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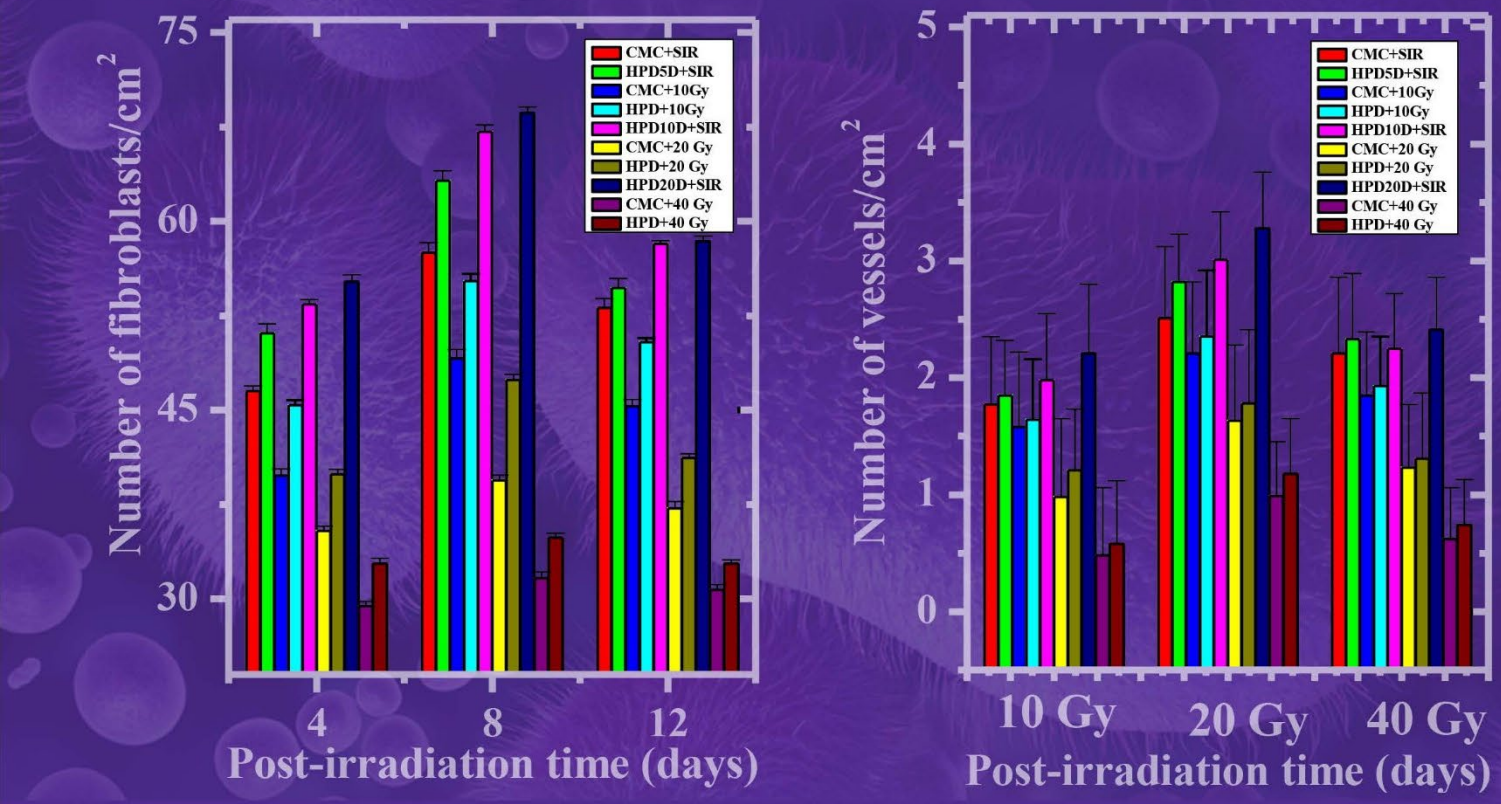

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ABOUT JCTR

Aims and scope

The Journal of Clinical and Translational Research (JCTR) is an open access, peer-reviewed, multidisciplinary scientific journal that publishes studies with at least an ex vivo, in vivo, or clinical component. The published research is centered on any clearly defined clinical problem, which may comprise a disease or the basis of disease, a form of therapy or intervention, and clinical diagnostics or prognostics. Articles (original research, reviews, technical reports, medical hypotheses, commissioned articles, special issue articles, and editorials) are published continuously online and bimonthly in print. Studies performed in cells only will generally not be accepted unless they contain critical data that are in line with the scope of the journal. Some examples of such studies include molecular pathways that lie at the basis of a disease, novel biotechnological approaches for e.g., the production of drugs, or new techniques that improve clinical diagnostics and prognostics. Articles that combine preclinical and clinical data are given priority. Contributions from academic institutions and industry are welcome.

The research areas that JCTR covers include but are not limited to:

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Nephrology
Dermatology
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Radiology and nuclear medicine
Clinical chemistry
Genetics and epigenetics
Global health
Nutrition
Immunology
Virology
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- No word count or reference restrictions
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- Rapid online publication of articles upon acceptance
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Indexing

The Journal of Clinical and Translational Research is currently indexed by Chemical Abstract Service, Google Scholar, CNKI, and Peking University Library, and is currently working towards being indexed (PubMed, Science Citation Index Expanded, BIOSIS, Scopus, etc.).

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EDITORIAL

Clinical and translational medicine in the heart of *JCTR*

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1. Introduction

The two main areas of medicine, namely clinical and translational medicine, are the primary focus of the *Journal of Clinical and Translational Research* (*JCTR*) – a journal that we wish would become the leading platform for dissemination and scientific exchange of medical discoveries and applications. The current issue features a collection of excellent articles covering the two above-mentioned areas, representative of the journal's scope. This editorial will present an overview of the papers included in the current issue, highlighting the significance of the authors' works.

2. Scope and spectrum of our journalistic activity

Rapidly developing biomedical research requires rapid worldwide dissemination, which aligns with *JCTR*'s main goal of delivering our content to the audience at the fastest speed possible. The content of our journal is very diverse since both key areas, namely clinical medicine and translational medicine, are covered by the journal. Moreover, we cover the whole spectrum of biomedical research from *in silico* and *in vitro* to *in viv*o and *ex vivo* studies conducted on both animal models and human patients. Epidemiologic studies also constitute a significant part of our articles, encompassing works at both the regional and global levels. We believe having a diverse scope, covering works from a variety of medical and clinical specialties, is our strength to meeting multiple challenges encountered at clinical settings.

3. What can we find in the current issue of *JCTR*?

The original article "*Dexamethasone in critical coronavirus disease-2019 cases: Insights from a cross-sectional study*" by Francine Johansson Azeredo and colleagues focuses on the survey of COVID-19 patients with comorbidities in Bahia, Brazil. Their findings contribute to the full description of the clinical course of SARS-CoV-2 infection.[1]

A commentary "*Reprogramming of lipid droplets by host cells as a defense mechanism against viral infection*" by Chunfu Zheng from the University of Calgary, Canada, deals with the not-yet-well-explored virus-host interactions consisting of host cells reprograming of lipid droplets as a response to viral infection. The model system referred to in this paper is the porcine reproductive and respiratory syndrome virus (PRRSV) that attacks the porcine alveolar macrophages (PAMs). This paper focuses on the role of the transcription factor YY1 in reprogramming lipid droplet content to promote resistance to viral infection.[2]

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The article "*Hesperidin enhances the repair and regeneration of full-thickness dermal wounds in mice exposed to fractionated γ-radiation*" by Ganesh Chandra Jagetia and KV.N. Mallikarjun Rao from Mizoram University, Aizawl, India, describes the effects of hesperidin on wound contraction and acceleration of mouse skin healing.[3]

"*Association between pesticide exposure and ischemic heart disease among adults who took part in the Rafsanjan Cohort Study*" is an article by Soheila Pourmasumi and colleagues from Iran, United Kingdom, and Greece. This paper presents an excellent epidemiological study correlating exposure to pesticides with cardiovascular disease.[4]

The review article "*Assessing the efficacy of cannabinoids in managing cannabis use disorder: A systematic review of randomized controlled trials with pharmacological emphasis*" by Fernanda Valeriano Zamora and colleagues from Brazil provides a review of the burning issues of cannabis use disorder and cannabis withdrawal syndrome in the face of a lack of proven effective medical treatments.[5]

In this issue, Iftekhar Mahmood from the USA contributed an article entitled "*Simple methods for predicting pediatric doses of antituberculosis medicines: Application of allometry and the Salisbury Rule,*" which presents his findings of two analytic methods used to predict doses of antituberculosis drugs for children. In light of the enormous importance of effectively combating tuberculosis on a global scale, his efforts should be of special interest to scientists and doctors engaged in delivering tuberculosis diagnoses and treatments.[6]

The article "*Prevalence and risk factors of osteoporosis in diabetic individuals above 50 years of age at a tertiary care hospital: An observational study*," on the coexisting diabetes mellitus and osteoporosis in the elderly population, is grounded in the arguments on whether diabetes mellitus affects the progression of osteoporosis. The major conclusion stemming from this paper is that patients with DM are more prone to developing osteoporosis.[7]

The first of the two case reports included in this issue "*Meropenem-induced cholestasis in a pyelonephritis patient: A case report and evaluation using the updated RUCAM scale*" by Shatavisa Mukherjee from the Calcutta School of Tropical Medicine, India, reports a case of a 57-year-old patient with a history of recurrent urinary tract infections, with a focus on the effects linked to the meropenem treatment.[8]

Another case report "*The importance of clinical and radiological follow-up after conservative treatment of iatrogenic type A aortic dissection: A case report*" by Lars Niclauss and colleagues from Switzerland focuses on a complicated case of 79-year-old patient who underwent endo-prosthetic treatment for an aneurysm. This paper highlights the importance of clinical and radiological follow-up.[9]

All of these articles will be of great interest not only for the experts in their respective fields but also for a broader audience interested in medical advancements. We hope that our readers in the medical practice will find these articles interesting and useful read.

4. Conclusion

The transfer of knowledge from the laboratory bench to the patient's bed is our mission. We want to do it in the best, professional and the fastest possible way. This issue of JCTR is just an example. It is your turn, Dear Reader, to judge our work.

Conflict of interest

Jacek Z. Kubiak is the Editor-in-Chief of this journal. The author declared that he has no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

1. Yamashita SR, Martins VLM, Patrício VISS, Noblat LACB, Azeredo FJ. Dexamethasone in Critical Coronavirus Disease-2019 Cases: Insights from a Cross-Sectional Study. *J Clin Transl Res*. 00036.

   doi: 10.36922/jctr.24.00036

2. Zheng C. Reprogramming of Lipid Droplets by Host Cells as a Defense Mechanism against Viral Infection. *J Clin Transl Res*. 00039.

   doi: 10.36922/jctr.24.00039

3. Jagetia GC, Rao KVNM. Hesperidin Enhances the Repair and Regeneration of Full-thickness Dermal Wounds in Mice Exposed to Fractionated Γ-Radiation. *J Clin Transl Res*. 00049.

   doi: 10.36922/jctr.24.00049

4. Pourmasumi S, Khalili P, Jamali Z, *et al*. Association between Pesticide Exposure and Ischemic Heart Disease among Adults who Took Part in the Rafsanjan Cohort Study. *J Clin Transl Res*. 00187.

   doi: 10.36922/jctr.22.00187

5. Yamada CM, Zamora FV, de Farias Santos ACF, *et al*. Assessing the Efficacy of Cannabinoids in Managing Cannabis use Disorder: A Systematic Review of Randomized Controlled Trials with Pharmacological Emphasis. *J Clin Transl Res*. 00066.

6. Mahmood I. Simple Methods for Predicting Pediatric Doses of Antituberculosis Medicines: Application of Allometry and the Salisbury Rule. *J Clin Transl Res*. 00070.

   doi: 10.36922/jctr.24.00070

7. Devi S, Sahu S, Subhiksha D, *et al*. Prevalence and Risk Factors of Osteoporosis in Diabetic Individuals above 50 Years of Age at a Tertiary Care Hospital: An Observational Study. *J Clin Transl Res*. 00062.

doi: 10.36922/jctr.24.00062

8. Mukherjee S. Meropenem-induced Cholestasis in a Pyelonephritis Patient: A Case Report and Evaluation using the Updated RUCAM Scale. *J Clin Transl Res*. 00072.

doi: 10.36922/jctr.24.00072

9. Niclauss L, Dulguerov F, Gunga Z, Nowacka A, Kirsch M. The Importance of Clinical and Radiological Follow-up after Conservative Treatment of Iatrogenic Type A Aortic Dissection: A Case Report. *J Clin Transl Res*. 00048.

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Journal of Clinical and Translational Research

REVIEW ARTICLE

Assessing the efficacy of cannabinoids in managing cannabis use disorder: A systematic review of randomized controlled trials with pharmacological emphasis

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Abstract

Background: Cannabis is the most used illicit drug worldwide. The increasing prevalence of cannabis use has raised concerns about the development of cannabis use disorder (CUD) and cannabis withdrawal syndrome (CWS). Thus far, however, no medications have been proven effective for treating these conditions. In this context, several cannabinoid (CB) preparations are being investigated as potential treatments. **Aim:** This systematic review aims to provide an overview of the key findings from medical CBs in the management of CUD and CWS, focusing on the efficacy of and users' tolerability to different CB formulations. **Conclusion:** The findings suggest that certain CB preparations, such as nabiximols and cannabidiol, may effectively reduce cannabis use and enhance abstinence rates, providing hope for patients struggling with CUD. Conversely, CB receptor agonists such as dronabinol and nabilone show limited therapeutic potential, indicating the need for further research to identify effective treatments for cannabis dependence. **Relevance for patients:** These findings offer potential treatment options to help patients reduce cannabis use and improve abstinence from cannabis dependence.

Keywords: Cannabis use disorder; Cannabinoids; Cannabis withdrawal syndrome

1. Introduction

Cannabis use has grown considerably at the global level in the past two decades, making it the most used illicit drug worldwide. In 2021, over 4% of the global population aged 15 to 64, roughly 218 million people reported using cannabis in the past year.[1] This increasing prevalence of cannabis use has raised concerns about potential negative

consequences, including the development of cannabis use disorder (CUD).[2]

As outlined in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-V), CUD is characterized by persistent cannabis use despite clinically significant impairment or distress, leading to functional impairment in various domains such as work, school, relationships, and daily activities.[3] Notably, it is estimated that one in five individuals who have ever used cannabis, and one in three among those who have used cannabis on a weekly basis for an extended duration develops CUD.[4] Among those who engage in recreational cannabis use, approximately 22% develop CUD.[5] Dependence is more common with earlier age of initiation and higher levels of use.[6]

Following the cessation of cannabis use, symptoms of cannabis withdrawal syndrome (CWS) would manifest within a specific time frame. According to the DSM-V,[3] CWS is characterized by the presence of at least three of the following symptoms developing within 7 days of reduced cannabis use: (1) irritability, anger, or aggression; (2) nervousness or anxiety; (3) sleep disturbance; (4) appetite or weight disturbance; (5) restlessness; (6) depressed mood; and (7) somatic symptoms, such as headaches, sweating, nausea, vomiting, or abdominal pain. While there is a common belief that marijuana carries a lower risk of physical dependency compared to other drugs, clinical evidence shows that regular marijuana smoking can lead to a specific withdrawal syndrome.[7] The estimated prevalence of CWS ranges from 11.1% to 94.2%.[7] It has been reported that CWS occurs in up to one-third of regular cannabis users in the general population and 50 – 95% of individuals among heavy users based on treatment data or research studies.[6]

The physiological processes underlying CUD involve the dysregulation of the endocannabinoid system, commonly observed in individuals with regular cannabis use. Repeated exposure to marijuana leads to notable desensitization and downregulation of cannabinoid receptor type 1 (CB1) in the limbic system and neocortex, along with reduced levels of endocannabinoids such as anandamide (AEA) and 2-arachidonoylglycerol.[8] This dysregulation manifests through the development of cravings, tolerance, and withdrawal symptoms.

Studying animals has been useful in understanding the potential mechanisms underlying and the risk factors for CWS occurrence, as rodents show both dependence and tolerance after long-term use of CBs.[9] Repeated exposure to either marijuana smoke or tetrahydrocannabinol (THC) injections in mice leads to similar physical withdrawal symptoms, such as paw tremors and head twitches. This has been linked to increased adenylyl cyclase activity in the cerebellum, which contrasts with the acute effects of CBs that typically inhibit adenylyl cyclase activity. Both CUD and CWS demonstrate moderate heritability, indicating that both genetic and environmental factors play a role in their development.[9]

In Europe, cannabis was the most cited problem drug by new treatment clients, representing 45% of all first-time treatment entrants on the continent.[10] However, the use of treatments specifically designed for CUD is relatively low. Available treatments primarily involve psychosocial interventions such as cognitive-behavioral therapy, motivational enhancement therapy, and contingency management. Nevertheless, these therapies are expensive, and the rates of abstinence are only modest and tend to decline after treatment ends.[2] Approximately 80% of individuals who undergo CWS tend to relapse due to regular cannabis use within 1 – 6 months.[11] At present, there are no medications that have been proven to be effective for treating CUD or cannabis withdrawal. Due to these findings, medical CB use as a possible treatment approach to improving endocannabinoid function has gained traction.

In this context, several CBs and CB preparations have attracted significant attention. Dronabinol (a synthetic THC), nabilone (a synthetic derivative of THC), cannabidiol (CBD), nabiximols (a 1:1 THC/CBD combination), and PF-04457845 (a synthetic compound that inhibits the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) were investigated as potential treatments for CUD and CWS.[12]

The use of CBs is associated with potential side effects and adverse events, and interactions with medications are important considerations. Understanding and addressing these risks is crucial for ensuring the safe and effective use of medical CBs in clinical practice.[12] This systematic review aims to provide a concise yet comprehensive overview of the key findings from medical CB research, specifically focusing on their pharmacological management of CUD, a major public health concern.

2. Methods

2.1. Eligibility criteria

This systematic review was performed and reported following the Cochrane Collaboration Handbook for Systematic Review of Interventions and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement guidelines. Inclusion was restricted to studies that met all the following eligibility criteria: (1) randomized trials; (2) enrolling patients

diagnosed with CUD; and (3) comparing CB receptor agonists (i.e., dronabinol and nabilone) or modulators of endocannabinoid activity (i.e., nabiximols and CBD) with placebo.

2.2. Search strategy and data extraction

We systematically searched PubMed, Scopus, and Cochrane Central Register of Controlled Trials from inception to May 2024 with the following search strategy: (cannabidiol OR CBD OR sativex OR nabiximols OR dronabinol OR nabilone OR cesamet OR FAAH OR "fatty acid amide hydrolase") AND ("cannabis use disorder" OR "cannabis dependence" OR "cannabis withdrawal"). The references from all included studies, previous systematic reviews, and meta-analyses were also searched manually for any additional studies. Two authors independently extracted the data following predefined search criteria and quality assessment.

The study protocol was registered in the International Prospective Register of Systematic Reviews on May 27, 2024, as CRD42024547431.

2.3. Endpoints

We extracted the following data from individual studies: (1) study characteristics: authors, study design, intervention, dose, route of administration, treatment duration, and follow-up days; (2) patient characteristics: gender, mean age, cannabis use in grams per day, days of use in the past 30 days, Marijuana Craving Questionnaire and Hamilton Depression scale; (3) outcomes: weekly cannabis use, cannabis withdrawal scale, cannabis cravings, number of adverse effects and 21-day consecutive abstinence.

2.4. Risk of bias and quality assessment

According to the recommendations from the Cochrane Handbook for Systematic Reviews of Interventions, we used the revised Cochrane risk-of-bias tool for randomized trials (RoB-2) to assess the risk of bias in RCTs.[13] Disagreements were resolved through consensus after discussing reasons for the discrepancy. The information was presented as a risk of bias graph and a risk of bias summary figure.

2.5. Statistical analysis

This systematic review was designed following the PRISMA.[14] The studies included in our analysis exhibited significant heterogeneity, rendering meta-analysis unfeasible. This was due to the use of disparate effect measures, including correlation coefficients, odds ratios, and regression coefficients, coupled with insufficient data to standardize effect sizes uniformly across all studies. To facilitate a comprehensive interpretation of results across studies, we opted to generate albatross plots encompassing all included studies.[15]

An albatross plot depicts a scatter plot wherein each study's sample size is plotted against its respective two-sided *P*-values derived from effect estimates. This visual representation enables the interpretation of estimated standardized effect sizes (in this case, standardized to correlation coefficients) within the context of both study size and significance level for each individual study, as well as for the collective relationship observed across all studies.[15]

To gather the necessary data for constructing these plots, we retrieved the *P*-values, sample sizes, and effect estimates from each study. In instances where studies did not explicitly provide *P*-values, we calculated them based on the sample size and effect magnitude (such as Pearson's correlation coefficient).[16]

3. Results

3.1. Study selection

The initial search yielded 1,317 results. After the removal of duplicate records and ineligible studies, 14 remained and were fully reviewed based on inclusion criteria. Of these, a total of eight studies met the final eligibility criteria. As two of the eight articles refer to one study, a total of seven studies were included in the systematic review, comprising 597 patients. The PRISMA flow diagram is illustrated in Figure 1.

3.2. Study characteristics

A total of 317 patients (53%) received CB receptor agonists (i.e., dronabinol and nabilone) or modulators of endocannabinoid activity (i.e., nabiximols and CBD), as monotherapy or in combination with another medication, with concomitant psychotherapy. All the studies included met the final eligibility criteria; however, there was significant variation in terms of population characteristics, interventions, and outcomes.

All studies were randomized, double-blind, and placebo-controlled clinical trials with a parallel design. All studies included participants with CUD, as defined by the diagnostic criteria of the DSM-IV,[17-19] DSM-IV-TR,[20,21] DSM-V[22], and the International Statistical Classification of Diseases and Related Health Problems, 10th edition (ICD-10).[23,24]

The mean age of the participants ranged from 26.4 to 37.65 years, with a male predominance of 74.2% across all the studies. Cannabis use at baseline was reported in different ways: (1) according to the number of days of use, ranging from 28 to 30 days in the past 30 days[17,18,20] or 25.7 days in the past 28 days;[23] (2) according to cannabis weight, ranging from 0.55 to 3.28 g/day.

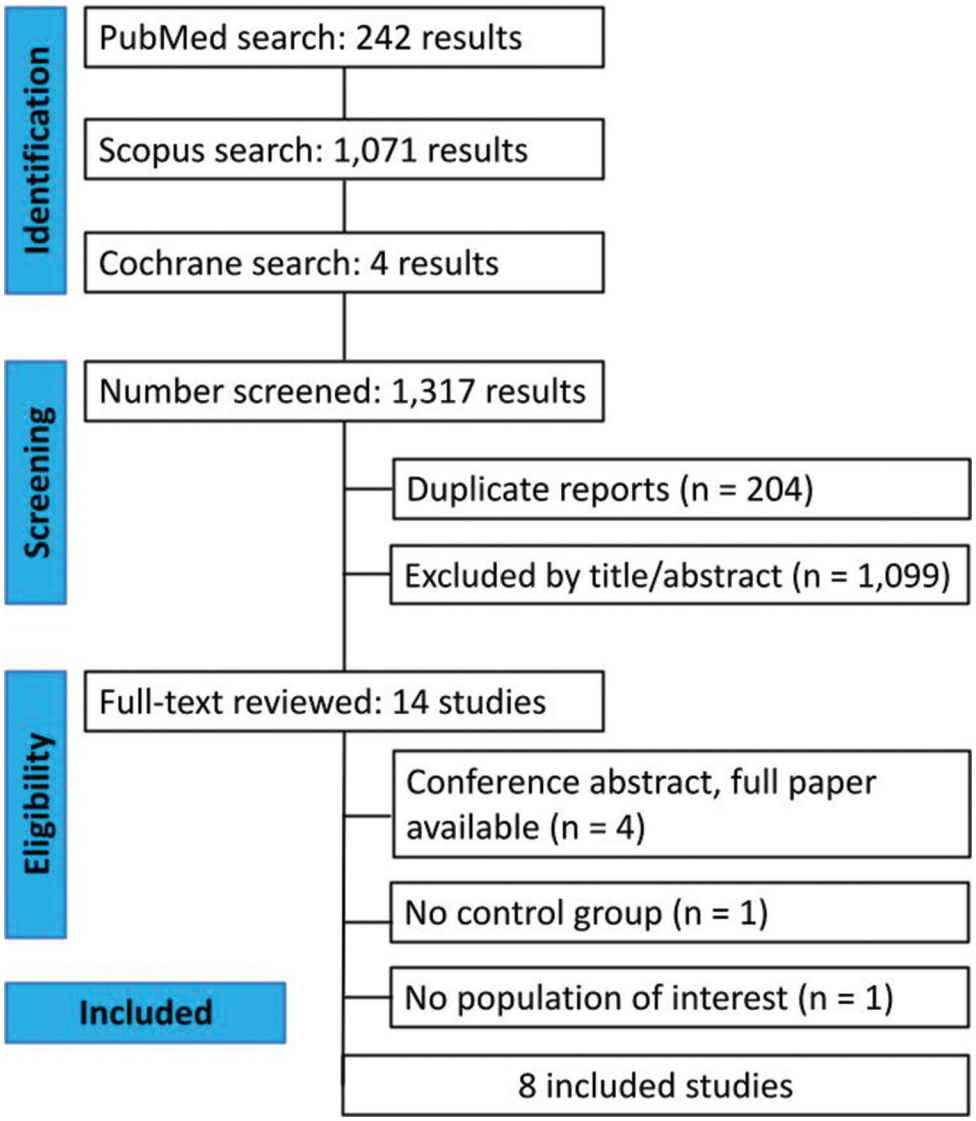

Figure 1. PRISMA flow diagram of study screening and selection

The studies used five different CB preparations as interventions: nabiximols (n = 3), CBD (n = 1), nabilone (n = 1), dronabinol (n = 1), and a combination of dronabinol and lofexidine (n = 1). All participants received supplementary psychosocial therapy during the active treatment period in all trials.

