol consumption, systemic disease, medication use, family history of hair loss, baseline shedding, or baseline symptom presence (all p>0.05).

DISCUSSION

Non-invasive transdermal delivery is limited by the barrier function of the stratum corneum; however, iontophoresis enhances the penetration of hydrophilic and charged molecules through low-intensity electrical current.[11] In the context of hair disorders, this approach may increase the follicular bioavailability of topically applied bioactive compounds.

Experimental studies support this mechanism. Gelfuso et al.[12] demonstrated significantly greater follicular accumulation of minoxidil sulphate with iontophoresis compared to passive delivery, with findings further supported by *in vivo* evidence. Additional studies have shown that combining optimized formulations with iontophoresis enhances follicular targeting.[12,13]

Clinical investigations have reported improvements in hair density and diameter following growth factor-based therapies delivered via iontophoresis and in split-scalp designs where iontophoresis enhanced transdermal absorption of growth factor cocktails.[10,14] Similarly, favorable outcomes and high tolerability have been reported with iontophoresis-assisted adjunctive protocols.[15]

Against this background, in this real-world retrospective cohort, daily hair shedding significantly decreased after four sessions ($p<0.001$). After Bonferroni correction for the three diagnostic subgroup comparisons (adjusted $\alpha=0.0167$), the reduction remained statistically significant in the TE subgroup ($p=0.002$), whereas the reductions observed in the AGA ($p=0.030$) and combined AGA + TE ($p=0.025$) subgroups did not meet the corrected significance threshold. Notably, no patients remained in the ≥200 hairs/day categories post-treatment. Although shedding categories represent patient-reported outcomes, the overall reduction and the direction of change observed across diagnostic subgroups suggest a pattern of improvement. A reduction in patient-perceived daily hair shedding may represent an important early treatment outcome, even in the absence of objective evidence of hair regrowth. Excessive hair shedding is one of the most common reasons patients seek medical attention, and is frequently associated with anxiety and a reduced quality of life. Therefore, a perceived reduction in shedding may improve patients' reassurance, treatment adherence, and overall satisfaction; however, these subjective findings should be interpreted cautiously until confirmed by objective assessments. However, because the study lacked a control or sham-treated group, these changes cannot be attributed solely to iontophoresis-assisted growth factor therapy.

The intervention evaluated in this study consisted of a standardized multimodal protocol that included controlled microdermal stimulation, radial pressure waves, iontophoresis-assisted delivery of a growth-factor-containing gel, and red-LED exposure. Consequently, the observed clinical improvements should not be attributed to any individual component of the protocol, including iontophoresis, but should rather be interpreted as the overall effect of the combined adjunctive intervention delivered in routine clinical practice.

While classical AGA is primarily characterized by progressive follicular miniaturization rather than pronounced daily shedding, increased hair shedding is commonly reported in routine clinical practice, particularly in early-stage disease or in mixed phenotypes such as AGA combined with TE. Therefore, changes in patient-reported shedding categories in our cohort should be interpreted within this clinical context rather than as a direct surrogate marker of hair regrowth. In addition, the natural course of TE, which may improve spontaneously after correction of triggering factors or over time, should be considered when interpreting the reduction in shedding observed in TE patients.

Moreover, because all patients received standard-of-care management according to their underlying diagnosis, including topical minoxidil for AGA and correction of reversible causes of TE when indicated, the relative contribution of the adjunctive multimodal intervention cannot be determined in the absence of a comparator group.

Symptom improvement was analyzed separately from reduction in shedding. Patients with TE demonstrated greater symptom improvement than those with isolated AGA, potentially reflecting underlying pathophysiologic differences. A moderate correlation between shedding reduction and patient satisfaction ($r=0.48$, $p=0.004$) indicates that perceived clinical improvement translates into meaningful patient benefit. Nevertheless, patient-reported outcomes are susceptible to recall bias and expectation effects, particularly in uncontrolled real-world studies.

The observed clinical findings are biologically plausible, as growth factor-based interventions have been shown to promote hair regeneration and anagen-related gene expression, and data have identified IGF-1 as an important regulator of follicular growth.[16,17] Multiple growth factors involved in follicular cycling and microenvironment modulation have been described.[18] Our peptide-rich formulation aligns with this regenerative framework.

Our findings are consistent with prior regenerative topical and growth factor-based studies reporting reduced shedding and improved hair parameters.[1,19,20] Across these modalities, enhanced follicular delivery appears central to clinical improvement.

The present study reflects routine clinical practice in which iontophoresis-assisted growth factor therapy was applied as an adjunct to standard management. While this design limits the ability to isolate of its independent effect, the overall reduction in patient-reported shedding, favorable tolerability profile, and association between clinical response and satisfaction provide supportive real-world evidence suggesting the feasibility of this adjunctive approach in hair loss management.

Study Limitations

Several limitations should be acknowledged. The retrospective design and absence of a control or sham-treated group preclude causal inference and limit the ability to account for placebo effects or regression to the mean. In addition, only patients who completed the planned four-session treatment protocol and met the eligibility criteria were included in the final analysis. Consequently, attrition and completer bias cannot be excluded, and treatment tolerability, patient satisfaction, and favorable patient-reported outcomes may have been overestimated. The intervention was administered alongside standard-of-care treatments, including topical minoxidil in AGA and etiologic management in TE, which limited the ability to isolate the independent contribution of iontophoresis-assisted therapy. In addition, detailed patient-level data regarding micronutrient supplementation were not consistently available. Furthermore, this was a single-center study with a relatively small and clinically heterogeneous sample, including patients with AGA, TE, and mixed AGA + TE. These characteristics may limit the external validity (generalizability) of the findings to other patient populations, clinical settings, or treatment protocols. In addition, the relatively small sample size within diagnostic subgroups reduced statistical power for subgroup analyses. Missing data for some variables may also introduce selection bias.

In addition, objective outcome measures, such as standardized global photography, phototrichograms, hair density measurements, hair shaft diameter analysis, and blinded trichoscopic assessments, were

not available. Consequently, treatment response relied primarily on patient-reported outcomes, which are inherently susceptible to recall and expectation biases. Furthermore, clinical outcomes were assessed only immediately after the fourth treatment session; therefore, the durability of the observed improvements remains unknown.

Nevertheless, the observed reduction in patient-reported hair shedding and its association with treatment satisfaction support the feasibility of this adjunctive multimodal approach in routine clinical practice and justify further prospective evaluation. These findings should therefore be interpreted as real-world observational evidence supporting feasibility rather than definitive evidence of therapeutic efficacy.

CONCLUSION

Adjunctive multimodal therapy incorporating iontophoresis-assisted growth factor delivery was associated with improvements in patient-reported hair shedding and treatment satisfaction among patients in this real-world retrospective cohort who completed the treatment protocol. Although the retrospective, uncontrolled design and concomitant standard therapies preclude attribution of an independent treatment effect, these findings support the feasibility of incorporating this adjunctive multimodal treatment protocol into routine clinical practice.

