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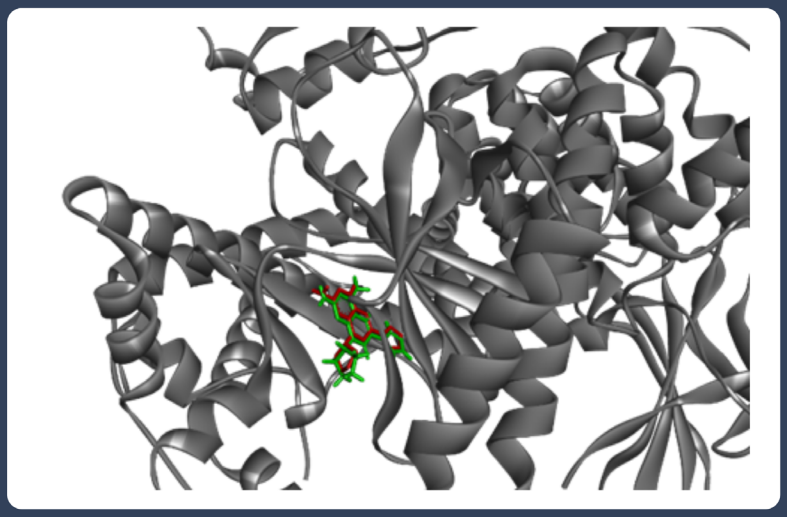
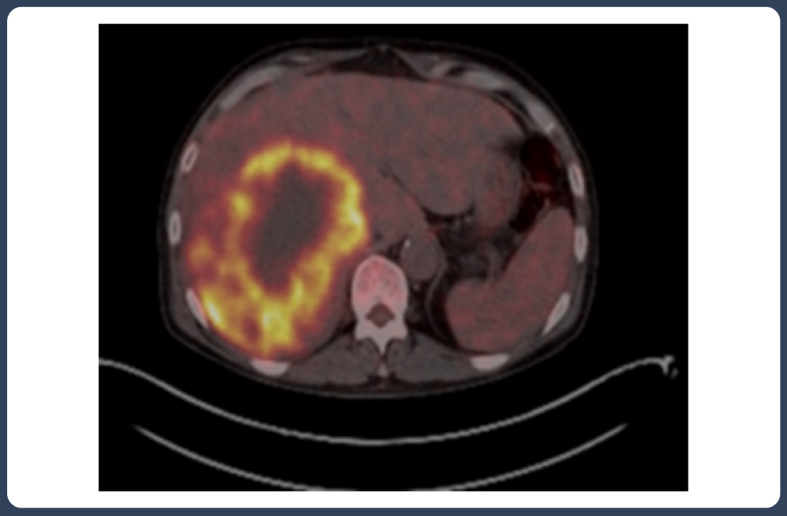
The Official Journal of the Inonu Liver Transplantion Institute



Molecular Docking Analysis Reveals Potential Dual Targeting of FGFR4 and PI3K by Atorvastatin in Hepatocellular Carcinoma



Remarkable Response to Immune Checkpoint Inhibitor Therapy in Advanced-Stage Hepatocellular Carcinoma

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Journal of Inonu Liver Transplantation Institute

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Aim and Scope: The Journal of Inonu Liver Transplantation Institute is a peer-reviewed open-access e-only publication in the field of liver transplantation publishing research articles on clinical, experimental liver transplantation, combined liver and other organ transplantation, and liver diseases. The journal welcomes original research articles, reviews, meta-analyses, case reports, and letters.
Average Duration of the First Review Round: 2 months
Type of Publications: Research Article, Review Article, Meta-Analyses, Case Report, Letter to the Editor
Language of Publication: English
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Journal of Inonu Liver Transplantation Institute

JILTI

Aim and Scope

Aim

The Journal of Inonu Liver Transplantation Institute is a peer-reviewed open-access e-only publication in the field of liver transplantation publishing research articles on clinical, experimental liver transplantation, combined liver and other organ transplantation, and liver diseases. The journal welcomes original research articles, reviews, meta-analyses, case reports, and letters.

Overview

Journal of Inonu Liver Transplant Institute has been founded and established by Inonu Liver Transplant Institute in order to form a source of high-quality research in diseases and therapy of the liver and biliary tract. Both clinicians and basic science researchers are the target population of our journal.

Scope

Hepatobiliary disorders are a complex spectrum of diseases, usually requiring a multi-disciplinary approach that involves interventional radiologists, hepatologists, oncologists, hepatobiliary-transplant surgeons and translational researchers. The Journal of Inonu Liver Transplant Institute (JILTI) is internationally peer reviewed and provides a source for articles on prevention, diagnosis and cutting-edge therapy of hepatobiliary diseases and cancers which also includes liver transplantation, complex hepatobiliary surgical procedures, medical and immune therapies. In accordance with our aims, basic and translational research as applied to these diseases have utmost importance for our journal.

Keywords: Hepatobiliary diseases and cancers, liver surgery, liver transplantation, advanced therapy of hepatobiliary diseases, basic and translational research on hepatobiliary diseases.

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Drafting the work or revising it critically for important intellectual content; AND
Final approval of the version to be published; AND
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.
In addition to being accountable for their own work, authors should have confidence in the integrity of the contributions of their co-authors and each author should be able to identify which co-authors are responsible for other parts of the work.
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Ethics Policy

The Editorial Board of the Journal of Inonu Liver Transplantation Institute and the Publisher adheres to the principles of the International Council of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE), the US National Library of Medicine (NLM), the World Medical Association (WMA) and the European Association of Science Editors (EASE).
In accordance with the journal's policy, an approval of research protocols by an ethics committee in accordance with international agreements "WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (last updated: October 2013, Fortaleza, Brazil)" , "Guide for the care and use of laboratory animals (8th edition, 2011)" and/or "International Guiding Principles for Biomedical Research Involving Animals (2012)" is required for all research studies. If the submitted manuscript does not include ethics committee approval, it will be reviewed according to COPE's guideline (Guidance for Editors: Research, Audit and Service Evaluations). If the study should have ethical approval, authors will be asked to provide ethical approval in order to proceed the review process. If they cannot provide ethical approval, their manuscript will be rejected and also their institutions and when needed, the related bodies in their country will be informed that such studies must have ethics committee approval. If they provide approval, review of the manuscript will continue.
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All authors are required to declare what support they received to carry out their research. Declaring funding sources acknowledges funders' contributions, fulfills funding requirements, and promotes greater transparency in the research process.
Each author must individually declare all sources of funding received for the research submitted to the journal. This information includes the name of granting agencies, grant numbers, and a description of each funder's role. If the funder has played no role in the research, this must be stated as well.
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Journal of Inonu Liver Transplantation Institute

Information for the Authors

THE JOURNAL

The Journal of Inonu Liver Transplantation Institute (The Journal) is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of the Inonu Liver Transplantation Institute, and it is published in April, August and December, three times a year. The publication language of the journal is English.

The Journal aims to contribute to international literature by publishing high-quality manuscripts in the field of diseases and therapy of the liver and biliary tract. The journal's target audience includes academics and expert physicians working in transplantation surgery specialists.

REVIEW PROCESS

Manuscripts submitted to the Journal will undergo a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their field in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation process of manuscripts submitted by editors or by the editorial board members of the journal. The editor-in-chief is the final authority in the decision-making process for all submissions.

Reviews are typically completed within one month of submission to the journal. Authors will be sent constructive reviewer comments intended to be useful. In general, the instructions, objections, and requests made by the reviewers should be followed. The revised manuscript should clearly and precisely indicate every step taken in accordance with the reviewers' notes. A list of responses and the corrections made to each comment should be provided.

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Drafting the work or revising it critically for important intellectual content; AND

Final approval of the version to be published; AND

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.

In addition to being accountable for their own work, authors should have confidence in the integrity of the contributions of their co-authors and each author should be able to identify which co-authors are responsible for other parts of the work.

All of those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criteria should be acknowledged on the title page of the manuscript.

The Journal requires that corresponding authors submit a signed and scanned version of the authorship contribution form (available for download through www.jilti.org) during the initial submission process in order to appropriately indicate and observe authorship rights and to prevent ghost or honorary authorship. If the editorial board suspects a case of "gift authorship," the submission will be rejected without further review. As part of the submission of the manuscript, the corresponding author should also send a short statement declaring that they accept all responsibility for authorship during the submission and review stages of the manuscript.

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Key Features:

- The "Letter to the Editor" should be unstructured and should not include an abstract, keywords, tables, figures, images, or other media.
- The manuscript being commented on must be properly cited within the "Letter to the Editor."
- Our journal considers all feedback on published articles. However, we emphasize that comments should be scientifically relevant and meaningful to the discussion. Irrelevant or unfounded comments may be rejected.

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Our journal adheres to the guidelines set forth by the ICMJE (International Committee of Medical Journal Editors). According to ICMJE, "Letters to the Editor" should be a platform for responsible debate, critique, and discussion. These letters may raise substantial criticisms or questions about previously published articles, and authors of the discussed articles are expected to respond to these criticisms.

ICMJE also notes that editors have the right to edit these letters for length, grammar, and style. However, all letters should contribute constructively to the academic discussion and critique, and those deemed irrelevant or unfounded may be rejected.

You can view the ICMJE guidelines on "Correspondence" here.

Table 1. Limitations for each manuscript type.

Type of manuscript	Wordlimit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	4000-5000	350-400	40-50	6	6
Review Article	5000-6000	350-400	50-60	6	10
Meta analysis	5000	350	50	6	10
Case Report	1500	200	20	No tables	5
Letter to the Editor	1000	No abstract	10	No tables	1

Title page: A separate title page should be submitted with all submissions and this page should include:

The full title of the manuscript as well as a short title (running head) of no more than 50 characters

Name, affiliation, ORCID ID number, and highest academic degree of the author(s)

Funding and other material support

Name, address, phone number(s), fax number, and email address of the corresponding author

Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfill the authorship criteria

Manuscripts that have been presented orally or as a poster should include the name, date and place of the event

Abstract: An English-language abstract is required with all submissions except editorial comments, images, and letters to the editor. Systematic reviews and original articles should contain a structured abstract of maximum 350*400 words with the subheadings of objective, methods, results, and conclusion.
Keywords: Each submission must be accompanied by a minimum of three and a maximum of six keywords for subject indexing included at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (https://www.nlm.nih.gov/mesh/MBrowser.html).
Tables: Tables should be uploaded as separate files and not embedded in the main text. They should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the table with footnotes, even if they are defined within the main text. Tables should be created using the "insert table" command of the word processing software and they should be designed for easy reading. Data presented in tables should not be a repetition of the data presented within the main text but should support the main text.
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All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition. Units should be prepared in accordance with the International System of Units (SI). When a drug, device, hardware, or software program, or other product is mentioned within the main text, the name of the product, the manufacturer/copyright holder of the product (not simply the vendor), and city and the country of the company (including the state, if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric Co., Boston, MA, USA)"
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Limitations, drawbacks, and shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.
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Journal article: van Erk MD, Dam-Vervloet AJ, de Boer FA, Boomsma MF, van Straaten H, Bosschaart N. How skin anatomy influences transcutaneous bilirubin determinations: an in vitro evaluation. Pediatr Res 2019;86:471-7.
Epub ahead-of-print article: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead-of-print].
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Original Research

Protective Effects of Vinpocetine on Methotrexate-Induced Hepatic Oxidative Stress in Rats

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Abstract

Objectives: Methotrexate (MTX) is a potent antineoplastic and immunosuppressive drug; nevertheless, its therapeutic use is limited by hepatotoxicity, mostly driven by oxidative stress (OS). This research sought to examine the possible preventive impact of vinpocetine (VPC) against MTX-induced liver injury in rats.

Methods: Thirty-two female Wistar albino rats were randomly allocated into four groups: Control, MTX (20 mg/kg, single dose, intraperitoneally), VPC (10 mg/kg/day, intraperitoneally for 7 days), and MTX+VPC. Upon conclusion of the experiment, liver tissues and serum samples were obtained. Hepatic OS indicators, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI), were assessed. Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed. Histopathological evaluation focused on sinusoidal dilation and congestion.

Results: The treatment of MTX markedly elevated liver levels of MDA, TOS, and OSI, while concurrently diminishing SOD, CAT, GPx, and TAS values, signifying substantial OS. VPC co-administration markedly reduced MTX-induced oxidative imbalance, as shown by decreased MDA and OSI levels and a partial recovery of antioxidant enzyme activity. Serum AST and ALT levels exhibited no significant differences among the groups. Histopathological examination revealed that MTX caused considerable sinusoidal dilatation, which was somewhat reduced by VPC, but this reduction did not achieve statistical significance.

Conclusion: VPC offers partial protection against MTX-induced hepatic OS and early histopathological changes without influencing serum transaminase levels. These data indicate that VPC may function as a possible supplementary treatment to alleviate MTX-induced hepatotoxicity.

Keywords: Methotrexate, vinpocetine, hepatotoxicity, oxidative stress, rat model

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Methotrexate (MTX) is an antifolate drug widely used in the treatment of several malignancies, including acute lymphoblastic leukemia and non-Hodgkin lymphoma, and is also commonly prescribed for chronic inflammatory conditions such as rheumatoid arthritis and psoriasis.[1,2] Although clinically successful, prolonged use of MTX is often linked to significant side effects, including hepatotoxicity. MTX-induced hepatic injury presents via several pathogenic mechanisms, including oxidative stress (OS), inflammation, mitochondrial dysfunction, apoptosis, and, in some instances, fibrosis and cirrhosis.[3,4]

The pathophysiology of MTX-induced hepatotoxicity is marked by elevated production of reactive oxygen species (ROS), diminished endogenous antioxidant defenses like glutathione (GSH), and increased expression of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β). These mechanisms together induce hepatocyte injury and necrosis, resulting in compromised liver function, as shown by increased serum transaminase levels and significant histological damage.[3,5] Consequently, the identification of safe and efficacious medicines that may safeguard the liver against MTX-induced toxicity has emerged as a significant objective in both clinical and preclinical research.

Vinpocetine (VPC; ethyl apovincaminate), a semi-synthetic derivative of the vinca alkaloid vincamine, is best known for its cerebral vasodilatory, neuroprotective, and nootropic properties.[6] The pharmacological actions include the inhibition of phosphodiesterase-1 (PDE-1), blocking of voltage-gated sodium channels, and anti-inflammatory activity predominantly via the suppression of the nuclear factor kappa B (NF-κB) signaling pathway.[7-9] VPC has significant antioxidant capabilities by neutralizing ROS and enhancing the activity of essential antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT).[9] Experimental investigations have shown VPC's protective benefits in models of ischemia-reperfusion damage, diabetic nephropathy, and hepatic ischemia.[7,10] VPC augments antioxidant enzyme activity, diminishes lipid peroxidation, and inhibits inflammatory pathways. VPC enhances protective factors such as nuclear factor erythroid 2–related factor 2 (Nrf2) and heme oxygenase 1 (HO-1), while inhibiting apoptosis-related proteins, leading to better liver histology and function in rats subjected to MTX exposure [11].

We hypothesize that VPC may mitigate MTX-induced hepatic injury by reducing OS and preserving liver architecture. The findings of this study may support the potential repositioning of VPC as an adjunct hepatoprotective agent in patients receiving MTX-based therapy.

Methods

A total of 32 female Wistar albino rats (300–350 g), sourced from the Inonu University Laboratory Animal Production and Research Center, were randomly assigned to four experimental groups. The animals were kept under regulated laboratory settings, with a controlled ambient temperature of 21±2 °C, relative humidity of 60±5%, and a 12-hour light/12-hour dark photoperiod. All rats were provided with a regular pellet diet and had unrestricted access to tap water throughout the research period.