The treatment duration ranged from 9 to 84 days. One study was conducted within a 4-week assessment interval[17,18,20] and five studies had an assessment frequency of 1 – 2 times per week.[17-20,22] One study included an inpatient treatment phase of up to 9 days.[21] Two studies had a follow-up assessment after 28 days,[18,21] one trial included multiple follow-up assessments up to 168 days,[22] and one study conducted follow-up 84 days after treatment.[24] The study characteristics are summarized in Table 1.

3.3. Study outcomes

3.3.1. Cannabis use

Nabiximols significantly reduced self-reported cannabis use compared to placebo in 3 studies[19,23,24] and had no significant effect in another study. Treatments of daily CBD 400 mg and 800 mg showed greater effectiveness in reducing cannabis use over a 30-day treatment period compared to a placebo, as evidenced by a decrease in urinary THC-COOH: creatinine ratios[22]. However, the 400 mg dose decreased THC-COOH: creatinine ratio by 94.21 ng/mL, whereas the 800 mg dose decreased by 72.02 ng/mL, suggesting that the 400mg dose might be slightly more effective[22]. Nabilone and dronabinol did not affect the magnitude of cannabis use compared to placebo.[18,20]

Table 1. Baseline characteristics of the included studies

Study	Intervention	Dose[$]	Route of administration	Treatment duration	Follow-up (days)	Patients	Male, *n* (%)	Age, mean (years)	Cannabis use, mean (g/day)	Days of use[†]	MCQ score	HDS
Allsop *et al.* (2014)[21]	Nabiximols	THC 86.4 mg CBD 80 mg	Oromucosal spray	6 days 3-day washout	28	51	39 (76)	35.39	3.28	N/A	N/A	N/A
Freeman *et al.* (2020)[22]	CBD	200 mg 400 mg 800 mg	Capsules, p.o.	30 days	140	82	59 (72)	26.55	N/A	N/A	N/A	N/A
Hill *et al.* (2017)[18]	Nabilone	2 mg	Capsules, p.o.	70 days	28	18	12 (67)	26.4	N/A	28.1	51.9	N/A
Levin *et al.* (2011)[20]	Dronabinol	40 mg	Capsules, p.o.	7 days for placebo 63 days for treatment 14 days for lead-out	0	156	128 (82)	37.65	0.55	30	4.1	5.3
Levin *et al.* (2016)[17]	Dronabinol+ Lorefexidin	Dronabinol 60 mg Lofexidine 1.8 mg	Capsules, p.o.	7 days for placebo 70 days for treatment 7 days for lead-out	0	122	84 (68.8)	35.1	1.65	28	41.25	5.3
Lintzeris *et al.* (2019)[23] and Lintzeris *et al.* (2020)[24]	Nabiximols	THC 86.4 mg CBD 80 mg	Oromucosal spray	84 days	84	128	98 (76.5)	35	2.3	N/A	N/A	N/A
Trigo *et al.*, (2018)[19]	Nabiximols	THC 113.4 mg CBD 105 mg	Oromucosal spray	84 days	0	40	29 (72.5)	33	0.86	N/A	N/A	2.85

Notes: [$]Maximum dose/mg/day; [†]in the past 30 days.

Abbreviations: CBD: Cannabidiol; HDS: Hamilton Depression scale; MCQ: Marijuana Craving Questionnaire; N/A: Not available; p.o.: Per os, a Latin term meaning "by mouth"; THC: Tetrahydrocannabinol.

3.3.2. Abstinence

Only one study using nabiximols reported a higher rate of abstinence in those who received treatment.[24] Nevertheless, there was a higher proportion of participants of the nabiximols group than the placebo group of the same study sample who reported abstinence in the previous 4 weeks at the week-24 follow-up.[24]

CBD 400 mg and 800 mg increased the number of days per week with abstinence from cannabis compared to placebo, as assessed by self-reports.[22] However, 400 mg dose increased abstinence from cannabis by 0.48 days per week, whereas 800 mg increased by 0.27 days per week with no statistical significance, suggesting that 400 mg dose could be more effective.[22] Both dronabinol monotherapy and combination treatment of dronabinol with lofexidine failed to demonstrate any difference in the abstinence rates compared to placebo.[17,20]

3.3.3. Withdrawal symptoms

Only one study using nabiximols reported a reduction in withdrawal symptoms during a 6-day treatment compared to placebo.[21] Only CBD 800 mg was effective in reducing withdrawal symptoms compared to placebo.[22] Dronabinol showed a significant reduction in withdrawal symptoms during 84 days of study; however, dronabinol with lofexidine showed no significant effect on the withdrawal scores.[17,20] Nabilone did not differ from placebo in the reduction of cannabis withdrawal symptoms.[18]

3.3.4. Craving

One study with nabiximols reported a significant reduction in cannabis cravings[21] and another study also reported a reduction during the treatment, but with no statistical significance compared to placebo.[19]

Nabilone did reduce cravings during the 70-day treatment but did not affect cravings after the 28-day follow-up.

3.3.5. Retention in treatment

Only one study reported a higher rate of treatment retention using nabiximols.[21] Dronabinol also reported significantly higher retention in treatment compared to placebo. However, dronabinol combined with lofexidine did not show a difference in retention rate.

3.3.6. Adverse effects

Overall, there was no significant difference in adverse events between intervention and placebo reported in any study. No serious adverse event related to the study procedure was recorded in the intervention group. The study outcomes are summarized in Table 2.

Table 2. Outcomes of included studies

Study	Intervention	Cannabis use	Abstinence	Withdrawal symptoms	Cravings	Treatment retention	Adverse effects
Allsop *et al.* (2014)[21]	Nabiximols	=	N/A	↓	↓	↑	=
Freeman *et al.* (2020)[22]	CBD 200 mg	=	=	N/A	N/A	N/A	=
	CBD 400 mg	↓	↑	=	N/A	N/A	=
	CBD 800 mg	↓	↑	↓	N/A	N/A	=
Hill *et al.* (2017)[18]	Nabilone	=	N/A	=	=	N/A	=
Levin *et al.* (2011)[20]	Dronabinol	=	=	↓	N/A	↑	=
Levin *et al.* (2016)[17]	Dronabinol+Lofexidine	N/A	=	=	N/A	=	=
Lintzeris *et al.* (2019)[23] and Lintzeris *et al.* (2020)[24]	Nabiximols	↓	=	=	=	=	=
Trigo *et al.* (2018)[19]	Nabiximols	↓	↑	N/A	N/A	N/A	N/A
Allsop *et al.* (2014)[21]	Nabiximols	↓	=	=	↓	N/A	=

Notes: ↑, a significant increase compared to placebo; =, Non-significant effect compared to placebo; ↓, a significant reduction compared to placebo.
Abbreviations: CBD: Cannabidiol; N/A: Not available.

3.3.7. Albatross plot

To analyze the size effect of interventions on various outcomes, we utilized albatross plots, a graphical method that visually represents approximate effect sizes through superimposed contours.[15]

In Figure 2A, we present an albatross plot focusing on cannabis cravings among patients with CUD who utilized cannabis as an adjunct treatment. Most studies depicted correlations ranging between 0.25 and 0.8, indicating a moderate to strong relationship between the intervention and the reduction in cravings.[19,21,23] Notably, only one study reported correlations below 0.25, suggesting variability in the effectiveness of cannabis as an adjunct treatment for managing cravings in CUD.[18]

Conversely, Figure 2B and Figure 2C illustrate a different scenario when analyzing weekly cannabis use. Here, the albatross plot indicates no significant correlation among the studies regarding the intervention's impact on weekly cannabis consumption. This lack of correlation suggests that the effectiveness of the intervention in reducing weekly cannabis use varied widely across the studies analyzed.

In summary, albatross plots provided a nuanced visual representation of the effect sizes observed in different outcomes related to the intervention for CUD. They highlighted both the promising impact on cannabis cravings in Figure 2A and the inconsistent results in Figure 2B and Figure 2C regarding weekly cannabis use. These graphical representations are instrumental in synthesizing and interpreting findings from systematic reviews, offering insights into the efficacy of interventions across various outcome measures.

3.3.8. Risk of bias

We assessed the risk of bias in every individual trial using the Cochrane Collaboration's Risk of Bias Tool (RoB-2) in randomized controlled trials and assigned a rating of "low" or "some concerns" risk to each of the five domains (randomization, deviations from intended intervention, missing outcome data, measurement of outcome, and reported results). Based on the number of domains classified as "low risk," we also created an "overall" risk of bias. The risk of bias assessment is depicted in Figure 3.

3.3.9. GRADE assessment

The eight studies analyzed had a low risk of bias, low inconsistency, no serious indirectness, and imprecision, and were of high quality.

4. Discussion

In this systematic review with an albatross plot presentation, we aim to summarize the main findings of the efficacy and safety of CBs in the treatment of CUD. We included seven studies, comprising 597 patients, from which 317 (53%) received CB receptor agonists (nabiximols, nabilone, and dronabinol) or modulators of endocannabinoid activity (CBD).

Previous clinical trials have proved that agonist substitution therapy is an effective treatment in various substance use disorders, particularly in nicotine- and opioid-dependent patients.[25,26] For that reason, agonist treatment is also studied as a viable approach to cannabis dependence, for its ability to reduce withdrawal symptoms, including irritability, anxiety, sleep disturbances, and mood swings, which often pose significant barriers to recovery.

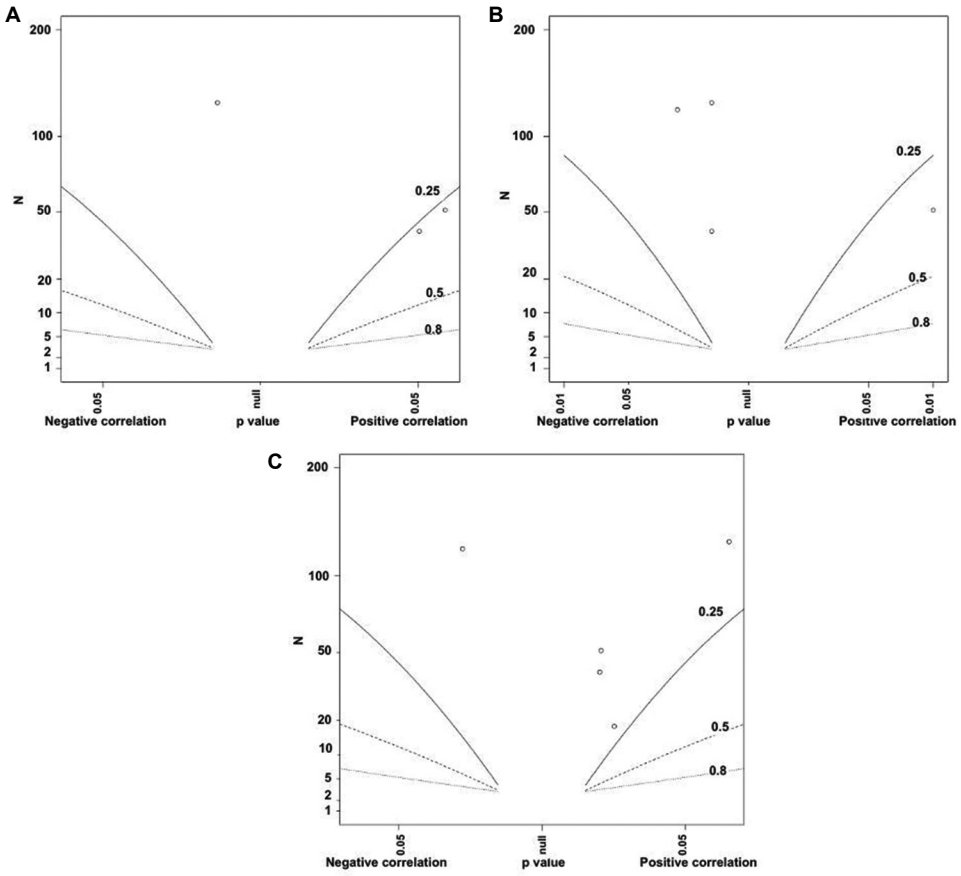

Figure 2. Albatross plot for studies of the approximate effect size of the use of cannabidiol for cannabis use disorder on outcomes: (A) cannabis cravings; (B) cannabis withdrawal scale; and (C) weekly cannabis use

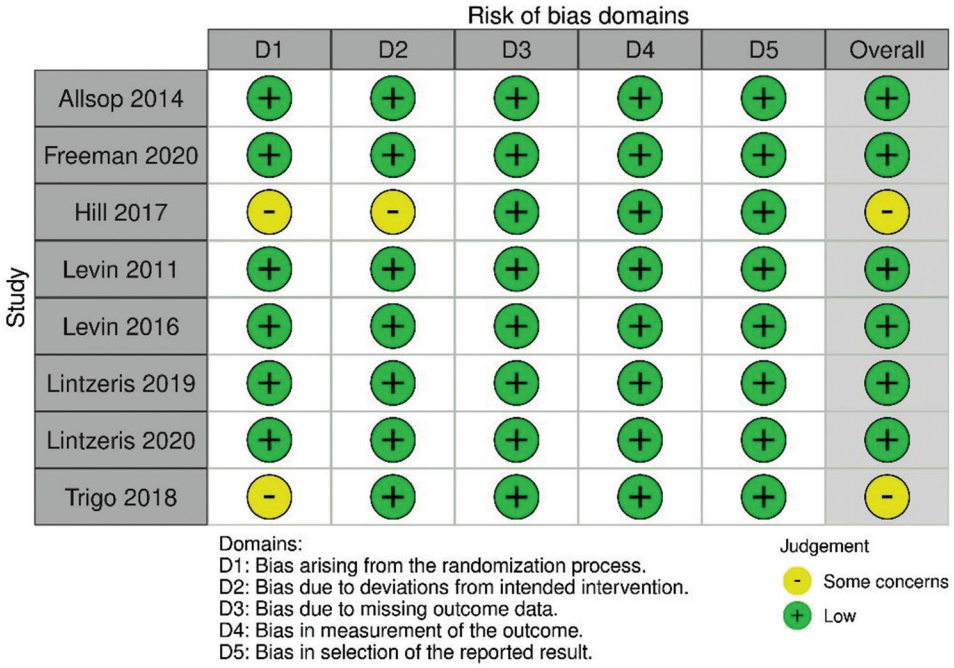

Figure 3. Risk of bias assessment of the included studies

Furthermore, by addressing cravings, these therapies might aid recovery efforts and help lower the rates of relapse.

Nabilone, a synthetic derivative of THC, and dronabinol, a synthetic form of THC, are currently used as a second-line treatment for patients with AIDS, cancer cachexia, and chemotherapy patients experiencing nausea or vomiting.[27,28] Both medications primarily act on the CB1 receptor. Nabiximols, a CB agonist containing approximately equal parts of THC and CBD, is used in the treatment of central neuropathic pain in multiple sclerosis and as an adjuvant analgesic in adults with advanced malignancy.[29] CB agonists, particularly those targeting CB1 and CB2 receptors, play a crucial role in the endocannabinoid system, influencing physiological processes such as pain sensation, mood, appetite, and memory. CB1 receptors, primarily located in the central nervous system, modulate neurotransmitter release through the inhibition of adenylyl cyclase, activation of the mitogen-activated protein kinase (MAPK) pathway, and modulation of ion channels, leading to effects such as analgesia, euphoria, and appetite stimulation. CB2 receptors, found mainly in peripheral tissues and immune cells, also inhibit adenylyl cyclase and activate the MAPK pathway, contributing to anti-inflammatory and neuroprotective effects. In the context of cannabis dependence, agonist substitution therapy leverages these mechanisms to stabilize neurotransmitter levels, which might reduce cravings, and manage withdrawal symptoms, thereby supporting recovery and reducing relapse rates.[30]

In this study, dronabinol reported significantly higher retention in treatment compared to placebo, and nabilone reduced cravings early in the treatment, but no improvements were seen after the 28-day follow-up. Nabiximols reduced self-reported cannabis use, whereas nabilone and dronabinol alone, or combined with lofexidine, did not affect the magnitude of cannabis use when compared with a placebo. Nabiximols were also effective in reducing withdrawal symptoms, reducing cannabis cravings, and had a higher rate of treatment retention. As hypothesized by Lintzeris *et al.*,[23] the significant reductions observed in cannabis cravings with nabiximols can be attributed to several factors: adjustable dosage schedule, pharmacokinetic characteristics such as higher bioavailability and quicker onset of action, and the combination of THC with CBD, which may provide benefits to nabiximols recipients.[31-34]

The modulation of the endocannabinoid system is a relatively new approach to treating CUD. CBD modulates the endocannabinoid system through a multifaceted mechanism involving indirect interactions with CB receptors, endogenous ligands, and enzymes. Unlike THC, CBD has a low affinity for CB1 and CB2 receptors but can act as an antagonist at CB1 receptors in the presence of THC, thereby mitigating its psychoactive effects. CBD enhances the signaling of the endogenous CB AEA by inhibiting its reuptake and degradation through the FAAH, leading to increased AEA levels in the brain, which contributes to its anxiolytic and anti-inflammatory effects. In addition, CBD interacts with other receptors such as transient receptor potential vanilloid-1 channel, involved in pain perception, and 5-hydroxytryptamine receptor 1A, also known as serotonin receptor 1A, which influences serotonin signaling and mood regulation. Through these interactions, CBD exerts therapeutic effects that are beneficial in treating CUD by stabilizing the endocannabinoid system, reducing cravings, and alleviating withdrawal symptoms, thereby supporting recovery and reducing relapse rates.[35]

According to our findings, CBD was effective in reducing withdrawal symptoms at a dose of 800 mg. Both 400 mg and 800 mg CBD doses lengthened the period of abstinence and reduced cannabis use, but the 400 mg dose might be slightly more effective. However, one cannot conclude that there is a dose-response association since the data are limited to only one study and should be analyzed carefully. Finally, regarding adverse events, there was no significant difference between intervention and placebo reported in any study. Overall, the treatment was well tolerated in all studies.

This study has some important limitations. First, the small number of patients included in the studies, the great heterogeneity of substances used, and the absence of data from standardized questionnaires to evaluate baseline characteristics and outcomes significantly increased bias due to selection effects. Second, there was inconsistency in follow-up periods and drug dosages across the included studies, leading to significant heterogeneity. In addition, most outcomes are self-reported, which can introduce reporting bias. However, all studies included urinary drug screening tests as a method to validate the self-report, confirming self-reporting as a reliable method in determining ongoing drug use on grounds of a strong association between the urinary test results and self-report outcomes. While our systematic review is updated and includes the albatross plot, which offers a stronger methodological approach,[36] it remains underpowered to support any specific medication, let alone clinical practice.

5. Conclusion

This systematic review found that three studies highlighted the therapeutic potential of nabiximols in reducing cannabis use. One study with nabiximols also reported

a significant reduction in cravings, and withdrawal symptoms, and an increase in treatment retention. One study suggests that CBD might reduce cannabis use and withdrawal symptoms, and increase abstinence. Other CB receptor agonists, such as dronabinol and nabilone, showed limited or no therapeutic retention. Moreover, CBs were well tolerated and may decrease cannabis use with no significant increase in adverse effects among cannabis users seeking treatment. However, the small number of studies summarized in this review need to be interpreted with caution. While there is interest in the clinical potential of CBs for this purpose, it is important to note that their effectiveness and clinical indication are not yet established. Further research is necessary to standardize assessment methods for this population, producing stronger evidence to support or not the therapeutic use of cannabis for treating CUD.

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Conflict of interest

All authors declare no relationships that could be considered conflicts of interest.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All data supporting the findings of this meta-analysis are available and can be provided by the corresponding author upon reasonable request.

References

1. UNODC. *World Drug Report 2023*. United Nations Publication; 2023. Available from: https://www.unodc.org/unodc/en/data-and-analysis/world-drug-report-2023.html [Last accessed on 2024 Aug 1].
2. Sherman BJ, McRae-Clark AL. Treatment of cannabis use disorder: Current science and future outlook. *Pharmacotherapy*. 2016;36(5):511-535.

   doi: 10.1002/phar.1747
3. American Psychiatric Association (APA). *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Washington, DC: American Psychiatric Association; 2022.
4. Leung J, Chan GCK, Hides L, Hall WD. What is the prevalence and risk of cannabis use disorders among people who use cannabis? A systematic review and meta-analysis. *Addict Behav*. 2020;109:106479.

   doi: 10.1016/j.addbeh.2020.106479
5. Dawson D, Stjepanović D, Lorenzetti V, Cheung C, Hall W, Leung J. The prevalence of cannabis use disorders in people who use medicinal cannabis: A systematic review and meta-analysis. *Drug Alcohol Depend*. 2024;257:111263.

   doi: 10.1016/j.drugalcdep.2024.111263
6. Hasin DS. US epidemiology of cannabis use and associated problems. *Neuropsychopharmacology*. 2018;43(1):195-212.

   doi: 10.1038/npp.2017.198
7. Bahji A, Stephenson C, Tyo R, Hawken ER, Seitz DP. Prevalence of cannabis withdrawal symptoms among people with regular or dependent use of cannabinoids: A systematic review and meta-analysis. *JAMA Netw Open*. 2020;3(4):e202370.

   doi: 10.1001/jamanetworkopen.2020.2370
8. Connor JP, Stjepanović D, Le Foll B, Hoch E, Budney AJ, Hall WD. Cannabis use and cannabis use disorder. *Nat Rev Dis Primer*. 2021;7(1):16.

   doi: 10.1038/s41572-021-00247-4
9. Schlosburg JE, Carlson BLA, Ramesh D, *et al*. Inhibitors of endocannabinoid-metabolizing enzymes reduce precipitated withdrawal responses in THC-Dependent mice. *AAPS J*. 2009;11(2):342-352.

   doi: 10.1208/s12248-009-9110-7
10. European Monitoring Centre for Drugs and Drug Addiction. *European Drug Report 2023*. Publications Office; 2023. Available from: https://data.europa.eu/doi/10.2810/161905 [Last accessed on 2024 Jun 22].
11. Bahji A, Meyyappan AC, Hawken ER, Tibbo PG. Pharmacotherapies for cannabis use disorder: A systematic review and network meta-analysis. *Int J Drug Policy*. 2021;97:103295.

doi: 10.1016/j.drugpo.2021.103295

12. Lees R, Hines LA, D'Souza DC, *et al*. Psychosocial and pharmacological treatments for cannabis use disorder and mental health comorbidities: A narrative review. *Psychol Med*. 2021;51(3):353-364.

doi: 10.1017/S0033291720005449

13. Sterne JAC, Savović J, Page MJ, *et al*. RoB 2: A revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019;366:l4898.

doi: 10.1136/bmj.l4898

14. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *BMJ*. 2009;339:b2535.

doi: 10.1136/bmj.b2535

15. Harrison S, Jones HE, Martin RM, Lewis SJ, Higgins JPT. The albatross plot: A novel graphical tool for presenting results of diversely reported studies in a systematic review. *Res Synth Methods*. 2017;8(3):281-289.

doi: 10.1002/jrsm.1239

16. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. *Introduction to Meta-Analysis*. 1st ed. United States: John Wiley and Sons, Ltd; 2009.

doi: 10.1002/9780470743386

17. Levin FR, Mariani JJ, Pavlicova M, *et al*. Dronabinol and lofexidine for cannabis use disorder: A randomized, double-blind, placebo-controlled trial. *Drug Alcohol Depend*. 2016;159:53-60.

doi: 10.1016/j.drugalcdep.2015.11.025

18. Hill KP, Palastro MD, Gruber SA, *et al*. Nabilone pharmacotherapy for cannabis dependence: A randomized, controlled pilot study. *Am J Addict*. 2017;26(8):795-801.

doi: 10.1111/ajad.12622

19. Trigo JM, Soliman A, Quilty LC, *et al*. Nabiximols combined with motivational enhancement/cognitive behavioral therapy for the treatment of cannabis dependence: A pilot randomized clinical trial. *PLoS One*. 2018;13(1):e0190768.

doi: 10.1371/journal.pone.0190768

20. Levin FR, Mariani JJ, Brooks DJ, Pavlicova M, Cheng W, Nunes EV. Dronabinol for the treatment of cannabis dependence: A randomized, double-blind, placebo-controlled trial. *Drug Alcohol Depend*. 2011;116(1-3):142-150.

doi: 10.1016/j.drugalcdep.2010.12.010

21. Allsop DJ, Copeland J, Lintzeris N, *et al*. Nabiximols as an agonist replacement therapy during cannabis withdrawal: A randomized clinical trial. *JAMA Psychiatry*. 2014;71(3):281-291.

doi: 10.1001/jamapsychiatry.2013.3947

22. Freeman TP, Hindocha C, Baio G, *et al*. Cannabidiol for the treatment of cannabis use disorder: A phase 2a, double-blind, placebo-controlled, randomised, adaptive Bayesian trial. *Lancet Psychiatry*. 2020;7(10):865-874.

doi: 10.1016/S2215-0366(20)30290-X

23. Lintzeris N, Bhardwaj A, Mills L, *et al*. Nabiximols for the treatment of cannabis dependence: A randomized clinical trial. *JAMA Intern Med*. 2019;179(9):1242.

doi: 10.1001/jamainternmed.2019.1993

24. Lintzeris N, Mills L, Dunlop A, *et al*. Cannabis use in patients 3 months after ceasing nabiximols for the treatment of cannabis dependence: Results from a placebo-controlled randomised trial. *Drug Alcohol Depend*. 2020;215:108220.

doi: 10.1016/j.drugalcdep.2020.108220

25. Stead LF, Perera R, Bullen C, *et al*. Nicotine replacement therapy for smoking cessation. *Cochrane Database Syst Rev*. 2012;11:CD000146.

doi: 10.1002/14651858.CD000146.pub4

26. Weiss RD. Adjunctive counseling during brief and extended buprenorphine-naloxone treatment for prescription opioid dependence: A 2-phase randomized controlled trial. *Arch Gen Psychiatry*. 2011;68(12):1238.

doi: 10.1001/archgenpsychiatry.2011.121

27. Gottschling S, Ayonrinde O, Bhaskar A, *et al*. Safety considerations in cannabinoid-based medicine. *Int J Gen Med*. 2020;13:1317-1333.

doi: 10.2147/IJGM.S275049

28. Pergolizzi JV, Taylor R, LeQuang JA, Zampogna G, Raffa RB. Concise review of the management of iatrogenic emesis using cannabinoids: Emphasis on nabilone for chemotherapy-induced nausea and vomiting. *Cancer Chemother Pharmacol*. 2017;79(3):467-477.

doi: 10.1007/s00280-017-3257-1

29. Darkovska-Serafimovska M, Serafimovska T, Arsova-Sarafinovska Z, Stefanoski S, Keskovski Z, Balkanov T. Pharmacotherapeutic considerations for use of cannabinoids to relieve pain in patients with malignant diseases. *J Pain Res*. 2018;11:837-842.

doi: 10.2147/JPR.S160556

30. Pertwee RG. The pharmacology of cannabinoid receptors and their ligands: An overview. *Int J Obes* (*Lond*). 2006;30(S1):S13-S18.

doi: 10.1038/sj.ijo.0803272

31. Morgan CJ, Freeman TP, Schafer GL, Curran HV. Cannabidiol attenuates the appetitive effects of delta 9-tetrahydrocannabinol in humans smoking their chosen cannabis. *Neuropsychopharmacology*. 2010;35(9):1879-1885.

doi: 10.1038/npp.2010.58

32. Crippa JAS, Hallak JEC, Machado-de-Sousa JP, *et al*. Cannabidiol

for the treatment of cannabis withdrawal syndrome: A case report. *J Clin Pharm Ther*. 2013;38(2):162-164.

doi: 10.1111/jcpt.12018

33. Hermann D, Sartorius A, Welzel H, *et al*. Dorsolateral prefrontal cortex N-acetylaspartate/total creatine (NAA/tCr) loss in male recreational Cannabis users. *Biol Psychiatry*. 2007;61(11):1281-1289.

doi: 10.1016/j.biopsych.2006.08.027

34. Hermann D, Schneider M. Potential protective effects of cannabidiol on neuroanatomical alterations in cannabis users and psychosis: A critical review. *Curr Pharm Des*. 2012;18(32):4897-4905.

doi: 10.2174/138161212802884825

35. Pertwee RG. The diverse CB_1 and CB_2 receptor pharmacology of three plant cannabinoids: Delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br J Pharmacol*. 2008;153(2):199-215.

doi: 10.1038/sj.bjp.0707442

36. Higgins JPT, Thomas J, Chandler J, *et al*., editors. Cochrane *Handbook for Systematic Reviews of Interventions Version 6.4*. United Kingdom: Cochrane; 2023. Available from: https://www.training.cochrane.org/handbook [Last accessed on 2024 Aug 1].