Prospective, randomized, sham-controlled studies incorporating objective outcome measures, including standardized global photography, blinded trichoscopic assessment, phototrichogram-derived hair density, and hair-shaft diameter measurements, and longer follow-up, are warranted to determine the independent therapeutic contribution of iontophoresis-assisted growth factor delivery within this multimodal treatment protocol.

MAIN POINTS

- Adjunctive multimodal therapy incorporating iontophoresis-assisted growth-factor delivery was associated with a significant overall reduction in patient-reported daily hair shedding; after Bonferroni correction, the reduction remained statistically significant in the telogen effluvium subgroup.
- After four sessions, no patients remained in the ≥200 hairs/day shedding categories, indicating a favorable change in patient-reported hair shedding.
- A reduction in patient-reported hair shedding correlated with higher treatment satisfaction, which supports the clinical relevance of this adjunctive multimodal approach in real-world practice.

ETHICS

Ethics Committee Approval: The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Bahçeşehir University Non-Interventional Clinical Research Ethics Committee (approval number: 2026-01/06, date: 07.01.2026).

Informed Consent: Written informed consent was obtained from all participants prior to treatment. Due to the retrospective design of the study, no additional consent was required for data analysis.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ü.A., E.C., Concept: Ü.A., E.C., N.F.İ., Design: Ü.A., E.C., Y.H., N.F.İ., Data Collection and/or Processing: Ü.A., Y.H., Analysis and/or Interpretation: Ü.A., Y.H., Literature Search: Ü.A., Y.H., N.F.İ., Writing: Ü.A., Y.H.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors. The device manufacturer had no role in the study design, data collection, data analysis, manuscript preparation, or the decision to submit the manuscript for publication. The authors have no financial or commercial relationship with the manufacturer of the device evaluated in this study.

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CASE REPORT

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Kleefstra Syndrome-2 Case with a Novel Mutation and Multiple Disorders

İlke Beyitler[1], Yeliz Engindereli[2], Mahmut Çerkez Ergören[3]

[1]Department of Pediatrics, Near East University Faculty of Medicine, Nicosia, Cyprus
[2]Department of Child and Adolescent Psychiatry, Near East University Faculty of Medicine, Nicosia, Cyprus
[3]Department of Medical Genetics, Near East University Faculty of Medicine, Nicosia, Cyprus

Abstract

Kleefstra syndrome-2 (KLEFS2) is an extremely rare autosomal dominant condition characterized by a neurodevelopmental disorder and multisystem involvement. The main clinical manifestations include intellectual disability, autistic features, hypotonia, and dysmorphic facial features. We describe a unique case of KLEFS2 in a patient with multiple disorders, treated with anakinra for colchicine-resistant familial Mediterranean fever, who also has delta-beta thalassemia, dyserythropoietic anemia, and butyrylcholinesterase deficiency. A diagnostic dilemma arose because of several abnormalities and distinctive features. This KLEFS2 case exhibited a mild phenotype without neurological deformities. These findings suggest a possible polygenic influence or a convergence of pathways contributing to the complex phenotype. When several abnormalities lead to a diagnostic dilemma, polygenic etiology should be considered.

Keywords: Kleefstra syndrome 2, familial Mediterranean fever, thalassemia, butyrylcholinesterase deficiency

INTRODUCTION

Kleefstra syndrome is a rare autosomal dominant condition mainly characterized by intellectual disability, autistic features, hypotonia and dysmorphic face. Several organs including heart, skelatal and urogenital system may also be affected leading to high phenotypic heterogenity. Hyperactivity, aggressiveness, epilepsy, sleeping disorders, automutilation, insensitivity to pain, abnormal gait, strabismus, hydrocephalus, hypoplasia of cereballar vermis, constipation and dry skin are defined in reported cases. In addition to *euchromatic histone lysine methyltransferase 1* (*EHMT1*) mutations, variants of four more genes have been defined in *MBD5*, *SMARCB1*, *NR1I3*, and *KMT2C*. While there are more than 100 cases of Kleeftsra syndrome-1 (KLEFS1, OMIM:610253) due to *EHMT1* defect that was initially defined in 1999, reported [Kleeftsra syndrome-2 (KLEFS2), OMIM:617786] due to *KMT2C* mutation cases are 130. Although interventions aim to improve behavioral disorders as well as the growth and development of patients, data on long-term prognosis are still lacking.[1-3]

Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disease endemic in the Mediterranean and the Middle East, causing recurrent attacks of fever and serosal inflammation, and the devastating complication of AA amyloidosis, which can lead to renal failure if left untreated. *MEFV* gene mutations cause dysregulation of the interleukin-1 (IL-1)-mediated inflammatory response, which is successfully controlled by colchicine treatment in the majority of cases.[4]

Thalassemia is a heterogeneous autosomal recessive disorder that is common in the Mediterranean area. Some mutations in the *HBB* gene are inherited in an autosomal dominant manner and lead to a rare form of delta-beta (δ/β) thalassemia that causes mild anemia.[5]

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ORCID IDs of the authors: İ.B. 0000-0002-7758-9015; Y.E. 0009-0003-1868-0841; M.Ç.E. 0000-0001-9593-9325.

Corresponding author: İlke Beyitler
E-mail: ilkebeyit82@yahoo.com
ORCID ID: orcid.org/0000-0002-7758-9015

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Autosomal dominant dyserythropoietic anemia type III, on the other hand, is a very rare condition that has been reported in one American and one Swedish family with a unique variant c.2747C>G (p.P916R) in the *KIF23* gene. The following are defined: absent or moderate anemia, hemolysis, multinucleated erythroblasts, and increased risk of monoclonal gammopathy.[6]

Butyrylcholinesterase deficiency caused by *BCHE* mutations is characterized by prolonged postanesthetic apnea. Slow metabolic degradation of choline esters results in prolonged awakening after general or local anesthesia.[7]

We reported a unique case of multiple rare disorders: KLEFS2, FMF, δ/β thalassemia, dyserythropoietic anemia, and butyrylcholinesterase deficiency, presenting with recurrent inflammatory attacks characterized by fever, joint complaints, increased acute-phase reactants, colchicine resistance, behavioral disorders, intellectual disability, a history of anemia, urticarial rash, cervical and mesenteric lymphadenopathy. A diagnostic dilemma arising from several abnormalities and distinctive features led to consideration of a polygenic etiology.

CASE REPORT

A fourteen-year-old female patient has been followed up at our hospital since the age of 9. She presented with dysuria, eye blinking, hand clapping, and insistence on sameness.