Randomization methods were used for both group allocation and for data collection and analysis, conducted by investigators unaware of the treatment conditions. The experimental methodology adhered to the National Institutes of Health criteria for animal research and conformed to the ARRIVE reporting requirements.[12] Ethical permission was obtained from the Inonu University Faculty of Medicine Animal Experiments Local Ethics Committee (approved date: October 11, 2018; meeting number: 2015/A-37). A simple randomization approach guaranteed an impartial allocation of animals among the experimental groups. The required minimum sample size was determined a priori using G*Power software (version 3.1.9.7, Heinrich Heine University, Düsseldorf, Germany). Based on effect sizes reported in previous experimental studies investigating MTX-induced hepatic OS and antioxidant interventions, a large effect size was assumed (f=0.40). The calculation was performed for a one-way ANOVA with four independent groups, with a significance level (α) of 0.05 and a statistical power (1−β) of 0.80. Under these assumptions, the minimum total sample size required was 28 animals (7 per group). To compensate for potential experimental losses and to increase the robustness of the statistical analysis, 32 rats were included in the study (n=8 per group).

32 female Wistar albino rats were randomly divided into four groups:

- Control Group (n=8): The vehicle solution (0.5 mL) was administered intraperitoneally (i.p.) once daily for seven days.
- MTX Group (n=8): On day 1, a single dose of MTX (20 mg/kg) was administered i.p., followed by daily i.p. administration of 0.5 mL of the vehicle solution for seven days.
- VPC Group (n=8): A single daily dose of 10 mg/kg VPC was administered i.p. for 7 days.
- MTX+VPC Group (n=8): On the first day, a single dose of 20 mg/kg MTX was administered i.p., followed by a single daily dose of 10 mg/kg VPC administered i.p. for 7 days.

The dosages, administration routes, and dosing regimens for MTX (Methotrexate DBL, 500 mg/20 mL; Koçak Farma, Istanbul, Türkiye) and VPC (CAS No. 42971-09-5; Sigma-Aldrich, St. Louis, MO, USA) were determined according to prior research conducted by Samdanci et al. and Ristić et al., respectively.[13,14] On the eighth day of the experimental phase, the body weights of the rats were documented. Animals were anesthetized with urethane (1.2 g/kg, intraperitoneal; CAS No. 51-79-6; Sigma-Aldrich St. Louis, MO, USA), followed by the collection of blood samples from the inferior vena cava. Euthanasia was conducted surgically to guarantee exsanguination.

Subsequent to sacrifice, liver tissues were removed, irrigated with 0.9% NaCl isotonic saline to eliminate leftover blood, and weighed with a precision scale. Blood samples were centrifuged at 2000 rpm for 7 minutes to isolate serum. The harvested liver tissues were divided symmetrically for biochemical and histopathological assessments; one portion was fixed in 10% formaldehyde for histological evaluation, while the remaining liver tissue and serum samples were appropriately packaged and stored at −80 °C until biochemical analyses were conducted.

Tissue Biochemical Analysis

Upon the commencement of the analyses, the tissues were washed by submerging them in a beaker filled with Tris-HCl. Their weights were subsequently measured and documented. Tissues were homogenized for one minute in glass tubes using an IKA-WERKE T 25 B device with the addition of pH 7.4 Tris-HCl buffer. Homogenization was completed by including an additional quantity of buffer solution and homogenizing for an additional minute. A segment of the resultant homogenate was reserved for examination. The residual homogenate was subjected to centrifugation at 4000 rpm for 45 minutes at +4 °C using a Hettich D 78532 chilled centrifuge. The transparent supernatant was isolated for examination. Spectrophotometric measurements were conducted with SHIMADZU UV-160A and BİOTEK SYNERGY LX multi-mode reader instruments.

Malondialdehyde (MDA)

MDA was measured according to the method of Uchiyama and Mihara [15]. The results obtained were expressed in nmol/g tissue.

Superoxide Dismutase (SOD)

SOD activity was determined using the method of Sun et al.[16] Results were calculated in U/mg protein.

Protein Quantification

Protein quantity analysis was performed using the modified Lowry method to calculate the data for the other markers studied.[17] The results were calculated according to the standard graph obtained and expressed in µg/mL.

Catalase (CAT)

CAT activity was determined according to Aebi's method.[18] Results were reported as K/g protein.

Glutathione Peroxidase (GPx)

GPx activity was measured using the Paglia and Valentine method.[19] The activity calculated from the observed absorbance change during this process was given as U/mg protein.

Total Antioxidant Status (TAS), Total Oxidant Status (TOS), and Oxidative Stress Index (OSI)

The TAS measurement was determined in the supernatant using the Erel method.[20] The unit is mmol Trolox equivalent per liter. TOS was quantified colorimetrically using the Erel technique, utilizing the supernatant produced.[21] The unit is µmol H_2O_2 equivalent/L. The OSI was calculated by dividing the TOS by the TAS. The unit is arbitrary unit (AU).[22]

Serum Biochemical Analysis

Serum samples were extracted from the deep freezer one day before biochemical analysis and allowed to defrost prior to processing at the Inonu University Turgut Özal Medical Center Central Laboratory.

Histopathological Analysis

Upon completion of the experiment, liver tissues were preserved in 10% formaldehyde. After standard tissue preparation, paraffin-embedded blocks were created, and sections measuring 4–5 µm in thickness were acquired. The sections were stained with hematoxylin and eosin (H&E) for comprehensive histological evaluation.

Histopathological evaluation focused on sinusoidal dilatation and congestion. Tissue injury was assessed using a semi-quantitative scoring system as follows: 0, no pathological change; 1, mild injury; 2, moderate injury; and 3, severe injury. For each animal, ten randomly selected, non-overlapping microscopic fields were examined at ×20 magnification. Scoring was performed by an experienced histopathologist who was blinded to the experimental groups in order to minimize observer bias. The mean score for each animal was used for statistical analysis.

All histological analyses were performed using a Leica DFC-280 research microscope alongside the Leica Q Win Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

Statistical Analysis

Statistical analyses were performed using statistical software developed by the Department of Biostatistics and Medical

Informatics, Faculty of Medicine, Inonu University [23]. Data distribution was assessed using normality tests, and since the variables did not follow a normal distribution, non-parametric tests were applied. Overall group comparisons were performed using the Kruskal–Wallis test. When a significant difference was detected, pairwise comparisons were conducted using the Mann–Whitney U test. To control for inflation of Type I error due to multiple comparisons, a Bonferroni correction was applied, and the adjusted significance threshold was set at $p<0.0083$ (0.05/6). Data were expressed as median (minimum–maximum), and p values below the adjusted threshold were considered statistically significant.

Results

Body and Liver Weights of Rats

Table 1 demonstrates that liver weights exhibited no significant differences across the experimental groups ($p>0.05$), suggesting that neither MTX nor VPC treatment resulted in a notable alteration in liver mass. The ultimate body weight of rats in the MTX group was considerably lower than that of the Control group ($p<0.05$). The VPC therapy alone did not significantly affect the final body weight in comparison to the Control group. The co-administration of VPC with MTX considerably mitigated the body weight loss generated by MTX, resulting in final body weights that were markedly greater than those in the MTX group ($p<0.05$).

Tissue Biochemical Findings

As shown in Figure 1 and Table 2, MTX administration caused a marked disruption of oxidative balance in liver tissue, as indicated by significant increases in MDA, TOS, and OSI levels, along with significant decreases in antioxidant defense parameters, including SOD, CAT, GPx, and TAS, compared with the Control group ($p<0.05$).

Treatment with VPC alone induced considerable alterations in OS indicators, shown by increased MDA, TOS, and OSI levels, and diminished CAT and GPx activity compared to the Control group ($p<0.05$), although SOD and TAS levels were similar. Co-administration of VPC with MTX markedly reduced MTX-induced oxidative damage, as shown by decreased MDA and OSI values and a partial recovery of antioxidant enzyme activity relative to the MTX group ($p<0.05$), while some parameters continued to deviate from Control values.

Table 1. Body and liver weights of rats

Groups	Liver weight (g)	End of experiment rat weight (g)
Control	9.46 (5.73-11.16)	279 (242-340)[a]
MTX	9.05 (6.25-10.95)	229.5 (217-300)
VPC	8.17 (6.56-9.04)	265.5 (215-310)
MTX+VPC	8.17 (6.03-10.12)	252.5 (229-303)[a]

a: There is a statistically significant difference according to the MTX group ($p<0.05$). MTX: Methotrexate-treated group; VPC: Vinpocetine-treated group; MTX+VPC: Methotrexate- and Vinpocetine-treated group.

Serum Biochemical Findings

As shown in Table 3, serum liver enzyme levels did not differ significantly among the experimental groups. Neither MTX administration nor VPC treatment, whether individually or in conjunction, resulted in a statistically significant alteration in serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels compared to the Control group ($p>0.05$), signifying the lack of pronounced hepatocellular damage under the experimental conditions employed.

Histopathological Findings

Liver injury was assessed for sinusoidal dilatation and congestion. In the Control and VPC groups, the liver had a normal histological appearance, with the exception of modest alterations. In these groups, hepatocyte cords exhibiting anastomoses surrounding the central veins and the sinusoids situated between these cords were clearly visible (Fig. 2a and b). In the MTX group, sinusoidal congestion paralleled that of the Control group; however, a substantial increase in sinusoidal dilatation was seen ($p<0.001$) (Fig. 2c). The MTX+VPC group exhibited a little reduction in sinusoidal dilatation; yet, this reduction was not statistically significant in comparison to the MTX group (Fig. 2d). The scores for histopathological assessment are shown in Table 4.

Discussion

The present study investigated the protective effects of VPC against MTX-induced liver injury in rats, focusing on OS parameters, serum liver enzymes, and histopathological alterations. The main findings show that MTX administration induced a marked oxidative imbalance in liver tissue, characterized by increased lipid peroxidation and oxidant status along with a significant reduction in endogenous antioxidant defenses. Co-administration of VPC partially attenuated these changes, supporting its antioxidant and hepatoprotective potential; however, this effect was not fully reflected in serum transaminase levels or in complete normalization of histological alterations.

OS is widely recognized as a central mechanism underlying MTX-induced hepatotoxicity.[5,24] Excessive production of ROS and depletion of antioxidant enzymes such as SOD, CAT, and GPx contribute to lipid peroxidation, mitochondrial dysfunction, and hepatocellular injury [25,26]. Consistent with previous studies, MTX-treated rats in the present study exhibited sig-

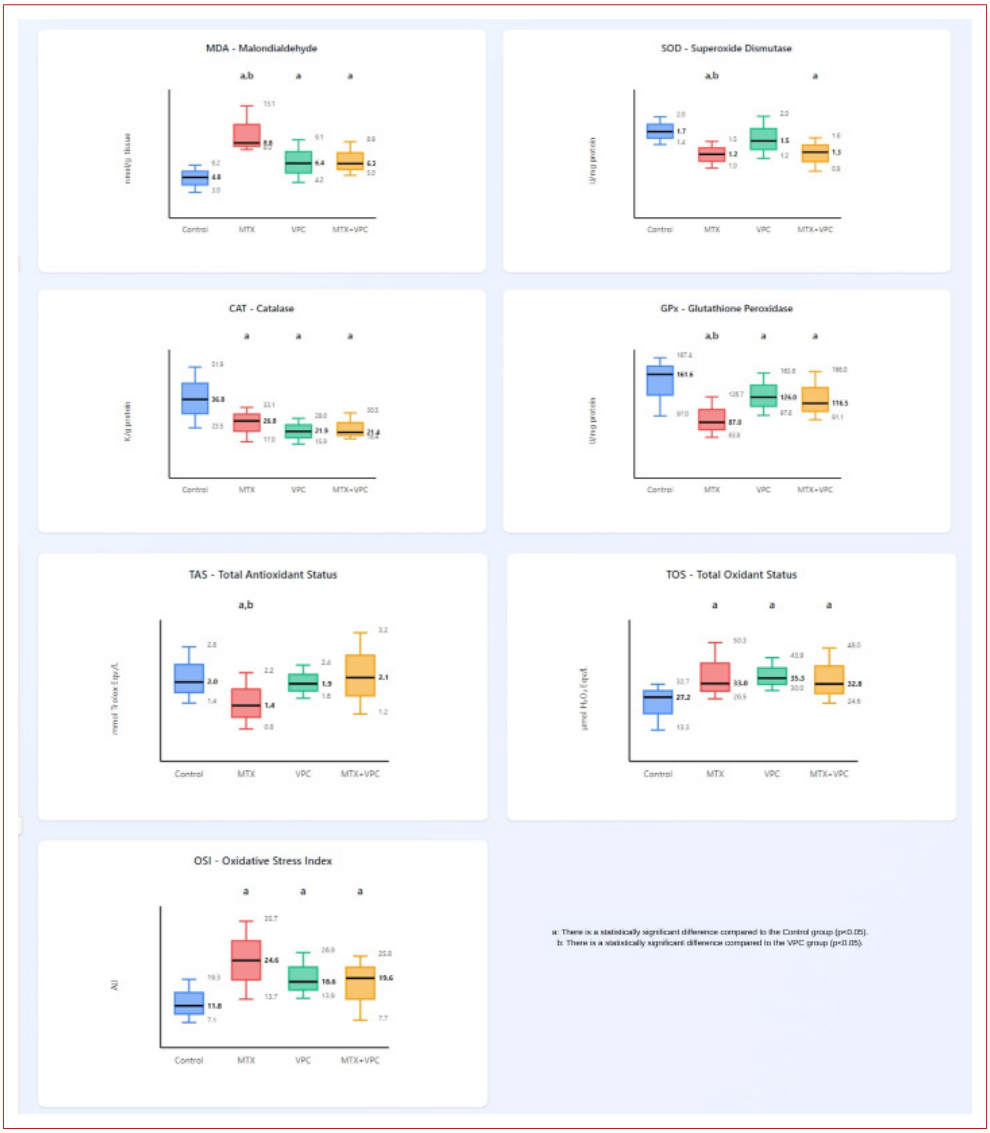

Figure 1. Liver tissue oxidative stress parameters.

nificantly elevated hepatic MDA, TOS, and OSI levels, along with decreased SOD, CAT, GPx, and TAS values, confirming the establishment of an oxidative injury model.[25,27,28] These findings align with earlier reports demonstrating MTX-induced redox imbalance as a key driver of liver damage.

