pISSN: 2733-6581
eISSN: 2733-659X

JEND

Journal of Electrodiagnosis and Neuromuscular Diseases

VOL. 26
NO. 3
DECEMBER
2024

JEND
Journal of Electrodiagnosis and Neuromuscular Diseases
VOL. 26, NO. 3 DECEMBER 2024

Pages 41-68

JEND
Journal of Electrodiagnosis and Neuromuscular Diseases

KOREAN ASSOCIATION OF EMG
ELECTRODIAGNOSTIC MEDICINE

KOREAN ASSOCIATION OF EMG
ELECTRODIAGNOSTIC MEDICINE

e-jend.org

JEND
Journal of Electrodiagnosis and Neuromuscular Diseases

e-jend.org

pISSN 2733-6581
eISSN 2733-659X

Vol. 26, No. 3 December 2024

Aims and Scope

Journal of Electrodiagnosis and Neuromuscular Diseases (*J Electrodiagn Neuromuscul Dis*, JEND) is a peer-reviewed journal concerning both normal and abnormal functioning of the muscle, the neuromuscular junction, and the peripheral motor, sensory and autonomic nerves. The journal publishes clinical studies, reviews, and case reports in the fields of electrophysiology, electrodiagnosis, imaging studies including ultrasonography, and management, about neuromuscular diseases. The journal is aimed to provide an open forum for original research in basic science and clinical research that will improve our fundamental understanding and lead to effective treatments of neuromuscular diseases.
JEND is the official journal of the Korean Association of EMG Electrodiagnostic Medicine.

Subscription

Korean Association of EMG Electrodiagnostic Medicine will send JEND for free to some relevant individuals and institutions. Full text PDF files are also available at the official website (http://www.e-jend.org). To order a subscription to JEND, please contact our editorial office.

Open Access

Publisher
Korean Association of EMG Electrodiagnostic Medicine

Editor-in-Chief
Dong Hwee Kim, Korea University, Korea

Editorial Office
Department of Physical Medicine and Rehabilitation, Korea University Ansan Hospital,
123 Jeokgeum-ro, Danwon-gu, Ansan 15355, Korea
Tel: +82-31-412-5330 Fax: +82-31-412-4215 E-mail: editjend@gmail.com

Printing Office
M2PI
#805, 26 Sangwon 1-gil, Seongdong-gu, Seoul 04779, Korea
Tel: +82-2-6966-4930 Fax: +82-2-6966-4945 E-mail: support@m2-pi.com

Published on December 31, 2024

JEND Journal of Electrodiagnosis and Neuromuscular Diseases

Editorial Board

pISSN: 2733-6581 · eISSN: 2733-659X

JEND Journal of Electrodiagnosis and Neuromuscular Diseases

Contents

Vol. 26, No. 3 December 2024

Review Articles

Case Reports

JEND Journal of Electrodiagnosis and Neuromuscular Diseases

REVIEW ARTICLE

pISSN: 2733-6581 · eISSN: 2733-659X
J Electrodiagn Neuromuscul Dis 2024;26(3):41-48
https://doi.org/10.18214/jend.2024.00066

Neuromuscular Junction Disease Modeling and Therapeutic Screening Using Zebrafish

Hui Su Jeong[1], Ji Eun Lee[1,2]

[1]Department of Health Sciences and Technology, Samsung Advanced Institute for Health Sciences & Technology, Sungkyunkwan University, Seoul, Korea
[2]Samsung Biomedical Research Institute, Samsung Medical Center, Seoul, Korea

Disorders of the neuromuscular junction (NMJ), which is crucial for signal transduction between nerve and muscle cells in the peripheral nervous system, ultimately lead to dysfunction in both nerves and muscles. Zebrafish have become a valuable model for studying peripheral neuropathy, owing to their high genetic similarity to humans and the transparency that allows for direct observation of NMJ formation and function *in vivo*. This review introduces various methods used to create zebrafish NMJ disease models, including genetic manipulation, chemical treatment, and physical damage induction. Additionally, we discuss experimental techniques such as immunostaining, behavioral analysis, and electrophysiological testing, which are used to assess NMJ structure and function in these models. We also explore how potential NMJ disease treatments have been applied and validated using zebrafish NMJ models, highlighting their significant benefits for high-throughput drug screening. In summary, this review aims to illustrate the utility of zebrafish as an *in vivo* platform for studying mechanisms and developing treatments for NMJ diseases.

Keywords: Neuromuscular junction diseases; Zebrafish; Disease modeling; Drug screening

Received: September 2, 2024
Revised: November 2, 2024
Accepted: November 8, 2024

Corresponding author:
Ji Eun Lee
Department of Health Sciences and Technology, Samsung Advanced Institute for Health Sciences & Technology, Sungkyunkwan University, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea
Tel: +82-2-3410-6129
Fax: +82-2-2148-7379
E-mail: jieun.lee@skku.edu

Introduction

The neuromuscular junction (NMJ) is a chemical synapse that is present between nerve cells and muscle cells in the peripheral nervous system (PNS), mainly deep within skeletal muscle tissue. Structurally, in addition to motor neurons (pre-synapse) and muscle cells (post-synapse), terminal Schwann cells in contact with them at the synaptic cleft also serve as major components of the NMJ. The presynaptic nerve terminals contain numerous synaptic vesicles filled with the neurotransmitter acetylcholine (ACh). The postsynaptic muscle membrane is densely packed with ACh receptors (AChRs) and has a highly folded structure due to specialized proteins such as agrin, MuSK, and rapsyn, which play important roles in NMJ formation and maintenance [1-3]. When an action potential reaches the nerve terminal, ACh is released into the synaptic cleft, which contains a special basal lamina that anchors acetylcholinesterase (AChE) and binds to AChRs on the muscle fiber. This interaction ultimately leads to muscle contraction. AChE quickly hydrolyzes ACh, thus terminating synaptic transmission and facilitating the recycling of neurotransmitter signals through the NMJ [4]. Peripheral neuropathies, including congenital myasthenic syndrome (CMS) and myasthenia gravis, are well-known diseases associated with the NMJ that result in abnormalities in neurotransmitter release or signal transmission [5]. Damage to the PNS is particularly severe, as it leads not only to neurological deficits but also to broader issues affecting bodily movement.

Studies using zebrafish as a model for NMJ research are in-

creasingly reported in biomedical literature [6,7]. The zebrafish model is particularly appealing for NMJ disease research because it allows for relatively easy observation of NMJ formation and function *in vivo* during embryonic and larval stages [8,9]. Above all, the high degree of similarity between human and zebrafish NMJ-related disease genes significantly strengthens its relevance [10-13]. These advantages make zebrafish an effective *in vivo* platform for high-throughput drug screening and for investigating disease mechanisms. By integrating advanced imaging technologies and genetic manipulation tools, research using the zebrafish model has accelerated our understanding of NMJ disease mechanisms and the development of targeted treatments. The aim of this review is to present practical methods for studying the functions of genes in NMJ diseases and assessing the efficacy of therapeutic candidates in zebrafish, drawing on data from recent publications. It also aims to identify and discuss necessary improvements to address the limitations of the zebrafish model.

Advantages of Zebrafish for Neuromuscular Junction Study

The zebrafish PNS shows structural and functional homology with humans, particularly in the organization and development of motor neurons, sensory neurons, and NMJs [14]. Both humans and zebrafish share a conserved basic structure of the NMJ, which consists of presynaptic motor neuron terminals, a synaptic cleft, and highly specialized postsynaptic muscle fibers. In addition to structural similarities, zebrafish are suitable for neuromuscular disease modeling due to their high similarity in NMJ component proteins and NMJ-related disease genes [6,15,16].

Despite these similarities, human NMJs generally exhibit mono-neuronal innervation during the early developmental stages, whereas zebrafish NMJs initially exhibit multi-neuronal innervation and undergo synaptic pruning during development [9,17]. Furthermore, the regenerative capacity of the nervous system, including the repair and remodeling of damaged NMJs, is generally more robust in zebrafish than in humans. This robustness provides a significant advantage for studies on neuronal regeneration and synaptic plasticity [18]. Therefore, zebrafish, with their similarities in human NMJ structure and function and their specific advantages as an experimental animal model, are attracting increasing attention in research on the pathogenesis of NMJ-related diseases and the development of treatments.

The experimental advantage of zebrafish in NMJ studies lies in the ease of *in vivo* visualization of NMJ development and function. This is achievable through both standard and advanced fluorescence microscopy techniques at embryonic and larval stages [9,19]. Additionally, real-time imaging of synaptic remodeling, axon guidance, and neurotransmission can be conducted in living organisms—experiments that are challenging to perform in mammalian models [20]. Another experimental advantage of zebrafish is that many offspring can be obtained through *in vitro* fertilization and embryo development is rapid. This allows large-scale genetic and chemical screening, enabling rapid and simple processing and screening of a variety of candidate therapeutics for NMJ-related diseases that currently lack targeted therapies.

Several examples of research on NMJ diseases using zebrafish models have been reported [12,21,22]. For instance, the use of zebrafish is increasing in research on the pathogenesis of Charcot-Marie-Tooth (CMT) disease [11-13,23,24]. Details related to NMJ disease modeling are discussed in detail in the next section. Previous studies have suggested that zebrafish are not only useful for studying disease mechanisms due to the anatomical similarity of the NMJ to humans, but also for evaluating drug efficacy, as drug responses in zebrafish are similar to those in humans [23,25]. Collectively, the benefits of embryonic optical transparency, rapid development, and species-specific regenerative capabilities, combined with genetic and anatomical similarities to humans, have established zebrafish as a preferred model for studying NMJ diseases. In this review, we aim to emphasize the utility of zebrafish in understanding the pathology of NMJ diseases at the molecular level, in verifying the physiological phenomena that underpin these mechanisms, and in their application as a disease model.

NMJ Disease Modeling and Analysis Using Zebrafish

Zebrafish have been widely used to genetically investigate the pathogenesis of various diseases, including those affecting the NMJ. Researchers have induced mutations in genes associated with NMJ diseases in zebrafish to better understand the functions linked to each disease mechanism and to explore their potential as targets for therapeutic intervention [26]. For instance, a zebrafish model has been developed that expresses mutations in genes associated with CMT disease, such as ganglioside-induced differentiation-associated protein 1 (*GDAP1*) and mitofusin 2 (*MFN2*) [11-13]. These genetically engineered zebrafish display marked motor deficits and NMJ abnormalities, a phenotype very similar to that of CMT patients [11-13]. Additionally, zebrafish models of CMS have been created by targeting genes involved in NMJ formation and function, such as docking protein 7 (*DOK7*) and *AGRIN* [27,28]. These zebrafish models effectively mimic the CMS phenotype, including reduced motility, altered NMJ morphology, and

https://doi.org/10.18214/jend.2024.00066

impaired synaptic transmission [27,28].

In addition to genetic models, zebrafish can also be used to elucidate the pathological mechanisms of NMJ diseases through chemical treatment and injury induction. Neurotoxins such as botulinum toxin, organophosphorus compounds, and caffeine have been effectively employed to induce transient NMJ dysfunction in zebrafish. This approach allows researchers to investigate the mechanisms of synapse destruction and subsequent recovery *in vivo* [29-32]. These toxin-based zebrafish models are valuable for studying the plasticity of the neuromuscular system and for screening potential therapeutics that could accelerate NMJ regeneration.

Zebrafish models of acquired myasthenia gravis have been developed through exposure to AChE inhibitors or by manipulating AChR expression using a chemical induction system [33]. These models have facilitated studies on antibody-mediated NMJ control mechanisms, akin to those conducted using mouse models [34,35]. Additionally, zebrafish models created by inducing physical injuries, such as spinal cord transections, are employed in research focused on NMJ remodeling and regeneration post-injury [36]. The unique nerve regeneration capabilities of zebrafish are particularly advantageous for exploring the molecular and cellular mechanisms that support NMJ repair and functional recovery.

A variety of sophisticated methods have been developed and further optimized to investigate NMJ dysfunction in zebrafish models. The combined use of these techniques enables a comprehensive analysis of NMJ structure, function, and dynamics: (1) Immunohistochemistry of NMJ: High-resolution imaging of NMJ structures is achieved through immunostaining techniques, such as fluorescently conjugated α-bungarotoxin for AChR visualization and antibodies against synaptic vesicle proteins for presynaptic staining [15,23,37]. These methods facilitate a detailed examination of NMJ structures, including the assessment of AChR cluster size, density, and distribution, as well as presynaptic terminal morphology. (2) Behavioral analysis: Quantitative analysis of motor function and coordination is conducted using a variety of complex behavioral tests, including touch-evoked escape response, spontaneous swimming analysis, and fine motor control assessment [23]. These analyses provide a valuable readout of NMJ function and can reveal subtle motor deficits associated with NMJ dysfunction. (3) Electrophysiological testing: NMJ function is assessed at the cellular level using advanced electrophysiological techniques, including patch clamp recordings and extracellular field potential measurements [38]. These methods allow accurate quantification of synaptic transmission parameters, such as miniature endplate potential frequency and amplitude, quantum content, and muscle fiber excitability [38]. Electrophysiological analysis is particularly useful in identifying subtle functional abnormalities that may not be apparent from morphological or behavioral studies. (4) *In vivo* live imaging: Transgenic zebrafish larvae expressing motor neuron- or muscle cell-specific fluorescent proteins can be used to observe axonal outgrowth during neural development or regeneration and muscle patterning in real time [39,40]. Advanced microscopy techniques, such as two-photon microscopy and light-sheet microscopy, enable long-term, high-resolution imaging of NMJ development, maintenance, and remodeling in living animals [39]. This approach could be particularly powerful for studying the progression of NMJ disorders and assessing the efficacy of therapeutic interventions in real time. (5) Molecular and biochemical analyses: RNA sequencing for transcriptome analysis, proteomics to identify alterations in protein expression and post-translational modifications, and clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 genome editing to generate precise genetic modifications are molecular and biochemical approaches for studying NMJ in zebrafish [41,42]. These approaches may provide important clues about the molecular pathways and processes resulting in NMJ disorders, thereby guiding the development of targeted treatment strategies.

By integrating various methods applicable to zebrafish models, it is possible to comprehensively investigate the multifaceted characteristics of NMJ disorders, ranging from genetic and molecular alterations to functional and behavioral abnormalities of the NMJ (Fig. 1).

Candidate Drug Screening for NMJ Diseases Using a Zebrafish Model

Zebrafish have been extensively utilized as an *in vivo* system for high-throughput drug screening to identify therapeutic candidates, as well as for physiological studies exploring various pathologies of hereditary motor neuron diseases caused by NMJ abnormalities [25,43-45]. Indeed, numerous studies have identified potential therapeutic candidates for NMJ diseases by testing a range of compounds that are effective in restoring NMJ formation or function in the zebrafish model. In this review, we summarize (1) the methods used to target causative genes for each NMJ disorder and the approaches taken to develop zebrafish disease models, and (2) the types of therapeutic candidates and *in vivo* delivery methods that have been employed in each zebrafish model (Table 1) [11-13,23,24,46-56].