She was treated with corticosteroids for hemolytic anemia at four months of age. She was diagnosed with FMF at 16 months of age because of recurrent fever, arthritis, arthralgia, abdominal pain, and elevated acute-phase reactants. Homozygous *MEFV*:p.E148Q mutation was detected, however she did not respond to colchicine. When she was 29 months old, she had minimal hepatosplenomegaly, cervical and axillary lymphadenopathy an urticarial rash, mild intellectual disability, and fever and joint complaints. She had a high hemoglobin F (HbF) level. She was screened in infancy for mutations associated with periodic fever syndromes: mevalonate kinase, tumor necrosis factor receptor-associated periodic syndrome, cryopyrin-associated periodic syndromes, and deficiency of the IL-1 receptor antagonist and no mutation was detected at another center. IL-1 receptor antagonist Anakinra was also initiated at another center because of persistent fever and elevated acute-phase reactants, which led to an immediate clinical response and normalization of C-reactive protein (CRP) levels. She was also prescribed aripiprazole for behavioral disorders. A mother of advanced maternal age became pregnant after in vitro fertilization treatment. The mother had the thalassemia trait (*HBB*:c.93-21G>A), and the father was healthy; the parents were not consanguineous.

Physical examination revealed short stature (weight at the 3rd percentile; height below the 3rd percentile) with normal pubertal development. She had down-slanting palpebral fissures, a nose with a mild saddle bridge and a bulbous tip, a thin upper lip, a mildly down-turned mouth, misaligned teeth, and a high palate. On psychiatric assessment, she had autism spectrum disorder, with limited social interactions and communication skills, and mild intellectual disability on Wechsler Intelligence Scale for Children-IV. She had a chronic motor tic disorder characterized by eye blinking and stereotypic hand-clapping movements that occurred when she was excited.

Laboratory results revealed leukocyte: 10200/mm^3, 65% lymphocytes, hemoglobine:13 gr/dL, platelet: 417000/mm^3, CRP:0,5 mg/dL, routine urine analysis: 8-10 leukocytes/each area, urine culture: 10^5 colony-forming unit *Escherichia coli*. Lymphocyte predominance was observed, and the lymphocyte panel showed a reversed CD4/CD8 ratio of 0.6. She had a urinary infection. On urinary ultrasound, she had bladder diverticula and bladder wall thickening measuring 5 mm. After treatment of urinary infection with trimethoprim/sulfamethoxazole, voiding cystourethrography was recommended, and voiding education and prophylactic nitrofurantoin were initiated. Bladder wall thickening normalized. Cranial magnetic resonance imaging was normal. A summary of laboratory results is shown in Table 1. Anakinra is continued, and she has normal acute-phase reactants. Aripiprazole is also used in combination with behavioral interventions, which improve but cannot thoroughly correct behavioral problems.

Whole-exome sequencing (WES) was conducted using an Illumina platform with high and uniform coverage (≥20× for 99.65% of targets). Variant detection and annotation were performed using a validated in-house pipeline incorporating VEP v94 and ACMG-based classification. The analysis was limited to a solo WES design without parental sequencing. However, findings and putative carrier status were confirmed by Sanger sequencing in the parents. WES was carried out, with particular focus on Fas mutation and Rett syndrome, both of which were negative. Recently, we detected KLEFS2, δ/β-thalassemia, dyserythropoietic anemia, and butyrylcholinesterase deficiency. WES analysis revealed a *de novo* heterozygous likely pathogenic (PVS1 and PM2) variant (NM_170606.3:c.6652dup; p.(Tyr2218Leufs*18) which is a novel mutation within the *KMT2C* gene. This variant, which causes a frameshift mutation, has not been registered in the NCBI ds database, gnomAD, ESP, 1000 G. Mutations in the *KMT2C* gene are associated with autosomal dominant Kleefstra syndrome type 2 (OMIM®: 617768). Moreover, WES analysis determined potentially relevant variants such as the heterozygous *HBB*:c.93-21G>A mutation causing autosomal dominant δ/β thalassemia/hereditary persistence of fetal hemoglobin (OMIM®: 141749), and the heterozygous *KIF23*:c.2140T>C; p.(Ser714Pro)

Table 1. Laboratory results of the case obtained during febrile attack and attack free periods

Age	8 months	11 years (during febrile atack)	14 years (atack free period)
Leukocyte/mm^3	17300	12140	8100
Hemoglobin (gr/dL)	6	13	12.5
CRP (mg/dL) (n: <0.5)	11.9	6.5	0.23
Erythrocyte sedimentation rate (mm/h) (n: <15)	87	35	14
Serum amyloid a protein (mg/L) (n: 0-7)	N/A	635	5

CRP: C-reactive protein.

causing autosomal dominant congenital dyserythropoietic anemia type IIIA (OMIM®:105600). The carrier status was also detected for a heterozygote likely pathogenic *BCHE*:c.293A>G; p.(Asp98Gly) variation that causes autosomal recessive butyrylcholinesterase deficiency (OMIM®:617936) (Table 2). Familial segregation analysis indicated that the *KMT2C*:c.6652 duplication variant was *de novo,* whereas the *KIF23*:c.2140T>C and *HBB*:c.93-21G>A variants were inherited only maternally. These findings overlapped with the patient's clinical phenotype. Therefore, we assessed putative gene-gene interactions using the publicly available software GeneMANIA (genemania.org) (Figure 1). The results showed that the genes *HBB*, *MEFV*, *KIF23*, and *KMT2C* interacted in the same pathways. Informed consent for publication was obtained from the parents of the case.

DISCUSSION

Kleefstra syndrome is a rare neurodevelopmental disorder with autosomal dominant inheritance. Although both forms of the syndrome are associated with multiple overlapping neurodevelopmental and psychiatric presentations, the clinical features of KLEFS1 and KLEFS2 are presented in Table 3.

Reported patients with KLEFS2 exhibited features including intellectual disability ranging from mild to severe; behavioral disorders (autism, hyperactivity, aggression); mild facial dysmorphisms; short stature; and, rarely, eczema most of which were similar to and present in our defined case.[1,2]

Other features as developmental delay, epilepsy, scoliosis, strabismus, microcephaly, plagiocephaly, recurrent respiratory infections, dry skin, bifid uvula, feeding difficulty, constipation, persistent weight loss, telalgia, hoarseness, inguinal hernia, insensitivity to pain, hydrocephalus, and hypoplasia of cerebellar vermis were differences as not observed in the current case.[1,8,9]

Although hypospadias and cryptorchidism have been reported in the literature, none of the KLEFS2 patients had urological abnormalities; however, the presented case had recurrent urinary tract infections, bladder wall thickening, and bladder diverticula requiring prophylaxis.[1,2] Tic disorders and hand clapping are also present in our patient, whereas they have not been reported in other KLEFS2 cases.