VPC administration alone produced moderate changes in certain OS parameters without causing marked histopathological damage or alterations in serum AST and ALT levels. This finding suggests that VPC does not exert overt hepatotoxic effects under the applied experimental conditions. Importantly, co-administration of VPC with MTX significantly reduced hepatic MDA and OSI levels and partially restored antioxidant enzyme activities compared with the MTX group. These results indicate that VPC mitigates MTX-induced oxidative injury, likely through its free radical scavenging capacity and enhancement of endogenous antioxidant systems. The observed effects are consistent with previous experimental studies reporting VPC-mediated upregulation of antioxidant enzymes and suppression of oxidative damage in various organ injury models, including hepatic ischemia-reperfusion and drug-induced toxicity. VPC, a synthetic vincamine derivative, shows consistent antioxidant and hepatoprotective effects across in vitro and animal liver models of toxic and metabolic injury. In human liver L02 cells, VPC (1–30 μM) showed strong, concentration-dependent radical scavenging in the ABTS assay (≈87% at 30 μM) and reduced H_2O_2- and paracetamol-induced ROS, while increasing intracellular GSH. VPC upregulated Nrf2 and HO-1, key regulators of endogenous antioxidant defenses, partly by competing with Nrf2 for kelch-like ECH-associated protein 1 (Keap1) binding and stabilizing Nrf2 protein.[30] In multiple rat models, VPC increased hepatic GSH and SOD and

Table 2. Liver tissue biochemical analysis results

Groups	MDA (nmol/g tissue)	SOD (U/mg protein)	CAT (K/g protein)	GPx (U/mg protein)	TAS (mmol Trolox Eqv./L)	TOS (μmol H_2O_2 Eqv./L)	OSI (AU)
Control	4.75 (2.99-6.18)	1.68 (1.43-1.97)	36.80 (23.50-51.90)	161.59 (96.97-187.39)	1.96 (1.44-2.83)	27.18 (13.25-32.72)	11.83 (7.11-19.29)
MTX	8.76 (7.99-13.10)[a,b]	1.24 (0.97-1.49)[a,b]	26.76 (17.03-33.10)[a]	87.02 (63.83-126.72)[a,b]	1.38 (0.80-2.19)[a,b]	32.98 (26.51-50.33)[a]	24.62 (13.68-35.72)[a]
VPC	6.38 (4.16-9.14)[a]	1.50 (1.16-1.98)	21.86 (15.95-27.96)[a]	126.04 (97.82-163.63)[a]	1.92 (1.56-2.38)	35.26 (30.03-43.91)[a]	18.62 (13.95-26.89)[a]
MTX+VPC	6.35 (4.98-8.90)[a]	1.28 (0.91-1.56)[a]	21.39 (18.41-30.52)[a]	116.53 (91.14-165.97)[a]	2.07 (1.17-3.18)	32.83 (24.65-48.05)[a]	19.62 (7.74-25.84)[a]

a: There is a statistically significant difference compared to the Control group ($p<0.05$). b: There is a statistically significant difference compared to the VPC group ($p<0.05$). MDA: Malondialdehyde; SOD: Superoxide Dismutase; CAT: Catalase; GPx: Glutathione Peroxidase; TAS: Total Antioxidant Status; TOS: Total Oxidant Status; OSI: Oxidative Stress Index; AU: Arbitrary Unit; MTX: Methotrexate-treated group; VPC: Vinpocetine-treated group; MTX+VPC: Methotrexate- and Vinpocetine-treated group.

reduced MDA and nitric oxide (NO/NOx), indicating reduced lipid peroxidation and nitrosative stress.[11,30-33]

Despite the clear biochemical evidence of oxidative injury and partial protection by VPC, serum AST and ALT levels did not differ significantly among groups. This finding suggests that the degree of hepatocellular damage induced by a single MTX dose in the present model may be subclinical or insufficient to elicit measurable elevations in circulating transaminases. Serum liver enzymes are known to be relatively insensitive in early or mild hepatic injury, particularly when oxidative damage precedes overt cell necrosis. OS can clearly precede overt hepatocyte necrosis and histologic liver injury, so standard serum liver enzymes [ALT, AST, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT)] may stay normal in early or mild oxida-

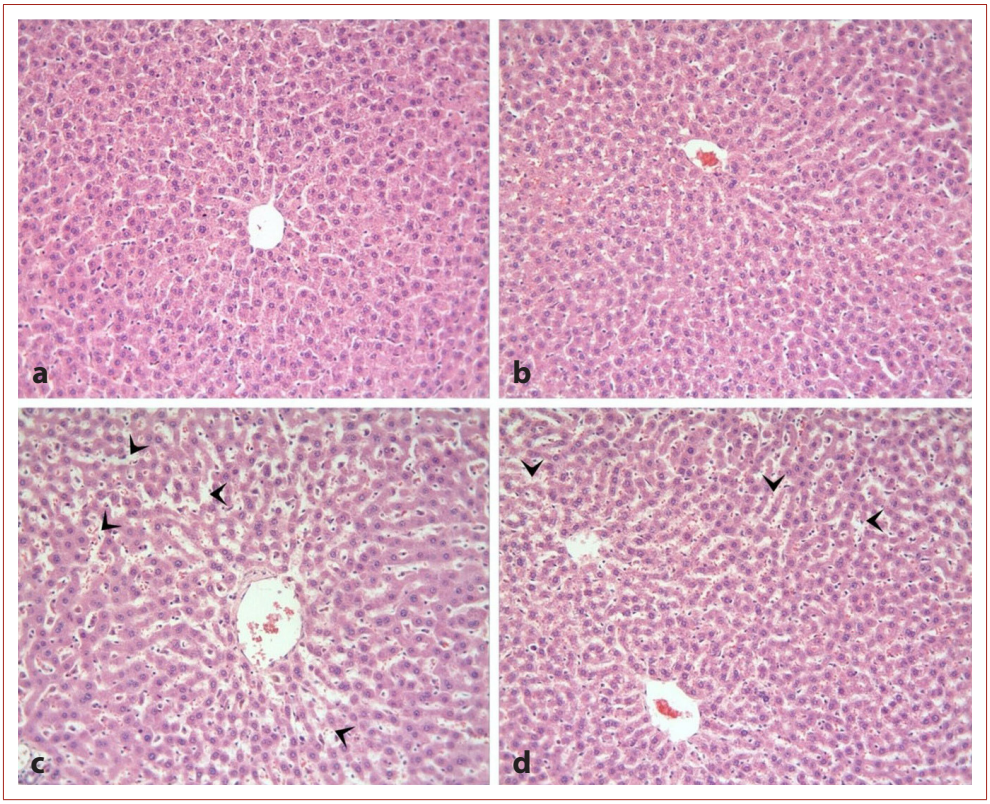

Figure 2. The liver shows a normal histological appearance in the control **(a)** and VPC **(b)** groups. In the MTX group **(c)**, sinusoidal dilatation (arrowheads) is noted. In the MTX+VPC group **(d)**, sinusoidal dilatation (arrowheads) persists, albeit to a lesser extent. H&E; 20x.

Table 3. Serum biochemical analysis results

Groups	AST (IU/L)	ALT (IU/L)
Control	177 (118-222)	35 (29-51)
MTX	164.5 (117-194)	35.5 (24-67)
VPC	182.5 (89-260)	30.5 (17-43)
MTX+VPC	122 (78-294)	26 (18-78)

There is no statistically significant difference between the groups (p>0.05). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; MTX: Methotrexate-treated group; VPC: Vinpocetine-treated group; MTX+VPC: Methotrexate- and Vinpocetine-treated group.

Table 4. Histopathological Evaluation Results

Groups	Sinusoidal dilatation	Sinusoidal congestion
Control	0.0 (0.0-1.0)	0.0 (0.0-0.1)
MTX	1.0 (0.0-3.0)[a]	0.0 (0.0-0.2)
VPC	0.0 (0.0-1.0)	0.0 (0.0-0.1)
MTX+VPC	1.0 (0.0-2.0)	0.0 (0.0-0.2)

a: Significant increase compared to the Control group (p<0.001). MTX: Methotrexate-treated group; VPC: Vinpocetine-treated group; MTX+VPC: Methotrexate- and Vinpocetine-treated group.

tive damage and do not reliably reflect its onset or severity. ALT/AST rises often correlate with necrosis or substantial membrane damage, not with the earliest oxidative events. OS and lipid peroxidation can occur days before necrosis in liver and other organs in dietary choline deficiency models.[34] Reviews of OS in acute liver injury and drug-induced liver injury emphasize that mitochondrial OS, GSH depletion, and ROS formation occur upstream of cell death and clinical enzyme release.[34-38] There is poor correlation at times between serum enzyme levels and true liver integrity; selective enzyme release can occur without necrosis, and necrosis can progress despite relatively modest enzyme changes.[37,39] Subclinical or mild chronic hepatitis in humans and dogs can have normal ALT despite histologic inflammation or copper-associated injury, highlighting insensitivity in early disease.[40] Therefore, the discrepancy between tissue OS markers and serum biochemistry underscores the importance of evaluating both biochemical and histopathological parameters when assessing hepatotoxicity. In this context, the lack of significant changes in serum AST and ALT, despite clear OS and histopathological alterations in liver tissue, suggests that the present MTX model represents an early or subclinical stage of hepatotoxicity. At this stage, intracellular oxidative injury and microstructural disturbances may occur before sufficient hepatocyte membrane damage develops to cause enzyme leakage into the circulation. Therefore, tissue OS markers and histological evaluation appear to be more sensitive indicators of early MTX-induced liver injury in this experimental setting.

Histopathological examination further supported the biochemical findings. MTX administration resulted in a significant increase in sinusoidal dilatation, indicating microcirculatory disturbance and early structural liver injury. VPC co-treatment produced a modest reduction in sinusoidal dilatation; however, this improvement did not reach statistical significance. VPC generally shows protective effects on hepatic structure, and in liver models, it can reduce histologic damage, but specific data on sinusoidal dilatation are limited, and any reduction is likely modest. In diethylnitrosamineinduced early hepatocellular carcinoma in rats, VPC improved liver ultrastructure, reduced OS and inflammatory signaling, and favorably modulated remodeling markers, indicating attenuation of structural liver damage and fibrosis.[33] In human hepatic L02 cells, VPC protected against H_2O_2 and acetaminopheninduced hepatotoxicity, reducing ROS and restoring antioxidant defenses through Nrf2/HO1 activation.[29] These effects would be expected to blunt microvascular/sinusoidal injury, though sinusoidal dilatation per se was not quantified. This partial histological protection may reflect the short duration of VPC treatment or the severity of MTX-induced injury, suggesting that longer treatment periods or different dosing regimens may be required to achieve more pronounced structural recovery.

Body weight loss observed in the MTX group further supports the systemic toxic effects of MTX, whereas partial restoration of body weight in the MTX+VPC group indicates an overall protective influence of VPC. Importantly, liver weights were not significantly altered, suggesting that functional and oxidative changes preceded gross morphological alterations in liver mass. In cyclosporine A–induced hepatotoxicity in rats, liver function markers and OS indices were markedly altered, with clear microscopic damage, despite preserved lobular architecture and no mention of early gross organ enlargement.[41] Fluoride exposure in mice produced significant OS, enzyme leakage, and ultrastructural damage (vacuolar degeneration, dilated endoplasmic reticulum, mitochondrial membrane damage) before advanced structural collapse, again emphasizing that functional and oxidative disruption precede massive morphologic change.[42] High-sugar/high-energy diets in young rats increased serum AST/ALT and oxidative/inflammatory markers along with early histopathological alterations, linking proinflammatory and OS mechanisms to early hepatic dysfunction before end-stage remodeling.[43] Reviews of liver OS emphasize that disturbed redox balance and mitochondrial dysfunction drive progression from simple steatosis to steatohepatitis, fibrosis, and hepatocellular carcinoma, acting upstream of gross fibrotic and mass changes.[44,45]

Several limitations of the present study should be acknowledged. The evaluation was limited to a short-term MTX exposure model, and inflammatory cytokines, apoptotic markers, or molecular signaling pathways. A limitation of the present study is that mechanistic signaling pathways involved in OS, inflammation, and cell death, such as the Nrf2/HO-1 and NF-κB pathways, inflammatory cytokines, and apoptotic markers, were not directly investigated. Although the observed biochemical and histopathological findings strongly support an antioxidant and hepatoprotective effect of VPC, the proposed molecular mechanisms are based on previous experimental evidence reported in the literature. Future studies incorporating molecular and protein-level analyses are warranted to clarify the precise signaling pathways responsible for the protective effects of VPC in MTX-induced hepatotoxicity. It should be noted that this study employed a short-term, single-dose MTX model that primarily reflects early or subclinical hepatotoxicity; therefore, future studies using longer-term or repeated-dose MTX protocols are warranted to better simulate chronic clinical exposure and to further clarify the long-term hepatoprotective potential of VPC.

Conclusion

The findings of this study demonstrate that MTX induces significant OS and early histopathological alterations in rat liver tissue without causing overt changes in serum liver enzymes. VPC co-administration partially attenuates MTX-induced oxidative damage and improves antioxidant capacity, supporting its potential role as an adjunctive protective agent against MTX-related hepatotoxicity. Further experimental and clinical studies are warranted to clarify its therapeutic relevance and optimal treatment strategies.

Disclosures

Ethics Committee Approval: This study was approved by The Inonu University Faculty of Medicine Animal Experiments Local Ethics Committee on October 11, 2018, at the meeting numbered 2015/A-37.

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Original Research

Molecular Docking Analysis Reveals Potential Dual Targeting of FGFR4 and PI3K by Atorvastatin in Hepatocellular Carcinoma

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Abstract

Objectives: Hepatocellular carcinoma (HCC) remains one of the leading causes of cancer-related mortality worldwide. Despite the availability of systemic therapies, treatment resistance and tumor progression remain major challenges in HCC management. Increasing evidence indicates that aberrant activation of the fibroblast growth factor receptor 4 (FGFR4) axis and the phosphoinositide 3-kinase (PI3K) signaling pathway plays a critical role in hepatocarcinogenesis, tumor proliferation, and therapeutic resistance. Drug repositioning strategies offer an efficient approach for identifying new therapeutic applications for widely used non-oncologic drugs. This study aimed to investigate the potential inhibitory interactions of commonly prescribed drugs—atorvastatin, metformin, and celecoxib—against FGFR4 and PI3Kα through molecular docking analysis and to compare their binding profiles with sorafenib, a reference drug used in HCC treatment.

Methods: The crystal structures of FGFR4 and PI3Kα were retrieved from the Protein Data Bank and prepared using AutoDockTools. The three-dimensional structures of atorvastatin, metformin, celecoxib, and sorafenib were obtained from the PubChem database. Molecular docking simulations were performed using AutoDock 4 employing the Lamarckian Genetic Algorithm. The docking protocol was validated by redocking the co-crystallized ligand into the ATP-binding pocket, and root mean square deviation (RMSD) values below 2.0 Å were considered acceptable. Binding energies (ΔG), ligand–protein interaction profiles, and pharmacokinetic properties were analyzed using SwissADME.

Results: Docking simulations revealed that atorvastatin exhibited the strongest binding affinity toward both FGFR4 (-9.94 kcal/mol) and PI3Kα (-9.10 kcal/mol), demonstrating binding energies comparable to or stronger than the reference inhibitor. Celecoxib also showed notable binding affinity toward PI3Kα (-8.79 kcal/mol), whereas sorafenib demonstrated moderate binding interactions. In contrast, metformin exhibited relatively weak binding energies for both targets. Interaction analysis revealed that atorvastatin formed stabilizing hydrogen bonds and hydrophobic contacts with key residues within the ATP-binding pockets of FGFR4 and PI3Kα. ADMET prediction indicated that all investigated compounds satisfied Lipinski's rule of five and displayed generally acceptable pharmacokinetic properties.

Conclusion: These findings suggest that atorvastatin may interact strongly with both FGFR4 and PI3Kα signaling proteins, highlighting its potential as a dual-target modulator in hepatocellular carcinoma. The results provide preliminary in silico evidence supporting the repositioning of commonly prescribed drugs in HCC therapy, warranting further experimental and clinical validation.