ACCSCIENCE PUBLISHING

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ORIGINAL ARTICLE

Association between pesticide exposure and ischemic heart disease among adults who took part in the Rafsanjan cohort study

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Abstract

Background: Cardiovascular disease (CVD) is recognized as a major cause of death worldwide. Sustained exposure to pesticides is a significant factor that can increase morbidity and mortality rates due to CVD. **Aims:** This study aimed to investigate the effects of pesticide exposure on CVD among adult participants in the Rafsanjan cohort study (RCS). **Methods:** Data were analyzed from 9,990 adult participants of RCS as a part of the Prospective Epidemiological Research Studies in IRAN. Data on personal habits, lifestyle, demographic characteristics, comorbidity history, physical activity, and pesticide exposure were collected via face-to-face questionnaires. The prevalence of ischemic heart disease (IHD) was assessed based on the medical history questionnaire of the participants. IHD was defined as a cardiac condition (either IHD or heart failure) diagnosed by a physician. **Results:** The mean age of participants with IHD was significantly higher than that of healthy participants ($P < 0.001$). Furthermore, the prevalence of IHD was higher in men compared to women ($P = 0.003$). In addition, the duration of pesticide exposure at home in men was significantly associated with IHD ($P = 0.047$). None of the other variables demonstrated a significant correlation with the prevalence of IHD. **Conclusion:** Pesticide exposure may increase the risk of IHD, as indicated by the results of the studied population. Implementing continuous, well-structured educational programs for individuals professionally exposed to pesticides is important. These programs should focus on personal protection and the safe use of pesticides to prevent or decrease the risk of CVDs. **Relevance for patients:** It is important for farmers, agricultural workers, and those living near agricultural areas to understand the risks that pesticides pose to cardiovascular health.

Keywords: Cardiovascular disease; Cohort study; Ischemic heart disease; Pesticides; Prospective epidemiological research studies in IRAN

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1. Introduction

Currently, pesticides are considered a pivotal tool that has been widely developed and used to meet the nutritional needs of the world's growing population. Agricultural development and the adoption of diverse pest control strategies are the main factors contributing to the increasing use of pesticides worldwide, especially in developing countries.[1] Historically, biological pesticides were applied as chemical warfare agents during the world wars. Although the use of many pesticides has been prohibited today, they have contributed to permanent environmental pollution worldwide.[2] Many pesticides degrade into several metabolites depending on their solubility and may persist in the environment for decades.[3] This issue is further compounded by the alarming rise pesticide utilization around the world, especially in industrial countries such as Iran. It has been reported that more than 3.5 million tons of pesticides have been used in 2020.[4] Table 1 illustrates the currently used pesticides worldwide and their respective modes of action.

Some studies have shown an association between pesticide exposure and an increased risk of cardiovascular disease (CVD).[5,6] Berg *et al.*[7] investigated the relationship between occupational exposure to pesticides and the incidence of CVD in Japanese American men. The results revealed that occupational pesticide exposure was associated with a higher incidence of coronary heart disease or cerebrovascular events.[7] Similarly, many agrochemical products have been linked to an increased risk of myocardial infarction (MI),[8] congestive heart failure,[7] stroke,[9] arrhythmias,[5] and sudden death[6] among pesticide factory workers and individuals exposed to pesticides.[10] A systematic review of agrochemicals also reported a close correlation between agrochemical use and CVD.[8]

According to the World Health Organization (WHO), CVD is recognized as one of the key causes of death worldwide. While many factors increase the incidence of CVD, pesticide exposure may exert harmful effects on the cardiovascular system, with evidence suggesting that higher pesticide utilization correlates with a greater risk of CVD over time.[11] In developed countries, CVD remains an important cause of mortality.[12] Iran, known as the second largest pistachio (member of the cashew family) producer in the world after the United States, heavily relies on agricultural pesticides, including organophosphates and herbicides, in its agricultural production, particularly in Rafsanjan.[13] While the biological mechanisms underlying the relationship between pesticide exposure and CVD are still unknown, further research on this topic is critical.

The purpose of this study is to determine the relationship between pesticide exposure and ischemic heart disease (IHD). While some studies have reported the negative effects of pesticides on heart health, limited data exist on the influence of personal habits, lifestyle, demographic characteristics, history of comorbidities, and physical activity in the context of pesticide exposure and heart disease. Therefore, in the present study, we investigated the effects of pesticide exposure on CVDs in the population that participated in the Rafsanjan cohort study (RCS).

2. Methods

2.1. The study population

This cross-sectional study aimed to investigate the association between pesticide exposure and the prevalence of IHD among participants aged 35 – 70 years in the RCS. RCS is part of the Prospective Epidemiological Research Studies in IRAN (PERSIAN),[14] and was launched in August 2015 in Rafsanjan, a region in southeastern Iran. The study was designed to recruit a total of 10,000 participants (2,500 from each of four pre-determined urban and suburban areas of Rafsanjan) from both genders, aged

Table 1. Types and modes of action of pesticides (according to the United States Environmental Protection Agency 2022)

Pesticide type	Mode of action
Algicides	Kill algae in lakes, canals, swimming pools, and water tanks.
Antifoulants	Kill or repel organisms that attach to underwater surfaces, such as barnacles on the boat bottoms.
Antimicrobials	Kill bacteria and viruses.
Attractants	Lure pests to a trap or bait.
Biocides	Kill microorganisms.
Biopesticides	Derived from animals, plants, bacteria, and certain minerals.
Defoliants	Cause leaves or foliage to drop from plants, usually to facilitate harvest.
Desiccants	Promote drying of living tissues, such as unwanted plant tops.
Disinfectants and sanitizers	Kill or inactivate disease-producting microorganisms on inanimate objects.
Fumigants	Produce gas or vapor intended to destroy pests, for example, in buildings or soil.
Fungicides	Kill fungi (including blights, mildews, molds and rusts).
Herbicides	Kill weeds and other plants that grow where they are not wanted.
Insecticides	Kill insects and other arthropods.
Insect growth regulators	Disrupt the molting, maturing from the pupal stage to adult, or other life processes of insects.
Microbial pesticides	Use microorganisms to kill, inhibit, or out-compete pests, including insects or other microorganism pests.
Miticides (Acaricides)	Kill mites that feed on plants and animals.
Molluscicides	Kill snails and slugs.
Nematicides	Kill nematodes on plant roots.
Ovicides	Kill egg of insects and mites.
Pheromones	Disrupt the mating of pests.
Plant growth regulators	Alter the expected growth, flowering, or reproduction rate of plants (do not include fertilizers).
Plant-incorporated substances	Substances from genetic material that has been added to the plant.
Protectants	Substances that plants produce from genetic material that has been added to the plant.
Repellents	Repel pests, including insects (such as mosquitoes) and birds.
Rodenticides	Control mice and other rodents.

35 – 70 years. The sample size was estimated by the PERSIAN Cohort Central Scientific Committee to ensure adequate statistical power. To reach the target population of 10,000 participants, the recruitment team randomly selected and invited 14,827 individuals from four pre-determined districts of Rafsanjan city using systematic clustering based on household numbers until the target sample size was reached. A total of 9,991 individuals aged 35 – 70 years from both genders willingly participated in the baseline phase of the RCS and signed informed written consent. Participants with missing data on the medical questionnaire were excluded from the study. Finally, 9,932 participants (5,310 women and 4,624 men) with completed medical questionnaires were included in the present study (Figure 1).

The study protocol was designed based on the Persian cohort study, and validated questionnaires approved by the PERSIAN Cohort Central Scientific Committee were used.[15] Ethical approval for this study was obtained from the Ethics Committee of Rafsanjan University of Medical Sciences (Ethical codes: ID: IR.RUMS.REC.1398.160).

2.2. Definition and measurements

In this study, data on participants' personal habits, lifestyle, demographic characteristics, comorbidity history, physical activity, and exposure to pesticides of the participants were collected through face-to-face questionnaires.

Smoking, alcohol drinking, and opium usage were self-reported. Participants were divided into two groups such as opium non-users and users. A participant was considered an opium user if they reported consuming opium at least once per week during the 6 months before the enrollment date.[16] Similarly, smoking was divided into three categories such as never, former, and current smokers. Alcohol drinkers included individuals who had consumed alcohol at least once in their lifetime. In addition, the prevalence of IHD was assessed based on the participants' self-reports recorded in the medical history questionnaire. IHD was

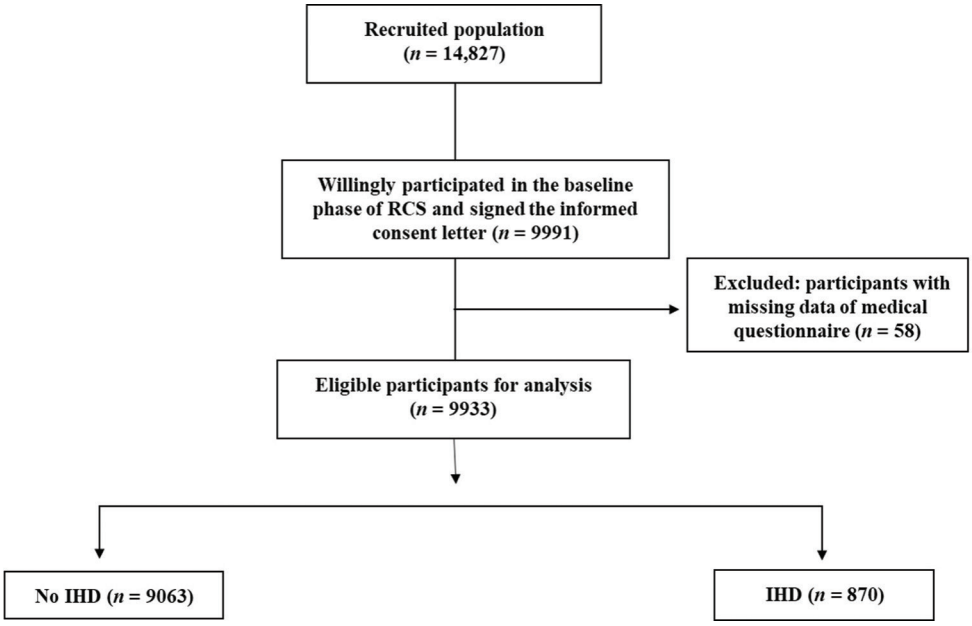

Figure 1. Flowchart of the study design of pesticides and ischemic heart disease in Rafsanjan cohort study
Abbreviations: RCS: Rafsanjan cohort study; IHD: Ischemic heart disease.

defined as a cardiac condition (IHD) diagnosed by a cardiologist.[15]

Blood samples were collected between 7:00 AM and 9:00 AM after 12 – 14 h of fasting. Serum levels of high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol, and triglycerides were measured using a CPALS analyzer (Coultronics, Margency, France) at the central laboratory of the cohort center. Dyslipidemia was defined according to the Third Report of the National Cholesterol Education Program (NCEP-Adult Treatment Panel III) as having any of the following: LDL cholesterol ≥130 mg/dL, total cholesterol ≥200 mg/dL, HDL cholesterol ≤40 mg/dL in men or ≤50 mg/dL in women, triglycerides ≥150 mg/dL, or the use of lipid-lowering drugs.[17]

Using a validated physical activity questionnaire, participants reported their usual daily activities, including the frequency and duration of time spent per week on these activities over the past 12 months. Physical activity levels were expressed as metabolic equivalent of task hours per day and categorized into three groups, including low (≤35.299), moderate (35.3 – 40.325), and high (≥40.326) based on the 25th and 75th percentiles.

The economic status of individuals was determined using the wealth score index (WSI), estimated by multiple correspondence analysis of variables such as access to a freezer, computer, internet, car, laptop, and the number of trips inside and outside Iran. Based on the 25th, 50th, and 90th percentiles, participants were categorized into four classes, including low class (≤−0.606), low-middle class (−0.607 – 0.0349), middle-high class (0.035 – 1.169), and high class (≥1.170).

Pesticide exposure was evaluated based on the detailed questionnaire that included the following parameters: proximity of the residential area to farming areas; exposure to pesticides in the farm, yard, and home; mixing and preparation of pesticides; entering work areas where pesticides had just been applied; cleaning equipment contaminated with pesticides; repairing pesticide equipment; handling stored pesticide products; and managing pesticide spraying. For each exposure, participants were asked separately about the frequency and duration of pesticide exposure and the use of personal protective equipment over the past year. The duration of each exposure was calculated in terms of minutes and categorized into three categories: no exposure, ≤mean minutes, and >mean minutes per year. The frequency differences between the total number of participants and some of the covariates were attributed to missing data.

2.3. Statistical analysis

Baseline characteristics of individuals were compared across the study groups using Chi-square (χ^2) tests for categorical variables and t-tests for continuous variables. Frequency (%) was used for categorical variables, and the mean ± standard deviation was used for the quantitative variables. In addition, dichotomous logistics regression

models were used to investigate the association between IHD and pesticide exposure. Crude and adjusted models were employed in the regression analysis. Confounding variables were identified using relevant epidemiological texts and subject matter knowledge. Potential confounding variables were sequentially entered into the models according to their hypothesized strength of association with IHD and pesticide exposure. Variables with a $P < 0.25$ were selected as confounders. The crude model was stratified by dietary antioxidant status. The adjusted model was adjusted for variables such as age (continuous), gender (male/female), education years (continuous), body mass index (BMI) (continuous), wealth status index (continuous), lifestyle-related variables (cigarette smoking, opium and alcohol consumption), physical activity level (continuous), and health conditions: diabetes (yes/no), hypertension (yes/no), and dyslipidemia (yes/no). All analyses were performed using Stata version 14. All *P*-values were two-sided.

3. Results

Table 2 shows the sociodemographic characteristics of the participants with IHD. The results showed that the mean age of males (58.13) and females (57.77) with IHD was significantly higher than that of healthy individuals ($P < 0.001$). On the other hand, the level of education and physical activity were lower in individuals with IHD compared to healthy individuals ($P < 0.001$). According to Table 2, the frequency of dyslipidemia was significantly higher in males with IHD (87.81%), and the frequency of hypertension (61.56%) and dyslipidemia (89.07%) was significantly higher in females with IHD compared to healthy individuals. In addition, the prevalence of cigarette smoking and opium consumption was significantly higher in individuals with IHD compared to those without IHD in both genders.

Table 3 demonstrates the prevalence of IHD according to pesticide exposure. Six variables showed a significant association with IHD in men, including the use of pesticides in the farm ($P = 0.032$), duration of exposure in the farm ($P = 0.020$), duration of exposure at home ($P = 0.047$), pesticide preparation ($P = 0.014$), and duration of exposure during pesticide preparation ($P = 0.039$).

In Table 4, the association between IHD and pesticide exposure is described. The odds ratios (ORs) for IHD associated with various factors in the univariate model were as follows: duration of exposure at home ≤ mean (OR 0.85, 95% CI 0.72 – 0.99), managing pesticide spraying (OR 1.27, 95% CI 1.05 – 1.54), and duration of exposure during pesticide spraying ≤mean (OR 1.26, 95% CI 1.01 – 1.06). On the other hand, the multivariate model was adjusted for confounding variables such as age, gender, education years, BMI, WSI, habits (cigarette smoking, opium and alcohol consumption), physical activity, and diabetes. According to the data in Table 4, none of the variables had a significant association with the prevalence of IHD.

4. Discussion

It is known that pesticides can exert deleterious effects on human health. The main goal of this study was to investigate the association between pesticide exposure and the prevalence of IHD in the Rafsanjan population, who participated in the Persian cohort.

The socio-demographic characteristics of the Rafsanjan population showed a significant association between the prevalence of IHD and variables, including age, sex, education, hypertension, physical activity, diabetes, dyslipidemia, smoking, and opium use. It is well known that hypertension, dyslipidemia, low physical activity, high-fat diet, and smoking are the risk factors for CVD.[18] Previous studies among farm workers have demonstrated that the risk of CVD is positively associated with older age, cigarette smoking, obesity, hypertension, and diabetes.[5,19]

Several cohort studies have assessed the association between long-term pesticide exposure and CVD.[20,21] For instance, Weichenthal *et al.*[10] in 2014 reported a positive relationship between CVD and agricultural pesticide exposure in farm workers. Recently, Rojas-Rueda *et al.*[22] reported that the rate of CVD mortality was higher in pesticide-exposure cases than that in non-pesticide-exposure cases. They also showed that pesticide exposure time was a main contributing factor to diseases including IHD, CVD, and diabetes, highlighting a positive relationship between pesticide exposure duration and CVD risk. Conversely, Dayton *et al.*[23] evaluated the association of pesticide exposure with CVD in female farm workers. They demonstrated that there was no significant relationship between pesticide exposure duration and CVD risk. However, they suggested that while pesticide exposure may not increase the risk of acute poisoning, it could still be a risk factor for CVD. In our results, after adjusting for confounding variables, none of the assessed variables had a significant association with the prevalence of IHD in women. Nevertheless, we agree with Dayton's findings that pesticide exposure could be a potential risk factor for IHD.

Zaller and Brühl[24] reported that the negative effects of pesticides were dose-dependent and correlated with exposure duration. On the other hand, various cardiovascular risk factors, including obesity, hypertension, stress, smoking, and dyslipidemia, were observed in pesticide exposure cases.[24]

Table 2. Selected characteristics related to ischemic heart diseases by gender among participants in the Rafsanjan cohort study

Characteristics	Male			Female		
	IHD (*n*=446)	No IHD (*n*=4177)	*P*-value	IHD (*n*=424)	No IHD (*n*=4886)	*P*-value
Age, years – *n* (%)			<0.001			<0.001
35 – 45	32 (7.17)	1689 (40.44)		27 (6.37)	1949 (39.89)	
46 – 55	112 (25.11)	1256 (30.07)		115 (27.12)	1577 (32.28)	
≥56	302 (67.71)	1232 (29.49)		282 (66.51)	1360 (27.83)	
Mean±SD	58.13±7.76	49.30±9.56	<0.001	57.77±7.35	49.06±9.22	<0.001
Education-*n* (%)			<0.001			<0.001
≤5 years of school	156 (34.98)	987 (23.64)		282 (66.51)	2059 (42.17)	
6–12 years of school	215 (48.21)	2302 (55.12)		123 (29.01)	2180 (44.64)	
≥13 years of school	75 (16.82)	887 (21.24)		19 (4.48)	644 (13.19)	
Mean±SD	8.20±5.11	9.65±4.87	<0.001	4.75±4.46	7.91±4.98	
Physical activity - *n* (%)			<0.001			
Low	193 (43.27)	1274 (30.49)		139 (32.78)	935 (19.14)	
Moderate	152 (34.08)	1496 (35.81)		238 (56.13)	3022 (61.85)	
Heavy	101 (22.65)	1408 (33.70)		47 (11.08)	929 (19.01)	
Mean±SD	37.71±6.49	40.26±8.52	<0.001	36.62±3.51	37.82±3.30	<0.001
BMI - *n* (%)			0.804			0.001
<25	178 (39.91)	17.5 (40.82)		51 (12.03)	934 (19.14)	
25 – 29.9	188 (42.15)	1773 (42.45)		171 (40.33)	1938 (39.70)	
≥30	80 (17.94)	699 (16.73)		202 (47.64)	2009 (41.16)	
Mean±SD	26.26±4.18	26.10±4.36	0.475	30.08±4.83	29.23±4.90	<0.001
WSI - *n* (%)			0.406			<0.001
Low	86 (19.28)	809 (19.38)		149 (35.14)	1291 (26.45)	
Low-middle	138 (30.94)	1175 (28.14)		149 (35.14)	1388 (28.44)	
Middle-high	184 (41.26)	1746 (41.82)		117 (27.59)	1928 (39.51)	
High	38 (8.52)	445 (10.66)		9 (2.12)	273 (5.59)	
Mean±SD	0.03±0.94	0.16±0.97	0.010	−0.39±0.97	−0.11±1.01	<0.001
Hypertension - *n* (%)			<0.001			<0.001
Yes	179 (40.13)	534 (12.78)		261 (61.56)	1261 (25.81)	
No	267 (59.87)	3644 (87.22)		163 (38.44)	3625 (74.19)	
Dyslipidemia - *n* (%)			<0.001			<0.001
Yes	389 (87.81)	2864 (68.91)		375 (89.07)	3620 (74.44)	
No	54 (12.19)	1292 (31.09)		46 (10.93)	1243 (25.56)	
Cigarette smoking - *n* (%)			<0.001			0.019
Current	178 (40)	2021 (48.55)		405 (95.52)	4767 (97.64)	
Former	140 (31.46)	1459 (35.05)		10 (2.36)	70 (1.43)	
Never	127 (28.54)	683 (16.41)		9 (2.12)	45 (0.92)	
Opium consumption - *n* (%)			<0.001			<0.001
Yes	272 (61.12)	1862 (44.73)		33 (7.78)	178 (3.65)	
No	173 (38.88)	2301 (55.27)		391 (92.22)	4704 (96.35)	
Alcohol consumption - *n* (%)			0.393			0.376
Yes	88 (19.78)	896 (21.52)		0 (0)	9 (0.18)	
No	357 (80.22)	3267 (78.48)		424 (100)	4873 (99.82)	

Abbreviations: IHD: Ischemic heart diseases, WSI: Wealth score index, BMI: Body mass index, SD: Standard deviation.

Table 3. Association between risk of ischemic heart diseases with pesticide exposures

Pesticide use	Male			Female		
	IHD (*n*=441)	No IHD (*n*=4162)	*P*-value	IHD (*n*=421)	No IHD (*n*=4860)	*P*-value
Use in farm - *n* (%)			0.032			0.318
Yes	149 (33.79)	1624 (39.01)		10 (2.38)	83 (1.71)	
No	292 (66.21)	2539 (60.99)		411 (97.62)	4777 (98.29)	
Duration of exposure in year (minutes) – *n* (%)			0.020			0.420
No	297 (66.59)	2554 (61.13)		414 (97.64)	4804 (98.32)	
≤Mean	124 (27.80)	1249 (29.89)		10 (2.36)	78 (1.60)	
>Mean	25 (5.61)	375 (8.98)		0 (0)	4 (0.08)	
Use in yard - *n* (%)			0.905			0.822
Yes	64 (14.51)	613 (14.72)		20 (4.75)	243 (5.00)	
No	377 (85.49)	3550 (85.28)		401 (95.25)	4618 (95.00)	
Duration of exposure in year (minutes) - *n* (%)			0.509			
No	382 (85.65)	3566 (85.35)		404 (95.28)	4643 (95.03)	
≤Mean	45 (10.09)	470 (11.25)		15 (3.54)	199 (4.07)	
>Mean	19 (4.26)	142 (3.40)		5 (1.18)	44 (0.90)	
Use at home- *n* (%)			0.063			0.385
Yes	270 (61.22)	2733 (65.67)		351 (83.37)	3970 (81.67)	
No	171 (38.78)	1429 (34.33)		70 (16.63)	891 (18.33)	
Duration of exposure in year (minutes) - *n* (%)			0.047			0.397
No	176 (39.46)	1448 (34.66)		73 (17.22)	917 (18.77)	
≤Mean	215 (48.21)	2270 (54.33)		274 (64.62)	3195 (65.39)	
>Mean	55 (12.33)	460 (11.01)		77 (18.16)	774 (15.84)	
Pesticide Preparation - *n* (%)			0.014			0.143
Yes	112 (25.40)	1292 (31.04)		10 (2.38)	71 (1.46)	
No	329 (74.60)	2870 (68.96)		411 (97.62)	4790 (98.54)	
Duration of exposure in year (minutes) - *n* (%)			0.039			
No	334 (74.89)	2887 (69.10)		414 (97.64)	4815 (98.55)	
≤Mean	93 (20.85)	1055 (25.25)		9 (2.12)	69 (1.41)	
>Mean	19 (4.26)	236 (5.65)		1 (0.24)	2 (0.04)	
Manage pesticide spraying - *n* (%)			26.08			
Yes	115 (26.08)	903 (21.69)		22 (5.23)	265 (5.45)	
No	326 (73.92)	3260 (78.31)		399 (94.77)	4596 (94.55)	
Duration of exposure in year (minutes) - *n* (%)			0.130			
No	331 (74.22)	3275 (78.39)		402 (94.81)	4621 (94.58)	
≤Mean	85 (19.06)	666 (15.94)		22 (5.19)	252 (5.16)	
>Mean	30 (6.73)	237 (5.67)		0 (0)	13 (0.27)	

Abbreviation: IHD: Ischemic heart diseases.

