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EVALUATION OF THE EFFECT OF NEBIVOLOL ON HIPPOCAMPUS IN EXPERIMENTAL TRAUMATIC BRAIN INJURY

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Abstract

Objective: Traumatic brain injury (TBI) remains a major cause of mortality and disability worldwide, with secondary injury mechanisms contributing significantly to neurodegeneration. The hippocampus is particularly vulnerable to these delayed processes, including oxidative stress, neuroinflammation, and astroglial activation. Nebivolol, a third-generation β_1-adrenergic antagonist with nitric oxide–mediated vasodilatory properties, has been proposed to exhibit therapeutic potential in neural injury models. This study aimed to evaluate the therapeutic effects of nebivolol on hippocampal injury following experimental TBI, with a specific focus on S100 protein expression as a marker of astroglial activation.

Methods: Twenty-four male Sprague–Dawley rats were randomly assigned to three groups (n=8): Control, TBI, and TBI + Nebivolol. TBI was induced via the weight-drop method (50 g/m). Nebivolol (10 mg/kg/day) was administered orally for 14 days. Hippocampal tissues were harvested on day 14 for hematoxylin–eosin (H&E) and S100 immunohistochemical analyses. Statistical evaluation was performed using one-way ANOVA or Kruskal–Wallis tests (p<0.05).

Results: The TBI group exhibited neuronal degeneration, nuclear pyknosis, vascular dilation, and disrupted cytoarchitecture, along with intense S100 expression in neurons and glial cells. Nebivolol treatment markedly preserved neuronal morphology, reduced vascular alterations, and decreased S100 immunoreactivity, indicating attenuated astrocytic activation.

Conclusion: Nebivolol exhibited a therapeutic effect on hippocampal injury after TBI by attenuating structural deterioration and suppressing astroglial activation. These findings indicate that nebivolol may modulate secondary injury processes; however, further molecular and behavioral studies are required to clarify the underlying mechanisms.

Keywords: *Traumatic brain injury, hippocampus, nebivolol, S100 protein, immunohistochemistry, neuroprotection*.

Introduction

Traumatic brain injury (TBI) is a major global health problem and remains a leading cause of morbidity, disability, and mortality across all age groups. Worldwide, nearly 69 million individuals suffer from TBI annually, which contributes to substantial neurological and psychosocial impairments as well as long-term complications.[1,2] The pathophysiology of TBI involves primary injury, resulting from the mechanical insult to brain tissue, and secondary injury, characterized by a cascade of processes such as oxidative stress, excitotoxicity, mitochondrial dysfunction, and neuroinflammation, which can persist for weeks or months after the initial trauma.[3,4] Recent studies published within the last five years further emphasize the critical role of neuroinflammation and microglial activation in driving progressive neuronal dysfunction after TBI.[5,6] Among brain regions, the hippocampus is especially vulnerable to traumatic injury due to its central role in memory consolidation, learning, and synaptic plasticity. Structural disruption of hippocampal neurons and axonal networks has been closely associated with post-traumatic cognitive decline.[7] Therefore, interventions that mitigate hippocampal damage and modulate secondary injury mechanisms are of particular interest in neurotrauma research.

Nebivolol, a third-generation β1-adrenergic receptor blocker, has emerged as a cardiovascular drug with additional antioxidant and vasoprotective properties.[8] Unlike conventional beta-blockers, nebivolol induces endothelium-dependent vasodilation by stimulating nitric oxide (NO) release via endothelial nitric oxide synthase (eNOS), thereby improving microcirculation and reducing oxidative stress.[9,10] Experimental studies have also demonstrated that nebivolol exerts anti-inflammatory and anti-apoptotic effects, attenuates reactive oxygen species (ROS) formation, and enhances endothelial as well as neuronal survival pathways.[11,12] These pleiotropic effects suggest that nebivolol may confer protection against TBI-induced secondary damage, particularly within the hippocampus, where oxidative stress and vascular dysfunction play central roles in neuronal degeneration.