Keywords: Hepatocellular carcinoma, FGFR4, PI3K, drug repositioning, molecular docking, Sorafenib

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Hepatocellular carcinoma (HCC) is the predominant form of primary liver cancer and continues to represent a substantial global health challenge.[1] It ranks among the leading causes of cancer-related mortality worldwide, largely due to late diagnosis, rapid disease progression, and limited therapeutic options.[2] Although several systemic therapies have been developed in recent years, the prognosis of advanced HCC remains poor, highlighting the need for novel therapeutic strategies and improved understanding of the molecular mechanisms underlying hepatocarcinogenesis. Among the molecular pathways involved in HCC development, the fibroblast growth factor receptor 4 (FGFR4) signaling axis has gained increasing attention.[3] FGFR4 is predominantly expressed in hepatocytes and plays a crucial role in regulating hepatic metabolism, cell proliferation, and survival.[4] Aberrant activation of the FGF19–FGFR4 pathway has been associated with tumor growth, angiogenesis, and poor clinical outcomes in HCC patients. Consequently, FGFR4 has emerged as a promising therapeutic target in liver cancer.[5]

Another key signaling pathway implicated in hepatocellular carcinoma is the phosphoinositide 3-kinase (PI3K)/AKT/mTOR pathway.[6] Activation of the PI3K pathway contributes to tumor cell growth, metabolic alterations, and resistance to anticancer therapies.[7] Mutations and overactivation of PIK3CA, the catalytic subunit of PI3K, have been reported in multiple cancers, including HCC, and contribute to tumor progression and therapeutic resistance. Drug repositioning has emerged as a promising strategy to identify new therapeutic applications for already approved drugs.[8] Compared with conventional drug discovery, repositioning approaches offer significant advantages, including reduced development time, lower cost, and well-characterized pharmacokinetic and safety profiles. Increasing epidemiological and experimental evidence suggests that certain widely used drugs—such as statins, antidiabetic agents, and non-steroidal anti-inflammatory drugs—may influence cancer development and progression. For instance, statins have been associated with a reduced incidence of hepatocellular carcinoma in several observational studies,[9] potentially through modulation of oncogenic signaling pathways. Similarly, metformin has demonstrated antitumor effects in multiple malignancies by influencing metabolic and PI3K-related pathways.[10] Celecoxib, a selective cyclooxygenase-2 inhibitor, has also been reported to exert anticancer properties through anti-inflammatory and antiproliferative mechanisms.[11] Despite these observations, the potential direct molecular interactions between these drugs and key signaling proteins involved in HCC progression remain incompletely understood. Computational approaches such as molecular docking provide a valuable framework for investigating potential ligand–protein interactions and identifying novel inhibitory mechanisms.[12,13]

Therefore, the present study aimed to evaluate the potential interactions of atorvastatin, metformin, and celecoxib with FGFR4 and PI3K using molecular docking analysis. Sorafenib, an approved multikinase inhibitor used in HCC treatment, was included as a reference compound to compare binding affinities and interaction profiles.

Methods

Protein Preparation

The three-dimensional crystal structures of fibroblast growth factor receptor 4 (FGFR4, UniProt ID: P22455) and phosphoinositide 3-kinase alpha (PI3Kα, UniProt ID: P42336) were retrieved from the Protein Data Bank (PDB). The crystal structures with PDB IDs 5JKG for FGFR4 and 4FA6 for PI3Kα were selected based on their structural resolution and suitability for molecular docking analysis. The structure 5JKG has a crystallographic resolution of 2.35 Å, whereas 4FA6 has a resolution of 2.70 Å. Protein preparation was performed using AutoDockTools (ADT, version 1.5.6).[14] All crystallographic water molecules were removed from the protein structures, and polar hydrogen atoms were added. Kollman united atom charges were assigned to the protein structures. The co-crystallized ligands present in the crystal structures were extracted and saved separately for docking protocol validation. Additionally, electrostatic properties and solvation-related parameters of the proteins were evaluated using the Poisson–Boltzmann electrostatics server (https://server.poissonboltzmann.org/). This analysis was performed to characterize the electrostatic environment of the binding pockets prior to docking simulations. Finally, the prepared protein structures were converted into PDBQT format for subsequent docking calculations using AutoDock.

Ligand Preparation

The three-dimensional structures of atorvastatin, metformin, celecoxib, and sorafenib were retrieved from the PubChem database. Ligand structures were subjected to energy minimization prior to docking analysis. The ligands were prepared using AutoDockTools by assigning Gasteiger charges and defining rotatable bonds. All ligands were then converted into PDBQT format for docking simulations.

Docking Protocol Validation and Grid Definition

To ensure the reliability of the docking procedure, the docking protocol was validated by redocking the co-crystallized ligands into their respective binding pockets. The

ligands originally present in the crystal structures of FGFR4 and PI3Kα were extracted and subsequently redocked using the same docking parameters applied for the docking simulations. The docking calculations were focused on the ATP-binding pocket of each protein. The predicted binding poses were compared with the experimentally observed ligand conformations obtained from the crystal structures. The accuracy of the docking protocol was assessed by calculating the root mean square deviation (RMSD) between the redocked ligand pose and the crystallographic ligand conformation. RMSD values below 2.0 Å were considered indicative of a reliable docking protocol. For docking simulations, grid maps were generated using AutoDockTools to cover the active binding sites of the target proteins. For FGFR4 (PDB ID: 5JKG), the grid box center coordinates were set to x=-41.358, y=-16.614, z=377.853, with grid dimensions of 50×48×44 points along the x, y, and z axes, respectively. For PI3Kα (PDB ID: 4FA6), the grid box center coordinates were defined as x=44.481, y=14.981, z=31.276, with grid dimensions of 50×40×38 points along the x, y, and z axes. A grid spacing of 0.375 Å was applied in all docking calculations to ensure adequate coverage of the binding pockets while maintaining computational efficiency.

Molecular Docking

Docking calculations were performed using the AutoDock 4 platform with the Lamarckian Genetic Algorithm as the search method. Additionally, to ensure full reproducibility of the docking protocol, all relevant parameters were explicitly defined. The grid box center coordinates and dimensions for both FGFR4 and PI3Kα were specified as described above. Each docking simulation was performed using 100 independent genetic algorithm runs, with a population size of 150, a maximum of 2,500,000 energy evaluations, and 27,000 generations. The mutation rate and crossover rate were set to the default AutoDock values of 0.02 and 0.80, respectively. All docking parameters were kept consistent across all ligand–protein complexes to ensure comparability of the results. The docking calculations were focused on the ATP-binding pockets of FGFR4 and PI3Kα, as defined by the grid box parameters described above. For each ligand–protein complex, 100 independent docking runs were performed to ensure adequate sampling of possible binding conformations and to account for the stochastic nature of the Lamarckian Genetic Algorithm. The population size was set to 150 individuals, with a maximum number of 2,500,000 energy evaluations and 27,000 generations. All other docking parameters were maintained at their default AutoDock settings. To assess the reproducibility and stability of the docking results, the binding energies obtained from the 100 independent runs were analyzed, and the mean binding energy along with the standard deviation (mean±SD) was calculated for each ligand–target complex.

Following the docking simulations, the resulting ligand conformations were clustered according to their positional similarity. The optimal binding pose for each ligand was selected based on the lowest predicted binding energy (ΔG) and the largest cluster size, which represents the most favorable and stable binding conformation.

Interaction Analysis

The molecular interactions between the docked ligands and the target proteins were analyzed to identify key binding features within the active sites. The best docking poses obtained from AutoDock were examined to determine hydrogen bonds, hydrophobic interactions, and other non-covalent interactions contributing to ligand binding stability. The ligand–protein interaction profiles were evaluated by analyzing contacts with amino acid residues located in the ATP-binding pockets of FGFR4 and PI3Kα. Two-dimensional (2D) and three-dimensional (3D) interaction diagrams were generated to visualize the binding modes of the docked complexes and to identify key residues involved in ligand recognition.

ADMET (absorption, distribution, metabolism, excretion, and toxicity) Prediction

The pharmacokinetic and drug-likeness properties of the investigated compounds were evaluated using the SwissADME web server (http://www.swissadme.ch).[15] The simplified molecular-input line-entry system (SMILES) structures of the ligands were retrieved from the PubChem database and used as input for the analysis. Several physicochemical and pharmacokinetic parameters relevant to drug development were assessed, including molecular weight (MW), lipophilicity (LogP), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), topological polar surface area (TPSA), and molar refractivity (MR). In addition, drug-likeness was evaluated according to Lipinski's rule of five. Predicted pharmacokinetic properties such as gastrointestinal (GI) absorption and blood–brain barrier (BBB) permeability were also analyzed to evaluate the suitability of the investigated molecules as potential drug candidates.

Statement on Ethics Committee Approval

This study was conducted entirely using in silico computational methods. No human participants, animal subjects, clinical data, or biological samples were used. All structural and chemical data were obtained from publicly available databases (Protein Data Bank and ChEMBL), which provide open-access and fully anonymized data. Therefore, ethics committee approval was not required for this study.

Results

Docking Protocol Validation

Docking protocol validation was performed by redocking the co-crystallized ligands into the ATP-binding pockets of FGFR4 and PI3Kα using the same docking parameters applied in the study. The predicted binding poses were compared with the experimentally determined crystallographic conformations.

The redocking procedure yielded RMSD values of 1.22 Å for FGFR4 and 0.57 Å for PI3Kα, both of which are well below the commonly accepted threshold of 2.0 Å. These results indicate excellent agreement between the predicted and experimental ligand conformations. The superposition of the crystallographic and redocked ligand conformations is shown in Figure 1, demonstrating a high degree of structural overlap and confirming the reliability of the docking protocol.

Binding Energy Analysis

The binding affinities of the investigated ligands toward FGFR4 and PI3Kα were evaluated based on their predicted binding energies (ΔG) obtained from AutoDock simulations. The calculated docking scores are summarized in Table 1.

To evaluate the reproducibility of the docking simulations, mean binding energies and standard deviations were calculated from 100 independent runs for each ligand–protein complex. The relatively low variability observed across runs supports the consistency of the predicted docking outcomes.

FGFR4 Docking Results

For the FGFR4 target, atorvastatin demonstrated the strongest binding affinity with a docking energy of -9.94 kcal/mol, which was even lower than that of the reference inhibitor LY-2874455 (-9.55 kcal/mol). This finding suggests a strong predicted interaction of atorvastatin within the FGFR4 binding pocket. Among the other investigated compounds, sorafenib (-7.62 kcal/mol) and celecoxib (-7.43 kcal/mol) showed moderate binding affinities toward FGFR4. In contrast, metformin displayed the weakest interaction with a docking score of -5.16 kcal/mol, indicating relatively low binding affinity for the FGFR4 active site.

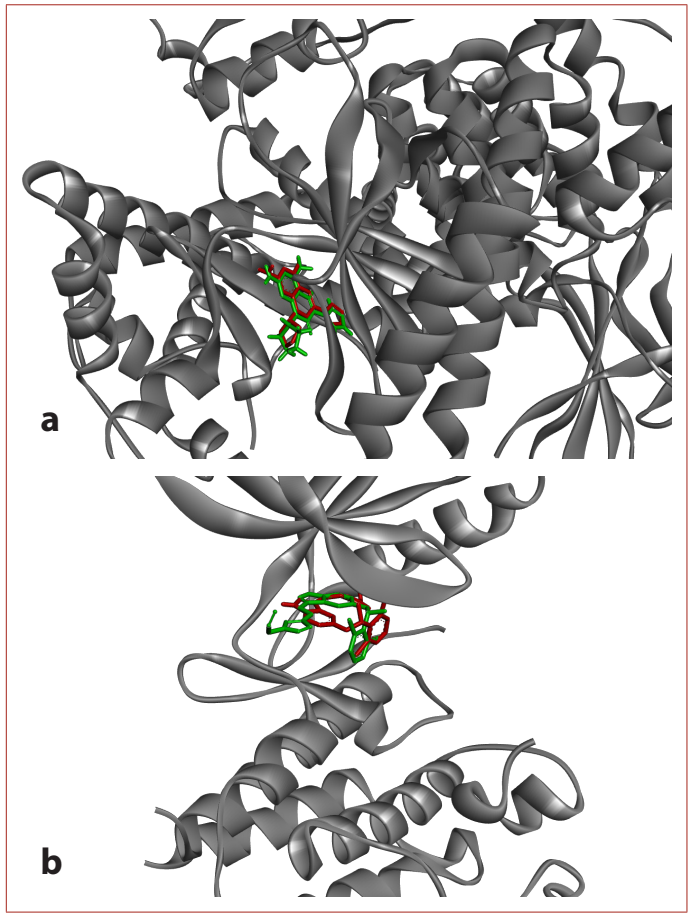

Figure 1. Docking protocol validation by redocking of co-crystallized ligands. **(a)** FGFR4 (PDB ID: 5JKG) and **(b)** PI3Kα (PDB ID: 4FA6). The superposition of the native (crystallographic) and redocked ligand conformations demonstrates strong agreement, with RMSD values of 1.22 Å and 0.57 Å, respectively.

PI3Kα Docking Results

Docking analysis against the PI3Kα target revealed that atorvastatin also exhibited the strongest binding affinity among the tested compounds with a docking energy of -9.10 kcal/mol, followed by celecoxib (-8.79 kcal/mol). The reference ligand demonstrated a binding energy of -8.92 kcal/mol, indicating that atorvastatin displayed a comparable or slightly stronger predicted interaction with the

Table 1. Table 1. Best docking scores and mean binding energy values (±SD) obtained from 100 independent docking runs against FGFR4 and PI3Kα

Ligand	FGFR4 (Best Score)	FGFR4 (Mean±SD)	PI3Kα (Best Score)	PI3Kα (Mean±SD)
Reference	-9.55	-8.341±0.431	-8.92	-8.803±0.065
Atorvastatin	-9.94	-9.5±0.3	-9.10	-8.8±0.2
Celecoxib	-7.43	-7.039±0.310	-8.79	-8.248±0.372
Sorafenib	-7.62	-7.248±0.373	-7.34	-6.822±0.284
Metformin	-5.16	-5.121±0.020	-4.7	-4.691±0.003

PI3Kα binding pocket. Sorafenib showed a moderate binding affinity with a docking energy of -7.34 kcal/mol, while metformin again demonstrated the lowest binding affinity toward PI3Kα with a docking score of -4.70 kcal/mol.

Overall, these findings indicate that atorvastatin exhibited the most favorable binding energies toward both FGFR4 and PI3Kα, suggesting a potential interaction with key signaling proteins involved in hepatocellular carcinoma.

Interaction Analysis

To further elucidate the structural basis of ligand binding, detailed interaction analyses were performed for the docked complexes within the active sites of FGFR4 and PI3Kα. Both two-dimensional (2D) interaction diagrams and three-dimensional (3D) binding pose visualizations were generated to characterize the molecular interactions. For FGFR4, atorvastatin exhibited a stable binding conformation within the ATP-binding pocket. The ligand formed a hydrogen bond with Arg483, which is located within the catalytic region of the kinase domain. In addition, multiple hydrophobic interactions were observed with key residues including Leu473, Val550, and Ala553, contributing to the stabilization of the ligand within the binding cavity. These residues are known to be involved in ligand recognition and kinase activity.

For PI3Kα, atorvastatin also demonstrated a favorable binding orientation within the catalytic site. Hydrogen bond interactions were identified with Lys833 and Asp841, while hydrophobic contacts were observed with residues such as Met804, Ile831, and Tyr867. These interactions support a stable ligand–protein complex and suggest effective accommodation of the ligand within the active site. Although no prominent π–π stacking interactions were observed, the combination of hydrogen bonding and hydrophobic interactions appears to play a dominant role in stabilizing the ligand within both binding pockets.