London *et al.*,[25] in 1998, showed that the risk of CVD was higher in the fruit production region than in other safe regions, suggesting that agricultural pesticide exposure could possibly be responsible for the high levels of IHD and mortality. Similarly, another study found that the rate of CVDs was higher in farm workers exposed to agriculture pesticides than in non-farm workers. The study highlighted that some pesticides, such as chlorpyrifos, coumaphos, and carbofuran, are acetylcholinesterase inhibitors.[26]

In an experimental study, Allon *et al.*[27] exposed rats to a pesticide that functions as an acetylcholinesterase inhibitor and assessed its effects on arrhythmias and mortality rates.

Table 4. Associations between pesticide use with ischemic heart diseases among participants in the Rafsanjan cohort study

Pesticide use	IHD	
	Univariate	Multivariate
	OR (95% CL)[a]	OR (95% CL)[b]
Use in farm		
No	1	1
Yes	0.97 (0.81 – 1.16)	0.92 (0.74 – 1.15)
Duration of exposure in year (minutes)		
No	1	1
≤Mean	1.05 (0.86 – 1.27)	0.94 (0.75 – 1.18)
>Mean	0.68 (0.45 – 1.03)	0.74 (0.47 – 1.18)
Use in yard		
No	1	1
Yes	1.03 (0.81 – 1.30)	0.98 (0.76 – 1.27)
Duration of exposure in year (minutes)		
No	1	1
≤Mean	0.94 (0.71 – 1.23)	0.87 (0.65 – 1.18)
>Mean	1.35 (0.88 – 2.07)	1.36 (0.85 – 2.17)
Use at home		
No	1	1
Yes	0.89 (0.76 – 1.04)	1.09 (0.92 – 1.29)
Duration of exposure in year (minutes)		
No	1	1
≤Mean	0.85 (0.72 – 0.99)	1.03 (0.87 – 1.23)
>Mean	1.02 (0.81 – 1.27)	1.24 (0.97 – 1.58)
Pesticide preparation		
No	1	1
Yes	0.93 (0.76 – 1.13)	0.91 (0.72 – 1.15)
Duration of exposure in year (minutes)		
No	1	1
≤Mean	0.93 (0.75 – 1.16)	0.89 (0.70 – 1.14)
>Mean	0.87 (0.54 – 1.37)	0.93 (0.56 – 1.53)
Managed pesticide spraying		
No	1	1
Yes	1.27 (1.05 – 1.54)	1.05 (0.84 – 1.30)
Duration of exposure in year (minutes)		
No	1	1
≤Mean	1.26 (1.01 – 1.06)	1.07 (0.84 – 1.36)
>Mean	1.29 (0.88 – 1.90)	0.93 (0.60 – 1.43)

Notes: [a]The univariate model is stratified on the status of pesticide use. [b]The multivariate is adjusted for the following confounding variables: age (continuous), gender (male/female), education years (continuous), BMI (continuous), wealth status index (continuous), habits (cigarette smoking, opium, and alcohol consumption), physical activity level (continuous), diabetes (yes/no), hypertension (yes/no), dyslipidemia (yes/no).
Abbreviations: IHD: Ischemic heart disease; OR: Odd ratio; CL: Confidence level; BMI: Body mass index.

They found that one-third of the rats died immediately after pesticide exposure, and several arrhythmias occurred 6 months later. Their findings confirmed that pesticides can act as acetylcholinesterase inhibitors, causing severe detrimental effects.

The human body can be exposed to pesticides through four common transmission routes such as skin, inhalation, ingestion, and eyes. Skin absorption, the main method of pesticide entry into the body, depends on factors such as the type of pesticide, exposure duration, skin health, moisture, and temperature. After inhalation, pesticides are rapidly transferred to the lungs, then into the bloodstream, where they circulate throughout the whole body. Ingestion of pesticides is not common, but it may occur accidentally. Like the skin, the eyes can also absorb pesticides during farm work.[9]

There are different mechanisms for the detoxification of pesticides, though pesticides infiltrate the human body in several ways. Sekhotha *et al.*[8] hypothesized that the skin is an important system for absorbing pesticides and agrochemicals. Once absorbed by the skin, pesticide particles can be transferred to the blood circulatory system and induce CVDs. In addition, inhalation of pesticide particles, often present in the air around farmworkers, is another route of entrance. In the respiratory system, particles of pesticides can adhere to the endothelial surface and migrate into the bloodstream, potentially lead to defects in prothrombotic and coagulant systems, fluctuations in blood pressure, artery inflammation, and increased oxidative stress. This cascade of effects may result in arrhythmias, atherosclerosis, and MI.[28] Furthermore, it is not only farm workers who are at risk of pesticide exposure but also their families, as they usually live near farms and may be exposed to contaminated shoes, gloves, and clothing. Thus, farm workers face different ways of pesticide exposure, making protective measures essential for farm workers. In the present study, we showed that pesticide spraying can increased the risk of IHD by 27% in the crude model. However, after adjusting for confounding variables, the association was not statistically significant.

A study published in 2017 highlighted that toxins could accumulate in adipose tissue, as they can aggregate into the fat and adipose cells.[29] Hence, based on our knowledge, some pesticides are fat-soluble, and this lipophilic characteristic is harmful to the human body. Pesticide droplets may aggregate when surrounded by lipid droplets, potentially leads to an elevated inflammatory response. This inflammation can have negative effects on the cardiac system.

Steenland *et al.*[30] demonstrated that pesticides can persist in the blood circulatory system for extended

periods, allowing them to circulate throughout the body and pose risks to the liver, heart, and other tissues. They evaluated both the short- and long-term side effects of pesticide exposure in farm workers, revealing that prolonged exposure had the most harmful effects to the heart and lungs. Interestingly, researchers speculated that pesticides may induce inflammation, leading to increased plasma fibrinogen levels.[30]

Arnal *et al*.[31] assessed the role of heavy metals in patients with Alzheimer's and Parkinson's diseases, demonstrating that heavy metals increase the level of ceruloplasmin in plasma, thereby increasing total oxidative stress. Recent research has documented that ceruloplasmin is a promoter and risk factor for coronary heart disease development.[32] These further suggest that farm workers exposed to pesticides, coupled with increased plasma ceruloplasmin levels, may be responsible for IHD development. This hypothesis aligns with the observed increase in IHD among farmers exposed to pesticides in our study population.

Another study[33] revealed a direct relationship between pesticide exposure, lower activity of diazoxonase, and higher levels of oxidized LDL. In addition, there was a direct association between pesticide exposure and decreased activity of paraoxonase (PON1).[33] PON1 is a mediator to hydrolyze pesticides and can protect the body from their harmful effects.[34] Therefore, low PON1 activity in pesticide-exposed farm workers is related to a higher prevalence of IHD and other CVDs.

Pesticides can potentially be a risk factor for peripheral vascular disease.[35] The association between pesticides and atherosclerosis was studied by Velmurugan *et al*.,[36] who identified pesticides as a potent risk factor for peripheral arterial disease. Furthermore, Aminov *et al*.[37] demonstrated that pesticides are directly involved in the increased synthesis of lipids, including cholesterol and triglycerides, which are the substances that constitute the main risk factors for CVD.[38] This suggests that some pesticides may be directly involved in CVD development, particularly with chronic exposure.

Researchers have hypothesized a mechanism for CVD development in cases of chronic pesticide exposure. They suggested that the estrogenic characteristic of pesticides and their ability to modify lipid metabolism contribute to CVD development.[39] Pesticides bind to lipoproteins, leading to changes in lipid profiles, such as increased LDL levels in serum.[40] These changes lead to inflammation and atherothrombosis, both of which are identified as risk factors for CVD. Furthermore, a positive relationship has been reported between pro-inflammatory interleukins (ILs), including IL-6 and IL-10, and CVD.[37] Therefore, pesticides can induce inflammation in human tissues, potentially resulting in IHD and CVD.

Consistent with our findings, other studies have also shown a link between pesticide exposure and IHD. The categorization of pesticides, exposure conditions, physical activity levels, biological parameters, and individual susceptibility are important factors for the incidence of CVD. Consequently, additional epidemiological studies are needed to evaluate the effects of different pesticides on CVD.

5. Study limitations

One of the limitations of our study is the lack of measurement of pesticide level in the participants. Moreover, the lack of a detailed questionnaire to determine the type of pesticides is an additional disadvantage. However, the advantages are the cohort-based study that can improve the quality, the number of participants, the detailed questionnaire about diseases and pesticide use, and a well-trained medical team.

6. Conclusion

Our findings demonstrate that pesticide exposure can increase the risk of IHD in the studied cohort population. In particular, farm workers bear a considerable burden of disease and morbidity, potentially due to the hard working nature of the agricultural sector. The widespread use of pesticides by farm workers, combined with the lack of accurate reporting on the prevalence of cardiovascular-caused mortality, poses a serious concern for public health professionals.

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Conflict of interest

The authors have no conflict of interest to declare.

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Ethics approval and consent to participate

This work has been approved by the Ethics Committee of Rafsanjan University of Medical Sciences (Ethical codes: ID: IR.RUMS.REC.1398.160). All participants signed written informed consent letter in the baseline phase of Rafsanjan cohort study.

Consent for publication

All participants gave consent to publish their data.

Availability of data

Data are available from the corresponding author upon reasonable request.

References

1. Singh BK, Walker A, Morgan JA, Wright DJ. Biodegradation of Chlorpyrifos by Enterobacter Strain B-14 and Its Use in Bioremediation of Contaminated Soils. *Appl Environ Microbiol.* 2004;70:4855-63.

   doi: 10.1128/AEM.70.8.4855-4863.2004

2. Delfino RT, Ribeiro TS, Figueroa-Villar JD. Organophosphorus Compounds As Chemical Warfare Agents: A Review. *J Braz Chem Soc*. 2009;20:407-28.

   doi: 10.1590/S0103-50532009000300003

3. Navarro S, Vela N, Navarro G. An Overview On the Environmental Behaviour of Pesticide Residues in Soils. *Span J Agric Res.* 2007;5:357-75.

4. Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GP, Handa N, *et al*. Worldwide Pesticide Usage and Its Impacts On Ecosystem. *SN Appl Sci.* 2019;1:1446.

   doi: 10.1007/s42452-019-1485-1

5. Zago AM, Faria NM, Fávero JL, Meucci RD, Woskie S, Fassa AG. Pesticide Exposure and Risk of Cardiovascular Disease: A Systematic Review. *Glob Public Health.* 2020;17:3944-66.

   doi: 10.1080/17441692.2020.1808693

6. Anakwue R. Cardiotoxicity of Pesticides: Are Africans At Risk? *Cardiovasc Toxicol*. 2019;19:95-104.

   doi: 10.1007/s12012-018-9486-7

7. Berg ZK, Rodriguez B, Davis J, Katz AR, Cooney RV, Masaki K. Association between Occupational Exposure to Pesticides and Cardiovascular Disease Incidence: The Kuakini Honolulu Heart Program. *J Am Heart Assoc*. 2019;8:e012569.

   doi: 10.1161/JAHA.119.012569

8. Sekhotha M, Monyeki K, Sibuyi M. Exposure to Agrochemicals and Cardiovascular Disease: *A Review. Int J Environ Res Public Health*. 2016;13:229.

   doi: 10.3390/ijerph13020229

9. Adeyemi JA, Ukwenya VO, Arowolo OK, Olise CC. Pesticides-induced Cardiovascular Dysfunctions: Prevalence and Associated Mechanisms. *Curr Hypertens Rev*. 2021;17:27-34.

   doi: 10.2174/1573402117666210111102508

10. Weichenthal S, Villeneuve PJ, Burnett RT, van Donkelaar A, Martin RV, Jones RR, *et al*. Long-term Exposure to Fine Particulate Matter: Association with Nonaccidental and Cardiovascular Mortality in the Agricultural Health Study Cohort. *Environ Health Perspect*. 2014;122:609-15.

    doi: 10.1289/ehp.1307277

11. Tuangratananon T, Wangmo S, Widanapathirana N, Pongutta S, Viriyathorn S, Patcharanarumol W, *et al*. Implementation of National Action Plans On Noncommunicable Diseases, Bhutan, Cambodia, Indonesia, Philippines, Sri Lanka, Thailand and Viet Nam. *Bull World Health Organ*. 2019;97:129-41.

    doi: 10.2471/BLT.18.220483

12. Wahab A, Hod R, Ismail NH, Omar N. The Effect of Pesticide Exposure On Cardiovascular System: A Systematic Review. *Int J Community Med Public Health*. 2016;3:1-10.

    doi: 10.18203/2394-6040.ijcmph20151542

13. Mehryar S, Sliuzas R, Sharifi A, Van Maarseveen MF. The Water Crisis and Socio-ecological Development Profile of Rafsanjan Township, Iran. *WIT Trans Ecol Environ*. 2015;199:271-85.

    doi: 10.2495/RAV150231

14. Poustchi H, Eghtesad S, Kamangar F, Etemadi A, Keshtkar AA, Hekmatdoost A, *et al*. Prospective Epidemiological Research Studies in Iran (The PERSIAN Cohort Study): Rationale, Objectives, and Design. *Am J Epidemiol*. 2018;187:647-55.

    doi: 10.1093/aje/kwx314

15. Hakimi H, Ahmadi J, Vakilian A, Jamalizadeh A, Kamyab Z, Mehran M, *et al*. The Profile of Rafsanjan Cohort Study. *Eur J Epidemiol*. 2021;36:243-52.

    doi: 10.1007/s10654-020-00668-7

16. Khalili P, Ayoobi F, Mohamadi M, Jamalizadeh A, La Vecchia C, Esmaeili-Nadimi A. Effect of Opium Consumption On Cardiovascular Diseases-A Cross-sectional Study Based On Data of Rafsanjan Cohort Study. *BMC Cardiovasc Disord*. 2021;21:2.

doi: 10.1186/s12872-020-01788-4

17. Kazemi M, Bazyar M, Naghizadeh MM, Dehghan A, Rahimabadi MS, Chijan MR, *et al*. Lipid Profile Dysregulation in Opium Users Based on Fasa PERSIAN Cohort Study Results. *Sci Rep*. 2021;11:12058.

    doi: 10.1038/s41598-021-91533-4

18. Krist AH, Davidson KW, Mangione CM, Barry MJ, Cabana M, Caughey AB, *et al*. Behavioral Counseling Interventions to Promote A Healthy Diet and Physical Activity for Cardiovascular Disease Prevention in adults with Cardiovascular Risk Factors: US Preventive Services Task Force Recommendation Statement. *JAMA*. 2020;324:2069-75.

    doi: 10.1001/jama.2020.21749

19. Linde K, Wright CY, Du Plessis JL. Subjective and Objective Skin Colour of a Farmworker Group in the Limpopo Province, South Africa. *Skin Res Technol*. 2020;26:923-31.

    doi: 10.1111/srt.12895

20. Sandoval-Insausti H, Chiu YH, Wang YX, Hart JE, Bhupathiraju SN, Mínguez-Alarcón L, *et al*. Intake of Fruits and Vegetables According to Pesticide Residue Status in Relation to All-cause and Disease-specific Mortality: Results from Three Prospective Cohort Studies. *Environ Int*. 2022;159:107024.

    doi: 10.1016/j.envint.2021.107024

21. Chen Y, Graziano JH, Parvez F, Liu M, Slavkovich V, Kalra T, *et al*. Arsenic Exposure from Drinking Water and Mortality from Cardiovascular Disease in Bangladesh: Prospective Cohort Study. *BMJ*. 2011;342:d2431.

    doi: 10.1136/bmj.d2431

22. Rojas-Rueda D, Morales-Zamora E, Alsufyani WA, Herbst CH, AlBalawi SM, Alsukait R, *et al*. Environmental Risk Factors and Health: An Umbrella Review of Meta-analyses. Int J Environ Res Public Health. 2021;18:704.

    doi: 10.3390/ijerph18020704

23. Dayton SB, Sandler DP, Blair A, Alavanja M, Freeman LE, Hoppin JA. Pesticide Use and Myocardial Infarction Incidence among Farm Women in the Agricultural Health Study. *J Occup Environ Med*. 2010;52:693-7.

    doi: 10.1097/JOM.0b013e3181e66d25

24. Zaller JG, Brühl CA. Non-target Effects of Pesticides On Organisms Inhabiting Agroecosystems. *Front Environ Sci*. 2019;7:75.

    doi: 10.3389/fenvs.2019.00075

25. London L, Nell V, Thompson M, Myers J. Health Status among Farm Workers in the Western Cape--collateral Evidence from A Study of Occupational Hazards. *S Afr Med J*. 1998;88:1096-101.

26. Stiernström E-L, Holmberg S, Thelin A, Svärdsudd K. A Prospective Study of Morbidity and Mortality Rates among Farmers and Rural and Urban Nonfarmers. *J Clin Epidemiol*. 2001;54:121-6.

    doi: 10.1016/s0895-4356(00)00287-0

27. Allon N, Rabinovitz I, Manistersky E, Weissman BA, Grauer E. Acute and Long-lasting Cardiac Changes Following A Single Whole-body Exposure to Sarin Vapor in Rats. *Toxicol Sci*. 2005;87:385-90.

    doi: 10.1093/toxsci/kfi263

28. Gress S, Lemoine S, Séralini GE, Puddu PE. Glyphosate-based Herbicides Potently Affect Cardiovascular System in Mammals: Review of the Literature. *Cardiovasc Toxicol*. 2015;15:117-26.

    doi: 10.1007/s12012-014-9282-y

29. Jackson E, Shoemaker R, Larian N, Cassis L. Adipose Tissue As A Site of Toxin Accumulation. *Compr Physiol*. 2017;7:1085-135.

    doi: 10.1002/cphy.c160038

30. Steenland K, Dick RB, Howell RJ, Chrislip DW, Hines CJ, Reid TM, *et al*. Neurologic Function among Termiticide Applicators Exposed to Chlorpyrifos. *Environ Health Perspect*. 2000;108:293-300.

    doi: 10.1289/ehp.00108293

31. Arnal N, Cristalli DO, de Alaniz MJ, Marra CA. Clinical Utility of Copper, Ceruloplasmin, and Metallothionein Plasma Determinations in Human Neurodegenerative Patients and their First-degree Relatives. *Brain Res*. 2010;1319:118-30.

    doi: 10.1016/j.brainres.2009.11.085

32. Arenas de Larriva AP, Limia-Pérez L, Alcalá-Díaz JF, Alonso A, López-Miranda J, Delgado-Lista J. Ceruloplasmin and Coronary Heart Disease-A Systematic Review. *Nutrients*. 2020;12:3219.

    doi: 10.3390/nu12103219

33. Lozano-Paniagua D, Gómez-Martín A, Gil F, Parrón T, Alarcón R, Requena M, *et al*. Activity and Determinants of Cholinesterases and Paraoxonase-1 in Blood of Workers Exposed to Non-cholinesterase Inhibiting Pesticides. *Chem Biol Interact*. 2016;259:160-7.

    doi: 10.1016/j.cbi.2016.04.008

34. Mahrooz A, Mackness M, Bagheri A, Ghaffari-Cherati M, Masoumi P. The Epigenetic Regulation of Paraoxonase 1 (PON1) As An Important Enzyme in HDL Function: The Missing Link between Environmental and Genetic Regulation. *Clin Biochem*. 2019;73:1-10.

    doi: 10.1016/j.clinbiochem.2019.07.010

35. Wang CH, Hsiao CK, Chen CL, Hsu LI, Chiou HY, Chen SY, *et al*. A Review of the Epidemiologic Literature On the Role of Environmental Arsenic Exposure and Cardiovascular

Diseases. *Toxicol Appl Pharmacol*. 2007;222:315-26.

doi: 10.1016/j.taap.2006.12.022

36. Velmurugan G, Swaminathan K, Mohanraj S, Dhivakar M, Veerasekar G, Alexander T, *et al*. Association of Co-accumulation of Arsenic and Organophosphate Insecticides with Diabetes and Atherosclerosis in A Rural Agricultural Community: KMCH-NNCD-I Study. *Acta Diabetol*. 2020;57:1159-68.

doi: 10.1007/s00592-020-01516-6

37. Aminov Z, Haase RF, Pavuk M, Carpenter DO. Analysis of the Effects of Exposure to Polychlorinated Biphenyls and Chlorinated Pesticides on Serum Lipid Levels in Residents of Anniston, *Alabama. Environ Health*. 2013;12:108.

doi: 10.1186/1476-069X-12-108

38. Morselli E, Santos RS, Criollo A, Nelson MD, Palmer BF, Clegg DJ. The Effects of Oestrogens and their Receptors on Cardiometabolic Health. *Nat Rev Endocrinol*. 2017;13:352-64.

doi: 10.1038/nrendo.2017.12

39. Wafa T, Nadia K, Amel N, Ikbal C, Insaf T, Asma K, *et al*. Oxidative Stress, Hematological and Biochemical Alterations in Farmers Exposed to Pesticides. *J Environ Sci Health B*. 2013;48:1058-69.

doi: 10.1080/03601234.2013.824285

40. Zhang B, Li XL, Zhao CR, Pan CL, Zhang Z. Interleukin-6 As A Predictor of the Risk of Cardiovascular Disease: A Meta-analysis of Prospective Epidemiological Studies. *Immunol Invest*. 2018;47:689-99.

doi: 10.1080/08820139.2018.1480034

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ORIGINAL ARTICLE

Dexamethasone in critical coronavirus disease-2019 cases: Insights from a cross-sectional study

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Abstract

Objective: The objective of the study is to describe the clinical and laboratory characteristics of critically ill coronavirus disease-2019 (COVID-19) patients treated with dexamethasone in an intensive care unit (ICU) to provide a support tool for clinical decision-making. **Design:** A survey was conducted among hospitalized patients from November 2020 to March 2021, with data collected through patient interviews, medical records, and laboratory tests. **Setting:** This is a large hospital serving as a reference center for COVID-19 care in Bahia, Brazil. **Patients:** A convenience sample of 22 patients admitted to the COVID-19 ICU who signed informed consent to participate in the study. **Methods:** A cross-sectional study of patients admitted to the ICU with COVID-19. Data were analyzed using statistical methods. **Results:** The most common comorbidities among patients were hypertension (54%), diabetes (36%), and cardiovascular disease (27%). Among the deaths recorded, 55% of patients had hypertension, 44% had diabetes and/or required insulin therapy, 33% had a history of cardiovascular disease (including atrial fibrillation and congestive heart failure), and 22% had a history of stroke. Renal dysfunction (elevated creatinine); liver function abnormalities (increased alanine aminotransferase and aspartate aminotransferase); and elevated levels of ferritin, fibrinogen, and D-dimer were identified as potential indicators of disease progression. Among these factors, only elevated creatinine demonstrated a statistically significant association with an increased mortality risk. **Conclusion:** These findings provide a better understanding of the clinical course of severe acute respiratory syndrome coronavirus 2 infections and suggest that laboratory medicine is crucial in supporting clinical decision-making and advancing scientific and healthcare knowledge during the early phases of the COVID-19 pandemic. **Relevance for patients:** Identifying key risk factors, such as renal dysfunction, can improve early intervention and personalized treatment for critically ill COVID-19 patients.

Keywords: Intensive care unit; SARS-COV-2; Hospitalized patients; Clinical and laboratory features

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1. Introduction

Coronavirus disease-2019 (COVID-19) is a severe acute respiratory syndrome caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first identified in Wuhan, China, in December 2019. On March 11, 2020, the World Health Organization (WHO) declared it a pandemic.[1] The disease manifestation in patients can range from asymptomatic cases to severe respiratory compromise requiring hospitalization, with intensive care and mechanical ventilation support being essential in critical cases.[2]

Early diagnosis and analysis of laboratory parameters are essential for diagnosing and predicting the disease, preventing its dissemination, controlling its progression more effectively, monitoring patient's outcomes, and performing therapeutic monitoring. The main laboratory findings in patients with COVID-19 include changes in C-reactive protein (CRP), leukocyte counts, albumin, hemoglobin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH).[3] The main routine tests requested for COVID-19 patients include blood counts, coagulation and fibrinolysis cascades (prothrombin time [PT], activated partial thromboplastic time [aPTT], and D-dimers), and inflammatory markers (CRP, ferritin, and procalcitonin). In addition, tests analyzing biochemical factors that represent the activities of vital organs such as the liver, kidneys, and heart are crucial, given the virus-induced impairments.[4]

The hematological studies among COVID-19 patients noted common changes including a decrease in lymphocytes, mild thrombocytopenia, increased PT, and prolonged aPTT. Elevated D-dimer levels further indicate coagulopathy and serve as an essential marker of disease progression. In addition, increases in erythrocyte sedimentation rate, CRP, and procalcitonin are frequently observed. Ferritin rises during the acute inflammation stage and even after viral infections, particularly in dialysis patients, making it a practical screening tool for assessing COVID-19 conditions.[5,6] Regarding disease prognosis, a recent report[7] recommends monitoring biomarkers such as LDH, AST, ALT, total bilirubin, creatinine, cardiac troponin, and serum albumin levels due to their association with the worsening clinical status of patients. Other studies have highlighted age, viral load, lung injury score, and blood biochemistry indices such as hypoalbuminemia, elevated D-dimer, CRP, LDH, and lymphopenia are associated with the severity of COVID-19.[8]

Given the current scenario, this study aims to describe the clinical and laboratory characteristics of critically ill COVID-19 patients treated with dexamethasone in an intensive care unit (ICU), serving as a support tool for clinical decision-making.