Currently, drug screening in zebrafish models primarily involves administering drugs directly into culture dishes or wells, leveraging the aquatic nature of zebrafish. This approach is effective for

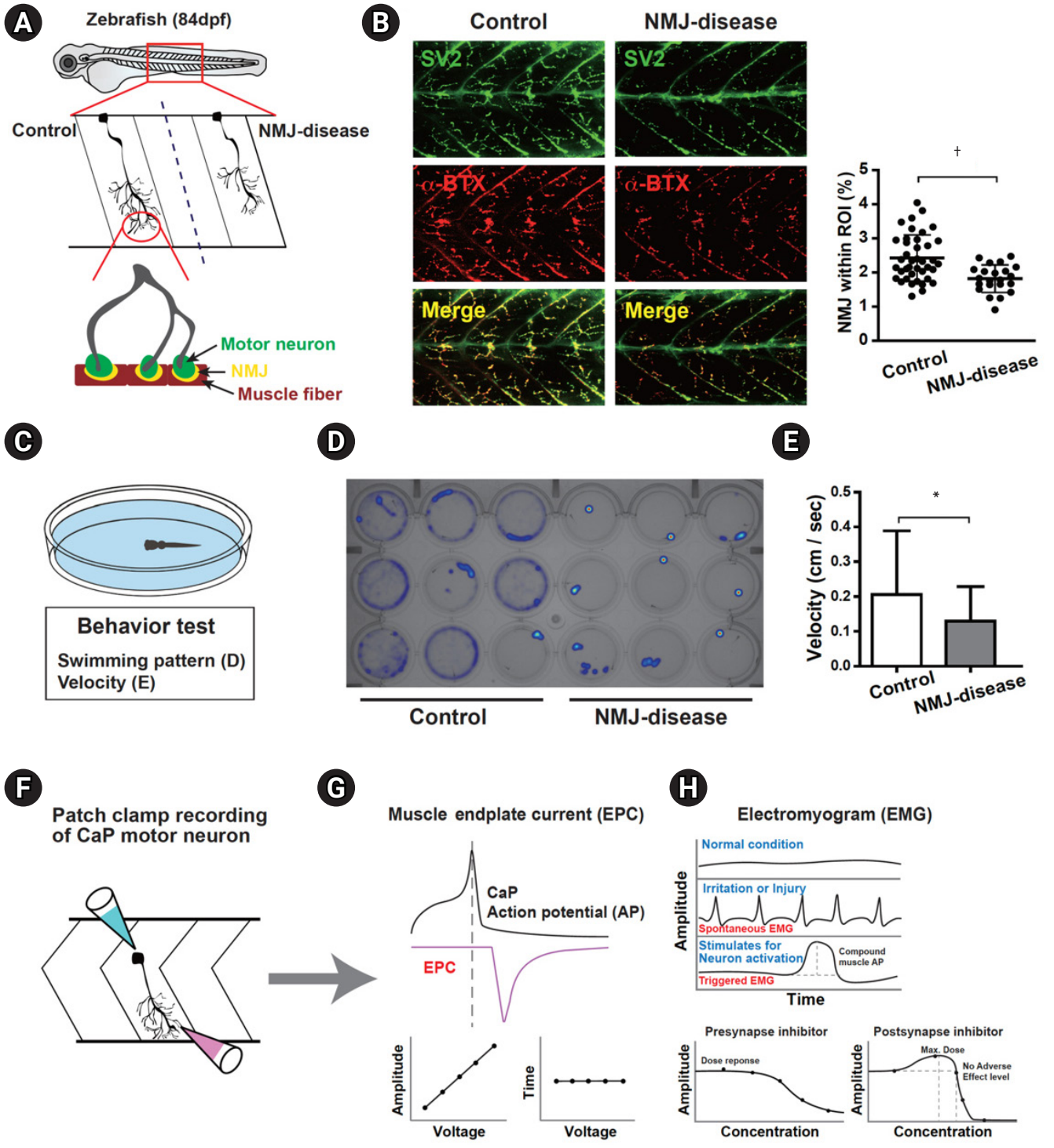

Fig. 1. Analytical methods in zebrafish neuromuscular junction (NMJ) disease models. (A) Diagram representing the NMJ regions analyzed in the zebrafish model. (B) Example of NMJ observation and analysis based on immunostaining. NMJs were visualized in merged colors by immunostaining with anti-synaptic vesicle 2 (SV2) and alpha-bungarotoxin (α-BTX) for pre- and post-synapse, respectively. (C) Diagram representing NMJ-related behavior tests performed using the zebrafish model. (D) Examples of behavioral tests including swimming patterns and velocity to examine NMJ function in zebrafish. The data presented in (B, D, E) are modified from a published study [37]. (F) Diagram representing the testing of NMJ-related functions through electrophysiological experiments in the zebrafish model. (G, H) Example of electrophysiological testing of zebrafish NMJ function using caudal primary (CaP) motor neuron activity. ROI, region of interest. *$p<0.01$; †$p<0.001$.

https://doi.org/10.18214/jend.2024.00066

Table 1. Methods of Genetic Modification to Generate Zebrafish NMJ Disease Models and Therapeutic Candidates Tested in the Zebrafish Model

NMJ disorder	Gene	Modification	Therapeutic chemicals/drugs	Methodology	Reference
Amyotrophic lateral sclerosis (ALS)	*C9orf72 (c9orf72)* *SOD1 (sod1)* *FUS (fus)* *TARDBP (tardbp/ tardbpl)*	Morpholino KD, MT, KO	Riluzole, edaravone (excitotoxicity inhibitor); ivermectin (antiparasitic drug)	Drug administration via waterborne exposure in multi-well plates	[46-48]
Charcot-Marie-Tooth (CMT2A)	*MFN2 (mfn2)*	N-ethyl-N-nitrosourea-induced MT, morpholino KD	ACY-738 (histone deacetylase inhibitor, HDACi)		[12,13,56]
Charcot-Marie-Tooth (CMT2D), distal hereditary motor neuropathy (dHMN5)	*GARS1 (gars)*	Morpholino KD, MT via yeast complementation assay	CKD504, tubastatin A, vorinostat, pomiferin (HDAC6i)		[23-24]
Congenital myasthenic syndromes (CMS)	*DOK7 (dok-7)*	Morpholino KD	Salbutamol (β2 adrenergic agonist)		[49]
Duchenne muscular dystrophy (DMD)	*DMD (dmd)*	KI MT	Oxamflatin and salermide (HDACi); aminophylline and sildenafil citrate (phosphodiesterase [PDE] inhibitors)		[50,51]
Spinal muscular atrophy (SMA)	*SMN1-UBA1 (smn1-uba1)* *CHODL (chodl)*	Morpholino KD, KI MT	Dipyridamole (non-selective phosphodiesterase, adenosine uptake inhibitor)		[52,53]
CMS	*MYO9A (myo9aa, myo9ab)*	Morpholino KD, CRISPR/Cas9 KD	NT1654 (agrin compound); Fasudil (Rho-associated protein kinase, ROCK inhibitor)	Injection at the single cell stage; drug administration via waterborne exposure	[54]
Charcot-Marie-Tooth (CMT2B)	*RAB40B (rab40b)*	KI MT	Investigational; further studies needed		[37]
Charcot-Marie-Tooth (CMT4A)	*GDAP1 (gdap1)*	Morpholino KD	Investigational; further studies needed		[11-55]

The names of human and zebrafish genes are indicated by capital and small letters, respectively.
NMJ, neuromuscular junction; KD, knock-down; MT, mutation; KO, knock out; KI, knock-in.

high-content screening as the drug passively diffuses into the organism. However, this method faces challenges in delivering drugs to specific target tissues, such as the NMJ of the PNS. These limitations can obscure the interpretation of phenotype restoration, making it difficult to determine whether the therapeutic effects of a drug are due to its direct action on NMJs or are the result of broader systemic effects. To address the shortcomings of these drug treatment methods in zebrafish NMJ disease models, several advanced drug delivery techniques may be employed. One promising approach involves the use of targeted drug delivery systems, such as nanoparticles or liposomes, that are engineered to specifically bind to NMJ-related proteins such as AChR [57-59]. These vehicles can be designed to encapsulate drugs and release them precisely at the NMJ, thereby minimizing off-target effects. Another strategy involves the direct administration of drugs into the PNS region of zebrafish using sophisticated microinjection techniques [60]. This method offers the advantage of being able to monitor the drug delivery process through real-time imaging, which helps predict the specific area affected by the drug. The implementation of an automated robotic injection system could further increase drug sensitivity and optimize the concentration for drug administration.

A final drug delivery method worth considering is photoactivated drug design, in which the drug remains inactive until it is activated by light of a specific wavelength. This approach allows for precise spatial and temporal control of drug activation and facilitates highly localized validation in the NMJ region. Integrating these advanced drug delivery methods could revolutionize drug screening and efficacy validation experiments using the zebrafish NMJ model.

Conclusion

In this review, we discuss the practical advantages and utility of zebrafish as a model for NMJ disease. Recent advances in gene editing technologies, ranging from morpholino oligomers to the CRISPR/Cas9 system, have enabled the modification of disease genes in zebrafish. The creation of these gene-targeted zebrafish models has enhanced our understanding of the pathology of gene-mediated NMJ diseases *in vivo* and has facilitated the development of targeted gene therapies. NMJ diseases, which often involve motility disorders due to abnormalities in nerve transmis-

sion, require *in vivo* models for accurate reproduction and verification of the pathology, as *in vitro* studies alone are insufficient. Zebrafish models allow for relatively easy induction of NMJ pathology compared to mammals, and the assessment of NMJ structure and function can be conducted quickly and simply.

Zebrafish has become a crucial animal model for high-throughput drug screening, favored for its small size, rapid development, and the simplicity of observing phenotypic changes. High-content screening utilizing this model has facilitated the identification of numerous promising compounds with potential applications in human medicine [23,25,43-45]. The similarity between the structure and function of the zebrafish NMJ and that of humans allows for the selection of candidate substances to treat NMJ diseases through *in vivo* experiments, providing an advantage over traditional *in vitro* methods alone.

The integration of advanced genetics and imaging techniques in zebrafish research has enabled the visualization of NMJ dynamics in real time. Transgenic lines facilitating neuron-targeted imaging in the PNS have allowed for real-time observation of NMJ disorders and immediate assessment of drug screening effectiveness [39]. These advancements in imaging and genetic modification have improved the analysis of NMJ formation and function, leveraging the practical strengths of the zebrafish model. The zebrafish fin represents a promising new frontier for future NMJ studies in the PNS. The extensive innervation and remarkable regenerative capabilities of the fin's dorsal structures offer a unique model for exploring NMJ formation, degeneration, and regeneration [21,61]. Therefore, expanding NMJ research from the zebrafish body to the fins could provide fresh insights into the mechanisms underlying NMJ diseases and foster the development of more innovative treatment strategies.

In conclusion, zebrafish are a widely used animal model that facilitates disease modeling through genetic manipulation, analysis of replicated disease phenotypes, and efficient large-scale drug screening. It is particularly valuable in studying the pathogenesis and developing treatments for peripheral neuropathy, including NMJ diseases. However, more sophisticated methods for analyzing NMJ structure and function, particularly physiological analysis at the molecular level, would make the zebrafish an even more effective model for understanding the complexities of NMJ disorders and advancing treatments for peripheral neuropathy.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

This work was supported by National Research Foundation, Korean government's MSIT RS-2024-00335208 and Ministry of Health and Welfare, Korean government's RS-2022-KH126084 to Ji Eun Lee.

ORCID

Hui Su Jeong, https://orcid.org/0000-0001-8419-4698
Ji Eun Lee, https://orcid.org/0000-0002-4898-741X

References

1. Denzer AJ, Hauser DM, Gesemann M, Ruegg MA: Synaptic differentiation: the role of agrin in the formation and maintenance of the neuromuscular junction. Cell Tissue Res 1997;290:357–365.
2. Wang Q, Zhang B, Xiong WC, Mei L: MuSK signaling at the neuromuscular junction. J Mol Neurosci 2006;30:223–226.
3. Glass DJ, Yancopoulos GD: Sequential roles of agrin, MuSK and rapsyn during neuromuscular junction formation. Curr Opin Neurobiol 1997;7:379–384.
4. Rotundo RL, Rossi SG, Kimbell LM, Ruiz C, Marrero E: Targeting acetylcholinesterase to the neuromuscular synapse. Chem Biol Interact 2005;157-158:15–21.
5. Vianello A, Racca F, Vita GL, Pierucci P, Vita G: Motor neuron, peripheral nerve, and neuromuscular junction disorders. Handb Clin Neurol 2022;189:259–270.
6. Singh J, Patten SA: Modeling neuromuscular diseases in zebrafish. Front Mol Neurosci 2022;15:1054573.
7. Brehm P, Wen H: Zebrafish neuromuscular junction: the power of N. Neurosci Lett 2019;713:134503.
8. Singh J, Pan YE, Patten SA: NMJ analyser: a novel method to quantify neuromuscular junction morphology in zebrafish. Bioinformatics 2023;39:btad720.
9. Lescouzères L, Bordignon B, Bomont P: Development of a high-throughput tailored imaging method in zebrafish to understand and treat neuromuscular diseases. Front Mol Neurosci 2022;15:956582.
10. Daikoku E, Saito M, Ono F: Zebrafish mutants of the neuromuscular junction: swimming in the gene pool. J Physiol Sci 2015;65:217–221.
11. Eijkenboom I, Vanoevelen JM, Hoeijmakers JG, Wijnen I, Gerards M, Faber CG, et al: A zebrafish model to study small-fiber neuropathy reveals a potential role for GDAP1. Mitochondrion 2019;47:273–281.

 https://doi.org/10.18214/jend.2024.00066

12. Vettori A, Bergamin G, Moro E, Vazza G, Polo G, Tiso N, et al: Developmental defects and neuromuscular alterations due to mitofusin 2 gene (MFN2) silencing in zebrafish: a new model for Charcot-Marie-Tooth type 2A neuropathy. Neuromuscul Disord 2011;21:58–67.
13. Chapman AL, Bennett EJ, Ramesh TM, De Vos KJ, Grierson AJ: Axonal transport defects in a Mitofusin 2 loss of function model of Charcot-Marie-Tooth disease in zebrafish. PLoS One 2013;8:e67276.
14. Fetcho JR: The utility of zebrafish for studies of the comparative biology of motor systems. J Exp Zool B Mol Dev Evol 2007;308:550–562.
15. Panzer JA, Gibbs SM, Dosch R, Wagner D, Mullins MC, Granato M, et al: Neuromuscular synaptogenesis in wild-type and mutant zebrafish. Dev Biol 2005;285:340–357.
16. Walker LJ, Roque RA, Navarro MF, Granato M: Agrin/Lrp4 signal constrains MuSK-dependent neuromuscular synapse development in appendicular muscle. Development 2021;148:dev199790.
17. Beattie CE, Hatta K, Halpern ME, Liu H, Eisen JS, Kimmel CB: Temporal separation in the specification of primary and secondary motoneurons in zebrafish. Dev Biol 1997;187:171–182.
18. Ohnmacht J, Yang Y, Maurer GW, Barreiro-Iglesias A, Tsarouchas TM, Wehner D, et al: Spinal motor neurons are regenerated after mechanical lesion and genetic ablation in larval zebrafish. Development 2016;143:1464–1474.
19. Panzer JA, Song Y, Balice-Gordon RJ: In vivo imaging of preferential motor axon outgrowth to and synaptogenesis at prepatterned acetylcholine receptor clusters in embryonic zebrafish skeletal muscle. J Neurosci 2006;26:934–947.
20. Wong HC, Drerup CM: Using fluorescent indicators for in vivo quantification of spontaneous or evoked motor neuron presynaptic activity in transgenic zebrafish. STAR Protoc 2022;3:101766.
21. Won SY, Choi BO, Chung KW, Lee JE: Zebrafish is a central model to dissect the peripheral neuropathy. Genes Genomics 2019;41:993–1000.
22. Ponomareva OY, Eliceiri KW, Halloran MC: Charcot-Marie-Tooth 2b associated Rab7 mutations cause axon growth and guidance defects during vertebrate sensory neuron development. Neural Dev 2016;11:2.
23. Jeong HS, Kim HJ, Kim DH, Chung KW, Choi BO, Lee JE: Therapeutic potential of CKD-504, a novel selective histone deacetylase 6 inhibitor, in a zebrafish model of neuromuscular junction disorders. Mol Cells 2022;45:231–242.
24. Malissovas N, Griffin LB, Antonellis A, Beis D: Dimerization is required for GARS-mediated neurotoxicity in dominant CMT disease. Hum Mol Genet 2016;25:1528–1542.
25. MacRae CA, Peterson RT: Zebrafish as tools for drug discovery. Nat Rev Drug Discov 2015;14:721–731.
26. Babin PJ, Goizet C, Raldúa D: Zebrafish models of human motor neuron diseases: advantages and limitations. Prog Neurobiol 2014;118:36–58.
27. Walogorsky M, Mongeon R, Wen H, Nelson NR, Urban JM, Ono F, et al: Zebrafish model for congenital myasthenic syndrome reveals mechanisms causal to developmental recovery. Proc Natl Acad Sci U S A 2012;109:17711–17716.
28. McMacken G, Cox D, Roos A, Müller J, Whittaker R, Lochmüller H: The beta-adrenergic agonist salbutamol modulates neuromuscular junction formation in zebrafish models of human myasthenic syndromes. Hum Mol Genet 2018;27:1556–1564.
29. Dubińska-Magiera M, Daczewska M, Lewicka A, Migocka-Patrzałek M, Niedbalska-Tarnowska J, Jagla K: Zebrafish: a model for the study of toxicants affecting muscle development and function. Int J Mol Sci 2016;17:1941.
30. Recidoro AM, Roof AC, Schmitt M, Worton LE, Petrie T, Strand N, et al: Botulinum toxin induces muscle paralysis and inhibits bone regeneration in zebrafish. J Bone Miner Res 2014;29:2346–2356.
31. Faria M, Garcia-Reyero N, Padrós F, Babin PJ, Sebastián D, Cachot J, et al: Zebrafish models for human acute organophosphorus poisoning. Sci Rep 2015;5:15591.
32. Rodriguez RS, Haugen R, Rueber A, Huang CC: Reversible neuronal and muscular toxicity of caffeine in developing vertebrates. Comp Biochem Physiol C Toxicol Pharmacol 2014;163:47–54.
33. Mott M, Luna VM, Park JY, Downes GB, Epley K, Ono F: Expressing acetylcholine receptors after innervation suppresses spontaneous vesicle release and causes muscle fatigue. Sci Rep 2017;7:1674.
34. Koneczny I, Herbst R: Myasthenia Gravis: pathogenic effects of autoantibodies on neuromuscular architecture. Cells 2019;8:671.
35. Mori S, Kubo S, Akiyoshi T, Yamada S, Miyazaki T, Hotta H, et al: Antibodies against muscle-specific kinase impair both presynaptic and postsynaptic functions in a murine model of myasthenia gravis. Am J Pathol 2012;180:798–810.
36. Arena KA, Zhu Y, Kucenas S: Transforming growth factor-beta signaling modulates perineurial glial bridging following peripheral spinal motor nerve injury in zebrafish. Glia 2022;70:1826–1849.
37. Son W, Jeong HS, Nam DE, Lee AJ, Nam SH, Lee JE, et al:

Peripheral neuropathy and decreased locomotion of a RAB40B mutation in human and model animals. Exp Neurobiol 2023;32:410–422.
38. Wen H, Brehm P: Paired motor neuron-muscle recordings in zebrafish test the receptor blockade model for shaping synaptic current. J Neurosci 2005;25:8104–8111.
39. Xiao Y, Faucherre A, Pola-Morell L, Heddleston JM, Liu TL, Chew TL, et al: High-resolution live imaging reveals axon-glia interactions during peripheral nerve injury and repair in zebrafish. Dis Model Mech 2015;8:553–564.
40. Limbach LE, Penick RL, Casseday RS, Hyland MA, Pontillo EA, Ayele AN, et al: Peripheral nerve development in zebrafish requires muscle patterning by tcf15/paraxis. Dev Biol 2022;490:37–49.
41. Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, et al: Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol 2013;31:227–229.
42. Hostelley TL, Nesmith JE, Zaghloul NA: Sample preparation and analysis of RNASeq-based gene expression data from zebrafish. J Vis Exp 2017;128:56187.
43. Pichler FB, Laurenson S, Williams LC, Dodd A, Copp BR, Love DR: Chemical discovery and global gene expression analysis in zebrafish. Nat Biotechnol 2003;21:879–883.
44. Steele SL, Prykhozhij SV, Berman JN: Zebrafish as a model system for mitochondrial biology and diseases. Transl Res 2014;163:79–98.
45. Eimon PM, Rubinstein AL: The use of in vivo zebrafish assays in drug toxicity screening. Expert Opin Drug Metab Toxicol 2009;5:393–401.
46. Oliveira NA, Pinho BR, Pinto J, Guedes de Pinho P, Oliveira JM: Edaravone counteracts redox and metabolic disruptions in an emerging zebrafish model of sporadic ALS. Free Radic Biol Med 2024;217:126–140.
47. Shaw MP, Higginbottom A, McGown A, Castelli LM, James E, Hautbergue GM, et al: Stable transgenic C9orf72 zebrafish model key aspects of the ALS/FTD phenotype and reveal novel pathological features. Acta Neuropathol Commun 2018;6:125.
48. Müller JS, Jepson CD, Laval SH, Bushby K, Straub V, Lochmüller H: Dok-7 promotes slow muscle integrity as well as neuromuscular junction formation in a zebrafish model of congenital myasthenic syndromes. Hum Mol Genet 2010;19:1726–1740.
49. Kawahara G, Karpf JA, Myers JA, Alexander MS, Guyon JR, Kunkel LM: Drug screening in a zebrafish model of Duchenne muscular dystrophy. Proc Natl Acad Sci U S A 2011;108:5331–5336.
50. Farr GH, Morris M, Gomez A, Pham T, Kilroy E, Parker EU, et al: A novel chemical-combination screen in zebrafish identifies epigenetic small molecule candidates for the treatment of Duchenne muscular dystrophy. Skelet Muscle 2020;10:29.
51. Boon KL, Xiao S, McWhorter ML, Donn T, Wolf-Saxon E, Bohnsack MT, et al: Zebrafish survival motor neuron mutants exhibit presynaptic neuromuscular junction defects. Hum Mol Genet 2009;18:3615–3625.
52. Oprişoreanu AM, Smith HL, Krix S, Chaytow H, Carragher NO, Gillingwater TH, et al: Automated in vivo drug screen in zebrafish identifies synapse-stabilizing drugs with relevance to spinal muscular atrophy. Dis Model Mech 2021;14:dmm047761.
53. O'Connor E, Cairns G, Spendiff S, Burns D, Hettwer S, Mäder A, et al: Modulation of agrin and RhoA pathways ameliorates movement defects and synapse morphology in MYO9A-depleted zebrafish. Cells 2019;8:848.
54. Nelis E, Erdem S, Van Den Bergh PY, Belpaire-Dethiou MC, Ceuterick C, Van Gerwen V, et al: Mutations in GDAP1: autosomal recessive CMT with demyelination and axonopathy. Neurology 2002;59:1865–1872.
55. Simões MG, Bensimon-Brito A, Fonseca M, Farinho A, Valério F, Sousa S, et al: Denervation impairs regeneration of amputated zebrafish fins. BMC Dev Biol 2014;14:49.
56. Butler L, Adamson KI, Johnson SL, Jestice LH, Price CJ, Stavish D, et al: HDAC6 inhibition partially alleviates mitochondrial trafficking defects and restores motor function in human motor neuron and zebrafish models of Charcot-Marie-Tooth disease type 2A [Preprint]. Posted 2022 Jul 5. bioRxiv https://doi.org/10.1101/2022.07.05.498819.
57. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R: Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov 2021;20:101–124.
58. Kim JY, Choi WI, Kim YH, Tae G: Brain-targeted delivery of protein using chitosan- and RVG peptide-conjugated, pluronic-based nano-carrier. Biomaterials 2013;34:1170–1178.
59. Liu P, Chen G, Zhang J: A review of liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives. Molecules 2022;27:1372.
60. Spaink HP, Cui C, Wiweger MI, Jansen HJ, Veneman WJ, Marín-Juez R, et al: Robotic injection of zebrafish embryos for high-throughput screening in disease models. Methods 2013;62:246–254.
61. Benedetti L, Ghilardi A, Rottoli E, De Maglie M, Prosperi L, Perego C, et al: INaP selective inhibition reverts precocious inter- and motorneurons hyperexcitability in the Sod1-G93R zebrafish ALS model. Sci Rep 2016;6:24515.

JEND Journal of Electrodiagnosis and Neuromuscular Diseases

REVIEW ARTICLE

pISSN: 2733-6581 · eISSN: 2733-659X
J Electrodiagn Neuromuscul Dis 2024;26(3):49-52
https://doi.org/10.18214/jend.2024.00080

TDP-43 as a Fluid Biomarker in Amyotrophic Lateral Sclerosis

Seok-Jin Choi[1,2]

[1]Center for Hospital Medicine, Seoul National University Hospital, Seoul, Korea
[2]Department of Neurology, Seoul National University Hospital, Seoul, Korea

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by the death of both upper and lower motor neurons in the brain, brainstem, and spinal cord. In approximately 95% of cases, ALS is associated with the nuclear-cytoplasmic mislocalization and aggregation of TAR DNA-binding protein 43 (TDP-43). The diagnosis of ALS is based solely on clinical assessments, including neurological examinations and electromyography studies, and currently, there is no reliable biomarker for diagnosing ALS using antemortem tissues. Additionally, while TDP-43 positron emission tomography ligands are being investigated, they are not yet widely available for assessing brain TDP-43 pathology. Therefore, a robust fluid biomarker that reflects pathological TDP-43 accumulation in the central nervous system is essential for confirming an ALS diagnosis. In this context, we provide a comprehensive summary of the current status of fluid biomarker development, focusing on TDP-43 pathology, and discuss the existing limitations as well as future directions for ALS biomarker discovery.

Keywords: Amyotrophic lateral sclerosis; Fluid biomarker; TDP-43

Received: September 19, 2024
Accepted: November 13, 2024

Corresponding author:
Seok-Jin Choi
Center for Hospital Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-2072-7603
Fax: +82-2-3672-7553
E-mail: seokjin83@gmail.com

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by the death of both upper motor neuron (UMN) and lower motor neuron (LMN) in the brain, brainstem, and spinal cord, ultimately leading to death from respiratory failure [1]. In the majority of cases (approximately 95%), excluding those with pathogenic variants in the *FUS* gene, the hallmark pathology is the nuclear-cytoplasmic mislocalization and aggregation of TAR DNA-binding protein 43 (TDP-43) [1,2]. Additionally, TDP-43 pathology is observed in patients with frontotemporal dementia (FTD), Alzheimer's disease, limbic-predominant age-related TDP-43 encephalopathy (LATE), and inclusion body myositis, indicating that it is not specific to ALS [2-5]. ALS is diagnosed based on the revised El Escorial or Awaji criteria, which incorporate neurological examination findings and electrophysiological studies. The diagnosis requires confirmation of UMN or LMN signs in each anatomical segment (bulbar, cervical, thoracic, and lumbosacral) [6,7]. Importantly, while electromyography can provide evidence for the degeneration of LMNs, current data do not support the use of imaging or neurophysiological modalities to establish UMN dysfunction [8]. Furthermore, although TDP-43 positron emission tomography ligands are under investigation [9], they are not yet widely available for evaluating brain TDP-43 pathology. Therefore, a robust fluid biomarker that reflects pathological TDP-43 accumulation in the central nervous system (CNS) would be highly useful for confirming ALS diagnoses.

This review comprehensively summarizes the current status of biomarker development, focusing on TDP-43 pathology, and discusses the existing limitations and future avenues for ALS biomarker discovery.

TDP-43

TDP-43 is a 43 kDa heterogeneous nuclear ribonuclear protein (hnRNP) composed of 414 amino acids that is encoded by the TAR DNA binding protein (*TARDBP*) gene located on chromosome 1 [2]. It plays a crucial role in regulating gene expression and various RNA processing activities, such as RNA splicing, mRNA turnover, RNA trafficking, and microRNA biogenesis. TDP-43 targets more than 4,000 different mRNA transcripts, including those associated with diseases and its own mRNA transcript through autoregulation.

TDP-43 is composed of an N-terminal domain, a nuclear localization signal, two RNA-recognition motifs, a nuclear export signal, and a C-terminal glycine-rich domain (Fig. 1) [2]. The protein also has an amyloidogenic core region (residues 311-360) with two alpha-helices that convert into beta sheets in TDP-43 aggregates. The N-terminus primarily functions to regulate the homodimerization of TDP-43, which is crucial for proper protein folding and mRNA splicing. The C-terminus plays a significant role in mRNA splicing and hnRNP interactions and is also believed to contribute to the formation of TDP-43 inclusions.

TDP-43 in Cerebrospinal Fluid and Plasma

TDP-43 pathology primarily develops in the CNS tissues of ALS patients, which initially directed research on TDP-43 fluid biomarkers toward the cerebrospinal fluid (CSF). In 2008, TDP-43 was first evaluated as a potential CSF biomarker for ALS [10]. Researchers found elevated levels of a 45 kDa band in the CSF of ALS patients compared to controls using a monoclonal antibody that targets amino acids 1 through 260 of recombinant TDP-43. Shortly afterward, another group measured TDP-43 levels in the CSF of ALS patients using a sandwich enzyme-linked immunosorbent assay (ELISA) with identical capture and detection antibodies [11]. Further analysis by immunoblotting revealed a 43 kDa band, indicating that the ELISA primarily detects full-length TDP-43. This study also demonstrated that CSF TDP-43 levels were significantly higher in individuals with sporadic ALS than in age-matched healthy or neurological disease controls. Subsequently, a different research group developed a TDP-43 sandwich ELISA using the same capture and detection antibodies [11]. In their study, CSF TDP-43 levels were significantly higher in ALS patients than in those with Guillain-Barré syndrome, showing a sensitivity of 84.6% and a specificity of 71.4% (cut-off, 1.16 ng/mL). These findings indicate that CSF TDP-43 measurements alone may not meet the standards for clinical application. In a later study using an ELISA, CSF TDP-43 levels were found to be elevated in ALS patients compared to those with neurodegenerative and inflammatory neurological diseases [12], aligning with previous findings [10,11]. The results showed a sensitivity of 59.3% and a specificity of 96% (cut-off, 27.9 ng/mL), suggesting that while levels below the cut-off may not exclude an ALS diagnosis, a positive result could aid in distinguishing ALS from other neurological conditions.

In a study involving 219 patients with ALS and 100 healthy controls, plasma TDP-43 levels measured by sandwich ELISA were significantly higher in the ALS cohort [13]. However, TDP-43 concentrations exceeded the assay's detection limit in only 28% of these patients, compared to 21% of controls, underscoring the need for a more sensitive assay. More recently, a commercial single-molecule array (SIMOA) assay, designed to detect both full-length and pathologically truncated TDP-43, was utilized [14]. Both CSF and plasma TDP-43 levels were significantly higher in patients with ALS than in controls within the discovery cohort. However, in the validation cohort, only CSF TDP-43 levels were found to be elevated in ALS patients compared to controls.

A recent study utilized sandwich ELISAs to measure both total TDP-43 and phosphorylated TDP-43 (pTDP-43) in CSF and plasma [15]. The results showed that plasma levels of TDP-43 and pTDP-43 were significantly elevated in ALS patients compared to healthy controls, whereas CSF levels did not exhibit a similar increase. Furthermore, ALS patients displayed significantly lower plasma pTDP-43/TDP-43 ratios, while their CSF pTDP-43/TDP-43 ratios did not significantly differ from those of the controls. In terms of diagnostic performance, plasma TDP-43 achieved a sensitivity of 91.3% and a specificity of 91.5%. In

Fig. 1. TAR DNA-binding protein 43 (TDP-43) protein structure. N, N-terminal domain; C, C-terminal domain; NLS, nuclear localization signal; NES, nuclear export signal; RRM, RNA-recognition motif.

https://doi.org/10.18214/jend.2024.00080

contrast, plasma pTDP-43 demonstrated a sensitivity of 82.6% and a specificity of 67.8%.

Interestingly, while pathological TDP-43 in biofluids is believed to originate from damaged motor neurons in the intrathecal compartment of ALS patients, it is important to note that the concentrations of total TDP-43 and pTDP-43 are significantly higher in plasma than in CSF [15]. This discrepancy could be attributed to active transport between the CSF and plasma or to the release from non-neuronal peripheral tissues or cells, which merits further investigation. In this context, platelet-rich plasma might be a significant source, as TDP-43 levels in platelets from ALS patients are markedly elevated compared to those in healthy controls [16].

Although no direct comparison studies have been conducted, TDP-43 levels in platelets appear to be substantially higher than those in plasma.

The real-time quaking-induced conversion reaction (RT-QuIC) has proven to be a robust technique for prion amplification in diseases such as Creutzfeldt-Jakob disease [17]. Recently, this technique was adapted to use TDP-43 as a substrate, exploiting its ability to amplify minute amounts of misfolded proteins for the detection of pathological TDP-43 species in the CSF of ALS and FTD patients [18]. The TDP-43 RT-QuIC method was able to detect as little as 15 pg of TDP-43 aggregates, successfully discriminating between patients affected by ALS or FTD harboring pathogenic variants in the *C9orf72*, granulin precursor (*GRN*), and *TARDBP* genes from age-matched controls, with a sensitivity of 94% and a specificity of 85%. These findings require further validation in larger cohorts of sporadic ALS.

Evidence indicates that cryptic exons arising from the loss of TDP-43 function play a crucial role in the pathogenesis of ALS [19]. Recent research utilizing a novel monoclonal antibody that targets a TDP-43-dependent cryptic epitope has shown that splicing repression by TDP-43 is compromised in ALS-FTD, including in presymptomatic carriers of *C9orf72* mutation [20]. This cryptic hepatoma-derived growth factor-related protein 2 (HDGFL2) accumulates in the CSF at significantly higher levels in familial ALS-FTD and sporadic ALS compared to controls, and it is elevated earlier than neurofilament light and phosphorylated neurofilament heavy chain levels in familial cases. These findings suggest that the loss of TDP-43 cryptic splicing repression occurs early in disease progression and that detecting the HDGFL2 cryptic neoepitope could serve as a potential diagnostic biomarker for ALS.

Limitations

Although CSF or plasma TDP-43 is considered a potential diagnostic biomarker for ALS, several limitations should be acknowledged. First, TDP-43 proteinopathy is not specific to ALS and is observed in other neurodegenerative diseases, including FTD, Alzheimer's disease, LATE, and inclusion body myositis. Second, the use of antibodies with varying sensitivities and specificities for immunoassays to detect different forms of TDP-43 has led to inconsistent results and challenges in comparing findings across studies. Third, TDP-43 is expressed ubiquitously in both the CNS and peripheral tissues, making it unclear whether soluble TDP-43 detected in biofluids specifically originates from the damaged CNS. Fourth, increases in TDP-43 levels in CSF or plasma might reflect neurodegeneration and the release of TDP-43 from damaged neurons, while decreases could be due to its sequestration in cytoplasmic aggregates. Although several studies have reported elevated TDP-43 levels in these fluids in ALS patients compared to controls, these findings have yet to demonstrate clear clinical utility at the individual patient level.