Table 2. Showing whole exome sequencing (WES) results and their characteristics

Gene	Variant	Zygosity	*In silico* parameters*	Allele frequencies**	Type & classification***
KMT2C	NM_170606.3: c.6652dup p.(Tyr2218Leufs*18) rs-	Heterozygous	PolyPhen: - Align-GVGD: N/A SIFT: : N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A	gnomAD: - ESP: - 1000 G: -	Frameshift likely pathogenic (class 2) PVS1, PM2,
HBB	NM_000518.4: c.93-21G>A p.? rs35004220	Heterozygous	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A	gnomAD: 0.00015 ESP: 0.00015 1000 G: 0	Effect unknown pathogenic (class 1) PM3, PM2, PP5
KIF23	NM_138555.3: c.2140T>C p.(Ser714Pro) rs-	Heterozygous	PolyPhen: Benign Align-GVGD: C0 SIFT: Tolerated MutationTaster: Disease causing Conservation_nt: moderate Conservation_aa: high	gnomAD: - ESP: - 1000 G: -	Missense uncertain significance (class 3) PM2
BCHE	NM_000055.3: c.293A>G p.(Asp98Gly) rs1799807	Heterozygous	PolyPhen: Possibly damaging Align-GVGD: C65 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high	gnomAD: 0.012 ESP: 0.014 1000 G: 0.0060	Missense pathogenic (class 1) PM2, PM1, PP2, PP5

Variant annotation based on OTFA (using VEP v94).
*AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function; splicing predictions: Ada and RF scores.
**Genome Aggregation database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G).
***Based on ACMG/ACP recommendations.

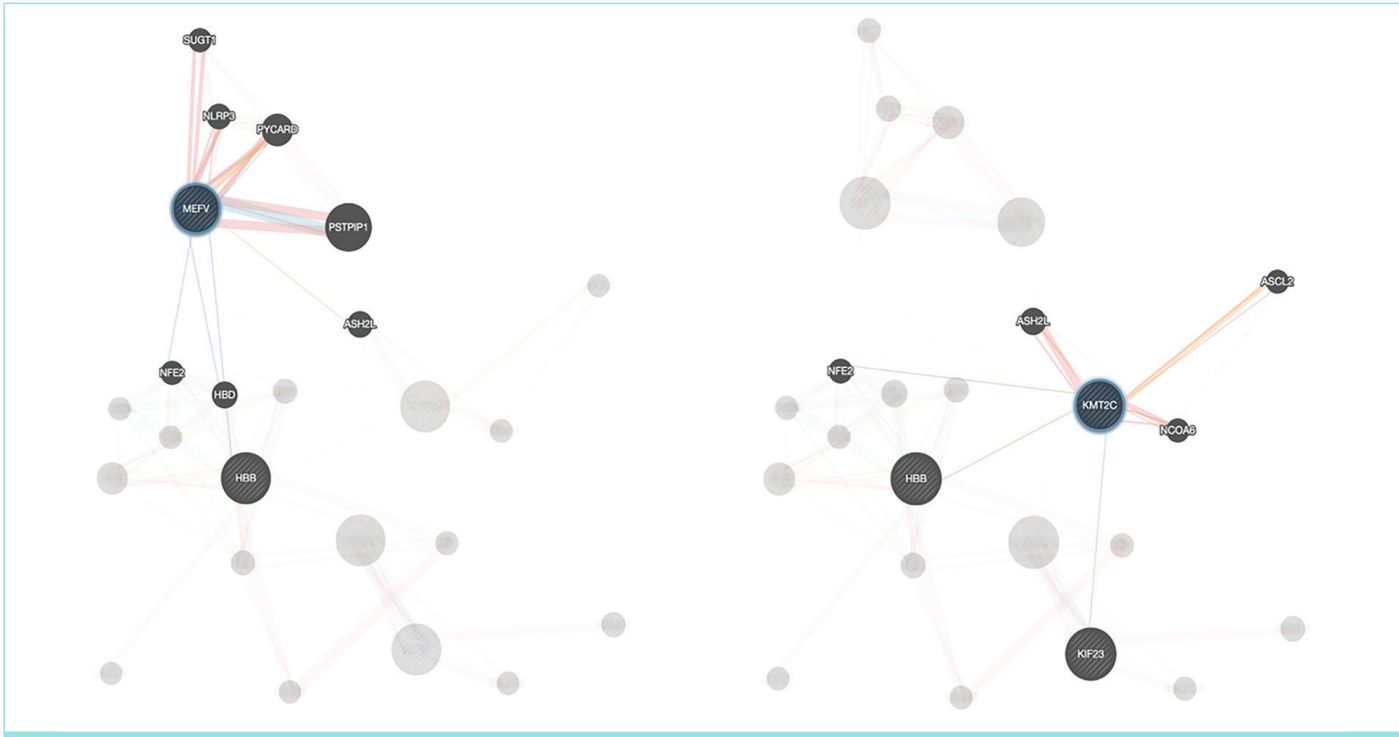

Figure 1. Putative gene-gene interactions of *HBB* and *MEFV* genes and *KIF23*, *HBB* and *KMT2C* interacting in the same pathways.

A 6-year-old female with a *de novo KMT2C* mutation leading to KLEFS2 was reported to have hypotonia, autism, hyperactivity, ventricular gliotic changes, and minor facial anomalies.[2] Another 9-year-old male with a *de novo KMT2C* mutation had developmental delay, anemia in infancy, hyperactivity, scoliosis, and facial features. He was the first case to have eczema, while our case was the second. He also had mild β-thalassemia, which caused anemia in infancy, and our case is the second case of KLEFS2 co-occurring with thalassemia.[1] The current case is distinct for having a rare autosomal dominant δ/β thalassemia and an autosomal dominant dyserythropoietic anemia. She and her mother had a history of anemia during infancy that resolved later in life. Unlike β-thalassemia, anemia is very mild in patients with δ/β-thalassemia. The case had elevated HbF levels, as expected for this disorder.[5] The revealed autosomal dominant dyserythropoietic anemia was also asymptomatic. The detected mutation in the *KIF23* gene is c.2140T>C (S714P), a variant of unknown significance.

Butyrylcholinesterase deficiency is a condition that may cause a prolonged wake-up time after anesthesia. Intellectual disability was also identified in one child, suggesting a connection with *BCHE* mutation. Regulation of dorsal root ganglia and the growth of axons, nerve terminals, and cones were related to *BCHE* in animal experiments. Lower functional *BCHE* activity was also associated with intellectual disability.[7] Investigation of *BHCE* in cancer and pregnancy demonstrated immunological differences in peripheral blood leukocyte populations and an inverse correlation with CD4 and CD8 levels.[10,11] Although the presented case had carrier status for the *BHCE* mutation, the inverse CD4/CD8 ratio may also be attributable to that mutation.