The 2D interaction diagrams and 3D binding poses presented in Figures 2 and 3 further illustrate these interactions and provide visual confirmation of the ligand–protein binding modes.

- **(A)** Three-dimensional representation of atorvastatin docked in the FGFR4 active site, illustrating its orientation within the catalytic region.
- **(B)** Two-dimensional interaction diagram showing key ligand–protein interactions. Atorvastatin forms a hydrogen bond with Arg483, while hydrophobic interactions are observed with residues including Leu473, Val550, and Ala553. These interactions contribute to the stabilization of the ligand within the binding pocket.

Further analysis of the two-dimensional interaction diagram revealed that atorvastatin formed a hydrogen bond

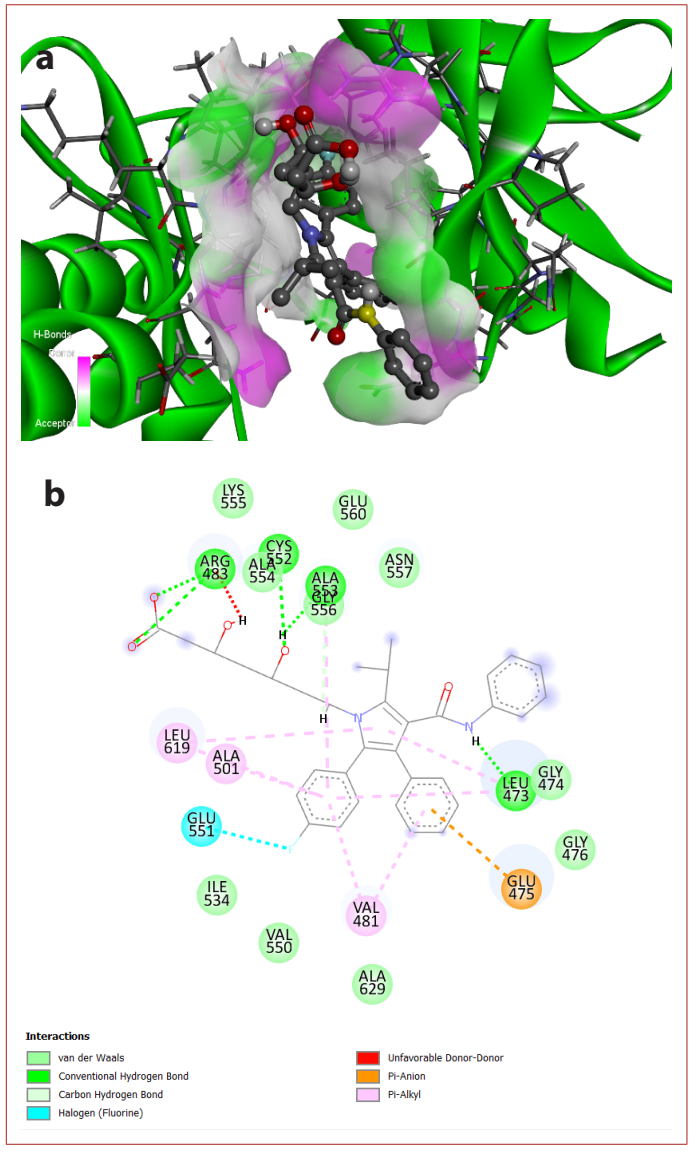

Figure 2. Interaction analysis of atorvastatin within the FGFR4 ATP-binding pocket.

with Arg483, while hydrophobic interactions were observed with residues such as Leu473, Val550, and Ala553 (Fig. 2b).

- **(A)** Three-dimensional visualization of atorvastatin positioned within the kinase active site, demonstrating its spatial orientation relative to surrounding residues.
- **(B)** Two-dimensional interaction diagram illustrating ligand–protein interactions. Atorvastatin forms hydrogen bonds with Lys833 and Asp841, along with hydrophobic contacts involving Met804, Ile831, and Tyr867, supporting stable binding within the catalytic pocket.

These residues are located within the ATP-binding region and are known to play a critical role in kinase activity. For PI3Kα, atorvastatin also demonstrated a favorable binding orien-

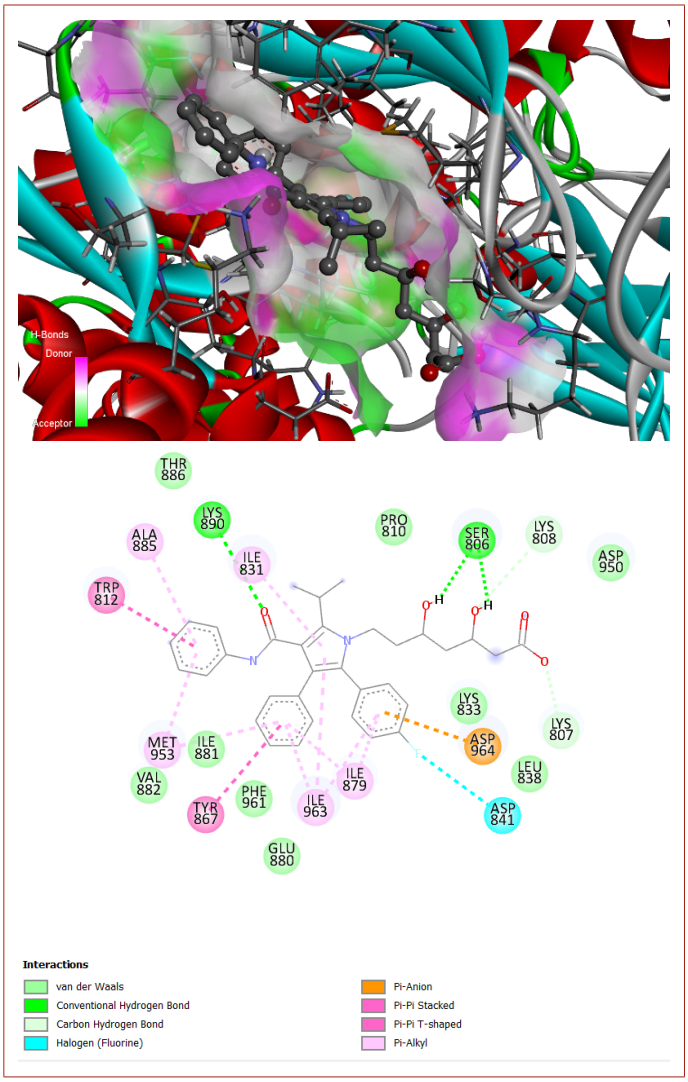

Figure 3. Interaction analysis of atorvastatin within the PI3Kα catalytic binding pocket.

tation within the catalytic pocket of the kinase domain. The ligand formed stabilizing interactions with residues lining the active site, suggesting a strong and stable binding configuration (Fig. 3a). The two-dimensional interaction diagram further revealed hydrogen bonding and hydrophobic contacts between atorvastatin and surrounding residues within the PI3Kα binding pocket (Fig. 3B). In particular, atorvastatin formed hydrogen bond interactions with Lys833 and Asp841, while additional hydrophobic contacts were observed with residues such as Met804, Ile831, and Tyr867, contributing to the stabilization of the ligand within the catalytic pocket.

ADMET Prediction

The predicted physicochemical and pharmacokinetic properties of the investigated ligands are presented in Table 2. All compounds satisfied Lipinski's rule of five, indicating acceptable drug-likeness profiles.

Among the investigated ligands, atorvastatin exhibited the highest molecular weight (558.64 g/mol) and the largest number of rotatable bonds (RB=13), suggesting greater molecular flexibility compared with the other compounds. In contrast, metformin showed the lowest molecular weight (129.16 g/mol) and fewer structural features, reflecting its smaller and more polar molecular structure. In terms of lipophilicity, the iLogP values ranged between 0.77 and 3.58, indicating moderate lipophilicity among the investigated ligands. Celecoxib demonstrated relatively balanced physicochemical parameters with moderate molecular weight, lipophilicity, and polar surface area. The TPSA values ranged from 86.36 to 111.79 Å^2, suggesting suitable polarity for potential oral bioavailability.

Regarding pharmacokinetic predictions, high gastrointestinal (GI) absorption was predicted for celecoxib and metformin, whereas atorvastatin and sorafenib were predicted to exhibit lower GI absorption. Additionally, none of the investigated compounds were predicted to cross the blood–brain barrier (BBB), indicating limited central nervous system penetration.

Overall, the ADMET analysis indicates that the investigated ligands possess generally acceptable physicochemical and pharmacokinetic properties according to the evaluated parameters.

Discussion

The present study investigated the potential interactions of commonly prescribed non-oncologic drugs with two key signaling proteins involved in hepatocellular carcinoma, FGFR4 and PI3Kα, using molecular docking analysis. The results demonstrated that atorvastatin exhibited the most favorable binding affinities toward both targets, with docking energies comparable to or stronger than those of the reference inhibitor. Interaction analysis further revealed that atorvastatin formed stabilizing hydrogen bonds and hydrophobic contacts within the ATP-binding pockets of both FGFR4 and PI3Kα. These findings suggest that atorvastatin may interact with critical residues located in the catalytic regions of these proteins and may potentially influence signaling pathways associated with HCC progression.

Aberrant activation of the FGFR4 signaling axis has been widely implicated in hepatocarcinogenesis, promoting tumor cell proliferation, survival, and resistance to therapy.[3,5,16] Similarly, dysregulation of the PI3K/AKT signaling pathway plays a central role in tumor growth, metabolic reprogramming, and resistance to anticancer therapies in hepatocellular carcinoma.[6,17] Targeting these signaling pathways has therefore emerged as an important therapeutic

Table 2. Physicochemical properties and ADME parameter values of ligands calculated with the SwissADME program

Properties name	Formula	MW (g/mol)	RB	HBA	HBD	iLogP	TPSA (Å^2)	MR	Lipinski rule	GI	BBBP
Reference (FGFR4)	$C_{21}H_{19}C_{l2}N_5O_2$	444.31	7	5	2	2.77	88.85	118.08	Yes	High	No
Reference (PI3Kα)	$C_{16}H_{18}N_6O$	310.35	2	4	2	1.82	102.48	89.13	Yes	High	No
Atorvastatin	$C_{33}H_{35}FN_2O_5$	558.64	13	6	4	3.58	111.79	158.26	Yes	Low	No
Celecoxib	$C_{17}H_{14}F_3N_3O_2S$	381.37	4	7	1	2.56	86.36	89.96	Yes	High	No
Sorafenib	$C_{21}H_{16}C_lF_3N_4O_3$	464.82	9	7	3	3.42	92.35	112.48	Yes	Low	No
Metformin	$C_4H_{11}N_5$	129.16	3	2	4	0.77	88.99	36.93	Yes	High	No

MW: Molecular Weight; RB: Rotable Bond; HBA: H-Bond acceptor; HBD: H-bond donor; TPSA: Topological polar surface area; MR: Molar Refractivity; GIA: Gastrointestinal absorption; BBBP: permeant. Ligands in bold indicate compounds that show better binding affinity than the reference ligand.

strategy in HCC management. The strong binding interactions of atorvastatin with both FGFR4 and PI3Kα observed in this study may indicate a potential modulatory effect on these oncogenic signaling pathways.

Accumulating evidence suggests that statins, particularly atorvastatin, may exert anticancer effects beyond their lipid-lowering properties. Several experimental and epidemiological studies have reported that statin use is associated with a reduced risk of hepatocellular carcinoma and improved clinical outcomes in patients with chronic liver disease.[9,18] Proposed mechanisms include inhibition of tumor cell proliferation, induction of apoptosis, and suppression of oncogenic signaling pathways such as PI3K/AKT.[19] In addition, statins have been reported to interfere with intracellular signaling cascades involved in tumor progression and angiogenesis. The binding interactions observed in the present docking analysis may provide a possible molecular explanation for these previously reported anticancer effects.

In addition to atorvastatin, celecoxib demonstrated relatively strong binding affinity toward PI3Kα in the present analysis. Celecoxib is a selective cyclooxygenase-2 (COX-2) inhibitor that has been widely investigated for its anti-inflammatory and potential anticancer properties. Previous studies have suggested that COX-2 inhibition may suppress tumor growth, angiogenesis, and metastasis in several malignancies.[20] The interaction of celecoxib with the PI3Kα binding pocket observed in this study may indicate a potential additional mechanism through which celecoxib could influence tumor-related signaling pathways.

As expected, sorafenib, a multi-kinase inhibitor commonly used in advanced hepatocellular carcinoma treatment, exhibited moderate binding interactions with the investigated targets. Sorafenib primarily acts by inhibiting multiple kinases involved in tumor proliferation and angiogenesis, including RAF kinases and vascular endothelial growth factor receptors.[21] The docking results obtained in this study are consistent with the known multi-target activity of sorafenib. In contrast, metformin demonstrated relatively weak binding affinities toward both FGFR4 and PI3Kα compared with the other investigated compounds. Although metformin has been reported to exert indirect anticancer effects through metabolic regulation and activation of the AMP-activated protein kinase (AMPK) pathway,[10] the docking results suggest that its potential interactions with these specific kinase targets may be limited.

In addition to the docking results, the pharmacokinetic properties of the investigated compounds were evaluated using SwissADME, providing further insight into their potential drug-likeness and clinical applicability. All compounds satisfied Lipinski's rule of five, suggesting favorable oral drug-like characteristics. Atorvastatin exhibited relatively higher molecular weight and lipophilicity compared to the other compounds, which may influence its absorption and distribution properties. Although predicted gastrointestinal (GI) absorption was lower for atorvastatin and sorafenib, their established clinical use indicates that such limitations may be mitigated by formulation or dosing strategies. Celecoxib demonstrated balanced physicochemical properties, including moderate lipophilicity and high predicted GI absorption, supporting its suitability as an orally active compound. In contrast, metformin, despite its favorable GI absorption and low molecular weight, showed high polarity, which may limit its ability to interact strongly with hydrophobic binding pockets, consistent with its weaker docking performance. Overall, the ADME analysis suggests that the investigated compounds possess acceptable pharmacokinetic profiles, supporting their potential as candidates for further investigation in drug repositioning strategies.

The findings of the present study also highlight the potential value of drug repositioning strategies in identifying novel therapeutic candidates for hepatocellular carcinoma. Drug repositioning offers several advantages compared

with traditional drug development approaches, including reduced development time, lower costs, and the availability of well-established safety profiles.[8] In this context, the strong binding interactions of atorvastatin with both FGFR4 and PI3Kα suggest that widely prescribed metabolic drugs may interact with oncogenic signaling pathways involved in HCC progression. The low RMSD values obtained from redocking further support the accuracy and reliability of the docking protocol used in this study. Despite the promising findings obtained from the molecular docking analysis, several limitations should be acknowledged. First, the present study was based solely on in silico computational methods, and the predicted ligand–protein interactions require validation through in vitro biochemical assays and in vivo experimental studies. Furthermore, molecular docking provides a static representation of ligand binding and may not fully capture the dynamic nature of protein–ligand interactions under physiological conditions. Despite the promising findings obtained from the molecular docking analysis, several limitations should be acknowledged. First, the present study was based solely on in silico computational methods, and the predicted ligand–protein interactions require validation through in vitro biochemical assays and in vivo experimental studies. Furthermore, molecular docking provides a static representation of ligand binding and may not fully capture the dynamic nature of protein–ligand interactions under physiological conditions. Importantly, molecular docking represents a predictive computational approach and does not directly demonstrate biological activity or therapeutic efficacy. Therefore, the findings of this study should be interpreted as hypothesis-generating rather than confirmatory. Further validation through in vitro biochemical assays, cell-based studies, and in vivo models is necessary to confirm the functional and therapeutic relevance of these interactions.