Conclusion

Numerous studies have investigated the diagnostic utility of CSF or plasma TDP-43 in ALS, frequently alongside other biomarkers like tau and neurofilament. However, there is currently no conclusive evidence to support the use of TDP-43 alone as a diagnostic biomarker for TDP-43 proteinopathy in ALS. Future research should focus on creating a comprehensive diagnostic strategy that integrates clinical data, genetic information, and a range of biomarkers—including fluid-based, electrophysiological, and imaging assessments—to enhance the accuracy of ALS diagnosis.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

ORCID

Seok-Jin Choi, https://orcid.org/0000-0001-6444-977X

References

1. Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, et al: Amyotrophic lateral sclerosis. Nat Rev

Dis Primers 2017;3:17071.
2. Tziortzouda P, Van Den Bosch L, Hirth F: Triad of TDP43 control in neurodegeneration: autoregulation, localization and aggregation. Nat Rev Neurosci 2021;22:197–208.
3. Meneses A, Koga S, O'Leary J, Dickson DW, Bu G, Zhao N: TDP-43 pathology in Alzheimer's disease. Mol Neurodegener 2021;16:84.
4. Nelson PT, Dickson DW, Trojanowski JQ, Jack CR, Boyle PA, Arfanakis K, et al: Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. Brain 2019;142:1503–1527.
5. Weihl CC, Temiz P, Miller SE, Watts G, Smith C, Forman M, et al: TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. J Neurol Neurosurg Psychiatry 2008;79:1186–1189.
6. Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases: El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000;1:293–299.
7. de Carvalho M, Dengler R, Eisen A, England JD, Kaji R, Kimura J, et al: Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol 2008;119:497–503.
8. Shefner JM, Al-Chalabi A, Baker MR, Cui LY, de Carvalho M, Eisen A, et al: A proposal for new diagnostic criteria for ALS. Clin Neurophysiol 2020;131:1975–1978.
9. Seredenina T, Vokali E, Dreyfus N, Chevallier E, Afroz T, Jaquier T, et al: Discovery and optimization of the first-in-class TDP-43 PET tracer. Alzheimers Dement 2023;19:e075525.
10. Steinacker P, Hendrich C, Sperfeld AD, Jesse S, von Arnim CA, Lehnert S, et al: TDP-43 in cerebrospinal fluid of patients with frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Arch Neurol 2008;65:1481–1487.
11. Kasai T, Tokuda T, Ishigami N, Sasayama H, Foulds P, Mitchell DJ, et al: Increased TDP-43 protein in cerebrospinal fluid of patients with amyotrophic lateral sclerosis. Acta Neuropathol 2009;117:55–62.
12. Noto Y, Shibuya K, Sato Y, Kanai K, Misawa S, Sawai S, et al: Elevated CSF TDP-43 levels in amyotrophic lateral sclerosis: specificity, sensitivity, and a possible prognostic value. Amyotroph Lateral Scler 2011;12:140–143.
13. Verstraete E, Kuiperij HB, van Blitterswijk MM, Veldink JH, Schelhaas HJ, van den Berg LH, et al: TDP-43 plasma levels are higher in amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2012;13:446–451.
14. Kasai T, Kojima Y, Ohmichi T, Tatebe H, Tsuji Y, Noto YI, et al: Combined use of CSF NfL and CSF TDP-43 improves diagnostic performance in ALS. Ann Clin Transl Neurol 2019;6:2489–2502.
15. Ren Y, Li S, Chen S, Sun X, Yang F, Wang H, et al: TDP-43 and phosphorylated TDP-43 levels in paired plasma and CSF samples in amyotrophic lateral sclerosis. Front Neurol 2021;12:663637.
16. Hishizawa M, Yamashita H, Akizuki M, Urushitani M, Takahashi R: TDP-43 levels are higher in platelets from patients with sporadic amyotrophic lateral sclerosis than in healthy controls. Neurochem Int 2019;124:41–45.
17. Candelise N, Baiardi S, Franceschini A, Rossi M, Parchi P: Towards an improved early diagnosis of neurodegenerative diseases: the emerging role of in vitro conversion assays for protein amyloids. Acta Neuropathol Commun 2020;8:117.
18. Scialò C, Tran TH, Salzano G, Novi G, Caponnetto C, Chiò A, et al: TDP-43 real-time quaking induced conversion reaction optimization and detection of seeding activity in CSF of amyotrophic lateral sclerosis and frontotemporal dementia patients. Brain Commun 2020;2:fcaa142.
19. Mehta PR, Brown AL, Ward ME, Fratta P: The era of cryptic exons: implications for ALS-FTD. Mol Neurodegener 2023;18:16.
20. Irwin KE, Jasin P, Braunstein KE, Sinha IR, Garret MA, Bowden KD, et al: A fluid biomarker reveals loss of TDP-43 splicing repression in presymptomatic ALS-FTD. Nat Med 2024;30:382–393.

https://doi.org/10.18214/jend.2024.00080

JEND Journal of Electrodiagnosis and Neuromuscular Diseases

CASE REPORT

pISSN: 2733-6581 · eISSN: 2733-659X
J Electrodiagn Neuromuscul Dis 2024;26(3):53-57
https://doi.org/10.18214/jend.2024.00024

Sciatic Nerve Injury after an Intramuscular Injection into the Gluteal Region

Sangpil Son, Sol Lee, Jae In You, Minji Chae

Department of Rehabilitation Medicine, Daejeon Eulji Medical Center, Eulji University School of Medicine, Daejeon, Korea

The sciatic nerve can be injured through various mechanisms, including direct compression and ischemia related to traumatic events. Reports of iatrogenic sciatic nerve injury caused by misplaced intramuscular injections are rare. We present a case involving a 5-year-old patient who developed motor weakness and hypesthesia in the left lower extremity following an intramuscular injection of diclofenac into the left buttock. An electrodiagnostic study diagnosed the patient with an injury to the left sciatic nerve, primarily affecting its peroneal division. This diagnosis was later confirmed by radiologic evaluation. Following several weeks of rehabilitation, which included gait pattern correction, verbal cueing, and electrical stimulation therapy, the patient showed improvement in sensory deficits and motor impairment. The peroneal portion of the sciatic nerve is more susceptible to injury than the tibial portion due to its structural characteristics. Additionally, the sciatic nerve follows various paths as it passes the piriformis muscle. Certain drugs, such as diclofenac, exhibit greater neurotoxicity than others. When neurologic deficits are observed, an electrodiagnostic study is recommended. This helps not only in identifying the etiology and precise location of the neural insult but also in predicting the prognosis and formulating a comprehensive treatment plan.

Keywords: Electrodiagnosis; Sciatic nerve; Injections, intramuscular

Received: July 1, 2024
Revised: August 8, 2024
Accepted: August 20, 2024

Corresponding author:
Minji Chae
Department of Rehabilitation Medicine, Daejeon Eulji Medical Center, Eulji University School of Medicine, 95 Dunsanseo-ro, Seo-gu, Daejeon 35233, Korea
Tel: +82-42-611-3630
Fax: +82-42-259-1125
E-mail: drcherry0911@eulji.ac.kr

Introduction

The sciatic nerve originates from the L4 to S3 spinal nerve roots and provides innervation to various muscles of the lower extremities. It directly innervates the posterior thigh muscles, including the biceps femoris, semimembranosus, and semitendinosus. Distally, it branches into the tibial and peroneal nerves, which further provide motor and sensory branches to the lower leg and foot [1]. Like other nerve injuries, sciatic nerve injury can result from stretching, compression, or ischemia [2]. These injuries are often linked to traumatic events such as falls and vehicle accidents, and are frequently associated with acetabular fractures or posterior hip dislocation [3]. Additionally, cases of iatrogenic sciatic nerve injury due to improperly placed intramuscular gluteal injections have been infrequently reported worldwide. The severity of symptoms from a misplaced injection injury can vary, depending on the extent of nerve involvement and the neurotoxicity of the administered medication [4]. We present a case of a patient with a sciatic nerve injury suspected to have occurred following an intramuscular gluteal injection of diclofenac.

Case Report

A 5-year-old female with no significant past medical history presented at the emergency department with persistent fever and sore throat. Following an evaluation that included a simple X-ray and laboratory tests, such as C-reactive protein levels and white

blood cell count, she was diagnosed with acute pharyngotonsillitis and admitted to the department of pediatrics. After reviewing the examination results, a 23-gauge needle was used to administer a prescribed dose of diclofenac into her left buttock via intramuscular injection for fever control. Approximately 30 minutes after the injection, she began to show motor weakness in her left ankle and toes, along with a dragging of her left foot while walking. The muscle strength in her left ankle dorsiflexor and big toe extensor was rated 2/5 according to the Medical Research Council grade. No motor weakness was observed in the other muscles of the left lower extremity. A sensory evaluation was also conducted to check for possible nerve injury, during which the patient reported hypesthesia in the lateral area of her left lower leg. She exhibited no bladder or bowel symptoms, and her deep tendon reflexes were normal.

An electrodiagnostic examination was conducted 3 weeks after the onset of symptoms to ensure more accurate results. At the time of the electrodiagnostic study, there were no neurological changes, including sensation and muscle strength, compared to the initial onset of symptoms. Sensory nerve conduction studies showed no response in the left sural nerve and the left superficial peroneal nerve. In motor nerve conduction studies, no response was observed in the left common peroneal nerve when recorded at the left extensor digitorum brevis muscle. Recording at the left tibialis anterior muscle revealed reduced amplitudes of compound muscle action potential. Similarly, reduced amplitudes of compound muscle action potential were also observed in the left tibial nerve when recorded at the left abductor hallucis muscle. Furthermore, the conduction velocity of the left common peroneal nerve was mildly slower compared to the opposite side (Table 1). In needle electromyographic studies, denervation potential was detected in the left tibialis anterior and peroneus longus muscles. A reduced recruitment pattern was also observed during voluntary contraction of these muscles (Table 2). Overall, the findings suggested an incomplete lesion of the left sciatic nerve with axonal loss, with the peroneal division being more affected. Four weeks after the onset of symptoms, magnetic resonance imaging of the left hip was performed to confirm the nerve lesion. The magnetic resonance imaging revealed diffuse thickening of soft tissue and increased signal intensity on the T2-weighted im-

Table 1. Initial and Follow-up Findings of Nerve Conduction Studies

Nerve and site	Initial			Follow-up		
	Latency (msec)	Amplitude	Conduction velocity (m/sec)	Latency (msec)	Amplitude	Conduction velocity (m/sec)
Motor nerve conduction						
Right peroneal nerve (EDB)	2.23	6.68	46.50	-	-	-
Right peroneal nerve (TA)	2.02	15.75	60.76	-	-	-
Right tibial nerve (AH)	2.62	23.85	40.34	-	-	-
Left peroneal nerve (EDB)	NR	-	-	3.44	4.46	41.97
Left peroneal nerve (TA)	2.42	2.72	50.53	2.19	8.39	64.00
Left tibial nerve (AH)	2.27	17.19	45.98	2.79	21.19	43.29
Sensory nerve conduction			-			-
Right superficial peroneal nerve	2.14	15.0		-	-	
Right sural nerve	1.56	37.1		-	-	
Left superficial peroneal nerve	NR	-		NR	-	
Left sural nerve	NR	-		1.77	21.5	

Amplitudes are measured in millivolts for motor nerves and microvolts for sensory nerves.
EDB, extensor digitorum brevis; TA, tibialis anterior; AH, abductor halluces; NR, no response.

Table 2. Initial and Follow-up Findings of Needle Electromyography

Muscle	Initial			Follow-up		
	PSW	MUAP	Recruitment	PSW	MUAP	Recruitment
Left tibialis anterior	1+	Normal	Markedly reduced	-	Normal	Reduced
Left peroneus longus	1+	Normal	Markedly reduced	-	Normal	Reduced
Left medial gastrocnemius	-	Normal	Normal	-	Normal	Normal
Left biceps femoris (short head)	-	Normal	Normal	-	Normal	Normal
Left biceps femoris (long head)	-	Normal	Normal	-	Normal	Normal
Left gluteus maximus	-	Normal	Normal	-	Normal	Normal
Left lumbar paraspinalis	-			-		

PSW, positive sharp wave; MUAP, motor unit action potential.

https://doi.org/10.18214/jend.2024.00024

age at the lateral side of the left sciatic nerve (Fig. 1). No space-occupying lesions, such as a hematoma or cyst, were observed.

The patient was transferred to the Department of Rehabilitation Medicine after 2 weeks of management for acute pharyngotonsillitis and underwent a follow-up evaluation for sensory and motor deficits. A new weakness in ankle eversion was detected, with a strength of 3/5 according to the Medical Research Council grade. The muscle strength of the left ankle dorsiflexor and big toe extensor had slightly improved to a 3/5 Medical Research Council grade. She exhibited a circumduction gait due to ankle dorsiflexion weakness. Additionally, during gait training, she tended to invert her ankle due to the weakness and decreased endurance of the ankle evertor. To correct the impaired gait pattern, visual and verbal cueing was used. Gait training was conducted barefoot, taking into account the weak dorsiflexor power of the ankle. Electrical stimulation therapy was administered to the left tibialis anterior, peroneus longus, and brevis muscles. Initially, the electrical stimulation therapy was applied to the left tibialis anterior; however, as the ankle dorsiflexion power rapidly recovered, the focus shifted to the peroneus longus and brevis. After several weeks of treatment, the patient was discharged in a significantly improved functional condition, capable of independent gait. Clinically, sensory deficits had fully resolved, and the muscle strength of the left ankle dorsiflexor and the left big toe extensor improved to 4/5 Medical Research Council grade. Follow-up electrodiagnostic studies were conducted about three months later. Given the patient's age and cooperation, only nerves that had shown abnormalities in previous tests were examined. The follow-up sensory nerve conduction study showed no response in the left superficial peroneal nerve, whereas the left sural nerve, which previously showed no response, now demonstrated sensory nerve action potential. In the motor nerve conduction study, there were reduced amplitudes of compound muscle action potential in the left peroneal nerve when recorded at the extensor digitorum brevis and tibialis anterior. Compared to the initial study, the left peroneal nerve now showed compound muscle action potential when recorded at the extensor digitorum brevis muscle. The needle electromyography results no longer showed denervation potential, indicating recovery (Tables 1, 2).

This study was approved by the Institutional Review Board of Daejeon Eulji Medical Center (IRB No.: 2024-06-010).

Discussion

We report a case of a sciatic nerve lesion initially diagnosed through an electrophysiological study, likely induced by an intra-

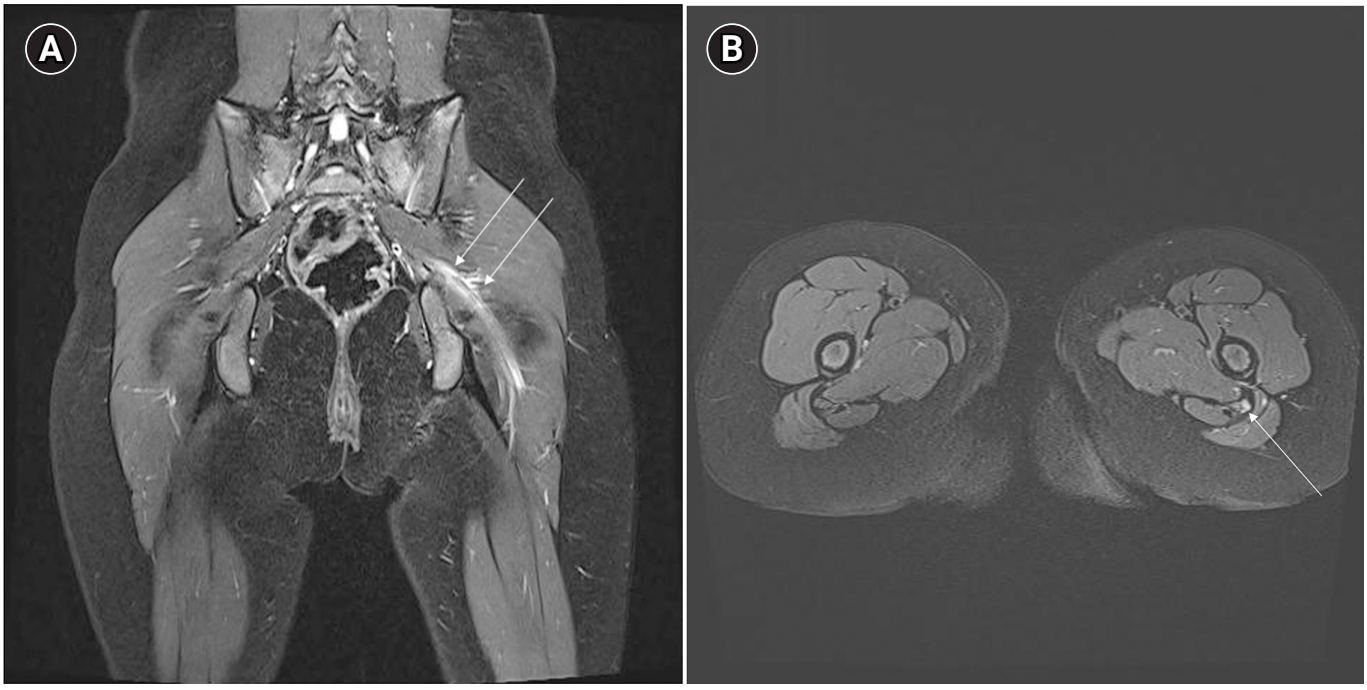

Fig. 1. Magnetic resonance imaging of the hip revealed an enlarged diameter of the left sciatic nerve and increased signal intensity in the peroneal portion of the left sciatic nerve on T2-weighted images (arrows). (A) Coronal view. (B) Axial view.

muscular injection. In this instance, the peroneal division of the sciatic nerve was predominantly affected, with clinical symptoms presenting solely as damage to the common peroneal nerve. Sensory changes were noted in the dermatome of the superficial peroneal nerve, and muscle weakness was observed in the ankle dorsiflexor, evertor, and big toe extensor. The initial needle electromyographic results showed no abnormal findings in the medial gastrocnemius and the long head of the biceps femoris, suggesting that the peroneal division was primarily affected. The short head of the biceps femoris muscle, which is innervated by the peroneal division of the sciatic nerve, is a critical muscle in the electrodiagnostic evaluation of sciatic neuropathy. However, the needle electromyographic examination of the short head of the biceps femoris muscle in this patient revealed no denervation potential. Considering the somatotopic organization, it can be hypothesized that the injection injury selectively affected only specific fascicles of the sciatic nerve. This hypothesis is further supported by the absence of motor impairment in the short head of the biceps femoris muscle in this patient.