Recurrent inflammatory attacks presenting with fever, arthralgia, increased levels of inflammatory markers, colchicine resistance, behavioral disorders, history of anemia, urticarial rash, cervical and mesenteric lymphadenopathies seemed as FMF with anE148Q mutation was not the only cause, as additional systemic findings led to challenge in diagnosis. Mesenteric lymphadenopathy is described in FMF, but hilar, paratracheal, axillary, pelvic and retroperitoneal lymphadepathies are extremely rare and defined in one case.[12] The inflammatory attacks in the current case were controlled after treatment with anakinra.

Although E148Q mutation of *MEFV* may very rarelycause amyloidosis, renal failure,and death, it should be considered when it is homozygous for timely follow-up and treatment.[13,14] *HBB* and *MEFV*, as well as *KIF23* and *KMT2C* interact in the same genetic pathways; hence, disruptions of these genes likely lead to combined, unexpected phenotypes.

NMDA receptor-dependent neural activity was altered, causing neuronal dysfunction in a mouse model of Kleefstra syndrome; this dysfunction was shown to recover after treatment with NMDA receptor antagonists. NMDAR inhibition also rescued neuronal network phenotypes in mature adults and represents a promising strategy.[15]

Moreover, the upregulation of inflammation-related genes, including IL-1β, which link to immune response, microglial activation and defects was reversed by the neuron-specific lysine methyltransferase G9a-like protein in a mouse model of Kleefstra syndrome. IL-1 receptor antagonist treatment was demonstrated to reverse the neurological phenotypes resulting from haploinsufficiency-induced activation of neuroinflammation via the caspase-1-IL-1β pathway in Kleefstra syndrome. Neurological abnormalities, hypoactivity, and autistic-like features normalized, while the behavioral phenotype was more difficult to correct, suggesting a better outcome with earlier supplementation.[16]

A recent study similarly reported that *KMT2C* mutations cause developmental delay, intellectual disability, behavioral and psychiatric

problems, convulsions, hypotonia, short stature, and facial features. However, they evaluated facial gestalt and deoxyribonucleic acid methylation signatures, suggesting that pathogenic variants in *KMT2C* are different from Kleefstra syndrome, so the condition should be renamed as *KMT2C*-related neurodevelopmental disorder.[17]

A limitation of this study is the lack of functional studies and gene expression analyses to determine the exact effects of alterations in these genes. Whole-genome analysis may be conducted in the future.

Table 3. Clinical features of Kleefstra syndrome variants

KLEFS 1	KLEFS 2
Developmental delay	Developmental delay
Learning difficulties	Small stature
Epilepsy	Hyperactivity
Sleeping disorder	Aggresive behavior
Sleep apnea	Hypotonia
Hearing loss	Autism spectrum disorder
Strabismus	Epilepsy
Hypermetropia	Sleeping disorder
Myopia	Automutilation
Astigmatism	Strabismus
Amblyopia	Cryptorchidism
Glaucoma	Bifid uvula
Cardiac valvular diseases	Inguinal hernia
Atrial fibrillation	Dry skin
Tachycardia	Hoarse voice
Asthma	Delayed puberty
Tracheomalacia	Constipation
Feeding difficulty in infancy	Torticollis
Pes planus	Hydrocephalus
Hypotonia	Hypoplasia of vermis
Scoliosis	Microcephaly
Recurrent infections	Macrocephaly
Hypothyroidism	Brachycephaly
Skin abnormalities	Plagiocephaly
Orofacial cleft	Coarse facies
Congenital kidney/urinary anomalies	Broad and rounded forehead
Behavioral and psychiatric disorders	Deep set eyes
Autism spectrum disorder	Hypertelorism
Anxiety disorder	Down-slanting palpebral fissures
Hyperactivity	Ptosis
Schizophrenia spectrum	Arched eyebrows
Bipolar disorder	Short nose
Depressive disorder	Nose with bulbous tip and saddle bridge
Obsessive-ompulsive trait	Narrow philtrum
Aggressive behavior	Thin upper lip, down-turned mouth
Apathy	Misaligned teeth
Small stature	High palate
Obesity	Preauricular tag
Microcephaly	Duplicated thumb
	Hearing loss
	Scoliosis
	Thoracal kyphosis

KLEFS1: Kleeftsra syndrome-1, KLEFS2 : Kleeftsra syndrome-2.

We presented a unique case with multiple disorders: the first case of Kleefstra syndrome treated with anakinra in which the patient did not have severe mental retardation, epilepsy, or scoliosis. Manipulation of epigenetic regulation has been suggested as a novel therapeutic target for Kleefstra syndrome and warrants further clinical investigation. In addition, this case highlights the possibility of a polygenic etiology and gene-gene interactions in colchicine-resistant FMF, with δ/β-thalassemia-associated *HBB* variation.

CONCLUSION

KLEFS2, a condition with high phenotypic heterogeneity, is a rare cause of neurodevelopmental disorder. Multiple disorders should be considered in atypical manifestations. Genetic testing is required in a timely manner when clinical findings are not clearly evident. Diagnosis is essential for informing prognosis, guiding management and providing genetic counseling in the prenatal or preimplantation setting. Behavioral interventions, with support from family, school, and society, are essential for a long period during the child's growth. Recent approaches may explore early intervention using inflammatory regulators to improve phenotypes.

MAIN POINTS

- Kleefstra syndrome-2 is characterized by a neurodevelopmental disorder with manifestations including intellectual disability, autistic features, hypotonia, and dysmorphic facial features.
- When multiple abnormalities leading to a diagnostic dilemma, a polygenic etiology should be considered.
- Genetic testing is essential when clinical presentation is challenging.

ETHICS

Informed Consent: The authors have written informed consent to publish from study participant's parents.

Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.B., Y.E., M.Ç.E., Concept: İ.B., Y.E., M.Ç.E., Design: İ.B., Y.E., M.Ç.E., Data Collection and/or Processing: İ.B., Y.E., M.Ç.E., Analysis and/or Interpretation: İ.B., Y.E., M.Ç.E., Literature Search: İ.B., Writing: İ.B.