Disclosures

Ethics Committee Approval: Ethics committee approval was not required for this study. This study was conducted entirely using in silico computational methods. No human participants, animal subjects, clinical data, or biological samples were used

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Use of AI for Writing Assistance: Not declared.

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Original Research

Comparative Diagnostic Performance of Non-Invasive Indices for Predicting Ultrasonographic Hepatic Steatosis in Morbidly Obese Patients: A Single-Center Retrospective Study

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Abstract

Objectives: Morbid obesity is strongly associated with non-alcoholic fatty liver disease (NAFLD), and hepatic steatosis is highly prevalent among candidates for bariatric surgery. Given the invasive nature of liver biopsy, there is a growing need for reliable non-invasive methods to assess hepatic steatosis. This study aimed to compare the diagnostic performance of insulin resistance–based indices (Homeostasis Model Assessment of Insulin Resistance [HOMA-IR] and Quantitative Insulin Sensitivity Check Index [QUICKI]) and biochemical scores (Hepatic Steatosis Index [HSI] and NAFLD Liver Fat Score [NAFLD-LFS]) in predicting ultrasonographically detected hepatic steatosis in patients with morbid obesity.

Methods: In this single-center retrospective observational study, 206 patients with morbid obesity who underwent primary laparoscopic sleeve gastrectomy between March 2024 and February 2026 and had available preoperative laboratory and abdominal ultrasonography data were included. Insulin resistance indices (HOMA-IR, QUICKI) and composite scores (HSI and NAFLD-LFS) were calculated. Hepatic steatosis was graded ultrasonographically as grade 1–3 and categorized as mild (grade 1) and moderate-to-severe (grade ≥2) for diagnostic performance analyses. Receiver operating characteristic (ROC) curve analysis was performed to evaluate diagnostic accuracy.

Results: Hepatic steatosis was classified as grade 1 in 28.2%, grade 2 in 59.2%, and grade 3 in 12.6% of patients. Steatosis grade showed positive correlations with HOMA-IR (r=0.244), HSI (r=0.354), NAFLD-LFS (r=0.297), HbA1c (r=0.274), transaminases, and glucose/insulin levels, and a negative correlation with QUICKI. In ROC analysis, HSI demonstrated the highest diagnostic performance (AUC=0.716), followed by HbA1c (AUC=0.656) and NAFLD-LFS (AUC=0.645). In multivariable analysis, age (OR=1.04), BMI (OR=1.23), and NAFLD-LFS (OR=1.42) were identified as independent predictors, while female sex was associated with lower risk (OR=0.34).

Conclusion: In patients with morbid obesity, hepatic steatosis is significantly associated with metabolic parameters and insulin resistance. Among non-invasive indices, HSI demonstrated the highest diagnostic performance. HSI and NAFLD-LFS may serve as practical tools in the preoperative assessment of bariatric surgery candidates.

Keywords: Hepatic steatosis, hepatic steatosis index, morbid obesity, NAFLD liver fat score, non-invasive indices

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Morbid obesity is a rapidly increasing global public health problem and is closely associated with metabolic, cardiovascular, and hepatic complications.[1] Among these hepatic complications, non-alcoholic fatty liver disease (NAFLD) is one of the most common conditions, recently redefined as metabolic dysfunction–associated steatotic liver disease (MASLD).[2,3] NAFLD is characterized by lipid accumulation in hepatocytes and encompasses a broad and progressive clinical spectrum, ranging from simple hepatic steatosis to non-alcoholic steatohepatitis, progressive fibrosis, and cirrhosis.[4]

NAFLD is highly prevalent, particularly in individuals with morbid obesity. Studies conducted in patients who are candidates for bariatric surgery have reported that the prevalence of NAFLD ranges between 60% and 90%.[4,5] This high prevalence reflects the strong pathophysiological relationship between obesity and hepatic fat accumulation. Insulin resistance is considered one of the main mechanisms underlying impaired hepatic lipid metabolism and increased triglyceride accumulation in hepatocytes.[6,7]

Liver biopsy is regarded as the gold standard for the assessment of hepatic steatosis; however, its invasive nature and potential risk of complications limit its use in routine clinical practice. Interest in non-invasive methods for evaluating hepatic steatosis has increased in recent years.[8] Insulin resistance-based indices and biochemical scores serve as alternative diagnostic tools for the assessment of hepatic steatosis.[9–11] However, studies evaluating the relationship between these indices and ultrasonographic hepatic steatosis in morbidly obese individuals, particularly in bariatric surgery candidates, are limited.[12,13] Demonstrating this relationship is clinically important for the non-invasive evaluation of hepatic steatosis.

In this study, we evaluated the relationship between insulin resistance-based indices and ultrasonographically detected hepatic steatosis in patients with morbid obesity, and investigated their potential clinical value in predicting ultrasonographically detected hepatic steatosis in candidates for bariatric surgery.

Methods

Study Design and Population

This study was designed as a retrospective observational study. The study protocol was approved by the Non-Interventional Clinical Research Ethics Committee of Elazığ Fethi Sekin City Hospital (date: March 26, 2026; decision no: 2026/26-01) and was conducted in accordance with the principles of the Declaration of Helsinki.

The clinical, laboratory, and radiological data of consecutive patients who met the inclusion criteria and were scheduled for bariatric surgery for morbid obesity between March 2024 and February 2026 in the General Surgery Department of Elazığ Fethi Sekin City Hospital were analyzed.

To ensure population homogeneity, only patients who underwent primary laparoscopic sleeve gastrectomy were included in the study. Patients who underwent gastric bypass or revision bariatric surgery were excluded. All surgical procedures were performed using a standardized laparoscopic sleeve gastrectomy technique.[14]

Inclusion Criteria:

Age ≥18 years

- Body mass index (BMI) ≥40 kg/m² or ≥35 kg/m² with obesity-related comorbidities
- Undergoing primary laparoscopic sleeve gastrectomy for morbid obesity
- Availability of preoperative laboratory data
- Preoperative assessment of hepatic steatosis by abdominal ultrasonography

Exclusion Criteria:

- History of alcohol consumption
- Presence of viral hepatitis
- Use of hepatotoxic medications
- Presence of chronic liver disease
- Missing clinical or laboratory data

Laboratory Measurements and Index Calculations

Preoperative laboratory data were obtained from the hospital information system. Fasting plasma glucose, fasting insulin, AST, ALT, HbA1c, and cortisol levels were recorded.

To evaluate insulin resistance, the homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were calculated.[15,16]

HOMA-IR was calculated using the following formula:[15]
HOMA-IR = [fasting glucose (mg/dL) × fasting insulin (μIU/mL)] / 405

QUICKI was calculated using the following formula:[16]
QUICKI = 1 / [log(fasting insulin (μIU/mL)) + log(fasting glucose (mg/dL))]

To assess hepatic steatosis, the Hepatic Steatosis Index (HSI) and NAFLD liver fat score (NAFLD-LFS) were calculated.[11,17]

The HSI was calculated as follows:[17]
HSI = 8 × (ALT / AST) + BMI

Additional +2 points were awarded for female sex and +2 points for the presence of diabetes mellitus.

NAFLD Liver Fat Score was calculated as follows:[11]
NAFLD-LFS = −2.89 + 1.18 × metabolic syndrome + 0.45 ×

diabetes + 0.15 × insulin + 0.04 × AST − 0.94 × (AST/ALT)

Metabolic syndrome was defined according to the International Diabetes Federation and harmonized criteria as the presence of ≥3 components.[18]

Data Collection and Variables

Demographic and clinical data of the included patients were obtained from electronic medical records. Age, sex, height, weight, and body mass index (BMI) were recorded. Comorbidities, including diabetes mellitus, hypertension, and hyperlipidemia, were also evaluated.

The primary outcome variable was the ultrasonographically determined grade of hepatic steatosis.

Ultrasonographic Assessment of Hepatic Steatosis

In all patients, the liver parenchyma was evaluated preoperatively using abdominal ultrasonography. Ultrasonographic examinations were performed by experienced radiologists who were blinded to the laboratory results.

Hepatic steatosis was graded based on ultrasonographic findings as follows: grade 1 (mild), grade 2 (moderate), and grade 3 (severe).

For the ROC analysis, hepatic steatosis was categorized into two groups: grade 1 (mild steatosis) and grade ≥2 (moderate-to-severe steatosis).[19] However, the number of radiologists was limited, and interobserver variability was not assessed, which should be considered a limitation of the study.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA).

The distribution of continuous variables was assessed using the Kolmogorov–Smirnov test and visual methods (histograms and Q–Q plots). Normally distributed variables were expressed as mean ± standard deviation, while non-normally distributed variables were presented as median (interquartile range). Categorical variables were expressed as numbers and percentages.

For comparisons between groups, the independent samples t-test was used for normally distributed variables, and the Mann–Whitney U test was used for non-normally distributed variables. Categorical variables were compared using the chi-squared test or Fisher's exact test.

The relationships between ultrasonographic hepatic steatosis grade and HOMA-IR, QUICKI, HSI, NAFLD-LFS, HbA1c, and other biochemical parameters were evaluated using Spearman's correlation analysis.

The diagnostic performance of these indices in predicting ultrasonographically detected hepatic steatosis was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) with 95% confidence intervals was calculated. Optimal cutoff values were determined using the Youden index.

Multivariate logistic regression analysis was performed to identify independent predictors of hepatic steatosis. Variables that were clinically relevant and had a p-value <0.10 in the univariate analysis were included in the model. Results were presented as odds ratios (ORs) with 95% confidence intervals.

Patients with missing data were excluded from the analysis, and a two-tailed p-value <0.05 was considered statistically significant.

Results

Patient Characteristics

A total of 206 patients were included in the study. The median age was 34 years (IQR: 27–45), and 62.6% of the patients were female. The median BMI was 42.97 kg/m² (IQR: 40.82–46.06). Ultrasonographic evaluation revealed mild steatosis in 28.2% and moderate-to-severe steatosis in 71.8% of the patients. The baseline characteristics are summarized in Table 1.

Comparison According to Steatosis Severity

Patients with moderate-to-severe steatosis had significantly higher BMI, glucose metabolism parameters, liver enzyme levels, HSI, and NAFLD-LFS values, whereas QUICKI values were significantly lower. Male sex was more frequent in this group (Table 2).

Table 1. Baseline characteristics of the study population

Variable	Total cohort (n=206)
Age, years	34 (27–45)
Female, n (%)	129 (62.6)
Male, n (%)	77 (37.4)
BMI, kg/m²	42.97 (40.82–46.06)
Length of hospital stay, days	4 (4–5)
Diabetes mellitus, n (%)	45 (21.8)
Hypertension, n (%)	19 (9.2)
Grade 1 steatosis, n (%)	58 (28.2)
Grade 2 steatosis, n (%)	122 (59.2)
Grade 3 steatosis, n (%)	26 (12.6)

BMI, body mass index; IQR, interquartile range. Continuous variables are presented as mean ± standard deviation or median (interquartile range), as appropriate. Categorical variables are presented as number (percentage).

Table 2. Comparison between patients with mild steatosis and moderate-to-severe steatosis

Variable	Grade 1 (n=58)	Grade ≥2 (n=148)	p
Age, years	29 (25–36)	36.5 (28–46)	0.002
BMI, kg/m²	41.45 (40.23–43.28)	43.94 (41.25–47.07)	<0.001
Fasting glucose, mg/dL	99.5 (87.25–110.0)	106 (91–132)	0.033
Fasting insulin, µIU/mL	21.05 (14.22–29.66)	24.39 (15.99–37.36)	0.045
HOMA-IR	5.11 (3.32–8.49)	6.38 (4.51–11.22)	0.01
QUICKI	0.302 (0.283–0.320)	0.293 (0.273–0.307)	0.01
AST, U/L	19 (17–21)	23 (19–30)	<0.001
ALT, U/L	21 (17.25–26)	30.5 (20–43.2)	<0.001
HbA1c, %	5.6 (5.4–5.9)	5.9 (5.6–6.6)	<0.001
HSI	52.85 (51.05–55.26)	55.78 (53.17–60.26)	<0.001
NAFLD-LFS	0.48 (-0.91–1.74)	1.35 (0.20–3.43)	0.001

BMI, body mass index; IQR, interquartile range; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA1c, glycated hemoglobin; HSI, hepatic steatosis index; NAFLD-LFS, nonalcoholic fatty liver disease liver fat score. Comparisons between groups were performed using the Mann–Whitney U test. Continuous variables are presented as median (interquartile range). p<0.05 was considered statistically significant.

Table 3. Correlation between steatosis grade and metabolic/biochemical parameters

Variable	Correlation coefficient (r)	p
HOMA-IR	0.244	<0.001
QUICKI	-0.244	<0.001
HSI	0.354	<0.001
NAFLD-LFS	0.297	<0.001
HbA1c	0.274	<0.001
Fasting glucose	0.170	0.015
Fasting insulin	0.195	0.005
AST	0.366	<0.001
ALT	0.354	<0.001
Cortisol	-0.002	0.982

HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; HSI, hepatic steatosis index; NAFLD-LFS, nonalcoholic fatty liver disease liver fat score; HbA1c, glycated hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Spearman correlation analysis was used. p < 0.05 was considered statistically significant.

Correlation and ROC Analysis

Steatosis grade was significantly positively correlated with HOMA-IR, HSI, NAFLD-LFS, and liver enzymes, whereas QUICKI was negatively correlated. Cortisol levels were not associated with steatosis grade (Table 3).

ROC analysis demonstrated that HSI had the highest diagnostic performance for predicting moderate-to-severe steatosis (AUC=0.716), followed by HbA1c (AUC=0.656) and NAFLD-LFS (AUC=0.645) (Table 4).

Multivariable Logistic Regression Analysis

In multivariable analysis, age (OR=1.04), BMI (OR=1.23), and NAFLD-LFS (OR=1.42) were identified as independent predictors of moderate-to-severe steatosis, whereas female sex was associated with lower odds (OR=0.34). Other indices were not independent predictors (Table 5).

Discussion

This study demonstrated that insulin resistance-related indices and biochemical parameters were significantly associated with ultrasonographic hepatic steatosis in patients with morbid obesity undergoing bariatric surgery. Patients with moderate-to-severe steatosis exhibited a markedly adverse metabolic profile, and HSI showed the highest diagnostic performance, whereas age, BMI, and NAFLD-LFS emerged as independent predictors. These findings suggest that hepatic steatosis in patients with morbid obesity represents a multifactorial metabolic condition that cannot be explained by insulin resistance alone.[20,21]

Table 4. Diagnostic performance of non-invasive indices for predicting moderate-to-severe steatosis: ROC analysis

Parameter	AUC	Cut-off	Sensitivity (%)	Specificity (%)
HOMA-IR	0.616 (0.536–0.696)	5.65	60.8	58.6
QUICKI	0.616 (0.536–0.696)	0.298	60.8	58.6
HSI	0.716 (0.642–0.790)	54.51	68.0	70.7
NAFLD-LFS	0.645 (0.565–0.724)	-0.051	79.7	43.1
HbA1c	0.656 (0.577–0.735)	5.8	63.3	62.1

AUC, area under the curve; CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; HSI, hepatic steatosis index; NAFLD-LFS, nonalcoholic fatty liver disease liver fat score; HbA1c, glycated hemoglobin. Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic performance of each parameter. p<0.05 was considered statistically significant.