The S100 protein family, especially S100B, serves as a sensitive biomarker of brain injury. While physiologic levels of S100B may support neuronal survival, its elevated expression reflects astrocytic activation and contributes to neuroinflammation and oxidative damage.[13,14] In line with recent literature, studies published in the last few years highlight that S100B overexpression correlates not only with glial activation but also with the severity of post-traumatic neurodegeneration.[15] Immunohistochemical evaluation of S100B expression provides valuable insights into the extent of neural tissue damage and potential therapeutic responses following TBI.[16]

Considering these aspects, the present study aimed to investigate the potential neuroprotective effects of nebivolol on hippocampal tissue following experimental TBI in rats. We focused on histopathological and immunohistochemical evaluation, particularly S100 expression, to determine whether nebivolol can mitigate neuronal and glial alterations associated with post-traumatic injury.

Methods

Experimental Animals

Twenty-four male Sprague–Dawley rats (250–300 g, 10–12 weeks old) were obtained from the Experimental Research Center of Dicle University. Animals were housed under standard laboratory conditions (12-h light/dark cycle, temperature 22±2°C, relative humidity 55±5%) with free access to food and water. All experimental protocols were approved by the Local Ethics Committee for Animal Experiments of Dicle University (Approval no: 2021/18) and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

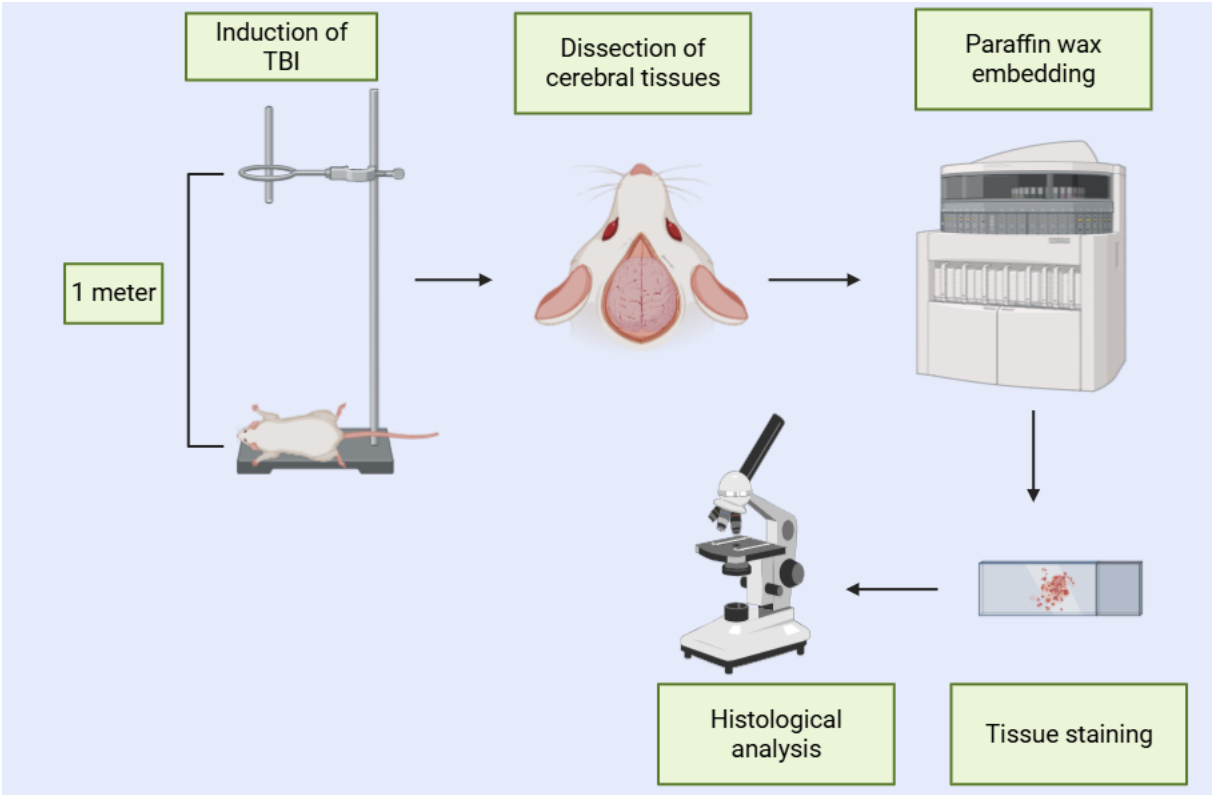

Figure 1. Experimental steps of methodology showing TBI induction and histological analysis. TBI, Traumatic Brain Injury.