2. Methodology

This research conducted a cross-sectional study on patients admitted to the COVID-19 ICU of a reference hospital in Salvador, Bahia, from November 2020 to March 2021. Blood samples were collected in EDTA tubes during their ICU stay after the first and subsequent administrations of dexamethasone to evaluate their laboratory profiles.

2.1. Inclusion criteria

Adult patients aged 18 years or older with confirmed SARS-CoV-2 infection who consented to participate in the study by signing an informed consent form. All patients were treated with dexamethasone, the only pharmacotherapy with proven efficacy during the study period.

2.2. Exclusion criteria

Patients under 18 years of age or those who declined to participate in the study were excluded from the study. Demographic data were obtained from electronic medical records at the time of admission.

The researchers conducted an initial descriptive analysis of the 22 study participants. For quantitative variables, results are presented with a 95% confidence interval (CI) and grouped distributions by class, showing both the observed counts and equivalent percentages. Relative risk (RR) analyses were applied to assess the increased mortality risk. Statistical significance was set at $\alpha = 0.05$, with two-tailed P-values of <0.05 considered significant.

The Research Ethics Committee of Hospital Santa Izabel - Santa Casa de Misericórdia da Bahia/Prof. Dr. Celso Figueirôa reviewed and approved this study. Informed consent was obtained from patients or their guardians through email, in person, or through Google Forms, allowing medical record data and biological samples to be used for research purposes.

3. Results

The clinical and laboratory features, as well as complications of COVID-19 patients, are reviewed in Table 1. The study included 22 patients, with 11 men and 11 women, having a mean weight and age of 79.32 kg and 62.14 years, respectively. The average length of stay in the ICU was 14.4 days, with 86.4% patients intubated (19/22). All patients who died were on mechanical ventilation. Table 1 presents the laboratory alterations studied. Only creatinine had a relative risk (RR 6.13; $P < 0.002$) considered significant.

Table 1. Clinical and laboratory features

Features	Patient number (%)	Demise	
		Yes *n* (%)	No *n* (%)
Sex Distribution - *n* (%)			
Female	11 (50.0)	5 (45.5)	6 (54.5)
Male	11 (50.0)	4 (36.4)	7 (63.6)
Age			
Mean, years (CI)	62.14 (54.6 – 69.7)	67.7 (54.0 – 80.0)	58.8 (48.5 – 69.1)
Distribution - *n* (%)			
<50	5 (22.7)	1 (20.0)	5 (80.0)
50 – 70	10 (45.5)	4 (40.0)	6 (60.0)
≥70	7 (31.8)	4 (57.1)	3 (42.9)
Hospitalization time			
Mean, years (CI)	14.4 (10.8 – 18.0)	11.8 (6.2 – 17.3)	16.2 (11.1 – 21.4)
Distribution - *n* (%)			
<10	9 (40.9)	5 (55.6)	4 (44.4)
10 – 20	7 (31.8)	3 (42.9)	4 (57.1)
≥20	6 (27.3)	1 (16.7)	5 (83.3)
BMI			
Distribution - *n* (%)			
Low weight <18.5	0 (0.0)	0 (0.0)	0 (0.0)
Normal 18.5 – 25	5 (22.7)	3 (60.0)	2 (40.0)
Overweight 25 – 30	6 (27.3)	3 (50.0)	3 (50.0)
Obesity ≥30	9 (40.9)	3 (33.3)	6 (66.7)
No collection	2 (9.1)	0 (0.0)	2 (100.0)
Creatinine (Reference range: 0.50 – 0.90 mg/mL)			
Highly elevated parameter - *n* (%)	8 (36.6)	7 (87.5)	1 (12.5)
AST (Reference range: Woman <35 U/L; Man <50 U/L)			
Highly elevated parameter - *n* (%)	14 (63.6)	8 (57.1)	6 (42.9)
No collection	2 (9.1)		2 (100.0)
ALT (Reference range: Woman <33 U/L; Man<41 U/L)			
Highly elevated parameter - *n* (%)	13 (59.1)	7 (53.8)	6 (46.2)
No collection	2 (9.1)		2 (100.0)
Ferriti*n* (Reference range: Woman 13 – 150 ng/mL; Man 30 – 400 ng/mL) - *n* (%)	20 (90.9)	8 (40.0)	12 (60.0)
No collection	2 (9.1)	1 (50.0)	1 (50.0)
Fibrinoge*n* (Reference range: 200 – 450 mg/dL) - *n* (%)	16 (72.7)	6 (37.5)	10 (62.5)
No collection	4 (18.2)	1 (25.0)	3 (75.0)
D-dimer (Reference range <500 ng/mL) - *n* (%)	12 (54.5)	5 (41.7)	7 (58.3)
No collection	6 (27.3)	3 (50.0)	3 (50.0)
Platelets			
Distribution - *n* (%)			
<150,000	2 (9.1)	2 (100.0)	0 (0.0)
150,000 – 450,000	19 (86.4)	7 (36.8)	12 (63.2)
>450,000	1 (4.5)	0 (0.0)	1 (100.0)
Glucose			
Distribution - *n* (%)			
<70	0 (0.0)	0 (0.0)	0 (0.0)
70 – 99	3 (13.6)	1 (33.3)	2 (66.7)
>99	19 (86.4)	8 (42.1)	11 (57.9)
Hemoglobin			
Distribution - *n* (%)			0 (0.0)
<5.7	0 (0.0)	0 (0.0)	0 (0.0)
5.7 – 6.4	0 (0.0)	0 (0.0)	13 (59.1)
>6.4	22 (100.0)	9 (40.9)	

(Cont'd...)

Table 1. ***(Continued)***

Features	Patient number (%)	Demise	
		Yes *n* (%)	No *n* (%)
Urea			
Distribution - *n* (%)			
<48.5	5 (22.7)	1 (20.0)	4 (80.0)
≥48.5	17 (77.3)	8 (47.1)	9 (52.9)

Abbreviations: AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; CI: Confidence interval; BMI: Body mass index.

The predominant chronic conditions indicated concomitant diseases. Table 2 presents the relevant chronic comorbidities. Among the deceased patients, 55% had been diagnosed with hypertension, 44% had diabetes and/or insulin therapy, 33% had a history of cardiovascular disease (including atrial fibrillation and congestive heart failure), and 22% had a history of stroke.

Table 2. Relevant chronic comorbidities

Comorbidities	*n*	Percentage
Hypertension	11	50.0
Diabetes/Insulin therapy	7	31.8
Cardiovascular disease (Atrial fibrillation, CHF)	4	18.2
Stroke history	3	13.6

Abbreviation: CHF: Congestive heart failure.

4. Discussion

Epidemiological data suggest that SARS-CoV-2 predominantly affects older adults with chronic diseases due to their weakened immune function, which aligns with the present findings, where the mean patient age was 62.14 years.[9] The lower susceptibility of women to viral infections may be linked to the protective role of the X chromosome and sex hormones, which enhance immune responses.[10] However, the number of infected females and males in this study was equivalent.

A retrospective cohort study conducted in Lombardy, Italy, among critically ill COVID-19 patients admitted to ICUs found that males with hypertension, cardiovascular disease, and diabetes were the most prevalent comorbidities and were associated with a high fatality rate. In this study, the most common comorbidities were hypertension (54%), diabetes (36%), and cardiovascular disease (27%). Similarly, the Lombardy study reported that 60.5% (95% CI, 58.9% – 62.2%) of patients had at least one comorbidity, with hypertension being the most frequent (42.1%; 95% CI, 40.5% – 43.6%), followed by hypercholesterolemia (16.5%; 95% CI, 15.3% – 17.8%) and heart disease (16.2%; 95% CI, 14.9% – 17.4%).[11]

Most COVID-19 studies have not identified obesity as a mortality risk factor, often due to the lack of body mass index (BMI) or total body weight data, as in this study. Without BMI data, the degree of obesity in the population could not be assessed.[12] However, obese patients are more likely to develop critical illness compared to nonobese individuals, and they frequently have other metabolic disorders, such as diabetes. This is significant because the cumulative risk of mortality increases with obesity-associated comorbidities. Metabolic syndrome and conditions related to obesity, such as diabetes, hypertension, and cardiovascular or cerebrovascular diseases, contribute substantially to this risk.[13]

Among the recorded deaths in this study, 50% of patients had hypertension, 31.8% had diabetes and/or required insulin therapy, 18.2% had a history of cardiovascular disease (including atrial fibrillation and congestive heart failure), and 13.6% had a history of stroke. In a cohort of 41 patients, 13 (32%) were admitted to the ICU due to the need for a high-flow nasal cannula or more advanced oxygen support to manage hypoxemia. The majority of patients were male (30 [73%]), and less than half had underlying conditions (13 [32%]), including diabetes (8 [20%]), hypertension (6 [15%]), and cardiovascular disease (6 [15%]).[13]

In an Italian cohort, the median length of ICU stay was 12 days (interquartile range 6 – 21; range 0 – 87), with 87% of patients requiring intubation.[11] The average ICU stay in this cohort was 14.4 days, with 86.4% of patients intubated. All patients who died were on mechanical ventilation. Consistent with previous findings, the length of hospital stay and the need for mechanical ventilation were identified as risk factors for coinfections.

Ten of the 22 patients in this study were infected with *Acinetobacter baumannii*, and a moderate correlation was found between the length of stay and *A. baumannii* infection (Spearman $\rho = 0.592$; $P < 0.005$). In addition, a strong correlation was observed between the number of days on mechanical ventilation and *A. baumannii* infection (Spearman $\rho = 0.740$; $P < 0.001$). This represents a significant increase compared to the historical rate

of *A. baumannii* infections in the ICU, which was approximately 0.62% during the same period the year before COVID-19 ($P < 0.0001$).

Another study conducted in Qom, Iran, examined 19 critically ill patients admitted to ICUs for COVID-19 treatment. Among these patients, 11 (58%) were male, and 8 (42%) were female, with an average age of approximately 67. The mean length of ICU stay was 15 days. Of the 19 patients, 18 (95%) died, while only one (5%) survived. All patients tested positive for bacterial infections, with 17 (90%) infected by *A. baumannii* and 2 (10%) infected by *Staphylococcus aureus*.[14]

In another study,[7] 83.2% of patients presented with lymphocytopenia, 36.2% with thrombocytopenia, and 33.7% with leukopenia at admission. Elevated CRP levels were typical, while abnormalities in ALT, AST, creatine kinase, and D-dimer were observed less frequently. Patients with severe COVID-19 displayed more significant laboratory abnormalities, including lymphocytopenia, leukopenia, hypoalbuminemia, elevated D-dimer, CRP, and LDH levels compared to those with nonsevere disease.

In this study, abnormalities in renal function (creatinine), liver function (ALT, AST), ferritin, fibrinogen, and D-dimer emerged as potential indicators of disease progression. However, only creatinine demonstrated a statistically significant association with mortality risk (RR 6.13; $P < 0.002$). Among patients with elevated creatinine levels suggestive of renal dysfunction, 87.5% succumbed to the disease.

Acute kidney injury (AKI) is a frequent complication among hospitalized patients with severe COVID-19. Although its incidence varies, studies report rates between 37% and 40%. In a retrospective observational cohort study in the U.S., 39.9% of 9,657 patients developed AKI. These patients had a higher prevalence of comorbidities, including diabetes mellitus, coronary artery disease, heart failure, and chronic kidney disease, as well as elevated inflammatory markers such as D-dimer, CRP, and serum ferritin. The mortality rate among patients with COVID-19 who developed AKI, particularly those requiring dialysis, was substantial, highlighting the importance of shared decision-making in the care of these critically ill patients.[15,16]

A retrospective analysis of 536 COVID-19 patients reported that although AKI was relatively uncommon (occurring in 36 cases), it was associated with a mortality rate of 91.7%.[17] Several mechanisms, including sepsis and direct viral infection of renal cells, contribute to the development of acute renal failure, particularly in critically ill patients. Early identification of AKI is crucial in initiating appropriate treatment to improve outcomes.[18]

Dexamethasone has emerged as a key therapeutic agent in the management of critically ill COVID-19 patients, especially those requiring oxygen support or mechanical ventilation. As a corticosteroid, it helps reduce the excessive inflammatory response caused by SARS-CoV-2, which can lead to severe lung injury and multiorgan failure. Studies have shown that dexamethasone can significantly reduce mortality in these patients by dampening the cytokine storm, a hyperinflammatory state triggered by the virus.[19] In this study, patients with severe COVID-19, many of whom presented with comorbidities such as hypertension and diabetes, benefitted from dexamethasone, which was administered as part of their treatment regimen to manage severe inflammation. However, its use must be carefully monitored, particularly in patients at risk for secondary infections like *A. baumannii*, as prolonged corticosteroid use can suppress immune function and potentially exacerbate infection risk.

5. Conclusion

It is essential to acknowledge the limitations of this study, mainly the relatively small sample size. The severe pandemic conditions and study design also precluded the inclusion of a control group of ICU patients without COVID-19 for comparison. However, this study prompts reflection on care and operational practices for handling these patients, especially during a pandemic.

Laboratory results from COVID-19 patients admitted to an ICU are essential to assess the disease prognosis. Comprehensive information on clinical and laboratory parameters of coronaviruses is crucial for implementing effective management and reducing their impact in future outbreaks. Emphasis should be placed on understanding the disease's pathophysiology, as well as on the early detection and isolation of suspected patients, which play significant clinical and social roles while enabling better adaptation of drug therapies. In this study, the laboratory parameters were closely integrated into the clinical staff's decision-making process, making a relevant contribution to scientific and healthcare knowledge during the initial outbreak of COVID-19.

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Conflict of interest

All authors report no conflicts of interest relevant to this article.

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Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Hospital Santa Izabel - Santa Casa de Misericórdia da Bahia/Prof. Dr. Celso Figueirôa. It was conducted in line with ethical principles. Patients or legal guardians provided written informed consent before participating in this research.

Consent for publication

The patients or their legal guardians gave consent to publish the data in this study.

Availability of data

The data supporting the study's results are included in the article. Additional information can be solicited from the corresponding author upon reasonable request.

References

1. World Health Organization. *Coronavirus Disease* (*COVID-19*) *Outbreak Situation*. Geneva, Switzerland: World Health Organization; 2020.

2. Huang JF, Wang XB, Zheng KI, *et al*. Letter to the Editor: Obesity hypoventilation syndrome and severe COVID-19. *Metabolism*. 2020;108:154249.

   doi: 10.1016/j.metabol.2020.154249

3. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. *Clin Chem Lab Med*. 2020;58(7):1131-1134.

   doi: 10.1515/cclm-2020-0198

4. Pourbagheri-Sigaroodi A, Bashash D, Fateh F, Abolghasemi H. Laboratory findings in COVID-19 diagnosis and prognosis. *Clin Chim Acta*. 2020;510:475-482.

   doi: 10.1016/j.cca.2020.08.019

5. Bataille S, Pedinielli N, Bergounioux JP. Could ferritin help the screening for COVID-19 in hemodialysis patients? *Kidney Int*. 2020;98(1):235-236.

   doi: 10.1016/j.kint.2020.04.017

6. Shoenfeld Y. Corona (COVID-19) time musings: Our involvement in COVID-19 pathogenesis, diagnosis, treatment and vaccine planning. *Autoimmun Rev*. 2020;19(6):102538.

   doi: 10.1016/j.autrev.2020.102538

7. Guan W, Ni Z, Hu Y, *et al*. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-1720.

   doi: 10.1056/nejmoa2002032

8. Liu Y, Yang Y, Zhang C, *et al*. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci*. 2020;63(3):364-374.

   doi: 10.1007/s11427-020-1643-8

9. Badawi A, Ryoo SG. Prevalence of comorbidities in the Middle East respiratory syndrome coronavirus (MERS-CoV): A systematic review and meta-analysis. *Int J Infect Dis*. 2016;49:129-133.

   doi: 10.1016/j.ijid.2016.06.015

10. Channappanavar R, Fett C, Mack M, Ten Eyck PP, Meyerholz DK, Perlman S. Sex-based differences in susceptibility to severe acute respiratory syndrome coronavirus infection. *J Immunol*. 2017;198(10):4046-4053.

    doi: 10.4049/jimmunol.1601896

11. Grasselli G, Greco M, Zanella A, *et al*. Risk factors associated with mortality among patients With COVID-19 in intensive care units in Lombardy, Italy. *JAMA Intern Med*. 2020;180(10):1345-1355.

    doi: 10.1001/jamainternmed.2020.3539. Erratum in: *JAMA Intern Med*. 2021;181(7):1021.

    doi: 10.1001/jamainternmed.2021.1229

12. Hussain A, Mahawar K, Xia Z, Yang W, El-Hasani S. Obesity and mortality of COVID-19. Meta-analysis. *Obes Res Clin Pract*. 2020;14(4):295-300.

    doi: 10.1016/j.orcp.2020.07.002. Retraction in: *Obes Res Clin Pract*. 2021;15(1):100.

    doi: 10.1016/j.orcp.2020.12.008

13. Huang C, Wang Y, Li X, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506.

    doi: 10.1016/S0140-6736(20)30183-5. Erratum in: *Lancet*. 2020;395(10223):496.

doi: 10.1016/S0140-6736(20)30252-X

14. Sharifipour E, Shams S, Esmkhani M, *et al*. Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. *BMC Infect Dis*. 2020;20:646.

    doi: 10.1186/s12879-020-05374-z

15. Chan L, Chaudhary K, Saha A, *et al*. AKI in Hospitalized Patients with COVID-19. *J Am Soc Nephrol*. 2021;32(1): 151-160.

    doi: 10.1681/ASN.2020050615

16. Ng JH, Hirsch JS, Hazzan A, *et al*. Outcomes among patients hospitalized with COVID-19 and acute kidney injury. *Am J Kidney Dis*. 2021;77(2):204-215.e1.

    doi: 10.1053/j.ajkd.2020.09.002

17. Chu KH, Tsang WK, Tang CS, *et al*. Acute renal impairment in coronavirus-associated severe acute respiratory syndrome. *Kidney Int*. 2005;67(2):698-705.

    doi: 10.1111/j.1523-1755.2005.67130.x

18. Yang X, Tian S, Guo H. Acute kidney injury and renal replacement therapy in COVID-19 patients: A systematic review and meta-analysis. *Int Immunopharmacol*. 2021;90:107159.

    doi: 10.1016/j.intimp.2020.107159

19. RECOVERY Collaborative Group, Horby P, Lim WS, *et al*. Dexamethasone in hospitalized patients with Covid-19. *N Engl J Med*. 2021;384(8):693-704.

    doi: 10.1056/NEJMoa2021436

ACCSCIENCE PUBLISHING

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ORIGINAL ARTICLE

Hesperidin enhances the repair and regeneration of full-thickness dermal wounds in mice exposed to fractionated γ-radiation

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(This article belongs to the *Special Issue: Innovations in wound healing*)

Abstract

Background and aim: Neoplasms are often clinically treated with radiation therapy, which is known for its therapeutic benefits. However, pre-surgical radiotherapy is associated with a high incidence of surgical wound healing complications, necessitating the exploration of new pharmacological approaches. This study investigates the effects of 100 mg/kg oral hesperidin on mice undergoing hemi-body irradiation with varying fractionated γ-radiation doses. **Methods:** Mice were exposed to 10, 20, or 40 Gy doses of fractionated γ-radiation (2 Gy/day) below the rib cage. Full-thickness excision wounds were created on their dorsum, and wound contractions were assessed periodically through video imaging. Biochemical profiles were analyzed by measuring collagen content, DNA synthesis, and nitric oxide (NO) levels in granulation tissue, as well as glutathione (GSH) levels, GSH peroxidase (GSHpx) activity, and lipid peroxidation (LPx) in the skin. Histological examinations were conducted at various post-irradiation intervals to assess skin regeneration. **Results:** Fractionated irradiation delayed wound contraction and prolonged mean wound healing time (MHT) in a dose-dependent manner, reducing collagen content, DNA synthesis, and NO levels in granulation tissue. In addition, irradiation decreased GSH concentrations and GSHpx activity while increasing LPx, with severity correlating to radiation dose. Fibroblast and vascular densities also declined following fractionated irradiation. In contrast, pre-treatment with hesperidin significantly enhanced wound contraction, reduced MHT, and increased collagen levels, DNA synthesis, and NO production. Furthermore, hesperidin treatment elevated GSH levels and GSHpx activity while decreasing radiation-induced LPx. In addition, hesperidin pre-treatment improved collagen deposition and fibroblast and vascular densities. **Conclusion:** This study demonstrates that hesperidin accelerates wound healing after fractionated radiation, suggesting hesperidin's potential as a therapeutic paradigm in managing irradiated wounds. **Relevance for patients:** The use of hesperidin is expected to mitigate the complications of surgical wounds arising after radiotherapy of cancer patients in a clinical setting.

Keywords: Mice; Fractionated irradiation; Wound; Collagen; DNA; Glutathione

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1. Introduction

Ionizing radiation is an important treatment modality for various neoplastic disorders, with approximately 50% of cancer patients undergoing fractionated radiotherapy as either a curative or palliative approach.[1] The therapeutic efficiency of radiation in cancer treatment is enhanced by administering 20 – 30 fractions, each delivering 2 – 3 Gy, over 5 – 6 weeks. Despite its significant therapeutic benefits, normal skin is invariably subjected to the cytotoxic effects of radiation.[2,3] In addition, fractionated radiotherapy is frequently utilized as a palliative treatment following the surgical resection of tumors.[3,4] Investigating the impact of irradiation on wound repair and regeneration is essential for improving cure rates among cancer patients. Wound healing involves a complex sequence of well-coordinated cellular and molecular events, encompassing inflammation, angiogenesis, fibroplasia, wound contraction, epithelialization, and matrix remodeling.[5-7] When wounded tissue is exposed to ionizing radiation, the normal physiological responses to injury are disrupted, resulting in an extended recovery period. The effects of ionizing radiation on the skin lead to multiple detrimental effects on wound healing processes. Specifically, ionizing radiation not only impairs inflammatory responses that are critical to wound repair, but also adversely affects connective tissue proliferation, granulation tissue formation maturation, collagen mRNA transcription, collagen secretion, and revascularization.[8-13] Moreover, ionizing radiation hinders collagen synthesis and delays the maturation of collagen fibers,[8-10,14] Infections resulting from radiation-induced bone marrow suppression can further complicate the recovery of irradiated wounds. The replicative capacity of fibroblasts within irradiated tissues may be diminished or selectively reduced due to the destruction of rapidly proliferating fibroblasts post-irradiation.[15-20] In addition, exposure to ionizing radiation has been reported to reduce the tensile strength of newly generated skin wounds.[21-23]

Several attempts have been made to identify potential therapeutic approaches aimed at enhancing the healing of irradiated wounds. Hydrogel and hydrocolloid gel dressings have been utilized to alleviate wound discomfort and reduce wound healing time in radiation-induced ulcers.[24] Sucralfate octasulfate, a persulfated disaccharide, has been reported to accelerate the repair and regeneration of irradiated wounds in animals while mitigating skin reactions in humans undergoing radiotherapy. Similar findings have been observed with pentoxifylline in patients receiving radiation therapy.[25] Phenytoin sodium has demonstrated the ability to enhance the stimulating effects of wound fluid on fibroblast proliferation and collagen synthesis following irradiation.[26,27] In addition, Vitamin A supplementation has been reported to inhibit acute radiation-induced defects in wound healing, likely by promoting an early inflammatory response and increasing the presence of monocytes and macrophages at the wound site.[28] Certain radioprotective compounds, including mercaptoethylamine, serotonin, and WR 2721, have also proven useful in managing combined injuries.[29] Furthermore, several growth factors and antimicrobial agents have been explored in animal models as potential options to improve wound healing in radiation-damaged skin.[30-33]

Wound-healing abnormalities can inflict significant physical and psychological trauma on affected patients, resulting in high treatment costs. Therefore, exploring new pharmacological agents that may help mitigate these wound-healing complications is essential. The use of nutraceuticals in the reconstruction of irradiated wounds presents a promising avenue, as they are widely accepted, well-tolerated, cost-effective, and can be safely utilized in human applications.[34-36] Hesperidin (hesperetin-7-rhamnoglucoside or hesperetin-7-rutinoside), a predominant citrus bioflavonoid found in sweet oranges (*Citrus sinensis*) and tangelos, has demonstrated various beneficial properties, including anti-inflammatory, analgesic, antihypertensive, diuretic, antibacterial, and antiviral effects across multiple studies.[37-43] Furthermore, it has been reported to inhibit tumor initiation and promotion while reversing the neoplastic transformation *in vitro* and *in vivo*.[40,44-46] Hesperidin has also been found to reduce human cholesterol levels and slow bone loss.[40,47,48] Deficiency of hesperidin has been associated with abnormal capillary leakage and exhibits beneficial effects on capillary permeability and fragility, protecting against various traumas, stresses, and cardiotoxicity.[49-51] Importantly, hesperidin is non-toxic in animals and humans.[52] In addition, it offers protection against chemical-induced toxicity.[52-56] Similarly, a micronized flavonoid fraction containing 90% diosmin and 10% hesperidin has been reported to protect against reactive oxygen radicals both *in vivo* and *in vitro*. This fraction has proven effective in promoting the healing of clean and infected wounds through oral and topical administration.[57] Hesperidin also exerts anticancer, antiobesity, antioxidant, and radioprotective actions.[38,58-62] Both hesperidin and its aglycone hesperetin are recognized for their wide range of pharmacological properties. Given its pleiotropic effects, non-toxic nature, and significant implications of fractionated radiation on wound repair in healthcare settings, we conceptualize the present study and investigate the efficacy of hesperidin treatment in mice partially exposed to varying doses of fractionated γ-radiation while subjected to additional trauma through an open deep dermal excision wound.