The peroneal portion of the sciatic nerve, characterized by larger funiculi and less supporting connective tissue, is more susceptible to injury compared to the tibial division [5]. This vulnerability was similarly observed in our case. Identifying the cause and location of the nerve damage was relatively straightforward in this instance. However, when the etiology and location of the damage are not clearly defined, there is a risk of misdiagnosis. Therefore, performing electrodiagnostic tests should be considered to accurately determine both the location and the cause of the nerve damage, as injuries to the proximal portion may present symptoms similar to those of injuries solely to the distal portion.

The sciatic nerve exhibits variability in its course, taking several forms. In most individuals, it typically runs beneath the piriformis muscle. However, in some cases, it bifurcates and passes above the muscle, and there are instances where the nerve traverses through or emerges between muscles [6]. Sciatic nerve injection injuries most frequently occur when the needle insertion site deviates medially or inferiorly from the recommended position in the upper outer quadrant of the buttock in normal individuals [4,7]. The proximity of the injection to the nerve is considered the most critical factor in determining the extent of nerve damage. Direct injection into the nerve is associated with the most severe outcomes [7]. Therefore, it is crucial to ensure precise injection into the upper outer quadrant, which is anatomically distant enough from the nervous structures, for intramuscular injections to the buttocks.

Nerve injuries associated with injections can result not only from direct physical damage caused by needlesticks but also from the injection of material into the surrounding tissue [8]. The drug may be injected between the nerve and its sheath or between the fascicles, making the neurotoxicity of the drug a relevant factor in the extent of the damage [9,10]. A histopathological study revealed that the neurotoxic effects of diclofenac on the sciatic nerve were more significant than those caused by other commonly used injection drugs, such as pethidine or morphine [10]. Therefore, physicians should consider alternative treatments, such as oral agents or intravenous drugs, for first-line symptom control. Additionally, further research could be beneficial in identifying more suitable alternative injection sites for diclofenac.

Electrodiagnostic examinations have clinical significance in both confirming the severity of neural damage and predicting the clinical prognosis. In cases where symptoms are mild, conservative treatment usually spans several weeks to months for neurapraxia. However, severe axonotmesis or neurotmesis, characterized by significant motor weakness and neuropathic pain, may require surgical intervention [7,9]. Alongside history taking, physical examination, and imaging studies, electrodiagnostic testing plays a crucial role in forecasting recovery patterns and identifying potential neurological complications.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

ORCID

Sangpil Son, https://orcid.org/0000-0001-7484-7034
Sol Lee, https://orcid.org/0009-0000-2132-5160
Jae In You, https://orcid.org/0000-0003-3345-2727
Minji Chae, https://orcid.org/0000-0002-0590-2113

References

1. Adibatti M, Sangeetha V: Study on variant anatomy of sciatic nerve. J Clin Diagn Res 2014;8:AC07–AC09.
2. Robinson LR: Traumatic injury to peripheral nerves. Muscle Nerve 2000;23:863–873.
3. Issack PS, Helfet DL: Sciatic nerve injury associated with acetabular fractures. HSS J 2009;5:12–18.
4. Kim HJ, Park SH: Sciatic nerve injection injury. J Int Med Res 2014;42:887–897.
5. Sunderland S: The relative susceptibility to injury of the me-

 https://doi.org/10.18214/jend.2024.00024

dial and lateral popliteal divisions of the sciatic nerve. Br J Surg 1953;41:300–302.
6. Beaton LE, Anson BJ: The relation of the sciatic nerve and of its subdivisions to the piriformis muscle. Anat Rec 1937;70:1–5.
7. Kline DG, Kim D, Midha R, Harsh C, Tiel R: Management and results of sciatic nerve injuries: a 24-year experience. J Neurosurg 1998;89:13–23.
8. Spinner RJ, Kline DG: Surgery for peripheral nerve and brachial plexus injuries or other nerve lesions. Muscle Nerve 2000;23:680–695.
9. Park CW, Cho WC, Son BC: Iatrogenic injury to the sciatic nerve due to intramuscular injection: a case report. Korean J Neurotrauma 2019;15:61–66.
10. Bostan H, Cabalar M, Altinay S, Kalkan Y, Tumkaya L, Kanat A, et al: Sciatic nerve injury following analgesic drug injection in rats: a histopathological examination. North Clin Istanb 2018;5:176–185.

JEND Journal of Electrodiagnosis and Neuromuscular Diseases

CASE REPORT

pISSN: 2733-6581 · eISSN: 2733-659X
J Electrodiagn Neuromuscul Dis 2024;26(3):58-61
https://doi.org/10.18214/jend.2023.00185

Isolated Conus Medullaris Infarction: Rare Cases Highlighting Diagnostic Challenges and Clinical Insights

Yuri Je[1], Seokho Choi[1], Han-Jin Cho[1,2], Young-Eun Park[1,2]

[1]Department of Neurology, Pusan National University Hospital, Pusan National University School of Medicine, Busan, Korea
[2]Medical Research Institute, Pusan National University Hospital, Busan, Korea

Spinal cord infarction that exclusively affects the conus medullaris is exceptionally rare. The dominant symptoms include flaccid paraparesis, sensory deficits with or without saddle anesthesia, and neurogenic bladder, all of which mimic cauda equina syndrome. We report two cases where patients initially presented with sudden onset of leg monoparesis, sensory deficits, and voiding difficulties. Although initially suspected of having lumbosacral radiculopathy or cauda equina syndrome, they were later diagnosed with conus medullaris infarction, as evidenced by their clinical course and spine magnetic resonance imaging findings. This report provides detailed clinical information about conus medullaris infarction, supplemented by a review of the literature, to aid in the diagnosis of this condition.

Keywords: Spinal cord; Infarction; Paraparesis; Urinary bladder, neurogenic; Cauda equina

Received: December 13, 2023
Revised: August 9, 2024
Accepted: September 12, 2024

Corresponding author:
Young-Eun Park
Department of Neurology, Pusan National University Hospital, 179 Gudeok-ro, Seo-gu, Busan 49241, Korea
Tel: +82-51-240-7819
Fax: +82-51-245-2783
E-mail: yepark407@gmail.com

Introduction

Spinal cord infarction is a rare condition, accounting for less than 1% of all stroke causes [1]. It typically results from vascular occlusion of the anterior spinal artery, which occurs more frequently than occlusion of the posterior spinal artery [2]. The anterior spinal arteries originate from the anterior segmental medullary arteries, with the artery of Adamkiewicz being the largest and most significant contributor to spinal cord infarction. This artery primarily supplies the lower two-thirds of the spinal cord, making infarctions more common in the lower thoracic and lumbar regions [2]. The onset of spinal cord infarction is rapid, typically reaching its most severe point within minutes to 12 hours. Symptoms often include motor weakness and a dissociative sensory deficit, characterized by a loss of pain and temperature sensation. While hyperreflexic deep tendon reflexes and pathological reflexes are frequently observed, there are instances where these are absent, and the presentation may resemble spinal shock [1,2]. In spontaneous cases of spinal cord infarction, most patients exhibit vascular risk factors such as hypertension and diabetes. The condition is also commonly associated with aortic dissection or aortic surgery [1].

The conus medullaris is the terminal segment of the spinal cord before it transitions into the cauda equina. Its blood supply is primarily derived from the artery of Adamkiewicz, yet isolated infarctions of the conus medullaris remain exceedingly rare. Typical symptoms of conus medullaris infarction include paraparesis, sensory deficits, and difficulties with voiding and defecation, which are also common in cauda equina syndrome [3]. These overlapping symptoms can lead to misdiagnosis, prompting unnecessary diagnostic and therapeutic interventions. It is crucial for physicians to understand the clinical presentation, etiology, and diagnostic approaches for conus medullaris infarction to avoid inappropriate treatment, although the scarcity of cases limits the availability of data.

This report describes two cases of conus medullaris infarction,

detailing their clinical manifestations, courses, and outcomes, and reviews the literature on conus medullaris infarction.

Case Reports

1) Case 1

A 68-year-old man presented with acute-onset weakness in the right lower leg on the day of admission. He also experienced difficulty urinating. His symptoms remained stable and did not worsen after their onset. Two years prior, he had suffered a left basal ganglia infarction, from which he partially recovered, allowing him to walk with the aid of a cane. Since then, he had been prescribed clopidogrel and aspirin. During the initial neurological examination, reduced strength was observed solely in the right distal lower limb; ankle dorsiflexion and plantar flexion were rated as 4 on the Medical Research Council (MRC) scale, and great toe flexion and extension were rated as 1. Decreased sensation to touch and temperature was noted on the outer side of the right lower leg and the dorsum of the right foot. Both urinary dysfunction and decreased anal tone were confirmed. Deep tendon reflexes were brisk only in the right knee, and no pathological reflexes were observed.

Lumbosacral radiculopathy was initially suspected due to neurological deficits localized to one side of the lower limb, accompanied by ipsilateral sensory disturbances indicative of a lesion in the right L5 root. Additionally, a combined cauda equina lesion was considered due to the patient's voiding difficulties. Consequently, lumbar magnetic resonance imaging (MRI) was performed. The MRI revealed high signal intensities in the conus medullaris on T2-weighted images, although there were no abnormalities in the cauda equina (Fig. 1A, B). Laboratory findings were generally unremarkable, except for an elevated erythrocyte sedimentation rate (35 mm/h; normal range 0 to 10). Both nerve conduction studies and needle electromyography showed normal results, with no abnormalities detected in somatosensory evoked potential testing.

Based on the acute onset and stable course of symptoms, along with MRI findings, the patient was diagnosed with conus medullaris infarction. He continued on his existing regimen of aspirin and clopidogrel while under observation. Subsequently, there was a rapid improvement in the muscle strength of the right ankle and toes, occurring within approximately 10 days of hospitalization, which enabled stable ambulation without assistance. However, bladder dysfunction persisted, as evidenced by sensory loss in the bladder during urodynamic testing.

2) Case 2

A 66-year-old man presented with sudden onset of weakness

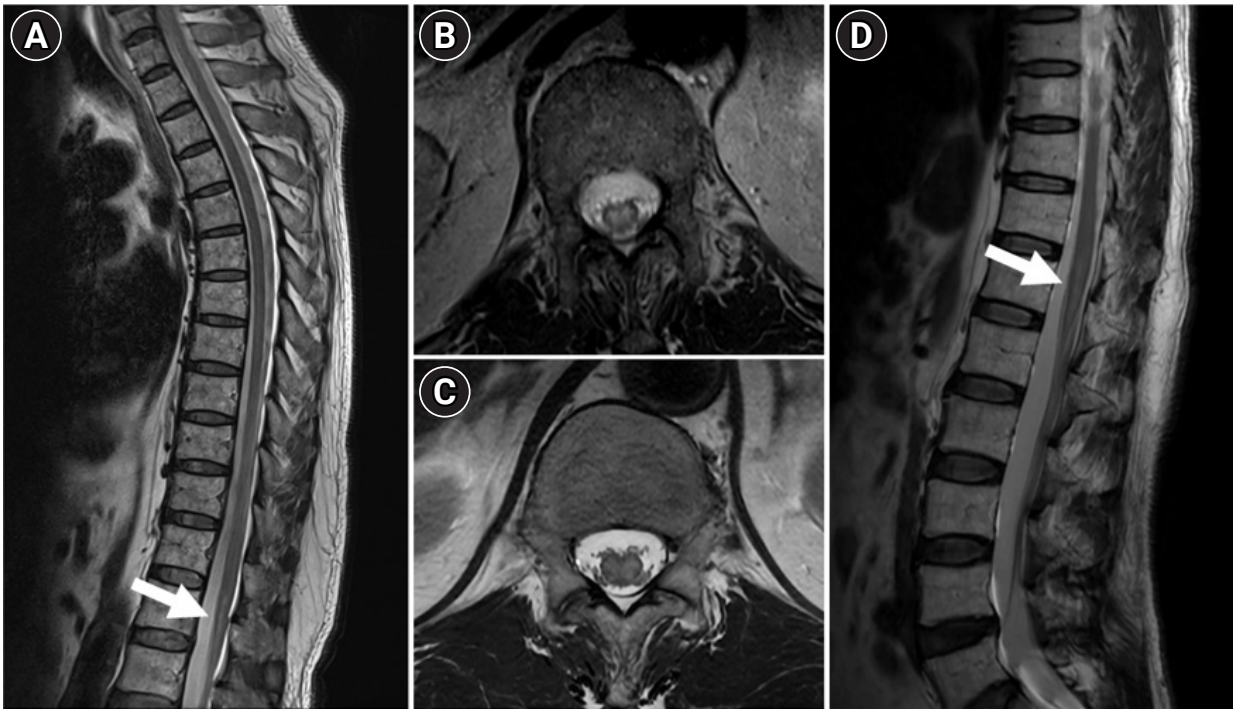

Fig. 1. Magnetic resonance images of the patients. (A) High signal intensity was observed at the distal end of the spinal cord (conus medullaris) in patient 1 (arrow). (B, C) Axial images of the conus medullaris in patients 1 (B) and 2 (C) demonstrated diffusely increased signal intensities. (D) High signal intensity at the conus medullaris was highlighted in patient 2 (arrow).

in his left lower limb one day prior to admission. There was no visible movement below the left ankle, and he experienced difficulty with urination. His symptoms did not worsen after their onset. Several hours before the symptoms appeared, he reported a dull, aching pain in his buttocks. His medical history included diabetes and hyperlipidemia. During the initial neurological examination, ankle dorsiflexion, plantar flexion, and extension and flexion of the great toe were all graded as 0. There was bilateral loss of touch and temperature sensation below the L5 level, along with loss of anal sphincter tone and bladder sensation. Deep tendon reflexes were normal, and no pathological reflexes were detected.

The initial suspicion of lumbosacral nerve root involvement was due to the motor weakness being limited to the unilateral distal leg. Additionally, the presence of urination difficulty led to the consideration of a combined cauda equina lesion. MRI of the lumbar spine showed high signal intensities in the conus medullaris, predominantly on the left side, with no contrast enhancement observed (Fig. 1C, D). Laboratory results were generally normal, except for an elevated blood glucose level (153 mg/dL; normal range 70 to 110). While nerve conduction studies and electromyography showed normal results, somatosensory-evoked potential testing indicated delayed cortical responses on both sides.

Based on the acute onset and progression of his symptoms, he was diagnosed with conus medullaris infarction, and treatment with clopidogrel was initiated. On the third day of hospitalization, there was a sudden decrease in the strength of his right ankle dorsiflexion and plantar flexion to grade 4, and great toe flexion and extension to grade 3. In response, aspirin was added to his treatment regimen, and no further deterioration has been reported since then. His deep tendon reflexes remained normoactive thereafter. Three months after discharge, he was able to walk with the aid of a cane, but only partial recovery of urinary dysfunction was observed.

This study was approved by the Institutional Review Board (IRB) of Pusan National University Hospital (IRB No. 2307-020-129) and granted an exemption from written consent by the IRB.

Discussion

The two cases are characterized by the sudden onset of unilateral weakness in the distal lower extremity, accompanied by sensory deficits and urinary dysfunction. These symptoms suggest involvement of the lumbosacral nerve roots or cauda equina. In the first case, sensory symptoms in the dermatome of the ipsilateral lower lumbar nerves led to a suspicion of lumbosacral radiculopathy or cauda equina syndrome. However, MRI of the lumbar spine ruled out cauda equina lesions in both cases. Importantly, the rapid progression to the nadir of symptoms at onset is a key indicator of vascular diseases. In this context, high signal intensities in the conus medullaris are indicative of conus medullaris infarction, and the neurological deficits observed in the two patients correlate with lesions in the conus medullaris. However, the presence of sensory symptoms on the same side as the weakness in the first patient casts some doubt on the diagnosis of conus medullaris lesions. This could suggest involvement of the right corticospinal tract and the left spinothalamic tract. Another possibility is that, in addition to the conus medullaris lesion, there may have been a concurrent nerve root infarction due to occlusion of radicular arteries, as reported in previous cases [4,5]. This was not confirmed in our case, but it is plausible given that radicular arteries, along with segmental medullary arteries, originate from segmental spinal arteries [4].