Study Design and Experimental Groups

Animals were randomly divided into three groups (n=8 in each);
Control group: Rats underwent anesthesia and surgical exposure of the calvarium without induction of trauma. The scalp was sutured, and animals received no further intervention.
TBI group: Rats were subjected to experimental traumatic brain injury (TBI) and received vehicle (0.9% saline, oral gavage) for 14 days.
TBI+Nebivolol group: Rats were subjected to TBI and subsequently treated with nebivolol (10 mg/kg/day, oral gavage) for 14 consecutive days. The dose was selected based on previous studies demonstrating antioxidant and neuroprotective effects of nebivolol in cerebral injury models.[17]

Induction of Traumatic Brain Injury

TBI was induced using the weight-drop method. Under general anesthesia (90 mg/kg ketamine hydrochloride and 8 mg/kg xylazine, intramuscular), a midline scalp incision was performed to expose the calvarium. A 50 g brass weight (18 mm diameter) was dropped from a height of 1 meter through a Plexiglas tube onto a steel disc placed over the exposed skull, producing a standardized closed head injury. The scalp was sutured, and animals were monitored daily during the follow-up period (Figure 1).[2]

Drug Administration

Nebivolol (Sigma-Aldrich, St. Louis, MO, USA) was freshly prepared in sterile distilled water before administration. It was administered orally via gavage at a dose of 10 mg/kg/day for 14 days following trauma induction. Control and TBI groups received an equal volume of vehicle solution.

Tissue Collection and Processing

On day 14, all animals were deeply anesthetized, and intracardiac blood samples were collected. Rats were then sacrificed, and brain tissues were rapidly removed. The hippocampal regions were dissected and fixed in zinc–formalin for 72 hours. Following fixation, tissues were embedded in paraffin, and 5 µm-thick sections were obtained using a rotary microtome. Sections were stained with hematoxylin–eosin (H&E) for histopathological evaluation.

Immunohistochemistry

Sections were deparaffinized, rehydrated in graded alcohols, and subjected to antigen retrieval in EDTA buffer (pH 8.0). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, followed by incubation with Ultra V Block (Thermo Fisher, USA). Slides were incubated overnight at 4°C with primary antibody against S100 protein (1:200; Santa Cruz Biotechnology, USA). After washing, sections were incubated with biotinylated secondary antibody and streptavidin–HRP. Immunoreactivity was visualized with 3,3'-diaminobenzidine (DAB), and nuclei were counterstained with Harris hematoxylin. Negative controls were prepared without primary antibody.[18]

Results

Nebivolol Treatment Improved Hippocampal Histopathology

In the Control group, the pyramidal cell layers of the hippocampus exhibited normal architecture. Pyramidal cell extensions were regular and extended toward the plexiform layer. Vessels showed only mild dilatation, and axon hillocks were clearly preserved. Axons in the plexiform layer appeared parallel and well organized (Figure 2A). In the TBI group, prominent histopathological alterations were observed. The cytoplasm of neurons in the alveus and pyramidal layers appeared vacuolated, with irregular membrane morphology suggestive of early apoptosis. Degeneration and nuclear loss were evident in pyramidal neurons. Vascular dilatation was noted. Synaptic degeneration was observed in the plexiform layer, with disrupted parallel orientation of axonal processes. Pyknosis was common in neuronal nuclei (Figure 2B). In the TBI+Nebivolol group, notable preservation of tissue morphology was detected. Membrane integrity of pyramidal neurons was largely maintained, with reduced cytoplasmic vacuolization compared to the TBI group. Vascular dilatation was markedly decreased. Axonal processes in the plexiform layer retained a parallel orientation. While pyknosis persisted in some pyramidal cells, several neurons exhibited preserved morphology and mild hyperplasia, indicating cellular regeneration (Figure 2C).