2. Materials and methods

2.1. Animals

Animal care and handling were conducted following the guidelines of the World Health Organization, the Indian National Science Academy, and the "Guide for the Care and Use of Laboratory Animals" (NIH publication #86-23, revised in 1985). Swiss albino mice, aged 8 – 10 weeks of either sex and weighing 30 – 36 g, were sourced from an inbred colony maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%), and light (12 h of light and dark cycle). The animals had free access to sterile food and water. The diet consisted of 50% cracked wheat, 40% Bengal gram, 4% milk powder, 4% yeast powder, 0.75% sesame oil, 0.25% cod liver oil, and 1% salt. Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. The study was approved by the institutional animal Ethical Committee of Manipal University, Manipal, India (Approval no.: IAEC/KMC/03/2003-2004).

2.2. Chemicals

Hesperidin (catalog no.: 12346-0050), hydroxyproline (catalog no.: H5534), chloramine T (catalog no.: C9887), glucosamine (catalog no.: G44875), acetylacetone (catalog no.: A3511), deoxyribonucleic acid (catalog no.: D4522), diphenylamine (catalog no.: D2385), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, catalog no.: D8130), ethylenediaminetetraacetic acid (EDTA, catalog no.: E4884), β-nicotinamideadenine dinucleotide phosphate (catalog no.: N1630), N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDD, catalog no.: N5889) and sulfanilamide (catalog no.: S9251) were procured from Sigma Chemical Co., United States. The trichloroacetic acid (TCA, catalog no.: 15213-5000) and ρ-dimethylaminobenzaldehyde (Ehrlich's reagent [catalog no.: 42363-0250]) were requisitioned from Across Organics, Belgium. Imidazole buffer (catalog no.: 38546) was procured from S.d. fine-chemicals Ltd., India. Ethanol (catalog no.: SIN 1170) was purchased from Hayman Ltd., England, and sterillium disinfectant solution was supplied by Bode Chemie, Germany. Perchloric acid, carboxymethylcellulose (CMC), diethyl ether, formalin, sodium hydroxide, sodium nitrate, sodium nitrite, phosphoric acid, hydrochloric acid (HCl), and sodium chloride were obtained from Ranbaxy Fine Chemical, India.

2.3. Preparation of drug and mode of administration

The required amount of hesperidin was suspended in 1% CMC, and the animals were administered orally with a single dose of 0.01 mL/g body weight of CMC or 100 mg/kg body weight hesperidin once daily, depending on the total fractionated irradiation dose. A gap of 2 days was consistently maintained between the delivery of the 6th, 11th, or 16th fractions of the radiation dose.

2.4. Experimental protocol

The effect of hesperidin treatment after delivery of different doses of fractionated radiation was investigated by performing the following experiments:

2.4.1. Experiment 1: Wound contraction

Wound contraction is an important aspect of wound repair and regeneration, which was studied by dividing an equal number of males and females into the following groups:

(i) CMC + sham-irradiation: Animals in this group received 0.01 mL/g body weight of 1% CMC once daily, consecutively for 5, 10, or 20 days before sham-irradiation.
(ii) Hesperidin + sham-irradiation: Animals in this group were administered 100 mg/kg body weight of hesperidin once daily, consecutively for 5, 10, or 20 days before sham-irradiation.
(iii) CMC + irradiation: This group received 0.01 mL/g body weight of 1% CMC once daily, consecutively for 5, 10, or 20 days before exposure to various doses of γ-radiation.
(iv) Hesperidin + irradiation: Animals in this group were administered 100 mg/kg body weight of hesperidin once daily, consecutively for 5, 10, or 20 days before exposure to various doses of fractionated γ-radiation.

2.4.1.1. Irradiation

One hour after each administration of CMC or hesperidin, each animal was placed into a specially designed well-ventilated acrylic restrainer. The lower half of the animals (below the rib cage) was exposed to a single dose of either 0 or 2 Gy once daily, delivered at a dose rate of 1.35 Gy/min from a ^{60}Co Teletherapy source (Theratron, Atomic Energy Agency, Canada). Treatments were given once daily for 5, 10, or 20 days, resulting in a total dose of 10 Gy (five fractions of 2 Gy each), 20 Gy (10 fractions of 2 Gy each), or 40 Gy (20 fractions of 2 Gy each). A gap of 2 days was consistently maintained between the delivery of the 6th, 11th, or 16th fractions of the radiation dose.

2.4.1.2. Production of full-thickness skin wound

The fur of the dorsum of each animal was removed with a cordless electric mouse clipper (Wahl Clipper Corporation, United States) before exposure to the last fraction of γ-radiation. A full-thickness skin wound was created on the dorsum (below the rib cage) as previously

described.[8-10] Briefly, the animals were anesthetized using diethyl ether, and the entire body was thoroughly cleaned and decontaminated using sterillium (Bode Chemie, Germany) disinfectant solution, followed by fur removal. The cleared dorsal surface of the skin was marked with a sterile circular stainless steel stencil (15 mm diameter). A full-thickness wound was created by removing the skin flap in an aseptic environment using sterile scissors and forceps. Each wounded animal was housed individually in a sterile polypropylene cage.

2.4.1.3. Wound contraction

Wound contraction was monitored by capturing video images of each full-thickness wound using a charge-coupled device camera connected to a computer.[8-10] The first image of each wound from different groups was obtained 1 day after wounding, which was designated as day 1. Subsequent images were captured on days 3, 6, 9, 12, and 15 post-wounding. The wound area was measured using Auto CAD R14 (Autodesk Inc., United States) software. Ten animals were used in each concurrent group for each radiation dose; however, for the 40 Gy radiation group, a minimum of 12 animals were used to account for potential radiation-related mortality. A total of 168 animals were utilized for this experiment.

2.4.2. Experiment 2: MHT

A separate experiment was performed to evaluate the effect of hesperidin on MHT after exposure to 0, 10, 20, or 40 Gy of fractionated γ-radiation. The grouping and other conditions were essentially similar to that described above. All the animals in each group were monitored until complete healing of wounds and the day each wound healed was recorded. The mean of all healing days was calculated and expressed as the MHT in days. Each concurrent group consisted of 10 animals for each radiation dose, except 40 Gy, where a minimum of 12 animals was used to account for potential radiation-related mortality. A total of 104 animals were used for this experiment.

2.4.3. Experiment 3: Biochemical estimations

2.4.3.1. Granulation tissue

A separate set of experiments was carried out to study the effect of hesperidin on various biochemical profiles of irradiated wounds after exposure to 0, 10, 20, or 40 Gy of fractionated γ-radiation. The grouping of animals and production of full-thickness excision wounds were similar to the procedures described for the wound contraction experiment. However, in this experiment, biopsies of regenerating wounds were collected on days 4, 8, and 12 post-irradiation and stored at −70°C until analysis.

2.4.3.1.1. Collagen

To assess the total collagen content, hydroxyproline concentration was determined, as described by Woessner.[63] The weighed granulation tissue was hydrolyzed in 6 N HCl for 3 h at 130°C, neutralized (pH 7) with 2.5 N sodium hydroxide, and diluted with Milli-Q water (18 MΩ). The diluted solution was mixed with chloramine-T reagent and incubated for 20 min at room temperature, followed by the addition of freshly prepared Ehrlich's reagent solution. The mixture was incubated for 15 min at 60°C. The absorbance of each sample was measured at 550 nm using an Ultraviolet (UV)-visible double-beam spectrophotometer (Shimadzu UV-260, Shimadzu Corp., Japan). The concentration of hydroxyproline was determined by comparing the absorbance of the samples to a standard curve. Total collagen content was determined by multiplying the hydroxyproline concentration with a factor of 6.94. The collagen content in granulation tissue was expressed as mg/g dry tissue weight.

2.4.3.1.2. DNA

The DNA content in the granulation tissue/s was measured using the method described by Burton.[64] Briefly, a known amount of dry granulation tissue was homogenized in 5% TCA, followed by centrifugation. The resulting pellets were washed with 10% TCA, resuspended in 5% TCA, and incubated at 90°C for 15 min. Following this incubation, the contents were centrifuged again, and the resultant supernatant was used to determine DNA content. The DNA was hydrolyzed with 60% perchloric acid at 80°C for 20 min. After hydrolysis, Burton's diphenylamine reagent was added and the mixture was incubated at room temperature overnight. Subsequently, 95% ethanol was added to the solution, and absorbance was recorded at 600 nm using a UV-visible double-beam spectrophotometer. The amount of DNA was determined by comparing the absorbance of the samples to a standard curve and was expressed as mg/g dry tissue weight.

2.4.3.1.3. NO

The stable end products of NO biosynthesis were determined by measuring nitrite levels in the granulation tissue of wounds. Granulation tissues were homogenized in hypotonic saline and centrifuged to obtain the supernatant. Nitrite concentrations were determined with the Griess reagent.[65] Briefly, the supernatant was mixed with freshly prepared Griess reagent (0.1% NEDD, 1% sulphanilamide, and 5% phosphoric acid in a 1:1:1 ratio), incubated at 37°C for 30 min, and the absorbance was recorded at 543 nm using a double beam UV-visible spectrophotometer. Sodium nitrite was used as the standard for comparison. Nitrite levels were expressed as μM/100 mg dry tissue

weight. Six animals were used for each radiation dose in each concurrent group at each interval, except for the 40 Gy group, where eight were used for each dose of radiation at each assay time. In total, 240 animals were used for this experiment.

2.4.3.2. Skin

A separate experiment was carried out to study the effect of hesperidin on the status of various antioxidants in the skin of wounded mice after exposure to 0, 10, 20, or 40 Gy of fractionated γ-radiation. The grouping of animals and production of full-thickness wounds were similar to those described in the wound contraction experiment, with skin biopsies collected at 0, 1.5, 3, 6, 12, 24, and 48 h after the last exposure. The skin was carefully separated from the *panniculus carnosus* muscle, flash-frozen in liquid nitrogen, and stored at −70°C until analysis. The skin samples were weighed and homogenized in phosphate-buffered saline. A minimum of four animals were used for each group and each dose of radiation at each post-irradiation time, resulting in a total of 280 animals used for this experiment.

2.4.3.2.1. Glutathione peroxidase (GSHPx)

GSHPx activity was estimated using the method described by Sazuka *et al.*[66] Briefly, 100 µL of homogenate was mixed with 200 µL each of EDTA, sodium azide, glutathione (GSH), H_2O_2, and 400 µL of buffer. The reaction mixture was incubated at 37°C for 10 min, followed by the addition of 10% TCA. After centrifugation, the supernatant was collected and mixed with 3 mL disodium hydrogen phosphate and 1 mL DTNB. The absorbance of the samples was recorded against the blank at 412 nm using a double-beam UV-visible spectrophotometer. The enzyme activity was expressed as µmol GSH/mg protein.

2.4.3.2.2. GSH

GSH concentration was measured using the method described by Moron *et al.*[67] Briefly, proteins were precipitated using 25% TCA, centrifuged, and the supernatant was collected. The supernatant was mixed with 0.2 moL sodium phosphate buffer of pH 8.0 and 0.06 mmoL DTNB, followed by incubation for 10 min at room temperature. The absorbance of the samples was read against the blank at 412 nm in a double-beam UV-visible spectrophotometer and the GSH concentration was calculated from the standard curve.

2.4.3.2.3. Lipid peroxidation (LPx)

LPx was measured using the method described by Buege and Aust.[68] Briefly, the tissue homogenate was mixed with TCA-thiobarbituric acid-HCl solution, butylated hydroxytoluene (3.5 mmoL, 0.1 mL), and diethylenetriamino-pentaacetic acid (70 µmoL, 0.1 mL). The mixture was heated for 15 min in a boiling water bath. After centrifugation, the absorbance was recorded at 535 nm using a double-beam UV-visible spectrophotometer. The LPx was expressed as malondialdehyde in nmol/mg protein.

2.4.3.2.4. Protein estimation

Protein contents were measured using the method described by Waterborg & Matthews (1994).[69] using bovine serum albumin as the standard.

2.4.4. Experiment 4: Histological studies

A separate experiment was conducted to evaluate the histological alterations during wound healing after exposure to 0, 10, 20, or 40 Gy fractionated γ-radiation. The grouping of animals and production of full-thickness wounds were similar to those described for the wound contraction experiment. Cross-sectional full-thickness biopsies of regenerating wounds from each group were collected on days 4, 8, and 12 post-irradiation. The samples were fixed in 10% buffered formalin, passed sequentially through different grades of alcohol to ensure complete dehydration, and then embedded in paraffin wax. Medial samples were sectioned to a thickness of 5 µm perpendicular to the surface, starting from the center of the wound, and stained with hematoxylin and eosin. Sections were assessed in a blinded manner under the light microscope using a planimeter for fibroblast proliferation, neovascularization, and collagen deposition. For collagen deposition studies, faint traces of staining reaction, hyalinization, and irregular arrangement of collagen bundles were considered as +, while the most intense reactions with compactly arranged collagen bundles were scored as +++. Two areas in each section were counted for neovascularization and fibroblast proliferation. Elongated spindle-shaped cells with purple nuclei and pink cytoplasm were identified as fibroblasts and scored accordingly. Blood vessels that were conspicuous with hematoxylin and eosin stains were scored for vascular repopulation studies. Four animals were used in each concurrent group at each interval for each radiation dose, resulting in a total of 120 animals used for this experiment.

2.5. Data analysis

Statistical significance between the treatments was determined using one-way Analysis of variance, while Student's *t-test* was used for biochemical estimations. Data analysis was conducted using Origin 8 (Origin Lab Corporation, United States). All data are presented as mean ± standard error of the mean, and $P < 0.05$ were considered statistically significant.

3. Results

3.1. Experiment 1: Wound contraction

The regeneration and repair of excision wounds can be effectively assessed through the periodic calculation of wound area as a measure of wound contraction. The area of each wound at specific time points is expressed as the percentage of its original size on day 1. The mean corresponding wound area for each group was plotted as a function of days post-wounding. The excision wound area progressively decreased over time, demonstrating a consistent contraction of the wound in both the CMC + sham-irradiation and hesperidin + sham-irradiation groups (0 Gy). Maximum wound contraction was observed on day 6 post-irradiation in both the CMC and hesperidin + sham-irradiation groups (Figure 1A). Hesperidin treatment resulted in a significant and dose-dependent enhancement in wound contraction at all post-irradiation times compared to the CMC + sham-irradiation group (Figure 1A). Furthermore, while the CMC + sham-irradiation group exhibited scab formation, this was relatively less pronounced in the hesperidin + sham-irradiation group.

Exposure to various doses of fractionated γ-radiation resulted in a dose-dependent delay in wound contraction (Figure 1A). Specifically, exposure to 10 Gy delayed wound contraction at days 6 ($P < 0.02$) and 9 ($P < 0.03$) post-irradiation (Figure 1B), accompanied by an earlier scab formation compared to the CMC + sham-irradiation group. The difference in wound contraction between CMC + irradiation (20 Gy) and CMC + sham-irradiation group was significant at days 3 ($P < 0.01$), 6 ($P < 0.005$), 9 ($P < 0.002$), 12 ($P < 0.005$), and 15 ($P < 0.01$) post-irradiation (Figure 1C). Exposure to 40 Gy significantly delayed wound contraction at all the post-irradiation times (Figure 1D), with scab formation being notably thick compared to the CMC + sham-irradiation group.

Hesperidin treatment before exposure to 10 Gy irradiation significantly reduced the radiation-induced delay in wound contraction on days 3 ($P < 0.03$), 6 ($P < 0.03$), 9 ($P < 0.04$), and 12 ($P < 0.05$) post-irradiation (Figure 1B), with thinner scab formation. Furthermore, hesperidin treatment before exposure to 20 Gy irradiation resulted in a significant increase in wound contraction on days 3 ($P < 0.05$), 6 ($P < 0.05$), and 9 ($P < 0.05$) compared to the concurrent CMC + irradiation group (Figure 1C). However, a significant increase in wound contraction after hesperidin treatment before exposure to 40 Gy radiation was detected only at days 6 ($P < 0.05$) and 9 ($P < 0.05$) post-irradiation in the hesperidin + irradiation group (Figure 1D).

3.2. Experiment 2: MHT

The complete closure of wounds was observed by day 18.0 ± 0.65 days in the CMC + sham-irradiation group. In contrast, treatment with hesperidin resulted in a significant, dose-dependent reduction in the MHT in the hesperidin + sham-irradiation group, with the maximum reduction recorded for the group receiving 20 fractions of hesperidin, where the MHT was 15 days. Exposure to different doses of fractionated γ-radiation resulted in a dose-dependent delay in wound closure, extending the MHT. An MHT of 21.0 ± 0.29, 24.0 ± 0.49, and 30 ± 0.33 days was observed in the animals exposed to 10, 20, and 40 Gy, respectively, in the CMC + irradiation group (Figure 2). Hesperidin treatment at a dose of 100 mg/kg before exposure to various doses of fractionated γ-radiation mitigated the radiation-induced delay in excision wound healing, resulting in MHTs of 19 ± 0.46, 22.0 ± 0.26, and 29.0 ± 0.37 days for exposures to 10, 20 and 40 Gy, respectively, in the hesperidin +irradiation group (Figure 2). This treatment advanced MHT by approximately 2 days for both 10 Gy and 20 Gy, while the reduction for 40 Gy was <2 days. The reductions in wound healing time were statistically significant ($P < 0.05$) for 10 and 20 Gy in the hesperidin + irradiation group.

3.3. Experiment 3: Biochemical estimations

The estimation of hydroxyproline levels in granulation tissues serves as an accurate indicator of collagen biosynthesis during wound repair and regeneration. Hydroxyproline content increased with time, peaking on day 8 post-irradiation in both the CMC and hesperidin + sham-irradiation groups. Following this peak, the synthesis of new collagen declined by 12 days post-irradiation; however, hydroxyproline levels on day 12 remained higher than those measured on day 4 post-irradiation in both groups (Figure 3). The pattern of collagen synthesis was almost similar in the CMC + irradiation group, although exposure to 10, 20, or 40 Gy resulted in a significant, dose-dependent decline in collagen synthesis at all post-irradiation times compared to the CMC + sham-irradiation and hesperidin + sham-irradiation groups (Figure 3). A significant reduction in collagen deposition was observed on days 4 ($P < 0.002$), 8 ($P < 0.001$), and 12 ($P < 0.002$) post-irradiation for the 40 Gy of CMC + irradiation group compared to the CMC + sham-irradiation group. Despite the overall reduction, maximum collagen synthesis was observed on day 8 post-irradiation in the CMC + irradiation group. In the hesperidin + irradiation group, the treatment with 100 mg/kg hesperidin before exposure to various doses of fractionated γ-radiation resulted in a significant elevation in collagen synthesis compared to the concurrent CMC

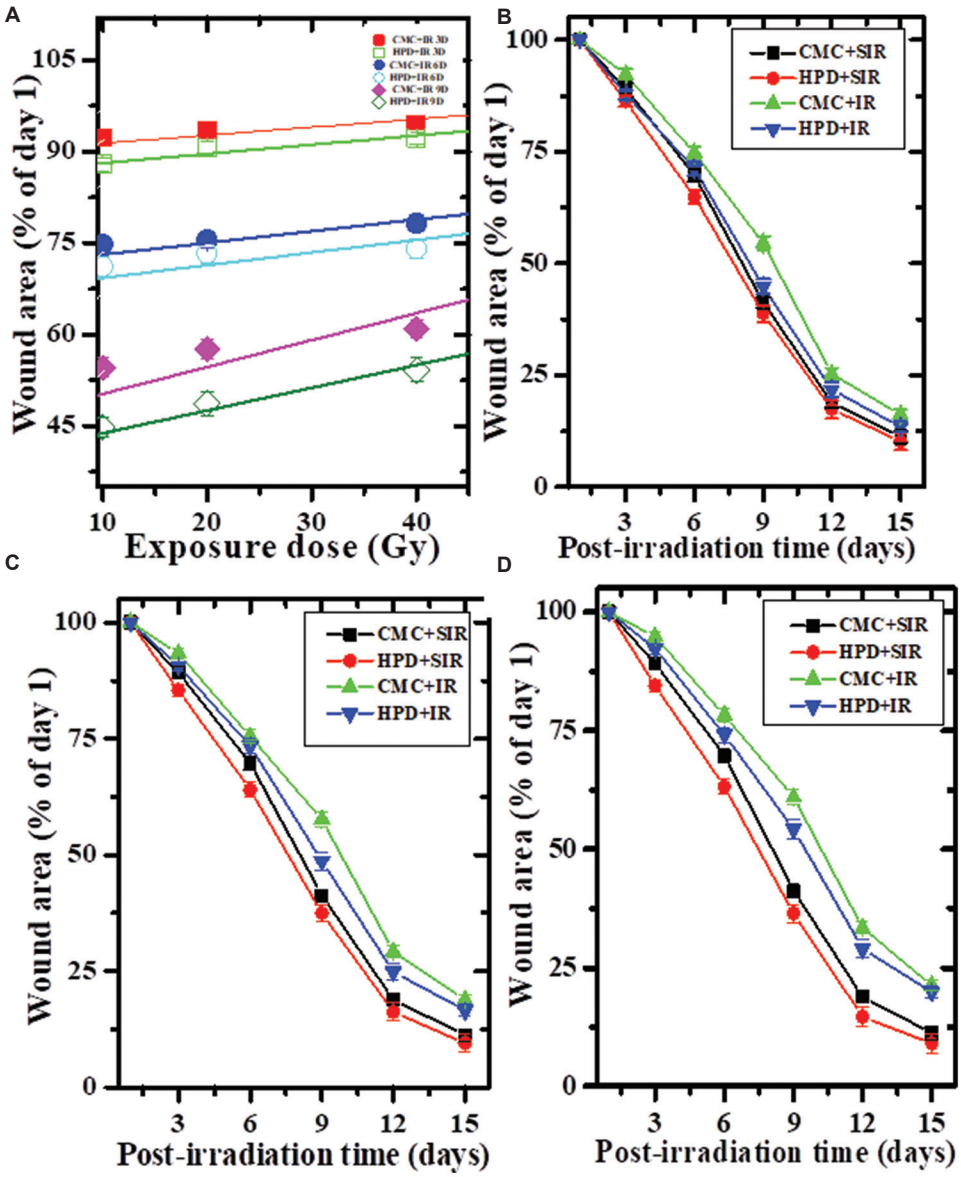

Figure 1. Effect of hesperidin treatment on contraction of excision wound in the skin of mice exposed to different doses of fractionated γ-radiation. Wound area percentage of day 1 in (A) a dose-response manner and after exposure to (B) 10 Gy, (C) 20 Gy, and (D) 40 Gy.
Abbreviations: CMC: Carboxymethylcellulose; HPD: Hesperidin; IR: Irradiation; SIR: Sham-irradiation.

+ irradiation group (Figure 3). Hesperidin pre-treatment was able to restore collagen levels nearly to baseline by day 8 post-irradiation, except for animals exposed to 40 Gy.

The increase in DNA content in treated wounds indicates cellular hyperplasia and wound regeneration. A rapid rise in DNA content was noted up to day 8 post-irradiation in both the sham-irradiation groups. Exposure to various doses of fractionated γ-radiation significantly reduced the DNA content at all post-irradiation times. However, hesperidin treatment before irradiation (10 and 20 Gy) resulted in a significant elevation of DNA content at days 4 and 8 post-irradiation in the hesperidin + irradiation group (Figure 4).

End products of NO synthesis, specifically nitrite levels, were elevated early at day 4 post-irradiation in granulation tissue but subsequently decreased substantially in both CMC and hesperidin + sham-irradiation groups. Exposure to various doses of fractionated γ-radiation considerably decreased nitrite contents in the granulation tissues at all post-irradiation times (Figure 5). The decrease in nitrite contents was significant only on day 8 ($P < 0.04$) after exposure to 10 Gy compared with the CMC + sham-irradiation group. In

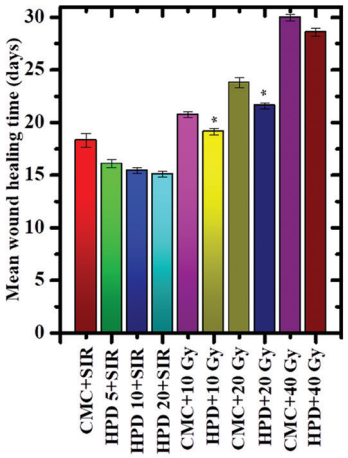

Figure 2. Effect of hesperidin treatment on the progression of wound closure with time (mean wound healing time) in mice exposed to various doses of fractionated γ-radiation. *$P < 0.05$ when comparing HPD groups to the CMC and SIR groups.
Abbreviations: CMC: Carboxymethylcellulose; HPD: Hesperidin; SIR: Sham-irradiation.

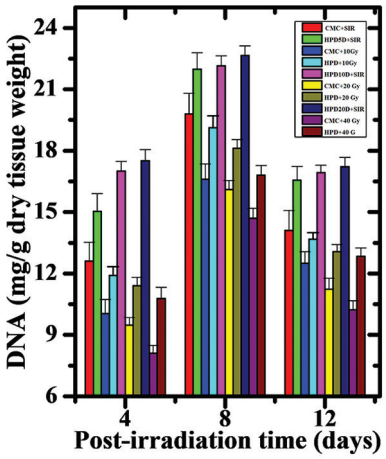

Figure 4. Effect of hesperidin on the DNA synthesis in the granulation tissue of regenerating deep dermal wound of mice exposed to different doses of fractionated γ- radiation at different post-irradiation times. HPD groups are compared to CMC and SIR groups.
Abbreviations: CMC: Carboxymethylcellulose; HPD: Hesperidin; SIR: Sham-irradiation.