Isolated conus medullaris infarction is exceedingly rare within the category of spinal cord infarctions. This rarity is attributed to the rich vascular anastomotic network between the anterior and posterior spinal arteries in the conus medullaris, which provides a protective environment against ischemic damage [6]. Due to this rarity, the literature on conus medullaris infarction is limited, making it a challenging diagnosis. According to previous reports, the most common presentation is bilateral flaccid paralysis with sensory deficits and a neurogenic bladder [3]. Although asymmetrical weakness was not uncommon (7/19, 36.8%), monoparesis as an initial presentation was reported to be quite rare (1/19, 5.3%) [7]. Furthermore, the monoparesis observed in our cases became a significant factor in initially overlooking conus medullaris infarction. Pain preceding neurological symptoms, as seen in the second case, was commonly reported in the buttocks, low back, and lower limbs [7,8]. The risk factors for conus medullaris infarction are often not apparent. However, the presence of preceding stroke, diabetes, and hyperlipidemia are considered underlying risk factors and are mentioned as influencing prognosis, with studies reporting a correlation with poor walking ability outcomes [3,7,8]. Our cases showed a relatively favorable prognosis in terms of walking recovery. However, it is notable that in both cases, improvement in bladder function was noted to be slow.

In general, spinal cord infarction on MRI may exhibit owl-eye or pencil-like hyperintensities on T2-weighted images, with approximately 30% of cases appearing normal in the early stages, according to various reports [9]. Diffusion-weighted imaging (DWI) can reveal significant diffusion coefficient restriction in

 https://doi.org/10.18214/jend.2023.00185

about two-thirds of cases, making it highly valuable for confirming the diagnosis [8,9]. Although DWI was not performed in this instance, as infarction was not initially suspected, the rapid diagnosis provided by this imaging technique is crucial for initiating appropriate treatment early, potentially preventing further symptom deterioration and improving prognosis. The authors present these cases to enhance the understanding of conus medullaris infarction as a rare cause of acute lower limb paralysis. Through a review of the literature and an analysis of clinical symptoms, progression, and outcomes, we aim to provide insights that may aid in future diagnoses.

Conflict of Interest

Young-Eun Park is an editorial board member of the journal, but she was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

Acknowledgements

This work was supported by a clinical research grant from Pusan National University Hospital in 2023.

ORCID

Yuri Je, https://orcid.org/0009-0005-0411-0981
Seokho Choi, https://orcid.org/0009-0007-6375-2989
Han-Jin Cho, https://orcid.org/0000-0001-7152-9225
Young-Eun Park, https://orcid.org/0000-0003-0887-1604

References

1. Sandson TA, Friedman JH: Spinal cord infarction: report of 8 cases and review of the literature. Medicine (Baltimore) 1989;68:282–292.
2. Masson C, Pruvo JP, Meder JF, Cordonnier C, Touzé E, De La Sayette V, et al: Spinal cord infarction: clinical and magnetic resonance imaging findings and short term outcome. J Neurol Neurosurg Psychiatry 2004;75:1431–1435.
3. Anderson NE, Willoughby EW: Infarction of the conus medullaris. Ann Neurol 1987;21:470–474.
4. Kamimura T, Nezu T, Aoki S, Ueno H, Hosomi N, Maruyama H: Conus medullaris infarction involving the paraspinal muscles and nerve roots. J Stroke Cerebrovasc Dis 2020;29:104983.
5. Willaume T, Felten R, Pijnenburg L, Lersy F, Bierry G: Spontaneous infarction of lumbar roots, vertebrae and paravertebral muscles. Am J Emerg Med 2021;46:796.e5–796.e7.
6. Martirosyan NL, Kalani MY, Lemole GM, Spetzler RF, Preul MC, Theodore N: Microsurgical anatomy of the arterial basket of the conus medullaris. J Neurosurg Spine 2015;22:672–676.
7. Tan YJ, Manohararaj N: Isolated infarctions of the conus medullaris: clinical features and outcomes. J Stroke Cerebrovasc Dis 2021;30:106055.
8. Weng YC, Chin SC, Wu YY, Kuo HC: Clinical, neuroimaging, and nerve conduction characteristics of spontaneous Conus medullaris infarction. BMC Neurol 2019;19:328.
9. Zalewski NL, Rabinstein AA, Krecke KN, Brown RD, Wijdicks EF, Weinshenker BG, et al: Characteristics of spontaneous spinal cord infarction and proposed diagnostic criteria. JAMA Neurol 2019;76:56–63.

JEND Journal of Electrodiagnosis and Neuromuscular Diseases

CASE REPORT

pISSN: 2733-6581 · eISSN: 2733-659X
J Electrodiagn Neuromuscul Dis 2024;26(3):62-67
https://doi.org/10.18214/jend.2024.00045

Spinal Cord Infarction Associated with Coronavirus Disease 2019: A Case Report with Magnetic Resonance Imaging Insights

Kyeongil Min, Myung Woo Park, Hyun Iee Shin, Byung Chan Lee, Du Hwan Kim

Department of Physical Medicine and Rehabilitation, Chung-Ang University Hospital, Chung-Ang University College of Medicine, Seoul, Korea

Coronavirus disease 2019 (COVID-19) has been associated with various neurological complications, including the rare occurrence of spinal cord infarction. In this report, we present the case of a 42-year-old man who developed sudden quadriplegia after being diagnosed with COVID-19. Initial magnetic resonance imaging (MRI) provided inconclusive results; however, subsequent imaging revealed diffusion restriction and vertebral body signal changes, indicative of ischemic changes in the spinal cord. The patient received anticoagulation and corticosteroid therapy followed by rehabilitation, resulting in partial recovery of motor function. This case illustrates the importance of considering spinal cord infarction in patients with COVID-19 who present with neurological symptoms. Furthermore, it highlights the crucial role of MRI, including diffusion-weighted imaging, in diagnosis.

Keywords: Spinal cord infarction; COVID-19; Diffusion-weighted imaging; Vertebral body infarction

Received: August 9, 2024
Revised: November 6, 2024
Accepted: December 11, 2024

Corresponding author:
Du Hwan Kim
Department of Physical Medicine and Rehabilitation, Chung-Ang University Hospital, Chung-Ang University College of Medicine, 102 Heukseok-ro, Dongjak-gu, Seoul 06973, Korea
Tel: +82-2-6299-1881
Fax: +82-2-6298-1866
E-mail: ripheonix@naver.com

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), presents with a wide range of clinical manifestations, from respiratory distress to multisystemic complications [1,2]. Growing evidence indicates a potential association between SARS-CoV-2 infection and various spinal cord disorders, including acute transverse myelitis, acute necrotizing myelitis, neuromyelitis optica spectrum disorder, spinal epidural abscess, and spinal cord infarction [1]. Although rare, spinal cord infarction poses a notable challenge, and its occurrence in the context of COVID-19 complicates the diagnosis and management of this condition.

Magnetic resonance imaging (MRI) is the preferred neuroimaging modality for suspected cases of spinal cord infarction. Since diffusion restriction can occur with both inflammatory etiologies and infarction, the limited spatial resolution of diffusion-weighted imaging (DWI) has historically hindered its clinical utility in evaluating spinal cord lesions. However, recent technological advancements in DWI have improved its capacity to reveal ischemic changes in the spinal cord [3,4].

Herein, we report a case of spinal cord infarction occurring post-COVID-19, emphasizing the rarity of this complication and the pivotal role of MRI in its diagnosis. This case underscores the importance of considering spinal cord infarction in the differential diagnosis of neurological complications associated with COVID-19, advocating for the early application of MRI in clinical practice.

Case Report

A 42-year-old man with no significant medical history present-

ed with symptoms of upper respiratory infection, including cough and sputum. He was confirmed to have COVID-19 through reverse transcriptase-polymerase chain reaction testing of a nasal swab. Five days later, he experienced a sudden tingling sensation in his neck, followed by weakness in both upper limbs. During transfer to the hospital, he also developed weakness in both lower limbs.

Upon arrival at the hospital, the patient demonstrated grade 1 muscle strength in both upper extremities and grade 0 in the lower extremities, as measured by the Medical Research Council (MRC) scale. Additionally, the patient exhibited sensory impairment below the C3 dermatome.

Initial brain computed tomography and MRI revealed no abnormalities. Likewise, his serum laboratory results were unremarkable: D-dimer, 0.16 μg/mL (reference, 0 to 0.5); fibrinogen, 438 mg/dL (reference, 200 to 495); fibrinogen degradation products, 0.72 μg/mL (reference, 0 to 3.0), prothrombin time, 12.5 seconds (reference, 10.3 to 13.6), and activated partial thromboplastin time, 31.6 seconds (reference, 25.0 to 37.0). Cerebrospinal fluid analysis and immunological testing showed no significant abnormalities, except a positive lupus anticoagulant screening test (1.28; reference < 1.20). The follow-up confirmatory test for lupus anticoagulant was also positive (1.30). Since all other autoantibody tests yielded negative results, this elevation was attributed to a transient response to the viral infection rather than an underlying hypercoagulable state.

Cervical spine MRI indicated high signal intensity and swelling in the anterior aspect of the spinal cord, spanning from C3 to C7, on T2-weighted images (Fig. 1A). At the lesion site, diffusion-weighted images (b 800) exhibited high signal intensity, while apparent diffusion coefficient images displayed low signal intensity. These findings are consistent with ischemic changes (Fig. 1B, C).

The patient was initiated on anticoagulation therapy, receiving 60 mg of enoxaparin subcutaneously every 12 hours. Concurrently, he was treated with 1 g/day of methylprednisolone for 5 days. Due to escalating difficulty with breathing and expectoration, a tracheostomy was performed.

On day 12, the patient's spinal MRI revealed high signal intensity on fat-suppressed images in the posterior one-third of the C7 vertebral body, indicative of bone infarction (Fig. 1D). Three months following the onset of symptoms, spinal MRI displayed persistent high signal intensity in the cervical cord on T2-weighted images (Fig. 1E). After 3 weeks of treatment with enoxaparin, the patient was transitioned to oral aspirin at a dosage of 100 mg/day.

The patient continued rehabilitation therapy, which included range-of-motion exercises, balance training, activities of daily living training, and gait training. At the 1-year follow-up, a slight improvement was noted in the strength of his distal upper extremities. His lower extremity strength had improved to grade 4 on the MRC scale, enabling him to walk with assistance. The patient's neurological status was classified as C5 grade D on the American Spinal Injury Association Impairment Scale. He was able to breathe spontaneously without the need for a tracheostomy or supplemental oxygen and could void on his own. However, intermittent catheterization was still necessary due to residual urine.

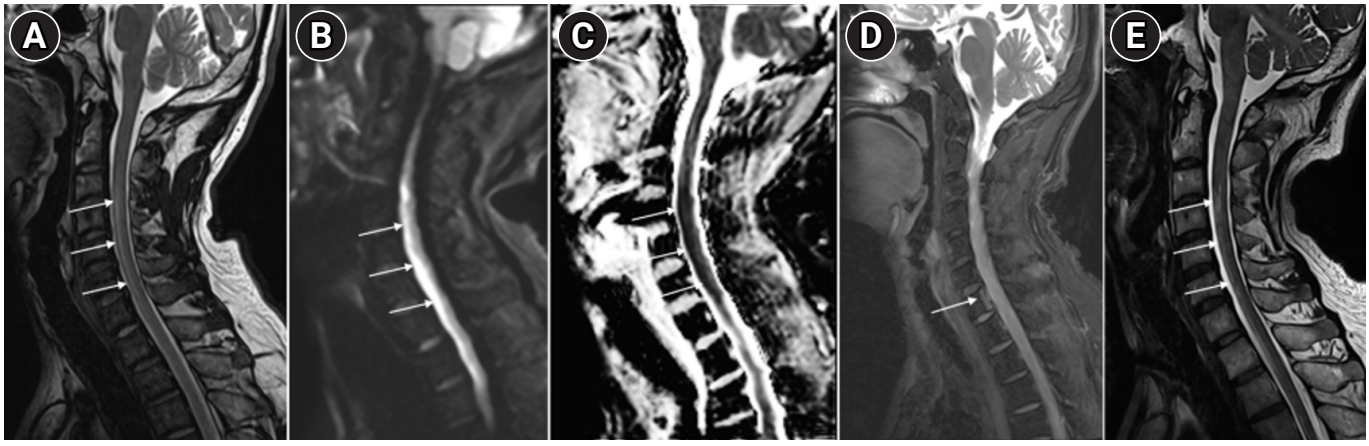

Fig. 1. Magnetic resonance imaging findings. (A) T2-weighted image reveals high signal intensity and swelling (arrows) in the anterior aspect of the spinal cord from C3 to C7. (B) The lesion site exhibits high signal intensity (arrows) on diffusion-weighted imaging (b 800). (C) Apparent diffusion coefficient image shows low signal intensity (arrows). (D) Fat-suppressed image demonstrates high signal intensity in the posterior one-third of the C7 vertebral body (arrow), indicative of bone infarction on day 12. (E) Three months after symptom onset, the cervical cord maintains high signal intensity (arrows) on T2-weighted imaging.

This study was approved by the Institutional Review Board Committee of Chung-Ang University Hospital (approval number: 2306-005-19473). Informed consent was waived by the board.

Discussion

The development of spinal cord infarction following COVID-19 infection underscores the potential of the virus to induce systemic vascular complications extending beyond the respiratory system. Although the pathophysiological mechanisms linking COVID-19 to spinal cord infarction are not fully understood, they may involve a multifaceted interaction of vascular endothelial damage, hypercoagulability, and inflammatory responses triggered by the viral infection [5].

The diagnostic challenges in identifying spinal cord infarction are compounded by its rarity and the variability of clinical presentations, which may resemble other neurological conditions. We conducted an extensive search of the PubMed and Google Scholar databases using the following search terms: 'COVID-19 OR SARS-CoV-2 AND spinal cord.' We identified eight isolated cases and summarized their clinical findings in Table 1. Although COVID-19 has been considered a global pandemic for the past 4 years, published cases of spinal cord infarction associated with COVID-19 are extremely rare [3,4,6-12]. COVID-19 is known to be linked to a hypercoagulable state; however, the causal relationship between COVID-19 and spinal cord infarction remains unclear due to its rare incidence. In contrast, increasing reports have been made of COVID-19-associated transverse myelitis, now recognized as a significant neurological complication of the disease [1]. Notably, differentiating spinal cord infarction from transverse myelitis is challenging due to their similar clinical fea-

Table 1. Literature Review of All Published Cases of Spinal Cord Infarction in the Context of COVID-19

Reference/ Country	Age (y)/ sex	Comorbidity	Respiratory and systematic symptoms	Interval from COVID-19 to spinal cord infarction	Evidence of hypercoagulable state	Neurological manifestations	Lesions on neuroimaging	Treatment for spinal cord infarction	Outcome
Sampogna et al. (2020) [9]/Italy	69/Male	Hypertension Diabetes mellitus	Fever Cough Anosmia Respiratory failure requiring mechanical ventilator Pulmonary embolism with deep vein thrombosis Infarction of the left middle third posterior renal cortex artery	Approximately 34 days	Elevated D-dimer levels	Paraplegia with T10 AIS B	Ischemia from T8 to the conus medullaris on conventional MRI	None	Minimal assistance at a wheelchair level
Eissa et al. (2021) [7]/ Qatar	41/Male	None	Fever Initially moderate respiratory symptoms with 95% oxygen saturation progressing to ARDS requiring tracheostomy	Near simultaneous	Not described	Headache Right-side weakness followed by weakness in all four limbs	Diffusion restriction at the cervical and dorsal levels on DWI Restricted diffusion in right cerebellar and medulla oblongata on DWI Occlusion of the distal right vertebral artery on MRA	None	Not described
Bax et al. (2021) [10]/ Italy	52/ Female	None	Fever Desaturation	Simultaneous	Elevated D-dimer Reduced fibrinogen	Quadriplegia with AIS C Urinary retention	T2-weighted anterior cord hyperintensity from C6 to T1	Acetylsalicylic acid Methylprednisolone	AIS D Walker ambulatory