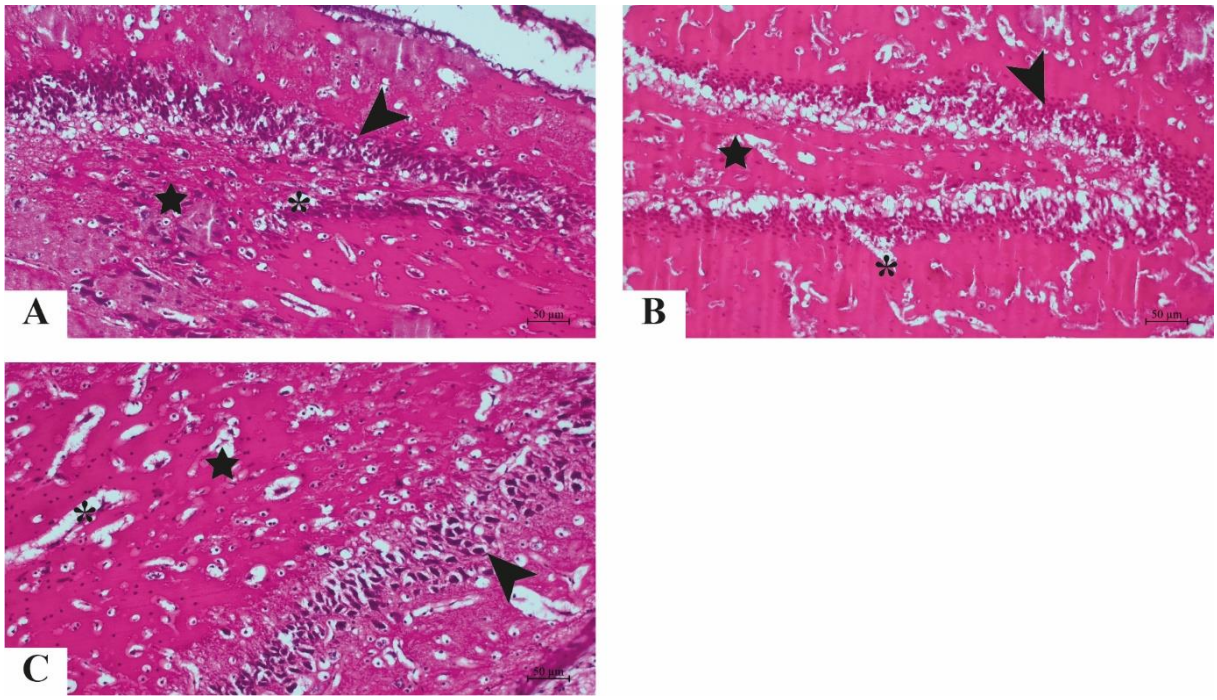

Figure 2. Histopathological findings in hippocampal tissues of the study groups. (A) Control group; (B) TBI group; C) TBI+Nebivolol group. Asterisk: Vascular dilatation, arrowhead: pyramidal neurons, star: Plexiform layer, Hematoxylin Eosin Staining, Scale Bar: 50 µm. TBI, Traumatic Brain Injury.

Nebivolol Downregulated S100 Expression After TBI Induction

In the Control group, S100 immunoreactivity was weak to moderate in the plexiform layer, while pyramidal and granule cells were largely negative. Vascular structures displayed regular morphology without strong immunostaining (Figure 3A). In the TBI group, strong positive S100 expression was observed in degenerating pyramidal neurons, glial cells, and vascular endothelium. Layer integrity was disrupted, with detachment in some regions. Intense S100 positivity was especially evident in synaptic regions and the plexiform layer, consistent with astroglial activation and traumatic injury (Figure 3B). In the TBI+Nebivolol group, S100 expression was reduced compared to the TBI group. Pyramidal neurons and granule cells showed negative to weak staining, while glial cells exhibited mild positivity. Vascular endothelial staining was less prominent than in the TBI group. S100 positivity persisted in axonal structures and the plexiform layer, but overall staining intensity at the cellular level was decreased, suggesting a protective effect of nebivolol against astrocytic overactivation (Figure 3C).