Table 5. Multivariable logistic regression analysis for moderate-to-severe steatosis

Variable	OR	95% CI	p
Age	1.04	1.01–1.08	0.022
Female sex	0.34	0.15–0.78	0.011
BMI	1.23	1.03–1.46	0.020
HOMA-IR	0.92	0.80–1.06	0.243
HSI	1.02	0.88–1.17	0.807
NAFLD-LFS	1.42	1.01–2.00	0.042
HbA1c	1.31	0.86–2.00	0.214

OR, odds ratio; CI, confidence interval; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; HSI, hepatic steatosis index; NAFLD-LFS, nonalcoholic fatty liver disease liver fat score; HbA1c, glycated hemoglobin. Multivariable logistic regression analysis was performed to identify independent predictors of moderate-to-severe steatosis. $p<0.05$ was considered statistically significant.

Although ultrasonography is widely used in clinical practice, it is an operator-dependent modality with limited sensitivity, particularly in detecting mild hepatic steatosis (<20% liver fat content). Therefore, the findings of this study should be interpreted within the context of ultrasonography-based assessment and reflect ultrasonographically detected hepatic steatosis rather than histopathologically confirmed hepatic steatosis.[8,19]

Insulin resistance is widely recognized as a central mechanism in the pathogenesis of hepatic steatosis, primarily through increased lipolysis, elevated free fatty acid flux, and enhanced hepatic de novo lipogenesis. In line with previous studies, HOMA-IR and QUICKI were significantly associated with steatosis severity in our cohort.[20,22] However, the loss of significance of HOMA-IR in multivariable analysis indicates that insulin resistance alone is insufficient to explain hepatic fat accumulation in morbid obesity. Additional mechanisms, including adipocyte dysfunction, chronic low-grade inflammation, and metabolic reprogramming of hepatocytes, likely play critical roles.[23,24]

A key finding of this study is the superior diagnostic performance of HSI compared to other parameters. Composite indices appear to outperform single biochemical markers, reflecting the multidimensional nature of hepatic steatosis. The superior performance of HSI in this cohort may be attributed to the high metabolic burden associated with morbid obesity, where indices incorporating anthropometric parameters provide additional discriminative value beyond insulin resistance alone.[20,25] The inclusion of BMI and transaminase ratios in HSI likely enhances its ability to capture both metabolic burden and hepatic injury.[26,27] Similarly, NAFLD-LFS remained an independent predictor, possibly due to its incorporation of insulin resistance and metabolic syndrome components.[20] The identified cut-off value of HSI may be useful in clinical decision-making, particularly for stratifying patients according to the risk of moderate-to-severe steatosis.

Some findings should be interpreted in the context of the underlying mathematical relationships. The identical diagnostic performance of HOMA-IR and QUICKI is expected, as they are inverse transformations of the same variables. Moreover, the lack of independent predictive value of HSI despite its strong ROC performance likely reflects collinearity with BMI and liver enzymes. Likewise, HOMA-IR and HbA1c lost significance after adjustment, whereas NAFLD-LFS retained its predictive value because of its composite structure. These observations highlight the complex and interrelated nature of the metabolic determinants of hepatic steatosis.

Previous studies in the general population have frequently identified the Fatty Liver Index (FLI) as the most accurate non-invasive marker; however, in high-risk populations such as morbidly obese individuals, HSI and NAFLD-LFS have also demonstrated strong diagnostic performance.[26,28] The prominence of HSI in our cohort suggests that indices that incorporate anthropometric and hepatic parameters may better capture the spectrum of steatosis in this specific population.[21,29]

Although non-invasive indices have certain advantages, biopsy-based studies indicate that these tools may have limited accuracy in quantifying hepatic fat content at the individual level.[21,28] Therefore, such indices should be considered complementary tools for screening and risk stratification rather than replacements for histopathological or advanced imaging methods.

From a clinical perspective, our findings support the use of simple, cost-effective, and non-invasive indices in the preoperative evaluation of bariatric surgery candidates. These tools may facilitate the early identification of high-risk patients and guide the selection of individuals who require further diagnostic evaluation. These findings suggest that non-invasive indices may help reduce the need for additional imaging or invasive diagnostic procedures, such as liver biopsy, in selected bariatric surgery candidates. In clinical practice, the use of HSI may reduce the need for additional imaging or invasive evaluation in selected bariatric surgery candidates with a high probability of steatosis.[30–32]

This study has several strengths, including a relatively large sample size, a homogeneous surgical population, and a comparative evaluation of multiple indices within the same cohort. However, certain limitations should be acknowledged. The retrospective design introduces poten-

tial selection bias. The use of ultrasonography instead of liver biopsy may limit diagnostic accuracy. Additionally, the restriction of the study population to morbidly obese patients undergoing bariatric surgery limits generalizability. The absence of detailed lipid profile parameters is another limitation.

Future studies should validate these findings using prospective, multicenter designs that incorporate histopathological confirmation. The development of combined predictive models and the integration of artificial intelligence–based approaches may improve the non-invasive assessment of hepatic steatosis. Population-specific scoring systems tailored to morbid obesity also represent a promising area for further research.[24,33,34] Taken together, these results support the clinical utility of non-invasive indices as accessible tools for risk stratification in bariatric populations.

Conclusion

HSI and NAFLD-LFS demonstrated superior diagnostic performance compared with single insulin resistance markers in the assessment of ultrasonographically detected hepatic steatosis. Age, BMI, and NAFLD-LFS were identified as independent predictors of moderate-to-severe steatosis, indicating that the disease is driven by multifactorial metabolic mechanisms beyond insulin resistance alone.

These indices may serve as practical and cost-effective tools in the preoperative evaluation of bariatric surgery candidates. However, prospective, multicenter studies with histopathological confirmation are required to validate these findings. The development of combined indices and artificial intelligence–based models may further improve diagnostic accuracy.

Disclosures

Ethics Committee Approval: This study was approved by The Elazığ Fethi Sekin City Hospital Non-Interventional Clinical Research Ethics Committee (Decision No: 2026/26-01;_26.03.2026).

Informed Consent: Informed consent was waived by the Institutional Review Board due to the retrospective design.

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Case Report

Liver Transplantation Experience in Two Children Diagnosed with Abernethy Type 1B Congenital Extrahepatic Portosystemic Shunt

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Abstract

Congenital extrahepatic portosystemic shunt (CEPS), also known as Abernethy malformation, is a rare vascular anomaly characterized by diversion of portal venous blood away from the liver into the systemic circulation. Type 1 malformations are defined by the complete absence of intrahepatic portal venous flow and require liver transplantation as definitive treatment. We report two pediatric patients diagnosed with Abernethy type 1B malformation. The first patient presented with progressive cholestasis, growth retardation, and impaired liver synthetic function. Due to clinical deterioration and absence of intrahepatic portal flow, living donor liver transplantation was performed with portal vein reconstruction using an interposition graft. The second patient presented with hyperammonemia and neurocognitive impairment. Imaging confirmed type 1B CEPS, along with a focal hepatic lesion consistent with a benign regenerative nodule. The patient underwent successful living donor liver transplantation with standard portal reconstruction. Both patients had uneventful postoperative courses. During follow-up (18 and 24 months), liver function normalized and growth parameters improved, with no vascular complications observed. Liver transplantation remains the only definitive treatment for Abernethy type 1B malformation. Early diagnosis and meticulous surgical planning, particularly regarding portal inflow reconstruction, are essential for optimal outcomes.

Keywords: Abernethy malformation, liver transplantation, pediatric, portosystemic shunt

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Congenital extrahepatic portosystemic shunts (CEPS), first described by Abernethy, are rare vascular malformations in which portal venous blood bypasses the liver and drains directly into the systemic circulation.[1] These anomalies may result in significant metabolic disturbances and multisystem involvement in pediatric patients.[2,3] CEPS are classified into two main types based on the presence or absence of intrahepatic portal venous flow.[1,4] Altered hepatic perfusion may lead to a variety of radiological findings and the development of hepatic nodules.[5] In type 1 malformations, there is a complete absence of intrahepatic portal venous flow, and liver transplantation is considered the only definitive treatment.[6] In contrast, type 2 malformations involve partial shunting with preserved portal perfusion and may be managed with alternative approaches depending on anatomical and clinical features.[7] Here, we present two pediatric cases of Abernethy type 1B malformation successfully treated with living donor liver transplantation.

Case Reports

Case 1

A pediatric patient was referred with persistent jaundice, growth retardation, and biochemical evidence of impaired liver function. Laboratory evaluation revealed elevated bilirubin levels, abnormal transaminases, and decreased albumin levels, indicating impaired hepatic synthetic function.

Imaging studies, including Doppler ultrasonography and contrast-enhanced computed tomography, demonstrated absence of intrahepatic portal venous branches and a portosystemic shunt consistent with Abernethy type 1B malformation.

Given progressive cholestasis, impaired liver function, and failure to thrive, liver transplantation was indicated. A living donor liver transplantation was performed.

Surgical Technique: Portal vein reconstruction was achieved using an interposition venous graft between the superior mesenteric vein–splenic vein confluence and the donor portal vein, ensuring adequate portal inflow.

The postoperative course was uneventful. Liver function normalized within weeks, and significant catch-up growth was observed during follow-up.

Case 2

The second patient presented with hyperammonemia and neurocognitive symptoms. Laboratory tests revealed elevated serum ammonia levels with mildly abnormal liver enzymes.

Radiological evaluation confirmed Abernethy type 1B malformation with absence of intrahepatic portal venous flow. A focal hepatic lesion detected on imaging was characterized as a benign regenerative nodule, likely secondary to altered hepatic perfusion.

Due to persistent metabolic abnormalities and risk of complications, living donor liver transplantation was performed with standard portal vein reconstruction.

The postoperative course was uneventful. Serum ammonia levels normalized, and neurocognitive symptoms improved. Follow-up imaging showed no recurrence of hepatic lesions.

Discussion

Abernethy malformation is a rare but clinically significant vascular anomaly of the portal venous system.[1,4] Its anatomical characteristics and embryological basis have been further detailed in anatomical studies.[8]

Patients may present with a wide spectrum of clinical manifestations, including cholestasis, hepatic dysfunction, hepatopulmonary syndrome, and neurocognitive impairment due to hyperammonemia.[2,3] In addition, altered hepatic perfusion may predispose patients to the development of benign regenerative nodules or malignant hepatic tumors.[5]

The classification of CEPS is essential for determining management strategies. In type 1 malformations, the absence of intrahepatic portal venous flow precludes shunt closure, making liver transplantation the only definitive treatment option.[6] Additional anatomical classifications have been proposed to guide surgical planning and optimize portal inflow reconstruction.[11] Liver transplantation restores hepatic function, corrects metabolic abnormalities, and reduces the risk of malignant transformation.[12,13,14] A critical technical aspect of transplantation in these patients is the establishment of adequate portal inflow, which may require advanced surgical techniques.[11] Various surgical approaches, including the use of interposition grafts, have been described to achieve optimal portal vein reconstruction.[12,13] Our cases support previous reports demonstrating excellent outcomes following liver transplantation in pediatric patients with type 1B CEPS.[15]

Conclusion

Abernethy type 1B malformation is a rare condition that requires early diagnosis and timely intervention. Liver transplantation remains the only definitive treatment. Careful preoperative evaluation and appropriate surgical techniques, particularly for portal inflow reconstruction, are essential to achieve successful outcomes.

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Case Report

Remarkable Response to Immune Checkpoint Inhibitor Therapy in Advanced-Stage Hepatocellular Carcinoma: A Case Report

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Abstract

Advanced hepatocellular carcinoma (HCC) with extrahepatic metastases has a poor prognosis. Immune checkpoint inhibitors have recently become an important treatment option for certain types of cancer. A 58-year-old Kyrgyz male patient with a history of hepatitis B virus (HBV)-related liver cirrhosis for five years was referred to our hospital for liver transplant evaluation. However, a PET-CT (positron emission tomography-computed tomography) scan performed prior to evaluation revealed metastases in the lungs and lymph nodes in different parts of the body. Dynamic liver MRI (magnetic resonance imaging) was consistent with multicentric hepatocellular carcinoma (HCC). The patient had no history of variceal bleeding, hepatic encephalopathy, or ascites. Liver function tests were not elevated, and the baseline AFP (alpha-fetoprotein) level was 463 ng/mL, with a Child-Pugh score of 6 (A). Due to the presence of extrahepatic disease, the patient was not eligible for liver transplantation, and systemic treatment was planned. Systemic treatment was initiated with nivolumab 3 mg/kg + ipilimumab 1 mg/kg at 21-day intervals. These doses were started due to toxicity concerns. The first response evaluation showed an 80% reduction in intrahepatic disease burden and a marked reduction in the size and activity of other metastatic lesions. No side effects beyond grade 1 were observed in the patient. Thus, treatment was continued. In the second treatment response evaluation, although there was an increase in the metabolic activity of some extrahepatic lesions, the reduction in liver lesions was 50% less than the previous one. The patient's general condition was good, and he had no symptoms. The dual therapy started in July 2025 was continued in January 2026 with nivolumab 240 mg every two weeks. This case highlights an extraordinary response to dual immune checkpoint inhibition in metastatic HCC and demonstrates the potential of immunotherapy without compromising efficacy, with some dose adjustments to account for toxicity in selected patients with advanced disease.

Keywords: Case report, Hepatocellular carcinoma, Immunotherapy, Ipilimumab, Nivolumab

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The most common form of primary liver cancer is hepatocellular carcinoma (HCC), which accounts for 90% of primary liver cancer cases. HCC is the sixth most common form of cancer diagnosed worldwide and the third leading cause of cancer-related mortality. Liver cancer can also present as cholangiocarcinoma, which is a form of cancer that occurs in the bile duct.[1] HCC predominantly affects individuals with pre-existing underlying conditions of chronic liver diseases and cirrhosis. Furthermore, the incidence of HCC is considerably higher in males compared to females. There are several risk factors that are considered critical in the development of hepatocellular carcinoma. These include viral infections, metabolic syndrome, toxic substances, and immune-related disorders. In addition, hepatocellular carcinoma is also resistant to chemotherapy and radiotherapy. The Barcelona Clinic Liver Cancer (BCLC) classification is universally accepted and used for the staging and management of HCC. Based on this algorithm, curative therapies such as surgical resection, local ablation, and liver transplantation are the first choice for the treatment of early-stage HCC. For the treatment of advanced-stage HCC, locoregional therapies such as radiofrequency ablation and transarterial chemoembolization are the first choice. For those who cannot be treated with locoregional therapies, systemic therapies are recommended. Finally, for the treatment of terminal-stage HCC, the best supportive care is recommended.[2]

Until 2008, there was no systemic therapy that demonstrated a clear overall survival benefit in advanced hepatocellular carcinoma (HCC). The multi-target tyrosine kinase inhibitor sorafenib was the first systemic agent to show a survival advantage in patients with advanced HCC in randomized phase III trials, leading to its approval for use in this setting. Subsequently, in 2018, another tyrosine kinase inhibitor, lenvatinib, was demonstrated in the phase III REFLECT trial to be non-inferior to sorafenib for overall survival in first-line treatment of unresectable HCC, thereby establishing lenvatinib as another first-line systemic therapy option.[3] Until the advent of these immune checkpoint inhibitor-based therapies, the median overall survival (OS) with first-line systemic therapies for advanced hepatocellular carcinoma (HCC) was generally in the range of 12-14 months with tyrosine kinase inhibitors. With the advent of these immunotherapy-based combinations, significant improvements in survival were demonstrated. In 2020, the combination of atezolizumab and bevacizumab was approved as a first-line therapy for unresectable HCC, showing that median OS can be extended up to 19.2 months with these therapies, as demonstrated in clinical trials, compared with previous therapies. In 2022, the combination of tremelimumab and durvalumab was approved as a first-line therapy for unresectable HCC, showing that median OS can be extended up to 16.4 months with these therapies, as demonstrated in clinical trials, compared with previous therapies. These immunotherapy-based combinations have been established as first-line therapy options with median OS ranging from 16-19 months.[4,5]

Nivolumab, a PD-1 inhibitor, in combination with CTLA-4 inhibitor ipilimumab, has demonstrated a significant OS advantage over lenvatinib or sorafenib in unresectable HCC in previously untreated patients. Moreover, this drug combination has demonstrated one of the best results in terms of ORR in this patient population, as demonstrated in the phase III CheckMate-9DW clinical trial, where the nivolumab + ipilimumab regimen demonstrated a median OS of 23.7 months compared with 20.6 months with lenvatinib or sorafenib, along with an ORR of 36% compared with 13% with tyrosine kinase inhibitors.[6] The nivolumab + ipilimumab regimen has been approved by the Food and Drug Administration (FDA) as a first-line therapy for adult patients with unresectable or metastatic hepatocellular carcinoma and has been increasingly used as a new standard of care for this patient population.