(Continued to the next page)

https://doi.org/10.18214/jend.2024.00045

Table 1. Continued

Reference/ Country	Age (y)/ sex	Comorbidity	Respiratory and systematic symptoms	Interval from COVID-19 to spinal cord infarction	Evidence of hypercoagulable state	Neurological manifestations	Lesions on neuroimaging	Treatment for spinal cord infarction	Outcome
Kahan et al. (2021) [3]/ USA	31/Male	None	Respiratory failure requiring intubation	Approximately 20 days	Elevated D-dimer and fibrinogen levels	Quadriplegia with motor level C5/C5 and sensory level T4/T4	T2 hyperintensity and enhancement of the cervical spinal cord spanning C4 through C6, with corresponding restricted diffusion	Enoxaparin Methylprednisolone	Not described
Amalia (2021) [6]/Indonesia	60/Male	Coronary artery disease Hypertension Taking aspirin 81 mg once daily, bisoprolol 2.5 mg once daily, and atorvastatin 20 mg once daily	Fever Upper respiratory tract infection	14 days	Elevated D-dimer and fibrinogen levels	Complete paralysis of lower limbs Loss of sensation below the T12 level Urinary retention	Drop in signal at the 12th thoracic level on MRA	Heparin	Improvement in lower limb motor strength (walking with assistance)
Braglia et al. (2022) [11]/ Italy	44/ Female	Hypertension Uterine fibromatosis Subacute thyroiditis Idiopathic elephantiasis	Cough Fever Respiratory failure requiring intubation	14 days	Elevated D-dimer Normal fibrinogen	Acute back pain Syncope Paraplegia below the T12 level	Normal spine MRI	Enoxaparin Steroid	Assisted walking
Oleson et al. (2023) [8]/ USA	70/ Female	Undifferentiated connective tissue disease	Cough Fever	7 days	Not described	Paraplegia with T3 AIS A Urinary retention Hypotension	T2 hyperintensity from T9 to the conus	Methylprednisolone	T6 AIS A
Xiao et al. (2024) [4]/ China	15/Male	Not described	Fever Dyspnea	Near simultaneous	Elevated D-dimer levels	Symmetric decreased muscle tension of limbs	T2 hyperintensity in medulla oblongata and cervical spinal cord and owl eye sign on conventional MRI	Not described	Not described
Present case	42/Male	None	Initially mild respiratory symptoms (i.e., cough and sputum) progressing to respiratory failure requiring tracheostomy	5 days	None	Quadriplegia with C3 AIS B	T2 hyperintensity of the cervical spinal cord spanning C3 through C7 with corresponding restricted diffusion	Enoxaparin Methylprednisolone	Quadriplegia with C5 AIS D

COVID-19, coronavirus disease 2019; AIS, American Spinal Injury Association Impairment Scale; MRI, magnetic resonance imaging; ARDS, acute respiratory distress syndrome; DWI, diffusion-weighted imaging; MRA, magnetic resonance angiography.

tures, such as the rapid onset of segmental neurological deficits and conventional MRI findings. These findings include T2 hyperintensities, which can appear longitudinally extensive or pencil-like on sagittal images and with an owl-eye appearance or extensive central involvement on axial images [3,4,9]. The clinical overlap between spinal cord infarction and transverse myelitis may contribute to an underdiagnosis of spinal cord infarction in the context of COVID-19. Among the nine cases of spinal cord infarction associated with COVID-19, including our case, six patients were male and three were female. Most spinal cord infarctions occurred within approximately 20 days following the onset of COVID-19 respiratory symptoms, with the infarctions located at the cervical or lower thoracic level.

In the present case, it is unclear whether COVID-19 infection directly caused the spinal cord infarction. A recent systematic review examining the link between COVID-19 and spinal cord ischemia identified coagulation abnormalities in five out of six patients. Our review found similar abnormalities in six of nine patients [12]. While coagulation-related abnormalities on laboratory tests may indirectly suggest a connection to spinal cord infarction, no specific biomarker currently exists for diagnosing spinal cord infarction caused by COVID-19. Classification criteria must be established for COVID-19-related spinal cord infarction, necessitating future research comparing the clinical characteristics, laboratory findings, and imaging features of spinal cord infarction cases associated with atherosclerosis and COVID-19.

Recent advances in DWI techniques for the spine have revolutionized diagnostic approaches, offering high sensitivity in detecting early ischemic changes within the spinal cord. This imaging modality enables the prompt initiation of therapies such as anticoagulation or antiplatelet treatment, which are crucial in mitigating irreversible neurological deficits. Theoretically, DWI is advantageous for detecting early ischemic changes because it can assess the restricted diffusion of water molecules in affected tissues. However, this diffusion restriction is not exclusive to infarcts; it can also occur under inflammatory conditions [3]. Other MRI findings of spinal cord infarction include signal changes within the vertebral bodies, which are diagnostically significant. Notably, infarction of the vertebral bodies at the same or adjacent levels as the spinal cord infarction is highly specific for diagnosing this condition, because the blood supply to the adjacent vertebral bodies and spinal cord originates from the same arteries. A recent study analyzing the MRI features of spinal cord infarction proposed that restricted diffusion and co-existing abnormalities of the vertebral body may represent key neuroimaging features for diagnosing spinal cord infarction [13]. Based on the clinical features and imaging findings, our patient was diagnosed with spinal cord infarction following COVID-19 infection.

In conclusion, recognition and early intervention in cases of spinal cord infarction following COVID-19 is crucial, despite the rarity of this complication, to optimize patient outcomes. Our case report underscores the importance of clinical awareness among healthcare providers regarding this uncommon sequela and emphasizes the critical role of MRI, including DWI, as a non-invasive, rapid, and reliable diagnostic tool for COVID-19-related spinal cord infarction. Ongoing research and further case reports are necessary to deepen our understanding of the underlying mechanisms and to improve diagnostic and therapeutic approaches in this evolving clinical scenario.

Conflict of Interest

Du Hwan Kim is an editorial board member of the journal, but he was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

ORCID

Kyeongil Min, https://orcid.org/0000-0002-4339-8077
Myung Woo Park, https://orcid.org/0000-0001-7531-8433
Hyun Iee Shin, https://orcid.org/0000-0002-9748-7585
Byung Chan Lee, https://orcid.org/0000-0001-8824-0639
Du Hwan Kim, https://orcid.org/0000-0002-9980-8549

REFERENCES

1. Garg RK, Paliwal VK, Gupta A: Spinal cord involvement in COVID-19: a review. J Spinal Cord Med 2023;46:390–404.
2. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, et al: Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 2020;77:683–690.
3. Kahan J, Gibson CJ, Strauss SB, Bronstein M, Winchell RJ, Barie PS, et al: Cervical spinal cord infarction associated with coronavirus infectious disease (COVID)-19. J Clin Neurosci 2021;87:89–91.
4. Xiao Q, Yang X, Wang S: Spinal cord and brainstem extensive infarction: a case with rare complication of COVID-19 infection. Neurol India 2024;72:451–452.
5. Lazzaroni MG, Piantoni S, Masneri S, Garrafa E, Martini G, Tincani A, et al: Coagulation dysfunction in COVID-19: the interplay between inflammation, viral infection and the coagulation system. Blood Rev 2021;46:100745.

 https://doi.org/10.18214/jend.2024.00045

6. Amalia L: Hypercoagulable state induced spinal cord stroke after coronavirus disease 19 infection. J Blood Med 2021;12:1057–1060.
7. Eissa M, Abdelhady M, Alqatami H, Salem K, Own A, El Beltagi AH: Spinal cord infarction in a 41-year-old male patient with COVID-19. Neuroradiol J 2021;34:245–248.
8. Oleson CV, Olsen AC, Shermon S: Spinal cord infarction attributed to SARS-CoV-2, with post-acute sequelae of COVID-19: a case report. World J Clin Cases 2023;11:8542–8550.
9. Sampogna G, Tessitore N, Bianconi T, Leo A, Zarbo M, Montanari E, et al: Spinal cord dysfunction after COVID-19 infection. Spinal Cord Ser Cases 2020;6:92.
10. Bax F, Gigli GL, Iaiza F, Valente M: Spontaneous spinal cord ischemia during COVID-19 infection. J Neurol 2021;268:4000–4001.
11. Braglia L, Vandelli G, Manenti A, Sacchi S: Acute spinal cord dysfunction following COVID-19 infection: a case report. Front Med Case Rep 2022;3:1–7.
12. Sourani A, Vahdat N, Bowers CA, Rezvani M, Foroughi M, Sourani A, et al: SARS-CoV-2 and spinal cord ischemia: a systematic review on clinical presentations, diagnosis, treatment, and outcomes. Spine J 2024;24:979–988.
13. Ke G, Liao H, Chen W: Clinical manifestations and magnetic resonance imaging features of spinal cord infarction. J Neuroradiol 2024;51:101158.

Instructions for Authors

JEND
Journal of Electrodiagnosis and Neuromuscular Diseases

2013. 9. 23 enacted
2015. 5. 29 revised
2015. 12. 14 revised
2019. 2. 23 revised
2020. 6. 24 revised
2021. 9. 28 revised

Journal of Electrodiagnosis and Neuromuscular Diseases (*J Electrodiagn Neuromuscul Dis*, JEND), an official journal of the Korean Association of EMG Electrodiagnostic Medicine, is published three times a year. It regards all aspects of EMG, electrodiagnostic medicine, and neuromuscular diseases, including clinical practice, experimental and applied research, and education, and its formal abbreviated journal name is J Electrodiagn Neuromuscul Dis.

The manuscript guidelines for JEND are based on the "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals" (http://www.icmje.org/recommendations/), and instructions which are not mentioned in the present guidelines are referred to the guidelines stated in the Recommendations.

Editorial Board will make the final decision on approval for the publication of submitted manuscripts and the publication order of accepted manuscripts. Editorial Board reviews ethics, rationality, originality, and scientific significance in accepting submitted manuscripts, and can request any further corrections, revisions, and deletions of the article if necessary.

1. General Guidelines

1-1. Qualifications for authorship

Authors should be limited to members of Korean Association of EMG Electrodiagnostic Medicine, associate members of Korean Association of EMG Electrodiagnostic Medicine or those who are associated with clinical practice, experimental and applied research, and education in the field of EMG, electrodiagnostic medicine, and neuromuscular diseases.

Authorship is credited to those who have direct involvement in the study and have made significant contributions to (a) conceptualization and design of the research, or acquisition, analysis, and interpretation of the data, (b) drafting of the manuscript or critical revision, and (c) approval of the submitted and final versions of the manuscript. The primary investigator is designated the first author of the study unless contested by the other authors. The corresponding author is directly responsible for communication and revision of the submitted manuscript.

In the case that more than one author contributed equally as the first author or the corresponding author, the acceptance of co-first or co-corresponding author should be determined through discussion of the Editorial Board. Everyone who is listed as coauthors should have made a substantial, direct, intellectual contribution to the work.

In the case of a change of authorship, a written explanation must be submitted. Change in either the first author or the corresponding author requires approval by the Editorial Board, and any changes of other authors require approval by the Editor-in-Chief.

1-2. Types of manuscript

Manuscripts include Original Articles, Case Reports, Brief communications, and Reviews, commissioned by the Editorial Committee on EMG, electrodiagnostic medicine, and neuromuscular diseases.

1-3. Duplicate or secondary publication

All submitted manuscripts should be original and should not be considered by other scientific journals for publication at the same time. No part of the accepted manuscript, including the table and the figure, should be duplicated in any other scientific journal without the permission of the Editorial Board. If duplicate publication related to the papers of this journal is detected, the manuscripts may be rejected.

But, if the authors have received approval from the editors of both journals (the editor concerned with secondary publication must have access to the primary version), secondary publication may be allowed only under the conditions for secondary publication stipulated in the "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals." The secondary version informs that the paper has been published in whole or in part elsewhere, and the secondary version cites the primary reference.

If the unauthorized duplicate publication is discovered, authors will be announced in the journal, and their institutes will be informed and are subject to penalties and/or unfavorable

outcomes including prompt rejection or prohibited submission.

1-4. Ethical considerations

For all studies involving human subjects, the principles embodied in the Declaration of Helsinki 2013; https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/) should be upheld, informed consent must be obtained from all participants, and must be approved by a recognized Institutional Review Board (IRB) or research ethics committee.

Any information that could have revealed subjects' identities, such as name and initials, should not appear in the text. If a photo is presented, proper measures should be taken not to reveal the subject's identity, or written consent must be presented for the photo and possible disclosure of the subject's identity.

Experiments involving animals should comply with the NIH guidelines for the use of laboratory animals and/or be reviewed by an appropriate committee (Institutional Animal Care and Use Committee, IACUC) to ensure the ethical treatment of animals in research.

All manuscripts should be written with strict adherence to the ethical guidelines recommended by the International Committee of Medical Journal Editors (http://www.icmje.org). If necessary, the Editorial Board could ask for providing patients' written consent and IRB's approval.

Issues of ethical misconduct, plagiarism, and duplicate/redundant publication will be judged and dealt with according to the "Good Publication Practice Guidelines for Medical Journals" (https://www.kamje.or.kr/board/view?b_name=bo_publication&bo_id=13&per_page=).

For the policies on the research and publication ethics not stated in this instructions, International standards for editors and authors (http://publicationethics.org/resources/international-standards-for-editors-and-authors) can be applied.

1-5. Copyright transfer

The Korean Association of EMG Electrodiagnostic Medicine is the owner of all copyright to papers published in JEND and has the right to publish, reproduce, distribute, and print the contents in other types of media.

1-6. Journal Publication and Manuscript Submission

This journal is published three times a year on April 30, August 31, and December 31, and submission is often allowed. Submitted manuscripts are initially examined for the format, and then appointed a submission date and a submission number. The day of the decision of the publication shall be the day when the manuscript is completed of its reviewing.

1-7. Submission of manuscripts

All submitted manuscripts must be accompanied by the official Copyright Transfer and Author Consent Form of JEND and must contain the title page, the title of the manuscript, manuscript, tables, and figures. The files of the title page, main text (the title of the manuscript, manuscript, and figure legends), tables, and figures must be submitted with the online submission system (https://submit.e-jend.org). The official Copyright Transfer and Author Consent Form must be submitted with the online submission system to the Editorial office. This form also should contain the title of the manuscript, date of submission, names of all authors, and written signatures. Note the corresponding author and provide his/her affiliation, email, telephone and fax numbers, and mailing address. Figures should be submitted as an original image (5x7 inches) or jpg file (at least 600 dpi, dots per inch).

Editorial office information (contact us):
Journal of Electrodiagnosis and Neuromuscular Diseases
Department of Physical Medicine and Rehabilitation, Korea University Ansan Hospital, 123 Jeokgeum-ro, Danwon-gu, Ansan 15355, Korea
Tel: 82-31-412-5330
Fax: 82-31-412-4215
E-mail : editjend@gmail.com

1-8. Review and revision of manuscripts

Submitted manuscripts will be reviewed by two peer reviewers selected from the Board's database of expert reviewers. Following review, the Editorial Board will decide whether the manuscript will be 1) accepted for publication 2) publication with minor revision, or accepted for publication following revision, 3) subject to major revision, or 4) denied publication.

For manuscripts which are either accepted for publication following revision or subject to major revision, the corresponding author must reply to reviewers' comments point by point and revise the manuscript with changes in red color and explain in detail what changes were made in the manuscript in "summary of revision" as soon as possible.

A manuscript that does not comply with the regulations for submission can be suggested to be adjusted or be reserved to be published or can be adjusted by the Editorial Board, if necessary, without affecting the original contents.

The reviewer and Editorial Board can request correcting English of the manuscript to a considerable level, and the author should accept it.

The manuscripts which are completed reviewing process shall be decided of its publication after reviewing of the Editorial Board, and a manuscript that does not comply with the regulations for submission can be rejected or delayed the acceptance.

When a manuscript is not resubmitted within two months of notification, it will be considered that the authors have withdrawn the manuscript from submission.

Manuscripts accepted for publication are generally published in order of submission, depending on the category of the manuscript and the date of acceptance for publication.

1-9. Charges for reviewing, publication and printing

There are no charges for reviewing, publication and printing, but illustrations that require extraordinary printing processes will be charged to the authors. The corresponding author is also charged a fee for the plate, English proof leading, offprints, and specialty printing.

2. Preparation of the Manuscript

2-1. Forms of the manuscript

Use Microsoft Office Word (versions after 2003) and ensure correct spelling and grammar. Set up the MS Word document for 1-inch margins on a letter or A4-sized paper. The manuscript must be written in 12-point font, and the sentences must be double-spaced including tables and figure legends. The length of the manuscript should not exceed 20 pages in original articles, 7 pages in the case report, and 30 pages in review article except for the tables and figures.

2-2. Use of language and unit

Draw up a manuscript in proper and clear English as per the orthography. When there is no appropriate translation of foreign medical terms, proper nouns, drug names, units, etc., use their originals in the manuscript. If foreign-language words are needed, capital and small letters should be clarified: in principle, proper nouns, place names, and names of persons should be written with a capital letter as the first letter and then small letters for the rest. If an original term has its translation whose meaning is unclear, place the original in a small parenthesis after its translation when it appears for the first time and then uses its translation alone.