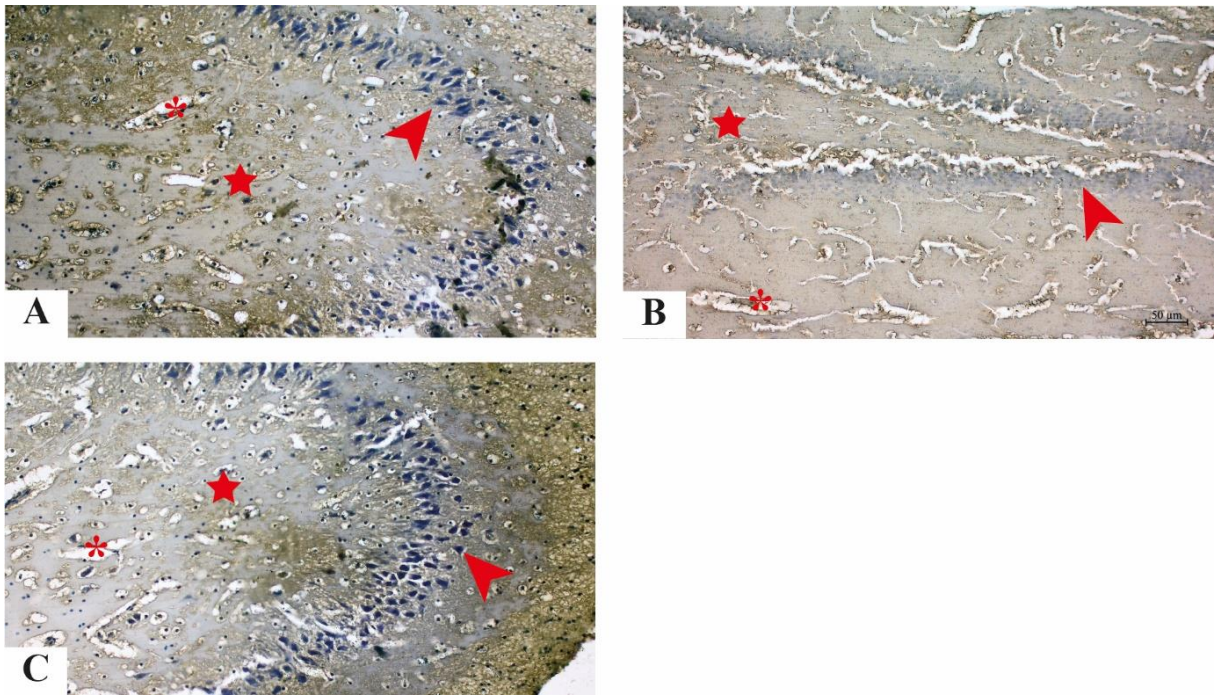

Figure 3. Immunohistochemical findings in hippocampal tissues of the study groups. (A) Control group; (B) TBI group; C) TBI+Nebivolol group. Asterisk: Vascular dilatation, arrowhead: pyramidal neurons, star: Plexiform layer, S100 immunostaining, Scale Bar: 50 µm. TBI, Traumatic Brain Injury.

Discussion

Traumatic brain injury (TBI) continues to be a major cause of mortality and long-term disability worldwide. The pathophysiology involves both primary mechanical injury and secondary processes such as oxidative stress, excitotoxicity, vascular dysfunction, and neuroinflammation, all of which exacerbate neuronal loss and cognitive decline. In particular, the hippocampus is highly vulnerable to TBI, and damage in this region is closely associated with impaired memory and learning functions.[7,19]

In the present study, histopathological evaluation showed that TBI induced vacuolization, nuclear pyknosis, vascular dilatation, and synaptic disorganization in the hippocampal pyramidal layers. Immunohistochemically, the trauma group demonstrated marked S100 expression in pyramidal neurons, glial cells, and vascular endothelium, reflecting astroglial activation and ongoing inflammatory response. In contrast, nebivolol-treated rats exhibited preserved neuronal and vascular structures, reduced cytoplasmic degeneration, and significantly attenuated S100 immunoreactivity, particularly in pyramidal neurons. These findings suggest that nebivolol exerts a protective effect against secondary brain injury by modulating astroglial responses.