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CASE REPORT

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Rare Isolated Bladder Metastasis in Breast Cancer

Ahmet Çamtosun[1], Ömer Faruk Yıldırım[1], Hasan Gökçe[2], Özlem Dalda[2], Murat Yılmaz[1], Harun Barutçu[1]

[1]Department of Urology, İnönü University Faculty of Medicine, Malatya, Türkiye
[2]Department of Pathology, İnönü University Faculty of Medicine, Malatya, Türkiye

Abstract

Breast cancer (BC) is the most common cancer in women, and approximately 317,000 new cases are expected to be diagnosed in the United States in 2025. Lymph nodes, bones, lungs, liver, and brain are common target organs for BC metastasis. Metastasis to the bladder is extremely rare, with approximately 70 cases reported in the literature. A 76-year-old female patient underwent a right total mastectomy by the general surgery team approximately 25 years ago for a mass in the right breast. The patient presented to the urology outpatient clinic with complaints of hematuria and dysuria. Imaging revealed a solid mass measuring approximately 6.5x3.5 cm, protruding into the bladder lumen from the left anterolateral wall, with a distinct soft-tissue component anteriorly extending into the perivesical fatty planes (possible bladder tumor). Based on this, we performed transurethral bladder tumor (TUR-MT). BC accounts for approximately 2.5% of all cases of metastatic bladder cancer in which the breast is the primary site of origin. Most secondary tumors arise by direct spread from another pelvic neoplasm, such as sigmoid colon, prostate, or cervical cancers. Metastases from distant organs are rarely reported in the literature; the most common are stomach, lung, and melanoma. Metastasis to the bladder after BC is rare and may occur many years later. Although the majority of cases are of the lobular subtype, metastasis can also occur in other subtypes. It may be asymptomatic or manifest as hematuria and voiding problems. Diagnosis involves imaging with ultrasound and magnetic resonance imaging, cystoscopic biopsy, and TUR-MT sampling.

Keywords: Bladder, breast cancer, metastasis

INTRODUCTION

Breast cancer (BC) is the most common cancer in women, and approximately 317,000 new cases are expected to be diagnosed in the United States in 2025.[1]

BC can be classified into four molecular subtypes: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-positive, and triple-negative BC. These subtypes have very different metastases, prognoses, and treatment methods.[2]

Lymph nodes, bone, lung, liver, and brain are common target organs for BC metastasis.[3] Metastasis to the bladder is extremely rare, with approximately 70 cases reported in the literature. In an autopsy series conducted in 1950, Abrams et al.[4] identified bladder metastasis in four patients after examining 167 cases of metastatic BC. In this case, which we identified approximately 25 years after diagnosis, we aimed to contribute to the literature by conducting a literature review.

CASE REPORT

A 76-year-old female patient underwent right total mastectomy by general surgery approximately 25 years ago due to a mass in the right breast. Pathology showed a ductal carcinoma with neuroendocrine differentiation (ER+, T2 N0 M0). Tumor dimensions were reported as T: 16x15x15 mm. She received 4 cycles of combination therapy consisting of cyclophosphamide, methotrexate, and 5-fluorouracil. In addition to this treatment, the patient received tamoxifen therapy. The patient was monitored with annual follow-ups. In 2015, while under follow-

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ORCID IDs of the authors: A.Ç. 0000-0002-5390-9088; Ö.F.Y. 0009-0003-8867-2193; H.G. 0000-0002-4658-9987; Ö.D. 0000-0002-7538-3507; M.Y. 0009-0009-7226-4850; H.B. 0009-0003-9253-8675.

Corresponding author: Ömer Faruk Yıldırım
E-mail: omer_yildirim_23@hotmail.com
ORCID ID: orcid.org/0009-0003-8867-2193

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up, the patient presented to the gynecology and obstetrics outpatient clinic with a complaint of vaginal bleeding. Ultrasound revealed a thick-walled cystic lesion with dense contents, measuring 5.5x3.5 cm, in the right ovarian bed. Based on these findings, the patient underwent a total hysterectomy with bilateral salpingo-oophorectomy in 2015.

Pathology: right adnexa: poorly differentiated malignant epithelial tumor (consistent with breast carcinoma/metastasis of high-grade ductal carcinoma). Uterus: poorly differentiated malignant epithelial tumor in the serosa/implant of high-grade breast ductal carcinoma. Left adnexa: reported as normal.

Following this surgery, she received 6 cycles of paclitaxel plus carboplatin in 2016. The patient was followed by medical oncology and had not received any additional treatment. The patient presented to the urology outpatient clinic with complaints of hematuria and dysuria. The imaging results revealed a solid mass measuring approximately 6.5x3.5 cm protruding from the bladder lumen on the left anterolateral wall of the bladder, with a distinct soft tissue component anteriorly extending into the perivesical fatty planes (bladder tumor?). Based on this, we performed a transurethral bladder tumor (TUR-MT) operation.

The TUR-MT pathology result was consistent with poorly differentiated breast carcinoma, with infiltration or metastasis and minimal malignant epithelial tumor involvement. Approximately 20-25% neuroendocrine differentiation in the carcinoma was observed.

Immunohistochemistry

1. In the TUR immunostaining, CK7 was patchy and variably positive; CK20 was negative (suboptimal technique, no external or internal controls); uroplakin was negative; thrombomodulin was negative; p40 was negative; e-cadherin was positive; mammaglobulin was negative; gcdfp-15 was negative; progesterone receptor was moderately to strongly nuclear positive in approximately 40% of cells; estrogen receptor was diffusely and strongly nuclear positive; c-erb-b2 was negative (score 0/suboptimal technique, no external control); CD10 was negative; synaptophysin was negative; chromogranin-A was positive in approximately 20-25% of cells; Ki-67 proliferation index was 70% (nuclear positive) (Figure 1).

The patient was subsequently scheduled by radiation oncology for definitive radiotherapy to the bladder and placed on follow-up.

DISCUSSION

BC accounts for approximately 2.5% of all metastatic bladder cancers as the primary site of origin.[5] Most secondary tumors arise from direct spread from another pelvic neoplasm, such as sigmoid, prostate, or cervical cancer. Metastases from distant organs are rarely reported in the literature, the most common being stomach, lung, and melanoma.[6]

Metastasis initially involves the outer layer of the bladder wall and progresses toward the mucosa. Therefore, the symptoms of bladder involvement appear at a later stage when the mucosa is also affected.[7]

Metastases from the lobular subtype of BC are generally seen either as nodules that spread to parenchymal organs or as a diffuse, sclerotic-like thickening involving serosal surfaces, the retroperitoneal space, or the walls of hollow and internal genital organs. Moreover, this "diffuse" pattern of metastatic spread in the retroperitoneum may cause bilateral hydronephrosis and not be detected for some time