Case Report

A 58-year-old male patient of Kyrgyz origin was referred to our center for evaluation for liver transplantation. The patient had been followed at an outside institution for the past five years due to hepatitis B virus (HBV)–related chronic liver disease. Dynamic contrast-enhanced computed tomography (CT) of the liver demonstrated lobulated hepatic contours consistent with chronic liver disease. Multiple space-occupying lesions compatible with hepatocellular carcinoma (HCC) were observed, the largest measuring up to 12.5 cm in diameter and almost completely occupying the right hepatic lobe. These lesions showed arterial phase hyperenhancement with venous phase washout and contained cystic and necrotic components.

^18F-FDG PET-CT revealed multiple metabolically active metastatic lymph nodes predominantly in the perihepatic and intra-abdominal regions, including the costophrenic angle and midclavicular line, as well as contrast-enhancing lesions in the lungs consistent with metastatic disease. Given the extent of the disease, the patient was not considered suitable for local ablative therapies or liver transplantation. As the radiological findings were typical for HCC, histopathological confirmation was not deemed necessary.

Baseline laboratory evaluation revealed an alpha-fetoprotein (AFP) level of 463 ng/mL. HBV DNA level was 21,395 copies/mL. Other laboratory parameters were as follows: total bilirubin 1.5 mg/dL, direct bilirubin 0.43 mg/dL, albu-

min 2.7 g/dL, prothrombin time international normalized ratio (INR) 1.0, AST 70 U/L, ALT 32 U/L, hemoglobin 12.2 g/dL, platelet count 234 $\times 10^3$/μL, and white blood cell count 14.2 $\times 10^3$/μL. The patient had a Child–Pugh score of 6 (class A) and an ALBI grade of 3. There was no prior history of hepatic encephalopathy or variceal bleeding. Physical examination revealed no evidence of ascites. The Eastern Cooperative Oncology Group (ECOG) performance status was 0. Apart from chronic hepatitis B infection, there was no significant comorbidity in the patient's medical history.

On July 10, 2025, combination immunotherapy with nivolumab at a dose of 3 mg/kg and ipilimumab at a dose of 1 mg/kg, administered every 21 days, was initiated. Approximately 2.5 months after treatment initiation, an FDG PET-CT performed on September 24, 2025, demonstrated an approximately 80% reduction in viable tumor burden, consistent with a marked partial response according to RECIST criteria (Fig. 1). At the same time, AFP had dropped to 10.5 ng/mL at the first interim evaluation. Combination therapy was therefore continued.

In the follow-up FDG PET-CT scan performed on January 22, 2026, increased metabolic activity was detected in some mediastinal lymph nodes. However, a marked reduction in the intrahepatic tumor burden persisted. The current AFP level had dropped to 6.3 ng/mL. This increase in activity in the mediastinal lymph nodes was interpreted as pseudoprogression. It was planned to continue treatment with nivolumab monotherapy at a dose of 240 mg every two weeks. No immune-related adverse events beyond grade 1 were observed during treatment. The patient received nivolumab 240 mg on January 31, 2026, without any complications (Fig. 1).

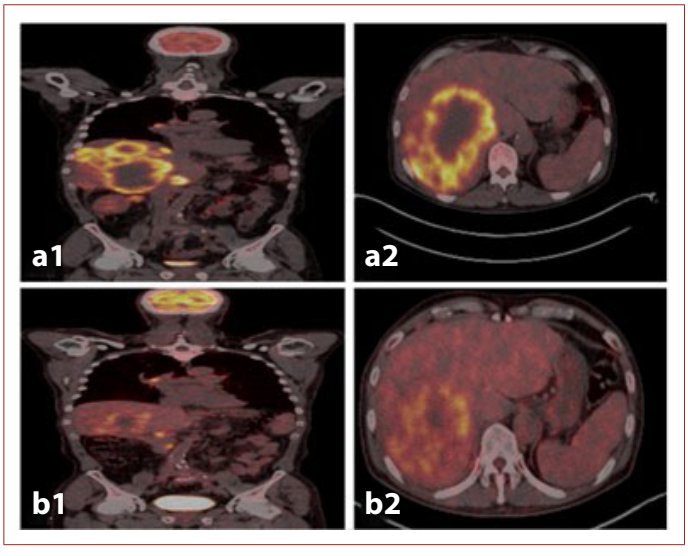

Figure 1. (a1, a2) Pre-treatment PET-CT image of the patient. **(b1, b2)** PET-CT image obtained 2,5 months after treatment.

Discussion

HCC is often asymptomatic during the early stages; therefore, the majority of cases are diagnosed at an advanced stage. Although the incidence is lower compared to other cancers, HCC ranks third among the cancers responsible for mortality worldwide. HBV infection is a major cause of HCC. Although the effectiveness of the HBV vaccine in preventing HBV-related HCC has been shown, especially in HBV-endemic areas such as East Asia, HBV remains a major cause of HCC worldwide. The management of hepatocellular carcinoma is considered challenging due to the innate drug resistance observed. Tyrosine kinase inhibitors were considered the standard treatment for patients with advanced-stage hepatocellular carcinoma until the early 2020s. However, recent treatment regimens targeting the immune system, either as monotherapy or in combination, have been established and are now considered the standard treatment regimens for the management of advanced-stage HCC. The immune system is considered an important regulator in controlling tumor progression. Liver cancer, like other cancers, uses natural physiological immune regulatory mechanisms to avoid an anti-tumor immune response by expressing immunosuppressive ligands on tumor and stromal cells. Multiple immune checkpoint pathways have been identified in this process, most notably programmed cell death protein 1 (PD-1) and its ligand PD-L1, cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte activation gene-3 (LAG-3), and several others. Dysregulation of these pathways contributes to immune tolerance within the tumor microenvironment and promotes tumor growth and progression. [7] In addition, a number of therapeutic agents targeting these immune checkpoints are currently in development. Against this background, combination immunotherapy with nivolumab, a PD-1 inhibitor, and ipilimumab, a CTLA-4 inhibitor, was initiated for our patient. This combination immunotherapy was granted regulatory approval in 2025 based on efficacy data from the CheckMate 9DW clinical trial.

In the CheckMate 9DW study, nivolumab was administered at a dose of 1 mg/kg in combination with ipilimumab at a dose of 3 mg/kg for four cycles. In the absence of disease progression, treatment was continued with nivolumab monotherapy at a fixed dose of 480 mg every four weeks. However, the incidence of treatment-related grade 3 or 4 adverse events was significantly higher in the nivolumab plus ipilimumab arm compared with the control arm, which consisted of sorafenib or lenvatinib.

The increase in toxicity among patients in the combination arm could also be explained by the increase in the dosage of ipilimumab, which is 3 mg/kg. It is worth noting that this

approach was previously tested in metastatic malignant melanoma and was included in treatment guidelines as a standard therapeutic approach. Previous studies have shown that ipilimumab is linked with low tolerability compared to nivolumab, supporting the claim that ipilimumab is a key contributor to toxicity.[8,9] In the CheckMate 9DW trial, the rationale for selecting nivolumab at a dose of 1 mg/kg in combination with ipilimumab at a dose of 3 mg/kg was based on results from a previously conducted multi-arm, multi-cohort phase II study, in which this regimen demonstrated the greatest improvement in overall survival. However, in the same study, objective response rates were found to be comparable between the nivolumab 1 mg/kg plus ipilimumab 3 mg/kg arm and the nivolumab 3 mg/kg plus ipilimumab 1 mg/kg arm.[10] Considering the toxicity profile, the treatment of the patient was initiated with nivolumab at a dose of 3 mg/kg combined with ipilimumab at a dose of 1 mg/kg administered every three weeks. After a period of six months with combination therapy, treatment with nivolumab monotherapy at a dose of 240 mg administered every two weeks was continued.

In fact, in the phase III CheckMate 9DW study, which led to this regimen being included as one of the standard treatment options, it was noted that the clinical benefits were more pronounced in patients who did not experience any disease progression or death during the initial six months of treatment. It is also important to note that there was an improvement in survival benefits, especially in those patients who experienced complete or partial responses. This is in line with our patient, who experienced a positive response within the initial six months, a response that has been ongoing. Importantly, there were no adverse effects associated with treatment, which led to its discontinuation or reduction in dosage.

One of the other key points to highlight in this case we are presenting is the ALBI and CHILD-PUGH scores. The ALBI score is an objective prognostic tool that predicts liver reserve in patients with HCC, calculated using albumin and bilirubin levels. The CHILD-PUGH score, on the other hand, is a prognostic scoring tool that combines both laboratory parameters and clinical findings. This scoring did not influence the dose selection we used in treatment. At the time of diagnosis, liver reserve was poor due to the excessive tumor burden in the liver, and the ALBI score was calculated as Grade 3. Consequently, a discordance may have arisen between the ALBI and CHILD-PUGH scores in this particular patient. In clinical practice, both scoring tools are utilized. The score changed following a good response to treatment. Initially an ALBI Grade 3, it had improved to Grade 2 at the first interim assessment. Our case demonstrates that this treatment is effective even in a patient with poor liver reserve.

Conclusion

In conclusion, the combination of nivolumab and ipilimumab represents an effective first-line systemic treatment option for selected patients with advanced-stage HCC. Moreover, individualized modifications to treatment scheduling and dosing, when applied within a reasonable clinical framework, may help preserve therapeutic efficacy while minimizing treatment-limiting toxicities, thereby improving overall treatment tolerability.

Disclosures

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Letter to the Editor

Comment on: Influence of Recipient Age on Outcomes After Liver Transplantation for Hepatocellular Carcinoma

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Dear Editor,

Any individual above the age of 65 years is defined as elderly, and it has been shown that major abdominal operations carry a high risk of morbidity and mortality when compared to patients of younger age.

Hepatocellular carcinoma (HCC) is the 6th most common cancer in both sexes and the 4th leading cause of cancer-related deaths.[1,2] HCC usually develops on a cirrhotic background, and therefore, liver transplantation (LT) is the only therapeutic modality that has the potential to cure both diseases simultaneously.[2] Diagnosis, treatment, and the course of HCC in the elderly are challenging. For this reason, we read the article by Gonultas et al.[3] with great interest. The authors analyzed 535 patients who underwent LT for HCC between April 2006 and March 2025, including 68 patients aged 65 years or older. Their data demonstrate a significant increase in elderly recipients over the last five years, rising from 2.5% (2002–2010) to 9.4% (2021–2025, p<0.001). Among HCC cases specifically, the proportion of elderly patients increased from 6.3% to 17.5% (p=0.039) over the same period.

While the survival rates in the elderly group were acceptable, they were significantly lower than those of their younger counterparts, with a mean overall survival of 2497.5 days versus 3793.5 days, respectively (p=0.012). The 1-, 5-, and 10-year survival rates for the elderly (81.5%, 52.8%, and 39.7%) were also significantly lower than those of younger patients.

A critical finding in the data provided by Gonultas et al.[3] is that 60% (n=41) of the elderly patients were beyond the Milan criteria. Of these, 28 patients (68% of advanced cases) exceeded the Malatya criteria, and 26 (63%) exceeded the Expanded Malatya criteria. Notably, 32% of these cases had a total tumor diameter exceeding 8 cm. Although the authors did not provide a specific analysis of these advanced cases—specifically regarding exact tumor diameters—the results remain acceptable despite the advanced stage of the disease. The inclusion of these advanced tumors may stem from the fact that these classifications were based on final pathology rather than pre-transplant imaging. Furthermore, the higher rate of patients beyond the Milan criteria in the elderly group may suggest a delay in diagnosis within this age group.

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The study also highlights the utility of the Malatya criteria, which incorporated an additional 13 patients beyond the Milan criteria (a ~25% expansion). In contrast, only two patients (7% of those beyond the Malatya criteria) met the Expanded Malatya criteria. In the younger cohort, 219 patients (46.9%) were beyond Milan; of these, 52 (24%) fell within the Malatya criteria and 27 (17%) within the Expanded Malatya criteria.

While these findings might suggest a difference in tumor biology or stage at diagnosis, the authors found no significant differences in AFP levels or tumor differentiation between the age groups, which argues against a distinct biological divergence. Recurrence rates were also similar, though the younger group showed higher hepatic recurrence, while the elderly showed more frequent distant metastasis. A notable limitation of the study is the lack of data regarding locoregional therapy (LRT) and subsequent response rates. Such information would have provided valuable insight into the biological behavior of HCC across these two age groups. The authors have found age to be a factor increasing the risk of mortality, and their results are supported by previous studies.[4]

Nevertheless, the treatment of HCC in patients is not governed by age. However, the outcome of LT in elderly patients has contradictory results.[5-8] Selection of elderly patients for liver transplantation should be individualized, as there are no clear selection criteria in this age group. [8] Frailty is a very important factor when treating elderly individuals. Frailty can be defined as the presence of chronic systemic diseases and nutritional deficiencies that significantly compromise the outcomes of surgery and other systemic treatments. This is particularly evident in oncological cases and major procedures such as liver transplantation.[9,10] There is not much that can be done for frail elderly patients other than offering the best supportive care.[4] It has been shown that in early-stage HCC, various treatment modalities such as local ablation or transarterial chemoembolization (TACE) resulted in comparable outcomes in elderly and non-elderly patients. Furthermore, in intermediate-stage tumors, surgery or TACE resulted in similar overall survival in both elderly and non-elderly patients.[11]

There are various differences in HCC in the elderly when compared to adolescents and young adults. Older patients are more likely to have Hepatitis C (HCV) infection or Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) (formerly NASH). In contrast, Hepatitis B (HBV) is less common in this age group.[12] Tumors in the elderly are frequently well-differentiated and may exhibit less aggressive biological behavior. They are more likely to emerge as single nodules rather than multinodular diseases. High rates of concurrent conditions such as diabetes, hypertension, and cardiovascular disease are common, which often complicate the treatment decisions. In adolescents and young adults, tumor biology is more aggressive.[12] On the other hand, the diagnosis of HCC with routine surveillance is harder in elderly patients.

Conclusion

In conclusion, the treatment of HCC requires a multidisciplinary and individualized approach, and this is especially true for elderly patients. While the majority of them are frail and best supportive care may be required, some may benefit from multimodal treatment, including liver transplantation or major hepatic resections.

Disclosures

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