Elevated S100B expression is widely used as an indicator of astroglial activation and secondary neuroinflammation in TBI models, consistent with previous studies reporting increased S100B following hippocampal injury.[20-22] S100B protein is a well-recognized marker of astrocytic activation. While low levels may have trophic functions, elevated extracellular S100B contributes to neurotoxicity through induction of proinflammatory cytokines and oxidative stress pathways. Previous experimental reports have indicated that treatments capable of reducing astroglial S100B expression correlate with improved neuronal survival after TBI.[23] In line with these data, our study demonstrated that nebivolol attenuated the TBI-induced increase in S100 expression, indicating suppression of astrocytic overactivation. Recent studies support these findings. In a rat diffuse TBI model, nebivolol treatment reduced neuronal degeneration, apoptosis of Purkinje cells, and vascular damage while restoring near-normal histological architecture; importantly, pro-inflammatory mediators such as TNF-α and metalloproteinases (ADAMTS-1) were downregulated. Similarly, in LPS-induced neuroinflammation models, nebivolol significantly decreased oxidative damage markers such as MDA while increasing antioxidant enzyme activity (SOD, CAT, GPx), consistent with reduced oxidative stress and neuronal apoptosis. These antioxidant and anti-inflammatory actions of nebivolol converge to mitigate glial overactivation and astrocyte-derived signals such as S100B, thereby protecting hippocampal neurons.[12,24]

The reduction of oxidative stress is another key mechanism underlying the beneficial effects of nebivolol. TBI is known to disrupt redox homeostasis, leading to lipid peroxidation and mitochondrial dysfunction. In our model, nebivolol likely exerted its neuroprotective actions through both β1-adrenergic

blockade and nitric oxide–mediated endothelial vasodilation, which have been shown to improve microcirculation and antioxidant defenses. Consistent with our findings, recent experimental work demonstrated that nebivolol decreased MDA levels while restoring glutathione-dependent antioxidant capacity, indicating enhanced resistance to oxidative injury after TBI.[11,25,26]

Taken together, our study and recent literature provide strong evidence that nebivolol reduces secondary brain injury by attenuating neuroinflammation, limiting oxidative stress, and suppressing astroglial overactivation. The parallel reduction in S100 expression observed in the nebivolol group suggests that this protein may serve not only as a biomarker of TBI severity but also as a useful indicator of therapeutic efficacy. While further investigations are required, especially at the molecular level, our findings indicate that nebivolol may represent a promising candidate for neuroprotection in TBI.

This study has several limitations that should be acknowledged. First, the sample size was relatively small, which may limit the generalizability of the findings. Second, the experimental design was restricted to a single dose and treatment duration of nebivolol; different doses or longer follow-up periods may yield additional insights into its neuroprotective potential. Third, only histopathological and immunohistochemical analyses were performed, focusing primarily on S100 expression as a marker of astroglial activation. Additional molecular evaluations, including assessments of oxidative stress parameters, inflammatory cytokines, and apoptotic signaling pathways, would provide a more comprehensive understanding of the underlying mechanisms. Furthermore, functional and behavioral outcomes such as cognitive performance and memory, which are directly related to hippocampal integrity, were not evaluated in this study. Finally, as an experimental rat model was used, the results may not fully reflect the complexity of human TBI pathology, and translational studies are required before clinical application can be considered.

Conclusion

Nebivolol exhibited a therapeutic effect on hippocampal injury after TBI by attenuating structural deterioration and suppressing astroglial activation. These findings indicate that nebivolol may modulate secondary injury processes; however, further molecular and behavioral studies are required to clarify the underlying mechanisms.

Histopathological analysis revealed preserved neuronal and vascular structures, reduced cytoplasmic degeneration, and improved axonal organization in nebivolol-treated rats compared to the TBI group. Immunohistochemically, nebivolol attenuated the trauma-induced increase in S100 expression, indicating suppression of astroglial activation. Taken together with recent preclinical evidence, our results suggest that nebivolol may represent a promising therapeutic candidate for mitigating secondary hippocampal damage after TBI. Further molecular and translational studies are required to clearly elucidate its mechanisms of action and to evaluate its potential for clinical application.

Conflict of Interest

The author declared that there was no conflict of interest during the cause of this study and producing and submitting this manuscript for publication.

Compliance of Ethical Statement

Ethical approval was taken from Dicle University, Animal Experiments Local Ethical Committee (date:07/04/2021 and number:2021/18).

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Author Contributions

F.A., H.A.: Study idea/Hypothesis; F.A., H.A.: Design; G.E.A.A, Z.T., A.S.A., E.Y.: Data Collection; M.Ö., M.Z.D.: Analysis; G.E.A.A., Z.T., E.Y.: Literature review; A.S.A., M.Ö., M.Z.D.: Writing; F.A., H.A.: Critical review.

Data Availability

All generated data were presented in this study.

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