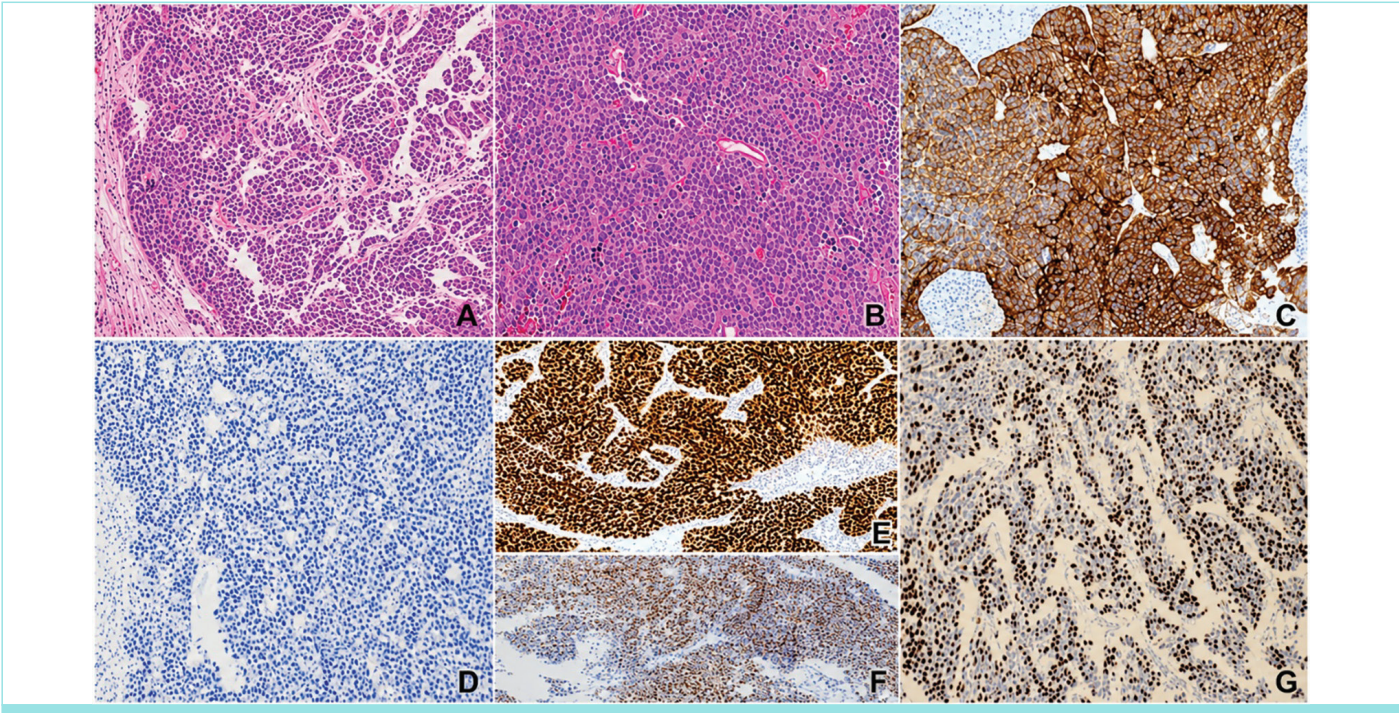

Figure 1. A) Tumor cells form solid layers and cords of varying sizes (H&E, 4x); **B)** Tumor lesion composed of cells with bizarrely shaped large vesicular/hyperchromatic nuclei in places and narrow eosinophilic cytoplasm (H&E, 100x); **C)** CK7 patchy, variable intensity positive; **D)** CK20 negative; **E)** Estrogen receptor diffusely strongly nuclear positive; **F)** Progesterone receptor moderately to strongly nuclear positive in approximately 40% of cells; **G)** Ki-67 proliferation index 70% nuclear positive (100x).

because of its appearance. By contrast, metastases of invasive ductal BC are seen almost exclusively as nodules and do not tend to involve the retroperitoneum or other organs.[8,9] In contrast, in our case the patient's primary diagnosis was reported as ductal carcinoma showing neuroendocrine differentiation.

The presentation of lower urinary tract-like symptoms across a broad clinical spectrum poses challenges for diagnosing isolated bladder metastasis. Clinically, symptoms may range from painless hematuria to stress and urge urinary incontinence, and may also include frequent urination, nocturia, and rarer findings such as dysuria and back pain.[10] That hematuria may be caused by factors such as cystitis, cyclophosphamide, stones, and bladder cancer contributes to diagnostic difficulty.[11] It is present in cases where hydronephrosis is reported as the initial reason for referral.[12,13] The presenting symptoms of some cases reported in the literature are listed in Table 1.

One of the key factors complicating diagnosis is the duration of metastases, which can extend from early to much later stages. While cases ranging from 1 to 17 years have been reported (Table 1), metastasis can occur at much later stages, as seen in our case. Therefore, this must be considered even during long-term follow-up.

Feldman et al.[14] described a patient who had negative cystoscopic findings despite significant evidence of bladder involvement based on symptoms, ultrasound, and CT scans; they stated that the evaluation should include further imaging studies. We supported our case by performing pelvic magnetic resonance imaging (MRI) (Figures 2 and 3). It has also been reported that positron emission tomography/computed tomography imaging is appropriate and contributes to the imaging evaluation of patients with bladder tumors (Figure 4).[15]

Immunohistochemical staining for various markers is routinely used in the diagnosis of undifferentiated tumors. Cytokeratins CK7, CK18, CK19, and CK20 are the main markers used in the diagnosis of epithelial differentiation. Other commonly used markers include estrogen receptor/progesterone receptor for endometrial and breast carcinomas, CA 19-9 for pancreaticobiliary malignancy, prostate-specific antigen for prostate carcinoma, thyroglobulin for thyroid, uroplakin III for urothelium, and HepPar I for hepatocellular carcinoma.[16] Although a review of the literature reveals reported cases of mammaglobin and GCDFP-15 positivity (Table 1), a study of 100 BC patients found Mammaglobin positivity in 57% of metastatic BC cases, compared with 27% for GCDFP-15.[17] In our case, both markers were reported as negative.

CONCLUSION

Metastasis to the bladder after BC is rare and may occur many years later. Although the majority of cases are due to the lobular subtype, metastasis can also occur in other subtypes. It may present asymptomatically or manifest with hematuria and voiding problems. Diagnosis involves imaging with ultrasound and MRI, biopsy with cystoscopy, and TUR-MT sampling. A definitive diagnosis is established by immunohistochemical examination. In patients with a history of BC, new urinary symptoms that appear years later should be evaluated for bladder metastasis.

MAIN POINTS

- Metastasis to the bladder from breast cancer (BC) is extremely rare and may mimic primary bladder tumors clinically and radiologically, leading to diagnostic delay.
- In patients with a history of BC, the development of hematuria and lower urinary tract symptoms, even many years after the initial diagnosis, should raise suspicion of metastasis to the bladder.
- Definitive diagnosis relies on immunohistochemical evaluation of cystoscopic and transurethral bladder tumor specimens in conjunction with imaging findings; and optimal management requires a multidisciplinary approach.