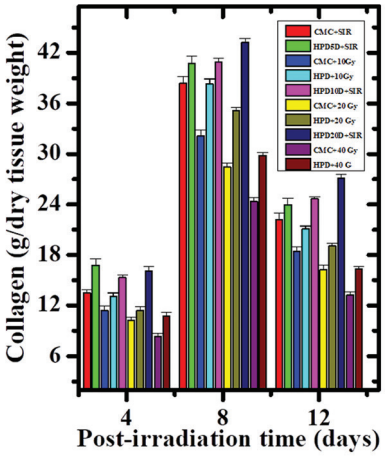

Figure 3. Effect of hesperidin on collagen synthesis in the granulation tissue of regenerating deep dermal wound of mice exposed to different doses of fractionated γ- radiation at different post-irradiation times. HPD groups are compared to the CMC and SIR groups.
Abbreviations: CMC: Carboxymethylcellulose; HPD: Hesperidin; SIR: Sham-irradiation.

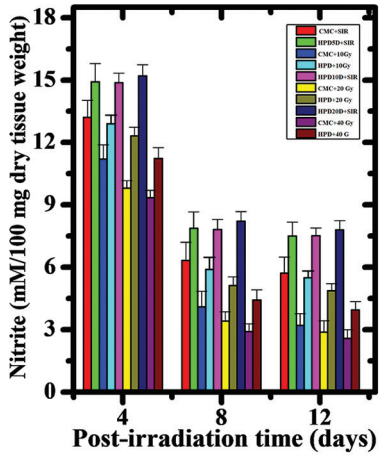

Figure 5. Effect of hesperidin on nitrate and nitric oxide biosynthesis in the granulation tissue of regenerating deep dermal wound of mice exposed to different doses of fractionated γ- radiation at different post-irradiation times. HPD groups are compared to the CMC and SIR groups.
Abbreviations: CMC: Carboxymethylcellulose; HPD: Hesperidin; SIR: Sham-irradiation.

addition, a significant decline in nitrite levels was observed on days 4 ($P < 0.005$), 8 ($P < 0.0004$), and 12 ($P < 0.005$) post-irradiation for 40 Gy compared to the CMC + sham-irradiation group. Hesperidin treatment resulted in an elevation in nitrite levels that were statistically significant at days 4 and 8 post-irradiation for 10 Gy (Figure 5).

3.3.1. GSH

GSH content started to decline immediately after wound creation, reaching its lowest point within 3 h. It then increased at 6 h post-irradiation in the CMC + sham-irradiation group, followed by a second decrement at 48 h post-irradiation. However, treatment with hesperidin before sham-irradiation resulted in a significant ($P < 0.05$) elevation in GSH levels at all post-irradiation times (Figure 6). Exposure to various doses of fractionated γ-radiation resulted in a drastic decline in the GSH contents compared to the CMC + sham-irradiation group. This decline was dose-dependent in both the CMC + irradiation and hesperidin + irradiation groups

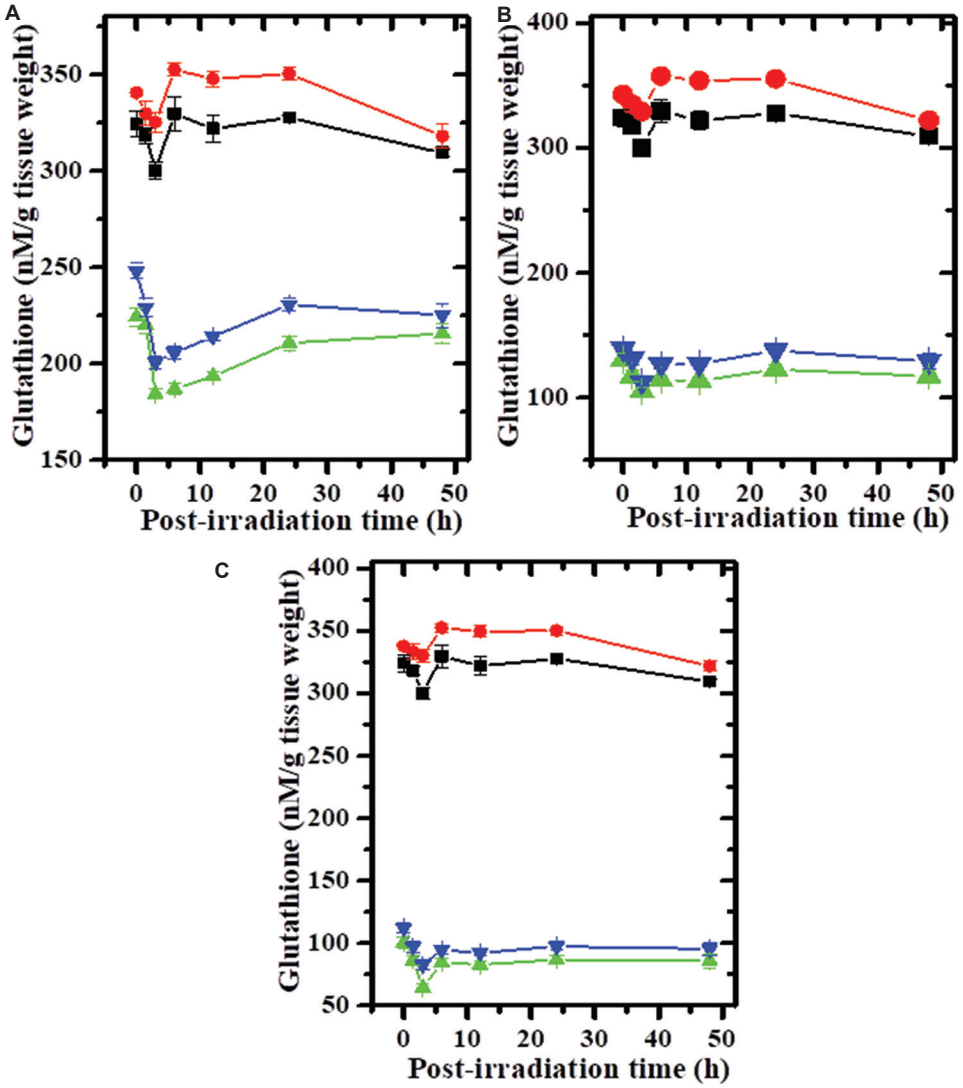

Figure 6. Effect of hesperidin on the glutathione contents in the skin of mice exposed to different doses of fractionated γ- radiation at different post-irradiation times. Glutathione levels in (A) 10 Gy, (B) 20 Gy, and (C) 40 Gy. Notes: Squares indicates: CMC+Sham-irradiation; Uptriangles indicates: Hesperidin+Sham-irradiation; Circles indicates: CMC+irradiation; Down triangles indicates: Hesperidin+irradiation.
Abbreviations: CMC: Carboxymethylcellulose; HPD: Hesperidin; IR: Irradiation; SIR: Sham-irradiation.

(Figure 6). Hesperidin treatment before irradiation caused a significant increase in the GSH contents compared to the concurrent CMC + irradiation group. However, even with hesperidin treatment, GSH levels could not be restored to normal by 48 h post-irradiation in either group (Figure 6).

3.3.2. GSHPx

The activity of GSHPx in the CMC + sham-irradiation group fluctuated over time, with the highest elevation detected at 1.5 h post-irradiation and a second elevation observed at 24 h that remained stable up to 48 h post-irradiation (Figure 7). Hesperidin administration before sham-irradiation resulted in a significant increase ($P < 0.05$) in GSHPx activity up to 48 h post-irradiation compared to the CMC + sham-irradiation group. Exposure to different doses of fractionated γ-radiation resulted in a dose-dependent decline in GSHPx activity in both the CMC + irradiation group and hesperidin + irradiation group compared to their respective sham-irradiation groups (Figure 7). However, the pattern of GSHPx activity fluctuations after radiation exposure was similar to that observed in the sham-irradiation group. The administration of hesperidin before exposure to various doses of γ-radiation caused a drastic increment in GSHPx activity, with the highest at 1.5 h ($P < 0.02$) followed by 24 h ($P < 0.03$) post-irradiation in the hesperidin + irradiation group (Figure 7).

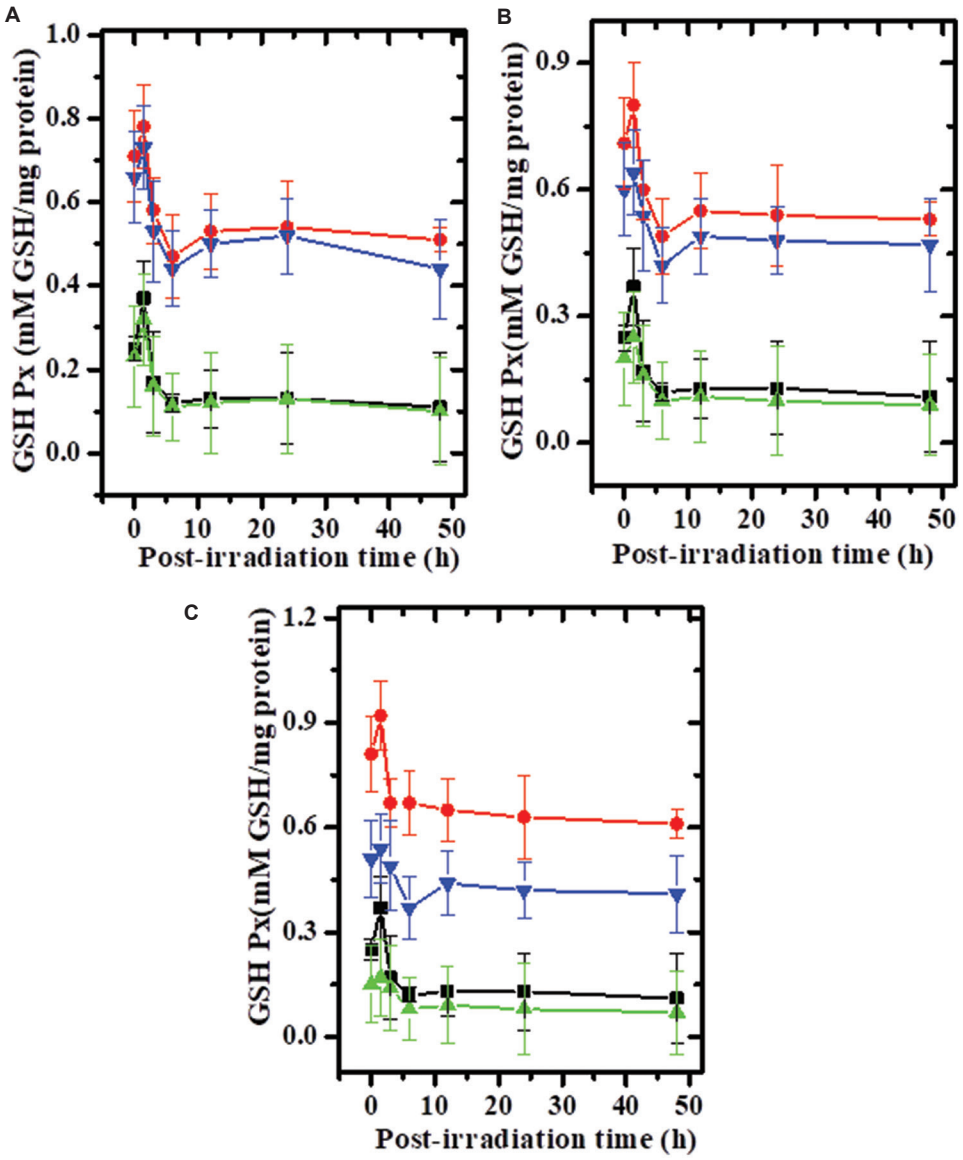

Figure 7. Effect of hesperidin on glutathione peroxidase in the skin of mice exposed to different doses of fractionated γ- radiation at different post-irradiation times. Glutathione peroxidase levels in (A) 10 Gy, (B) 20 Gy, and (C) 40 Gy. Notes: Squares indicates: CMC+Sham-irradiation; Uptriangles indicates: Hesperidin+Sham-irradiation; Circles indicates: CMC+irradiation; Down triangles indicates: Hesperidin+irradiation.
Abbreviations: CMC: Carboxymethylcellulose; GSH: Glutathione; HPD: Hesperidin; IR: Irradiation; SIR: Sham-irradiation.

3.3.3. LPx

The infliction of an excision wound caused an elevation in LPx levels in the CMC + sham-irradiation group. LPx levels fluctuated over time in both CMC + sham-irradiation and hesperidin + sham-irradiation groups, with the highest elevation observed at 3 h post-irradiation, followed by a gradual decline. Hesperidin treatment resulted in a significant ($P < 0.05$) decline in the baseline LPx levels (Figure 8). Radiation exposure increased LPx levels, with the highest levels observed at 40 Gy. This increase was also time-dependent in both the CMC + irradiation and hesperidin + irradiation groups, peaking at 3 h post-irradiation and declining steadily thereafter. Hesperidin pre-treatment provided significant protection ($P < 0.05$) against the radiation-induced LPx up to 24 h post-irradiation for the 40 Gy group. However, the baseline LPx levels could not be restored even by 48 h post-irradiation (Figure 8).

3.4. Experiment 4: Histological studies

Histological evaluation of wound biopsies at various post-irradiation times revealed that hesperidin treatment alone did not alter wound histology, except for an increase in

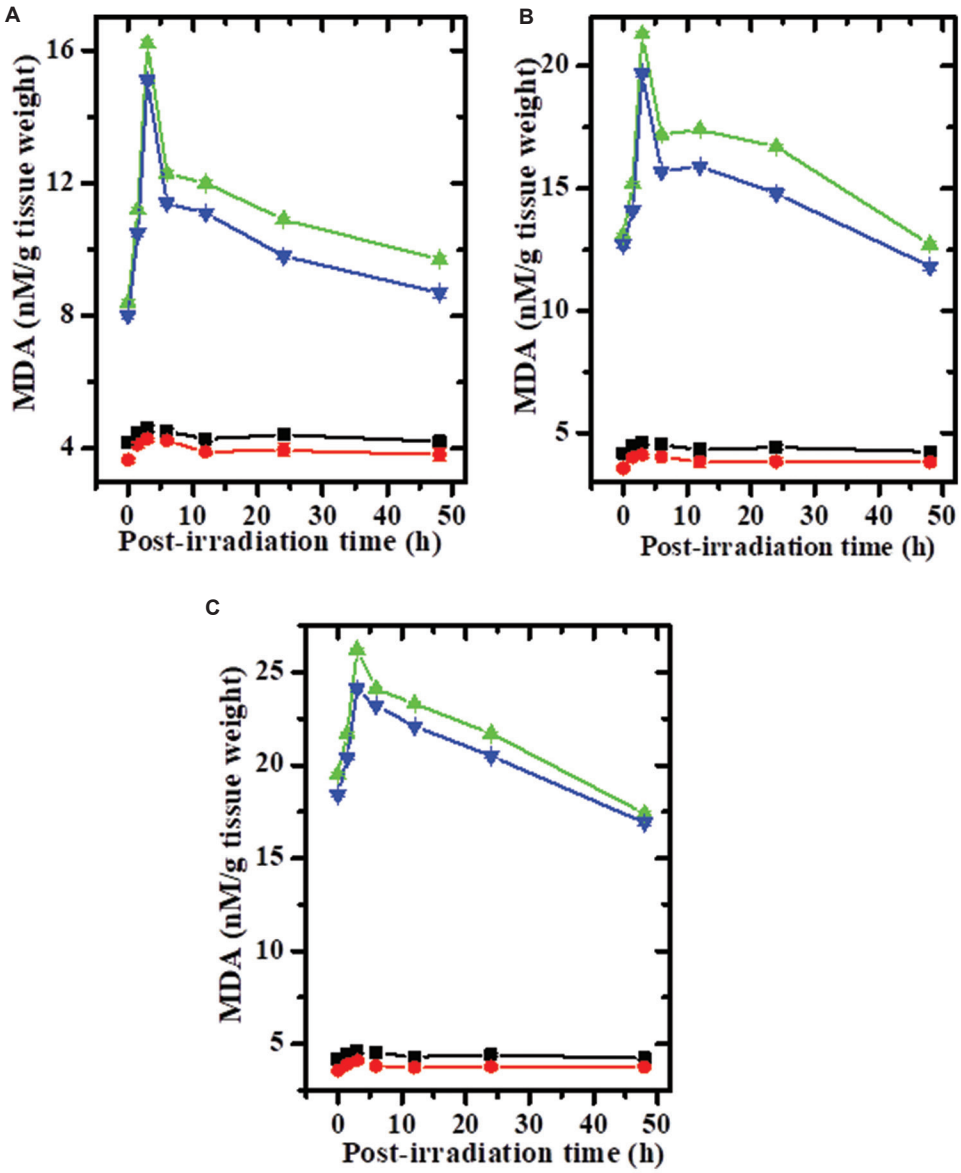

Figure 8. Effect of hesperidin on lipid peroxidation in the skin of mice exposed to different doses of fractionated γ- radiation at different post-irradiation times. MDA levels in (A) 10 Gy, (B) 20 Gy, and (C) 40 Gy. Notes: Squares indicates: CMC+Sham-irradiation; Uptriangles indicates: Hesperidin+Sham-irradiation; Circles indicates: CMC+irradiation; Down triangles indicates: Hesperidin+irradiation.
Abbreviations: CMC: Carboxymethylcellulose; HPD: Hesperidin; IR: Irradiation; MDA: Malondialdehyde; SIR: Sham-irradiation.

collagen deposition as well as fibroblast and vascular densities compared to the sham-irradiation group (Figure 9). Exposure to different doses of fractionated radiation caused degeneration and hyalinization of collagen bundles, which was more apparent at 20 and 40 Gy. At higher irradiation doses, isolated collagen fragments surrounded by empty spaces were observed, which were not stained. Pre-treatment with hesperidin increased and restored collagen bundles in irradiated mice. However, the density of fibroblasts declined after fractionated irradiation in a dose-dependent manner compared to the CMC + sham-irradiation group (Figure 9). Large, unusual stellate cells or "radiation fibroblasts" were visible in the dermis at higher radiation doses, especially at 20 and 40 Gy. Epidermal thickness varied with increasing radiation dose, and irregularly shaped blood vessels were seen in all exposure doses in the CMC + irradiation group. Hesperidin treatment protected mice against radiation-induced damage to fibroblasts and vasculature, as evidenced by increased fibroblasts and vascular densities (Figures 9 and 10). However, the normal histological picture was not restored.

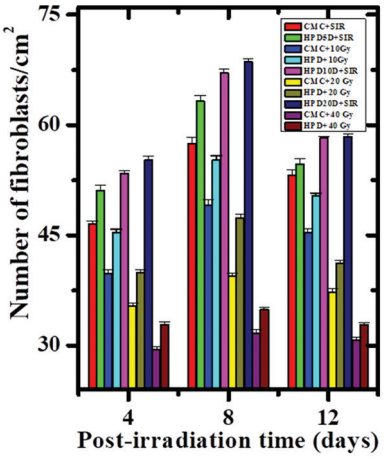

Figure 9. Effect of hesperidin on the fibroblast density in the granulation tissue of regenerating deep dermal wound of mice exposed to different doses of fractionated γ- radiation at different post-irradiation times. HPD groups are compared to the CMC and SIR groups.
Abbreviations: CMC: Carboxymethylcellulose; HPD: Hesperidin; SIR: Sham-irradiation.

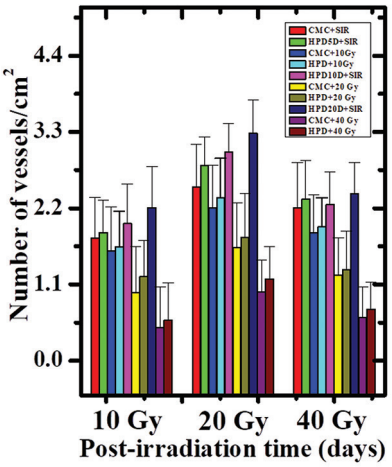

Figure 10. Effect of hesperidin on the blood vessel density in the granulation tissue of regenerating deep dermal wound of mice exposed to different doses of fractionated γ- radiation at different post-irradiation times. HPD groups are compared to the CMC and SIR groups.
Abbreviations: CMC: Carboxymethylcellulose; HPD: Hesperidin; SIR: Sham-irradiation.

4. Discussion

The increasing use of radioactive materials in various sectors, including industry, medicine, science, military, and localized areas of high radiation within nuclear facilities, has significantly increased the risk of large-scale, uncontrolled exposure to radiation, especially during accidents. In addition, the potential for deliberate misuse of radioactive material by terrorists cannot be dismissed.[8,9,70-72] Hemi-body irradiation, often administered in multiple fractionated doses, is frequently used alone or in combination with surgery or other modalities for treating neoplastic disorders. However, radiotherapy poses a critical challenge: it must balance the eradication of cancerous cells with the adverse effects on surrounding non-neoplastic tissues. This dichotomy raises significant concerns for surgeons regarding the malignant potential of irradiated tissue beds and wound-healing complications.[73-76] Given these challenges, it is imperative to explore new strategies that can mitigate the deleterious effect of ionizing radiation on normal tissue while enhancing wound repair and regeneration. Hesperidin, a dietary flavanone, has been investigated for its wound-healing potential in mice exposed to different doses of fractionated γ-irradiation and subsequently inflicted with full-thickness open excision wounds.

Wound contraction, defined as the centripetal movement of wound edges facilitating defect closure,[8,77,78] serves as a key indicator of wound healing progression. Periodic assessment of wound contraction allows for evaluating healing progress.[8,9,77,78] Exposure of mice to different doses of fractionated γ-radiation delays wound contraction and increases the MHT in a dose-dependent manner, with the maximum delay observed after 40 Gy irradiation. Previously, fractionated irradiation was found to delay wound contraction and MHT in a dose-dependent manner.[8,34,79] This dose-dependent delay in wound contraction and MHT suggests alterations in the local wound milieu, leading to impaired wound repair and regeneration.[8,9,34,79-82] Administration of 100 mg/kg body weight of hesperidin before exposure to fractionated radiation elicited enhanced regenerative responses, characterized by increased wound contraction and reduced MHT. Previous studies have shown that hesperidin enhances wound contraction and shortens MHT in deep dermal excision wounds of whole-body or hemi-body-irradiated mice. Similarly, topical application of hesperidin hydrogel has been effective in irradiated mice wounds.[9,10,83,84] Other compounds, such as ascorbic acid and curcumin, have also been reported to augment regenerative responses in irradiated wounds[8,34] by enhancing wound contraction and alleviating MHT in the deep dermal excision wound of mice.[79,80,85,86] A similar effect was discernible in the irradiated wounds of mice receiving topical application of curcumin hydrogel.[87] In addition, sildenafil citrate and antigen α-gal (Galα1-3Galβ1-(3)4GlcNAc-R) nanoparticles hydrogel gel have been shown to enhance wound contraction and tensile strength,[23,88] while Vitamin A supplementation has reduced radiation-induced delays in wound healing.[28]

Collagen plays a pivotal role in wound healing, as it is a principal component of connective tissue, providing a structural framework, strength, and milieu for regenerating

tissue.[89] It is essential for triggering inflammatory responses, mitogenesis, and cellular differentiation, as well as promoting the synthesis of inflammatory cytokines during wound healing.[90,91] A balance between collagen synthesis and degradation is crucial during normal wound healing as excess collagen formation can lead to keloid formation and hypertrophic scar formation, whereas insufficient collagen synthesis may result in wound dehiscence and chronic non-healing wounds/ulcers.[92,93] Collagen, primarily produced by fibroblasts, is a key molecule in the extracellular matrix of the wound bed, assisting in the acquisition of tensile strength during wound repair.[94] The reduction in hydroxyproline content in granulation tissue following fractionated irradiation indicates a depletion in collagen synthesis and retardation in wound repair and regeneration. An identical effect has been observed previously, where irradiation with different doses of fractionated γ-radiation resulted in a dose-dependent reduction in collagen synthesis.[8,34] Increasing doses of γ-radiation have been reported to progressively destroy native collagen synthesis.[79,80,85,86] Hesperidin administration before irradiation mitigated the radiation-induced decrease in collagen synthesis at all post-irradiation days. Similarly, ascorbic acid and curcumin have been shown to counteract the radiation-induced decline in collagen synthesis in regenerating wounds exposed to fractionated γ-irradiation.[8,34] Hesperidin treatment has been reported to increase collagen synthesis in excision wounds of mice.[9,10,83,84] Ascorbic acid, curcumin, and *Nigella sativa* extract have also been found to increase collagen synthesis in irradiated wounds.[36,79,80,86,95]

The estimation of DNA contents in wounds serves as a measure of cell proliferation, indicating regeneration and repair of wounds.[79,80,85,86] Fractionated irradiation causes a dose-dependent reduction in DNA synthesis.[8,34] A similar alleviation in DNA synthesis has been reported in regenerating wounds of mice exposed to different doses of γ-radiation.[79,80,85,86] Hesperidin treatment significantly increased DNA content at days 4 and 8 post-irradiation. Hesperidin administration has been shown to enhance DNA synthesis in regenerating wounds of mice created after whole-body or hemi-body irradiation.[9,10,83,84] Ascorbic acid, curcumin, or *Nigella sativa* methanol extract treatment of mice before whole/hemi-body or fractionated irradiation has been reported to increase DNA synthesis in granulation tissues of excision wounds.[8,36,79,80,85,86,95]

Inflammatory responses are critical for proper and smooth wound repair and regeneration, elicited immediately after injury through a series of well-orchestrated cellular and biochemical events. These events include increased synthesis of NO or nitrogen monoxide, a multifaceted signaling molecule that drives cellular metabolism, inflammation, and cell stress responses. NO exerts its effects through S-nitrosylation, binding to thiol proteins and non-protein molecules, which is essential for its protective or toxic effects.[96] Most evidence suggests that sufficient NO synthesis is required to promote processes central to wound healing, such as inflammation, angiogenesis, fibroblast synthetic function, epithelial cell proliferation, regulation of collagen formation, and wound contraction.[57,97,98] Estimation of nitrite or nitrate levels serves as a measure of NO production.[99] Nitrite levels, a stable end-product of NO synthesis, are elevated early and transiently in fluid obtained from sponges implanted in subcutaneous wounds.[58] The estimation of nitrate and nitrite levels in excision wounds of irradiated mice shows a reduction in NO synthesis. A similar effect has been reported in cutaneous excision wounds of mice receiving different doses of fractionated irradiation or acute whole-body or hemi-body exposure. The reduction in NO expression has been correlated with radiation-induced impairment of wound healing.[8,34,36,79,80,86,95,100] Administration of hesperidin before irradiation elevated nitrite levels. In earlier studies, hesperidin significantly increased nitrite and nitrate levels in wound granulation tissue of acute whole-body or hemi-body irradiated mice.[9,83,84] Similarly, ascorbic acid and curcumin increased nitrite and nitrate levels in open excision wounds of mice exposed to different doses of fractionated, acute whole, or hemi-body γ-irradiation, facilitating early healing of irradiated wounds.[8,34,79,80,86,95]