Numbers should be written in Arabic numerals, and measurements should be reported using the metric system, and hematologic and biochemical markers should be reported in the International System (SI) of Units. (http://physics.nist.gov/cuu/Units/index.html)

2-3. Use of abbreviations

The use of abbreviations should be minimized and restricted to those that are generally recognized. When using an abbreviated word, it should be spelled out in full on the first usage in the manuscript, followed by the abbreviation in parentheses.

2-4. Word-spacing

In manuscripts, leave one space for each side, using arithmetic marks as ±, =, +, - (minus), ×, etc. (ex. 25.3 ± 1.2). Leave no space for "-" (hyphen) between words (ex. post-stroke). Leave one space after ",", ";", "." and ":". Using parentheses, leave 1 space each side in English. And brackets in parentheses, apply square brackets. Ex) ([ ])

2-5. Order of manuscripts for original articles

The manuscript for original articles should be organized in the following order: 1) title page as a separate file, 2) Title of the manuscript 3) abstract and keywords, 4) introduction, 5) materials (or subjects) and methods, 6) results, 7) discussion, 8) conflict of interest, 9) acknowledgements (if necessary), 10) references, 11) figure legends 12) tables as separate files, and 13) figures as separate files.

Figures should be submitted with an online submission system as separate files, named as the number of figures of the text and figure legends in JPEG, TIFF, GIF format (ex: Fig1.jpg)..

Title page

The title page should be uploaded online as a separate file and should describe the title of the article, full names of authors, institutional affiliation(s) with each author. English names should not be described in initials. All authors' ORCIDs should be described.

If authors belong to different organizations, the chief research organization should be specified in the first place, and the other one's shoulder is specified in the order of Arabic numerals (e.g., 1,2,3).

In the title page, the corresponding author must be identified, and his or her contact information (postal address, e-mail, telephone, and fax numbers) should be listed, and if necessary, financial support might be described as a footnote. Running title with 50 spaces maximum should be described.

Title of the manuscript

The title of the manuscript page should contain the only title. Do not include author information on the title page for a blind peer review. The author names should not appear on this page.

The title should be short, specific, and informative to present clearly the objective of the study and should not use the expres-

sions, such as "study about---" or "clinical study about---." The title should contain less than 20 words. The first letter of words except article, preposition, and conjunction should be capitalized. Drug names in the title should be written with generic names, not product names.

Title of the manuscript

Abstract should summarize the content and should not exceed 250 words in the original article and 200 words in the case report. In the original article, a structured abstract with the headings of Objective, Methods, Results, and Conclusion must succinctly describe the paper. Use complete sentences and do not number the results. At the end of the Abstract, list up to 5 relevant Keywords which are in accordance with the Medical Subject Headings (MeSH) in the Index Medicus (http://www.nlm.nih.gov/mesh). Keywords should be written with a capital letter as the first letter and then small letters for the rest and separate each word by a semicolon (;). The abstract of the case report should be non-structured, with no more than 5 Keywords attached. Brief communications should not describe abstract and keywords.

Introduction

Introduction should clearly present the objective of the study, and a brief background to inform the readers of the relevance of the study may be necessary.

Materials & Methods

Describe the participants or research materials of the study, divided by subsection titles, and describe the experimental methods in a logical and systematic manner so that they can be reproducible by another investigator. Explain in detail the inclusion and exclusion criteria for both the experimental and control groups. Experimental drugs should be stated in the generic name. When proprietary brands are used, include the brand name and the name of the manufacturer in parentheses after the first mention of the generic name. When using experimental devices or other products, state the brand name then follow with the name of the manufacturer, city (state), and country in parentheses, e.g., Flow Cytometer (Coulter Electronics Inc., New York, NY, USA). To ensure anonymity during the peer review process, the authors' affiliations or the institutional setting of the study should not be revealed. Subsection titles should be listed in order to 1), (1), A), (A).

Precisely describe the statistical analysis methods, computer programs, and criteria for determining significance.

(Description of participants)

Ensure correct use of the terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors), and, unless inappropriate, report the sex or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex or gender. If the study was done involving an exclusive population, for example, in only one sex, authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should define how they determined race or ethnicity and justify their relevance.

Results

Results should be summarized and described logically the significant findings and trends observed in the results, giving the main or most important objective. Results can be sectioned by subsection titles listed in order to 1), (1), A), (A). Avoid extensive repetition of contents of the tables and figures in the text. In statistical expression, mean and standard deviation should be described as mean ± SD, and mean and standard error as mean ± SE. The letter 'p' in p-value is written in the lower case.

Discussion

Refrain from an excessive review of historical studies, textbook facts, or irrelevant references. Interpret the results with respect to the objective of the study, and describe differences with previous studies and significant findings, which lead to the deduction of the conclusion. Accentuate newly obtained observations from the study and include significant limitations of the study. Do not repeat the results in detail or other information that is given in the Introduction or the Results section.

Conclusion

Conclusions should avoid unqualified statements that are not adequately supported by the data and describe briefly novel findings of the study, according to the purpose of the study.

Acknowledgment

If necessary, persons who have made contributions to the study, but who are not eligible for authorship may be named in this section. Their contribution must be specified, such as data collection, financial support, statistical analysis, or experimentation.

References

References must be written only to the cited body. It is recommended that only important references are recorded, and the number of references is within 40. References should be numbered in order of appearance in the text using Arabic numerals in square brackets such as [1], [2-4], and [5,7,9]. A bracket is placed after the author's name, or before the period in a sentence. In case the author should be mentioned, write only "last name" and list

the first two author and add "et al." if the authors are more than three (e.g., one author: Kim, two: Park and Jeong, more than three: David et al.). The English name is written the last name in conjunction with capital letters of first and middle names. If the reference is Korean, then list the English version in the reference section. List all authors when they are six or fewer; when there are seven or more, list only the first six and add 'et al.". If an article has been accepted but not yet published, the assigned month to be published could be written. Journal titles should be abbreviated in style used in the Index Medicus. If the reference is not listed in Medicus, use the full name of the journal. All other references should be listed, as shown in the "Uniform Requirement for manuscripts submitted to Biomedical Journals" (2008).

Sample References

1) Journals:

Authors: full title of the article. journal name year;volume:the first and last page number.

(e.g., Curr A, Dietz: Traumatic cervical spinal cord injury: relation between somatosensory evoked potentials, neurologic deficit and hand function. Arch Phys Med Rehabil 1996;77:48–53.)

2) Book:

Authors: Book title. edition. place: publisher; year, the first and last page number.

(e.g., Cailliet R: Shoulder pain. 3th ed. Philadelphia: FA Davis; 1991, pp32–35.)

3) Book chapter

Authors: title of the chapter. In: editor. The book title. edition. place: publisher; year, the first and last page number.

(e.g., Kottke FJ: The neurophysiology of motor function. In: Kottke FJ, Lehmann JF, editors. Krusen's handbook of physical medicine and rehabilitation. 4th ed. Philadelphia: Saunders; 1990, pp234–269.)

4) Online resource

National Library of Medicine: Fact sheet: AIDS information resources [Internet]. Bethesda: National Library of Medicine; 2003 [cited 2007 Mar 26]. Available from: http://www.nlm.nih.gov/pubs/factsheets/aidsinfs.html.

Tables

Tables should be uploaded online as separate files and numbered in order of appearance in the main text (Table 1, Table 2, etc.). Table should be easy to understand and unique. The total number of tables should not exceed more than five. Title of table should be briefly written as a phrase or sentence. The first letter except article, preposition, and conjunction, should be capitalized. The title of table is written above the table, and footnote should be described below the table. All abbreviations should be spelled out in footnote in order of abbreviation, colon, and unabbreviated name (e.g., NCS: nerve conduction study). The symbols (*, †, ‡, §, ||, ¶, **, ††, ‡‡) should be superscripts and be used in the indicated sequence (e.g., * $p < 0.05$).

Figure legends

Figure legends must appear on a separate page at the end of the manuscript written in the Microsoft Word file. Write sentences to be understood fully without relying on the main text. Only the first sentence written in capital letters. The second sentence should be set on the starting line of the first sentence. Explain any abbreviation and symbol in the legend. Figures containing histologic slides should be accompanied by legends explaining tissue origin, stain method, and microscopic amplification.

Figures

Figures should be uploaded online as separate files and numbered in order of appearance in the main text (e.g., Fig. 1). If more than two figures are used in the same number, insert the alphabet after Arabic number (e.g., Fig. 1A, Fig. 1B) and record as a single file. Arrows should be inserted to be easily understood. All images should be saved in JPEG, TIFF, GIF or PPT format within 3 MB. The minimum resolutions required are 300 dpi. At online submission, set a file name as the same title as written in main text and legends (e.g., Fig1.jpg).

When already published figures or graphs are inserted, the written consent of the author should be attached and acknowledged in the manuscript.

2-6. Articles other than the original manuscript

The general guidelines abide by the original article section.

Invited review

An invited review is a contemplation focused on a certain topic appointed by the Editorial Board. The abstract is limited to less than 250 words, the number of main text less than 30 pages, and the references no more than 60.

Case Report

Case report deal with any unique features, novel diagnosis or treatment, or others accepted in Editorial Board. The abstract should be non-structured and limited to 200 words, with no more

than 5 keywords attached. Introduction should be briefly written about background and significance of the case. Main texts are composed of the course of clinical features, diagnosis, and treatment. Discussion should focus on the significance of the case, and tedious review should be avoided. The number of table and figure is limited to five in total, and the number of references should not exceed more than ten. The maximum word count is limited to 1,500 words, excluding references, tables, and figure legends.

Brief communication

Brief communication deal with already reported findings or cases, but with any unusual features, or features that are considered to be important. Abstract and keywords are not required. The text is limited to 700 words. Up to seven references should be listed. Only one table or figure is allowed, and acknowledgment should not be written.

3. Copyright Transfer and Author Consent Form

Copyright Transfer and Author Consent must be used the official form made by the Korean Association of EMG Electrodiagnostic Medicine (available and posted at the journal on 'www.kanem.or.kr' or 'www.e-jend.org'). In addition, the title of the manuscript, date of submission, names of all authors, affiliation, and address, and phone number must be recorded with the handwritten signature of all authors. Also, the name and email address of corresponding author should be recorded. Completed Copyright Transfer and Author Consent Form should be submitted at online submission system to the Editorial Office.

Research and Publication Ethics

JEND
Journal of Electrodiagnosis and Neuromuscular Diseases

The Journal of Electrodiagnosis and Neuromuscular Diseases adheres to the guidelines and best practices published by professional organizations, including ICMJE Recommendations and the Principles of Transparency and Best Practice in Scholarly Publishing (joint statement by the Committee on Publication Ethics [COPE], Directory of Open Access Journals [DOAJ], World Association of Medical Editors [WAME], and Open Access Scholarly Publishers Association [OASPA]; https://doaj.org/bestpractice). Further, all processes of handling research and publication misconduct shall follow the applicable COPE flowchart (https://publicationethics.org/resources/flowcharts).

Statement of Human and Animal Rights

Clinical research should be conducted in accordance with the World Medical Association's Declaration of Helsinki (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/). Clinical studies that do not meet the Helsinki Declaration will not be considered for publication. For human subjects, identifiable information, such as patients' names, initials, hospital numbers, dates of birth, and other protected health care information, should not be disclosed. For animal subjects, research should be performed based on the National or Institutional Guide for the Care and Use of Laboratory Animals. The ethical treatment of all experimental animals should be maintained.

Statement of Informed Consent and Institutional Approval

Copies of written informed consent should be kept for studies on human subjects. Clinical studies with human subjects should provide a certificate, an agreement, or the approval by the Institutional Review Board (IRB) of the author's affiliated institution. For research with animal subjects, studies should be approved by an Institutional Animal Care and Use Committee (IACUC). If necessary, the editor or reviewers may request copies of these documents to resolve questions regarding IRB/IACUC approval and study conduct.

Conflict of Interest Statement

The author is responsible for disclosing any financial support or benefit that might affect the content of the manuscript or might cause a conflict of interest. When submitting the manuscript, the author must describe the conflict of interest statement. Examples of potential conflicts of interest are financial support from or connections to companies, political pressure from interest groups, and academically related issues. In particular, all sources of funding applicable to the study should be explicitly stated.

Originality, Plagiarism, and Duplicate Publication

Redundant or duplicate publication refers to the publication of a paper that overlaps substantially with one already published. Upon receipt, submitted manuscripts are screened for possible plagiarism or duplicate publication using Crossref Similarity Check. If a paper that might be regarded as duplicate or redundant had already been published in another journal or submitted for publication, the author should notify the fact in advance at the time of submission. Under these conditions, any such work should be referred to and referenced in the new paper. The new manuscript should be submitted together with copies of the duplicate or redundant material to the editorial committee. If redundant or duplicate publication is attempted or occurs without such notification, the submitted manuscript will be rejected immediately. If the editor was not aware of the violations and of the fact that the article had already been published, the editor will announce in the journal that the submitted manuscript had already been published in a duplicate or redundant manner, without seeking the author's explanation or approval.

It is possible to republish manuscripts if the manuscripts satisfy the conditions for secondary publication of the ICMJE Recommendations (http://www.icmje.org/icmje-recommendations.pdf).

Authorship and Author's Responsibility

Authorship credit should be based on (1) substantial contributions to conception and design, acquisition of data, and analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; (3) final approval of the version to be published; and (4) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Authors should meet these four conditions.

- A list of each author's role should accompany the submitted paper.
- Correction of authorship: Any requests for such changes in authorship (adding author(s), removing author(s), or re-arranging the order of authors) after the initial manuscript submission and before publication should be explained in writing to the editor in a letter or e-mail from all authors. This letter must be signed by all authors of the paper. A copyright assignment must be completed by every author.
- Role of the corresponding author: The corresponding author takes primary responsibility for communication with the journal during the manuscript submission, peer review, and publication process. The corresponding author typically ensures that all of the journal's administrative requirements, such as providing the details of authorship, ethics committee approval, clinical trial registration documentation, and conflict of interest forms and statements, are properly completed, although these duties may be delegated to one or more coauthors. The corresponding author should be available throughout the submission and peer review process to respond to editorial queries in a timely manner, and after publication, should be available to respond to critiques of the work and cooperate with any requests from the journal for data or additional information or questions about the article.
- Contributors: Any researcher who does not meet all four ICMJE criteria for authorship discussed above but contributes substantively to the study in terms of idea development, manuscript writing, conducting research, data analysis, and financial support should have their contributions listed in the Acknowledgments section of the article.

Registration of Clinical Trial

Clinical trial defined as "any research project that prospectively assigns human subjects to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome" is recommended to be registered to the primary registry to be prior publication. ARM accepts the registration in any of the primary registries that participate in the WHO International Clinical Trials Portal (http://www.who.int/ictrp/en/), NIH ClinicalTrials.gov (http://www.clinicaltrials.gov/), ISRCTN Resister (www.isrctn.org), University Hospital Medical Information Network (www.umin.ac.jp/ctr/index/htm), Netherlands Trial Register (http://www.trialregister.nl/trialreg/index.asp) or The Clinical Research Information Service (http://cris.nih.go.kr/). The clinical trial registration number will be published at the end of the abstract.

Process for Managing Research and Publication Misconduct

When the journal faces suspected cases of research and publication misconduct, such as redundant (duplicate) publication, plagiarism, fraudulent or fabricated data, changes in authorship, undisclosed conflict of interest, ethical problems with a submitted manuscript, appropriation by a reviewer of an author's idea or data, and complaints against editors, the resolution process will follow the flowchart provided by COPE (http://publicationethics.org/resources/flowcharts). The discussion and decision on the suspected cases are carried out by the Editorial Board.

Editorial Responsibilities

The Editorial Board will continuously work to monitor and safeguard publication ethics: guidelines for retracting articles; maintenance of the integrity of academic records; preclusion of business needs from compromising intellectual and ethical standards; publishing corrections, clarifications, retractions, and apologies when needed; and excluding plagiarized and fraudulent data. The editors maintain the following responsibilities: responsibility and authority to reject and accept articles; avoid any conflict of interest with respect to articles they reject or accept; promote the publication of corrections or retractions when errors are found; and preserve the anonymity of reviewers.

Copyright Transfer and Author Consent Form

JEND
Journal of Electrodiagnosis and Neuromuscular Diseases

Title of manuscript: _____

_____

I agree to transfer the copyright of this article to the Korean Association of EMG Electrodiagnostic Medicine if it is published in the Journal of Electrodiagnosis and Neuromuscular Diseases.

I warrant that the article is original work that has not been published before and is not being considered for publication elsewhere in its final printed form or electronic form.

I certify that all authors contributed to this manuscript actually and intellectually and have responsibility to this manuscript.

I also declare that my institution has approved the protocol for any investigation involving human subjects or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

I further attest that we have disclosed any and all financial and other relationships that could be construed as a conflict of interest and that all funding sources supporting the work are disclosed in the manuscript.

Date: _____

***Corresponding author:** _____ **E-mail:** _____

Address: _____

TEL: _____ **FAX:** _____

Author's name (Korean)	Author's name (English)	Signature