Table 1. Previously reported cases of bladder metastasis from breast cancer and comparison with the present case

Author/year	Age	Histologic subtype	Interval from primary breast cancer	Presenting symptoms	IHC findings	Other metastatic sites	Notes
Kase et al.,[18] 2018	NR	Invasive ductal carcinoma	8 years	Urinary incontinence	ER (+), PR (-), GCDFP-15 (+)	Bone metastasis	Secondary bladder metastasis
Hanley et al.,[19] 2022	64	Not reported	13 years	Hematuria, urinary incontinence	ER (-), PR (-), HER2 (-), GATA3 (+), GCDFP-15 (+)	None	Isolated bladder metastasis
Oshiro et al.,[13] 2024	61	Invasive lobular carcinoma	7 years	Nocturia, bilateral hydronephrosis	CK7 (+), GCDFP-15 (+), GATA3 (+), CK20 (-), ER (-), PR (-), E-cadherin (-), HER2 (-)	Bone metastasis	Lobular pattern with urinary tract obstruction
Caputo et al.,[20] 2022	70	Invasive ductal carcinoma	1 year	Hematuria	AE1/AE3 (+), CK8/18 (+), ER (+), PR (+), chromogranin (+), focal synaptophysin (+), CK20 (-), HER2 (-), p63 (-)	Bone metastasis	Ductal carcinoma with neuroendocrine marker expression
Khan et al.,[21] 2021	63	Invasive ductal carcinoma	17 years	Hematuria	Hormone receptor positive (HR+)	None	Isolated bladder metastasis
Kleinmann et al.,[22] 2005	65	Invasive ductal carcinoma	2.5 years	Hematuria	CA15-3 (+), CK18 (+?)	None	Isolated bladder metastasis
Ahmet Çamtosun, 2026	76	Invasive ductal carcinoma with neuroendocrine differentiation	25 years	Hematuria, dysuria	CK7 (+, patchy), CK20 (-), ER (+), PR (+), mammaglobulin (-), GCDFP-15 (-), uroplakin (-), p40 (-), chromogranin-A (+), synaptophysin (-), E-cadherin (+), HER2 (-)	Prior ovarian/ uterine metastasis	One of the longest latency periods reported; neuroendocrine differentiation

IHC: Immunohistochemistry, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2.

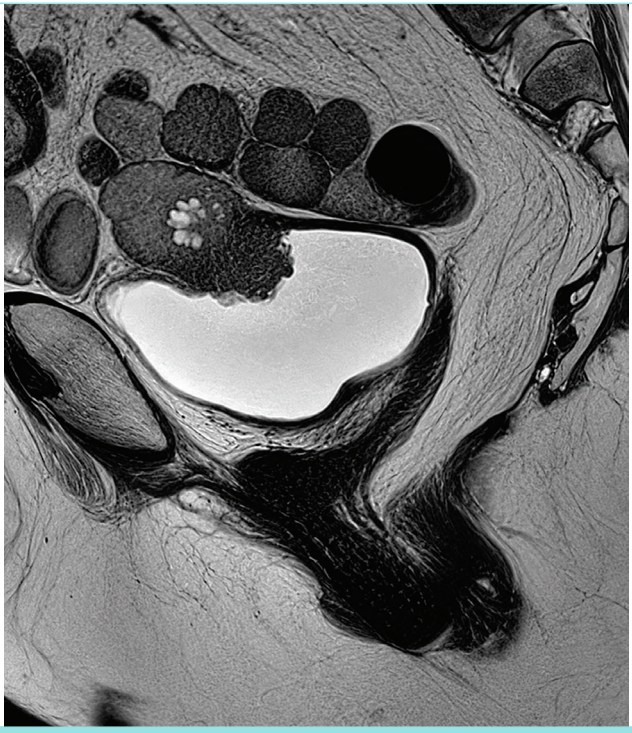

Figure 2. Pelvic MRI sagittal section showing a tumor invading the bladder.

MRI: Magnetic resonance imaging.

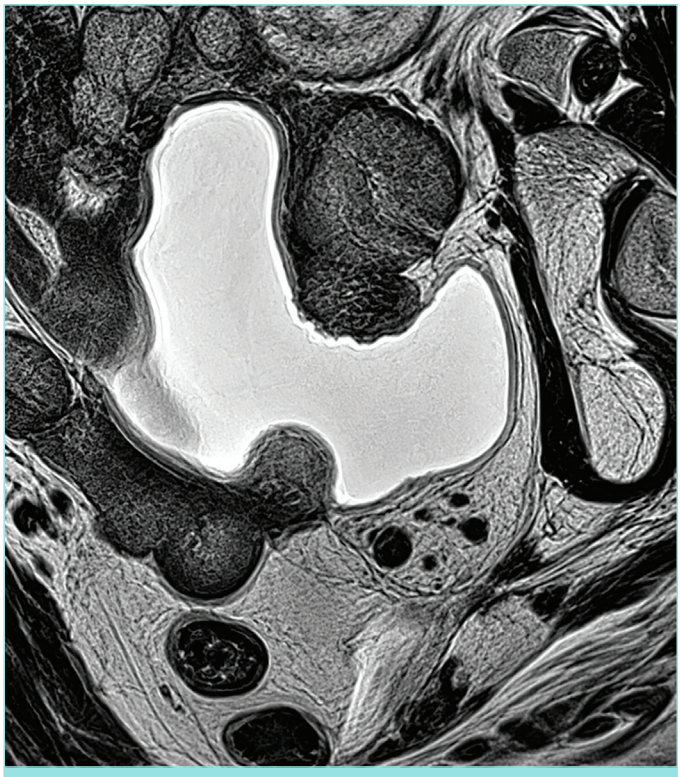

Figure 3. Pelvic MRI axial section showing a tumor invading the bladder.

MRI: Magnetic resonance imaging.

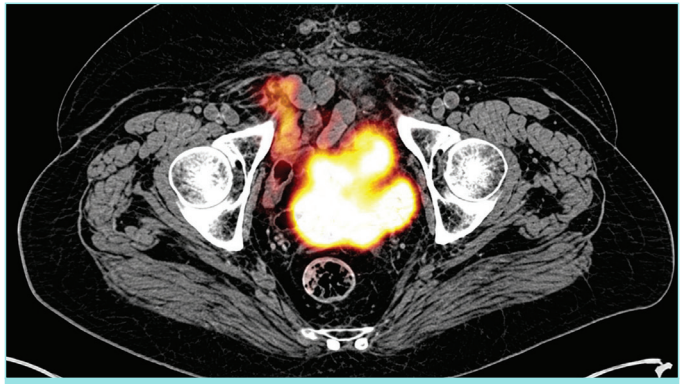

Figure 4. Bladder tumor image on PET/CT.

PET/CT: Positron emission tomography/computed tomography.

ETHICS

Informed Consent: Written informed consent was obtained from the patient for publication of this report and accompanying images.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.Ç., Design: H.B., Data Collection and/or Processing: H.G., Analysis and/or Interpretation: Ö.D., Literature Search: M.Y., Writing: Ö.F.Y.

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