Histological observations align with biochemical estimations, revealing a decrease in collagen deposition, which is further supported by reduced fibroblast proliferation – cells responsible for collagen synthesis. An identical effect has been observed previously, where exposure of mice to fractionated irradiation inhibited fibroblast proliferation, neovascularization, and collagen deposition in regenerating wounds.[8,34] Irradiation has been reported to impede fibroblast proliferation, neovascularization, and collagen deposition in regenerating wounds.[100] Similarly, total-body and hemi-body irradiation decreased cellular influx, vascularization, fibroblast density, and collagen deposition in mice.[21,79,80,85,95,101] Cell culture studies of fibroblasts exposed to fractionated ionizing radiation have also demonstrated a decline in fibroblasts.[102] Pre-treatment of mice with hesperidin improved collagen deposition, increased vascular and fibroblast density, and reduced hyalinization in the hesperidin + irradiation group. Ascorbic acid and curcumin have been reported to augment collagen deposition, and vascular and fibroblast densities, as well as reduce hyalinization in open regenerating excision wounds of mice receiving

different doses of fractionated γ-radiation.[8,34] Hesperidin treatment has been shown to enhance collagen deposition and vascular and fibroblast densities while decreasing hyalinization in excision wounds of mice exposed to various doses of whole-body or hemi-body γ-radiation.[9,10] Ascorbic acid and curcumin have shown similar effects in irradiated wounds of mice.[79,80,85,95]

The response of normal tissues to radiation can be viewed as two concurrent phenomena. The first shares common features with traumatic wound healing, perturbed by radiation treatment. The second involves temporary or permanent changes to extracellular and cellular components in the exposed area, potentially leading to progressive injury over time. Unlike physical trauma, fractionated radiation therapy produces a series of repeated insults to tissues, altering normal tissue responses with each dose fraction. Repetitive radiation exposure exacerbates, suppresses, or substantially alters cellular and molecular responses compared to single radiation exposure or traumatic injury. The detrimental effects of ionizing radiation on wound healing are multifaceted, including direct cytotoxic effects on various cellular and molecular components of wound repair and indirect effects through the production of free radicals, which cause DNA damage and alter proteins, lipids, carbohydrates, and other complex molecules involved in tissue repair and regeneration.[8-13,21,34,79,80,85,95] Even benign injuries may become lethal when exposed to repeated low doses of radiation. Ionizing radiation severely damages vital tissues, especially those with high cell division rates, such as the hematopoietic system.[103-105] The loss of significant bone marrow cells can lead to immunosuppression, making individuals susceptible to bacterial infections and complicating wound healing. Shielding bone marrow during acute whole-body X-irradiation has been reported to reduce mortality and enhance wound closure in rats.[106] These studies suggest that radiation-sensitive, bone marrow-derived cells are crucial for tissue repair. Another possibility includes delayed fixation of the wound edge to underlying tissue due to reduced fibroblast proliferation and synthetic function in the granulation bed. Wound contraction is a function of contractile fibroblasts, known as myofibroblasts.[107-109] Irradiation impairs wound healing in the skin by affecting fibroblasts through cytotoxic effects on fibroblasts and delaying cell cycle progression.[15,110,111] Radiation may also impact fibroblasts through bone marrow depression, as some fibroblasts in normal subcutaneous connective tissue participating in wound healing originate from the bone marrow.[17,112,113] Recent studies suggest that vascular cell progenitors residing in the bone marrow contribute to blood vessel formation during tissue repair and in other pathological conditions.[114] A similar effect cannot be ruled out in this study, as long bones of hind limbs were irradiated during fractionated irradiation.

Wounding and irradiation induce oxidative stress, and their combined effects can further complicate tissue conditions, as observed in this study. The evaluation of antioxidant status in irradiated wounds revealed an increase in LPx, consistent with previous studies that ionizing radiations induce LPx.[115,116] Fractionated irradiation negatively impacted antioxidant status, resulting in a significant decline in GSH concentration and GSHPx activities in this study. A similar effect has been reported earlier in the regenerating wounds of mice receiving fractionated irradiation.[115,116] Exposure to different doses of γ-radiation has been reported to reduce GSHPx activities and GSH concentration.[36,117] Hesperidin pre-treatment protected against radiation-induced LPx and mitigated the decline in GSHPx activity and GSH levels.[118] Ascorbic acid and curcumin have been reported to arrest the decline in GSHpx and GSH contents and reduce LPx after fractionated γ-radiation.[115,116] *Nigella sativa* has been found to suppress radiation-induced declines in GSH and GSHpx in regenerating excision wounds of mice exposed to whole-body γ-radiation and reduce LPx.

The exact mechanism by which hesperidin enhanced the healing of irradiated wounds is not clearly understood. However, the pleiotropic action of hesperidin may be responsible for early repair and regeneration of irradiated wounds. The acceleration in wound healing after hesperidin treatment may not be due to a single mechanism but rather an interplay of several mechanisms during wound healing. Reactive oxygen species play a fundamental role in tissue repair, and hesperidin may have reduced radiation- and wound injury-induced free radicals to optimal levels, facilitating early wound closure. Hesperidin passivates free radicals and elevates catalase, GSHPx, and superoxide dismutase, resulting in accelerated wound healing.[118-120] Hesperidin may have upregulated molecular events essential for wound repair, including collagen, NO, and DNA synthesis, leading to early repair. In this study, hesperidin was found to increase collagen, NO, and DNA contents in mice wounds. It acts as an antioxidant, anti-inflammatory, and antimicrobial agent, contributing to the early healing of irradiated wounds.[37,121,122] This contention is supported by the fact that hesperidin increased the antioxidant levels and reduced LPx in the present study. Hesperidin enhances fibroblast proliferation and vasculature, leading to early wound closure. Although the molecular mechanisms of hesperidin's actions on irradiated wounds were not investigated, it is hypothesized to modulate the NF-κB signaling pathway. NF-κB is a key regulatory molecule whose transcriptional activation

elicits inflammation required for wound regeneration by promoting cell proliferation, adhesion, and differentiation. However, prolonged inflammation delays wound repair, regeneration, and scar development.[123] NF-κB is upregulated after wounding and irradiation. Hesperidin blocks excessive transcriptional activation of NF-κB, accelerating the repair and regeneration of irradiated wounds observed in this study.[78,124,125] COX-2 is elevated after wounding, and hesperidin suppresses its expression to augment regenerative responses.[78,120,126]

The vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β) are essential in promoting the growth of fibroblasts, keratinocytes, and endothelial cells, thereby expediting the wound healing process. Although the levels of these growth factors were not measured, it is plausible that hesperidin could have increased the expression of VEGF, TNF-α, and TGF-β at a molecular level, leading to accelerated regenerative responses and faster wound repair in this study.[7,119,120] Wounding and irradiation activate a cascade of oxidative stress, which may be counteracted by stimulating vitagenes that aid in wound repair and regeneration through elevated expression of vitagenes. The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling plays a crucial role in countering oxidative stress by stimulating vitagenes, including heat shock proteins, heme oxygenase-1, NAD(P)H-quinone oxidoreductase, GSH transferase, GSHPx, sirtuin-1, and the thioredoxin system.[127,128] Plant polyphenols have been found to abate oxidative stress by stimulating Nrf2 signaling and vitagenes.[129] The increase in GSHPx and GSH concentrations is due to hesperidin's ability to upregulate Nrf2 and vitagenes, promoting wound healing by enhancing the antioxidant system.[45,120] Similarly, hesperetin, the aglycone form of hesperidin, has been reported to upregulate Nrf2 and vitagenes.[130] Matrix metalloproteinases (MMP), also known as gelatinase, play a crucial role in angiogenesis, matrix remodeling (MMP-2), and re-epithelialization of regenerating wounds (MMP-9). Hesperidin upregulates both MMP-2 and MMP-9 to heal irradiated wounds.[119] Poly-ADP ribose polymerase (PARP) regulates cell proliferation and is hyperactivated during wound healing, as it is involved in inflammation, cell proliferation, and migration and is co-activated with NF-κB.[131,132] Hesperidin treatment attenuated PARP activation and accelerated wound healing in this study.[45]

5. Conclusion

Most animal models of radiation-impaired wound healing involve irradiating the whole animal or a localized skin flap with a single large dose of radiation, which limits the applicability of these findings to humans, in which radiotherapy typically involves multiple fractions of smaller doses of radiation over an extended period. The present study holds clinical relevance as it employs a regimen similar to clinical radiotherapy to study the effect of fractionated radiation on wound repair and regeneration while exploring the application of hesperidin in this setting. Our study demonstrates that daily hesperidin treatment before exposure to fractionated γ-radiation expedites wound healing in mice, as evidenced by enhanced wound contraction, increased synthesis of collagen, DNA, and NO, as well as improved fibroblast proliferation and increased vasculature in the wound bed. The enhancement in wound repair and regeneration may be attributed to hesperidin's ability to increase the synthesis of crucial molecules, such as DNA, collagen, and NO while inhibiting or scavenging free radicals induced by wounding and irradiation. In addition, hesperidin's downregulation of PARP, NF-kB, and COX-2, and upregulation of VEGF, TNF-α, TGF-β, MMP-2, MMP-9, Nrf2, and vitagenes likely contributed to the enhanced regeneration and repair of irradiated wounds. Our findings suggest that strategies to modulate the pathophysiological environment of irradiated wounds using hesperidin may hold significant promise for improving radiation-impaired wound healing. Further studies are warranted to better understand the molecular mechanisms by which hesperidin initiates and supports tissue repair processes in irradiated wounds.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Ganesh Chandra Jagetia
Investigation: All authors
Methodology: Kota V. N. M. Rao
Writing – original draft: Ganesh Chandra Jagetia
Writing – review & editing: Ganesh Chandra Jagetia

Consent for publication

Not applicable.

Availability of data

All data are presented in the manuscript.

References

1. Reaz F, Traneus E, Bassler N. Tuning spatially fractionated radiotherapy dose profiles using the moiré effect. *Sci Rep*. 2024;14(1):8468.

doi: 10.1038/S41598-024-55104-7

2. Gil GOB, De Andrade WP, Diniz PHC, *et al*. A phase II randomized clinical trial to assess toxicity and quality of life of breast cancer patients with hypofractionated versus conventional fractionation radiotherapy with regional nodal irradiation in the context of COVID-19 crisis. *Front Oncol*. 2023;13:1202544.

doi: 10.3389/FONC.2023.1202544/BIBTEX

3. Lu Y, Hui B, Yang D, *et al*. Efficacy and safety analysis of hypofractionated and conventional fractionated radiotherapy in postoperative breast cancer patients. *BMC Cancer*. 2024;24(1):181.

doi: 10.1186/S12885-024-11918-2

4. Ryu S, Deshmukh S, Timmerman RD, *et al*. Stereotactic radiosurgery vs conventional radiotherapy for localized vertebral metastases of the spine: Phase 3 results of NRG Oncology/RTOG 0631 randomized clinical trial. *JAMA Oncol*. 2023;9(6):800-807.

doi: 10.1001/JAMAONCOL.2023.0356

5. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: A cellular perspective. *Physiol Rev*. 2019;99(1): 665-706.

doi: 10.1152/physrev.00067.2017

6. Wilkinson HN, Hardman MJ. Wound healing: Cellular mechanisms and pathological outcomes. *Open Biol*. 2020;10(9):200223.

doi: 10.1098/RSOB.200223

7. Peña OA, Martin P. Cellular and molecular mechanisms of skin wound healing. *Nat Rev Cell Mol Biol*. 2024;25:599-616.

doi: 10.1038/s41580-024-00715-1

8. Jagetia GC, Rajanikant GK. Acceleration of wound repair by curcumin in the excision wound of mice exposed to different doses of fractionated γ radiation. *Int Wound J*. 2012;9(1):76-92.

doi: 10.1111/j.1742-481X.2011.00848.x

9. Jagetia G, Rao KVNM. Hesperidin treatment abates radiation-induced delay in healing of deep cutaneous excision wound of mice hemi-body exposed to different doses of γ-radiation. *Clin Dermatol Dermat*. 2018;1(1):104.

10. Jagetia GC, Rao KVNM. Hesperidin, a citrus bioflavonoid potentiates repair and regeneration of deep dermal excision wounds of mice whole body exposed to different doses of ^{60}Co γ-radiation. *Clin Dermatol*. 2018;3(2):000147.

doi: 10.23880/cdoaj-16000147

11. Son B, Lee S, Kim H, *et al*. Low dose radiation attenuates inflammation and promotes wound healing in a mouse burn model. *J Dermatol Sci*. 2019;96(2):81-89.

doi: 10.1016/J.JDERMSCI.2019.10.004

12. Wijerathne H, Langston JC, Yang Q, *et al*. Mechanisms of radiation-induced endothelium damage: Emerging models and technologies. *Radiother Oncol*. 2021;158:21-32.

doi: 10.1016/J.RADONC.2021.02.007

13. Kameni LE, Januszyk M, Berry CE, *et al*. A review of radiation-induced vascular injury and clinical impact. *Ann Plast Surg*. 2024;92(2):181-185.

doi: 10.1097/SAP.0000000000003723

14. Pendleton MM, Emerzian SR, Liu J, *et al*. Effects of *ex vivo* ionizing radiation on collagen structure and whole-vertebrae. *Bone*. 2019;128:115043.

doi: 10.1016/j.bone.2019.115043

15. Rudolph R, Berg J Vande, Schneider JA, Fisher JC, Poolman WL. Slowed growth of cultured fibroblasts from human radiation wounds. *Plast Reconstr Surg*. 1988;82(4):669-675.

doi: 10.1097/00006534-198810000-00019

16. Kiang JG, Garrison BR, Burns TM, *et al*. Wound trauma alters ionizing radiation dose assessment. *Cell Biosci*. 2012;2(1):20.

doi: 10.1186/2045-3701-2-20

17. Green DE, Rubin CT. Consequences of irradiation on bone and marrow phenotypes, and its relation to disruption of hematopoietic precursors. *Bone*. 2014;63:87-94.

doi: 10.1016/J.BONE.2014.02.018

18. Lumniczky K, Impens N, Armengol G, *et al*. Low dose ionizing radiation effects on the immune system. *Environ Int*. 2021;149:106212.

doi: 10.1016/J.ENVINT.2020.106212

19. Nepon H, Safran T, Reece EM, Murphy AM, Vorstenbosch J, Davison PG. Radiation-induced tissue damage: Clinical consequences and current treatment options. *Semin Plast Surg*. 2021;35(3):181-188.

doi: 10.1055/S-0041-1731464

20. Loinard C, Ribault A, Lhomme B, *et al*. HuMSC-EV induce monocyte/macrophage mobilization to orchestrate neovascularization in wound healing process following radiation injury. *Cell death Discov*. 2023;9(1):38.

doi: 10.1038/S41420-023-01335-Y

21. Vegesna V, Withers HR, Holly FE, McBride WH. The effect of local and systemic irradiation on impairment of wound healing in mice. *Radiat Res*. 2005;135(3):434-433.

22. Ozbek N, Guneren E, Yildiz L, Meydan D, Cakir S, Coskun M. The effect of pre-operative conventional and hyperfractionated radiotherapy schedules on wound healing and tensile strength in rats: An experimental study. *Int J Oral Maxillofac Surg*. 2005;34(2):185-192.

doi: 10.1016/J.IJOM.2004.05.005

23. Kulshrestha S, Chawla R, Singh S, *et al*. Protection of sildenafil citrate hydrogel against radiation-induced skin wounds. *Burns*. 2020;46(5):1157-1169.

doi: 10.1016/J.BURNS.2019.11.020

24. Orsted H. Radiation skin reaction. *Can Nurse*. 1989;85(9): 30-31.

25. Olascoaga A, Vilar-Compte D, Poitevin-Chacón A, Contreras-Ruiz J. Wound healing in radiated skin: Pathophysiology and treatment options. *Int Wound J*. 2008;5(2):246-257.

doi: 10.1111/J.1742-481X.2008.00436.X

26. Song S, Cheng T. The effect of systemic and local irradiation on wound macrophages and the repair promoting action of phenytoin sodium. *Honghua Yi Xue Za Zhi*. 1997;77(1):54-57.

27. Song S, Cheng T, Lin Y. Influence of modulatory activity of wound fluid on the characteristics of fibroblasts from irradiation and the action of phenytoin sodium. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi*. 1999;13(1):47-50.

28. Levenson SM, Gruber CA, Rettura G, Gruber DK, Demetriou AA, Seifter E. Supplemental vitamin A prevents the acute radiation-induced defect in wound healing. *Ann Surg*. 1984;200(4):494-512.

doi: 10.1097/00000658-198410000-00011

29. Stromberg LR, Mclaughlin MM, Donati RM. Combined surgical and radiation injury. 3. The effect of pre-irradiation radioprotective drug treatment. *Proc Soc Exp Biol Med*. 1968;129(1):140-143.

doi: 10.3181/00379727-129-33270

30. Donati RM. Combined surgical and radiation injury. IV. Effect of antimicrobials on the wound healing pattern of the x-irradiated rat. *Arch Surg*. 1971;102(2):132-135.

doi: 10.1001/ARCHSURG.1971.01350020042011

31. Mustoe TA, Purdy J, Gramates P, Deuel TF, Thomason A, Pierce GF. Reversal of impaired wound healing in irradiated rats by platelet-derived growth factor-BB. *Am J Surg*. 1989;158(4):345-350.

doi: 10.1016/0002-9610(89)90131-1

32. Hom DB, Unger GM, Pernell KJ, Manivel JC. Improving surgical wound healing with basic fibroblast growth factor after radiation. *Laryngoscope*. 2005;115(3):412-422.

doi: 10.1097/01.MLG.0000157852.01402.12

33. Kinoshita N, Tsuda M, Hamuy R, *et al*. The usefulness of basic fibroblast growth factor for radiation-exposed tissue. *Wound Repair Regen*. 2012;20(1):91-102.

doi: 10.1111/J.1524-475X.2011.00758.X

34. Jagetia GC, Rajanikant GK, Rao SK. Evaluation of the effect of ascorbic acid treatment on wound healing in mice exposed to different doses of fractionated gamma radiation. *Radiat Res*. 2003;159(3):371-380.

doi: 10.1667/0033-7587(2003)159[0371:EOTEOA]2.0.CO;2

35. Jagetia GC, Venkatesha VA. Effect of mangiferin on radiation-induced micronucleus formation in cultured human peripheral blood lymphocytes. *Environ Mol Mutagen*. 2005;46(1):12-21.

doi: 10.1002/em.20124

36. Jagetia GC, Ravikiran PB. Acceleration of wound repair and regeneration by *Nigella sativa* in the deep dermal excision wound of mice whole body exposed to different doses of γ-radiation. *Am Res J Med Surg*. 2015;1:1-17.

37. Vabeiryureilai M, Lalrinzuali K, Jagetia GC. Determination of anti-inflammatory and analgesic activities of a citrus bioflavanoid, hesperidin in Mice. *Immunochem Immunopathol*. 2015;1(2):107.

doi: 10.4172/2469-9756.1000107

38. Yamamoto M, Suzuki A, Jokura H, Yamamoto N, Hase T. Glucosyl hesperidin prevents endothelial dysfunction and oxidative stress in spontaneously hypertensive rats. *Nutrition*. 2008;24(5):470-476.

doi: 10.1016/j.nut.2008.01.010

39. Iranshahi M, Rezaee R, Parhiz H, Roohbakhsh A, Soltani F. Protective effects of flavonoids against microbes and toxins: The cases of hesperidin and hesperetin. *Life Sci*. 2015;137:125-132.

doi: 10.1016/j.lfs.2015.07.014

40. Jagetia GC. A review on the anti-inflammatory activity of hesperidin, a bioflavonoid synthesized by citrus fruits. *J Immunol Inflamm Dis Ther*. 2018;1:10037.

doi: 10.31579/2637-8876/007

41. Anwar A, Masri A, Rao K, *et al*. Antimicrobial activities of green synthesized gums-stabilized nanoparticles loaded with flavonoids. *Sci Rep*. 2019;9(1):3122.

doi: 10.1038/s41598-019-39528-0

42. Rasquel-Oliveira FS, Manchope MF, Staurengo-Ferrari L, *et al*. Hesperidin methyl chalcone interacts with NFκB Ser276 and inhibits zymosan-induced joint pain and inflammation, and RAW 264.7 macrophage activation. *Inflammopharmacology*.

2020;28(4):979-992.

doi: 10.1007/s10787-020-00686-7

43. Valls RM, Pedret A, Calderón-Pérez L, *et al*. Effects of hesperidin in orange juice on blood and pulse pressures in mildly hypertensive individuals: A randomized controlled trial (Citrus study). *Eur J Nutr*. 2021;60(3):1277-1288.

doi: 10.1007/s00394-020-02279-0

44. Tanaka T, Makita H, Kawabata K, *et al*. Chemoprevention of azoxymethane-induced rat colon carcinogenesis by the naturally occurring flavonoids, diosmin and hesperidin. *Carcinogenesis*. 1997;18(5):957-965.

doi: 10.1093/carcin/18.5.957

45. Vabeiryureilai M, Lalrinzuali K, Jagetia GC. Chemopreventive effect of hesperidin, a citrus bioflavonoid in two stage skin carcinogenesis in Swiss albino mice. *Heliyon*. 2019;5(10):e02521.

doi: 10.1016/j.heliyon.2019.e02521

46. Hermawan A, Khumaira A, Ikawati M, *et al*. Identification of key genes of hesperidin in inhibition of breast cancer stem cells by functional network analysis. *Comput Biol Chem*. 2021;90:107427.

doi: 10.1016/j.compbiolchem.2020.107427

47. Kurowska EM, Spence JD, Jordan J, *et al*. HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia. *Am J Clin Nutr*. 2000;72(5):1095-1100.

doi: 10.1093/ajcn/72.5.1095

48. Chiba H, Uehara M, Wu J, *et al*. Hesperidin, a citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. *J Nutr*. 2003;133(6):1892-1897.

doi: 10.1093/jn/133.6.1892

49. Hou M, Man M, Man W, *et al*. Topical hesperidin improves epidermal permeability barrier function and epidermal differentiation in normal murine skin. *Exp Dermatol*. 2012;21(5):337-340.

doi: 10.1111/j.1600-0625.2012.01455.x

50. Dobiaš L, Petrová M, Vojtko R, Kristová V. Long-term treatment with hesperidin improves endothelium-dependent vasodilation in femoral artery of spontaneously hypertensive rats: The involvement of NO-synthase and Kv channels. *Phyther Res*. 2016;30(10):1665-1671.

doi: 10.1002/ptr.5670

51. Akamo AJ, Ojelabi AO, Somade OT, *et al*. Hesperetin-7-O-rhamnoglucoside ameliorates dichlorvos-facilitated cardiotoxicity in rats by counteracting ionoregulatory, ion pumps, redox, and lipid homeostasis disruptions. *Toxicol Reports*. 2024;13:101698.

doi: 10.1016/J.TOXREP.2024.101698

52. Li Y, Kandhare AD, Mukherjee AA, Bodhankar SL. Acute and sub-chronic oral toxicity studies of hesperidin isolated from orange peel extract in Sprague Dawley rats. *Regul Toxicol Pharmacol*. 2019;105:77-85.

doi: 10.1016/j.yrtph.2019.04.001

53. Mayumi K, Seiko T, Masa-Aki S, Masao H, Shoji F, Nobuyuki I. Subchronic toxicity study of methyl hesperidin in mice. *Toxicol Lett*. 1993;69(1):37-44.

doi: 10.1016/0378-4274(93)90143-L

54. Abdel-Raheem IT, Abdel-Ghany AA. Hesperidin alleviates doxorubicin-induced cardiotoxicity in rats. *J Egypt Natl Canc Inst*. 2009;21(2):175-184.

55. Merzoug S, Toumi ML. Effects of hesperidin on formaldehyde-induced toxicity in pregnant rats. *EXCLI J*. 2017;16:400-413.

doi: 10.17179/EXCLI2017-142

56. Caglayan C, Kandemir FM, Darendelioğlu E, Küçükler S, Ayna A. Hesperidin protects liver and kidney against sodium fluoride-induced toxicity through anti-apoptotic and anti-autophagic mechanisms. *Life Sci*. 2021;281:119730.

doi: 10.1016/J.LFS.2021.119730

57. Li W, Kandhare AD, Mukherjee AA, Bodhankar SL. Hesperidin, a plant flavonoid accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats: Role of TGF-ß/Smads and Ang-1/Tie-2 signaling pathways. *EXCLI J*. 2018;17:399-419.

doi: 10.17179/excli2018-1036

58. Pradeep K, Park SH, Ko KC. Hesperidin a flavanoglycone protects against γ-irradiation induced hepatocellular damage and oxidative stress in Sprague-Dawley rats. *Eur J Pharmacol*. 2008;587(1-3):273-280.

doi: 10.1016/j.ejphar.2008.03.052

59. Wang Y, Yu H, Zhang J, Gao J, Ge X, Lou G. Hesperidin inhibits HeLa cell proliferation through apoptosis mediated by endoplasmic reticulum stress pathways and cell cycle arrest. *BMC Cancer*. 2015;15(1):682.

doi: 10.1186/s12885-015-1706-y

60. Zhao W, Chen Y, Zhang X. Hesperidin-triggered necrosis-like cell death in skin cancer cell line A431 might be prompted by ros mediated alterations in mitochondrial membrane potential. *Int J Clin Exp Med*. 2018;11(3):1948-1954.

61. Xiong H, Wang J, Ran Q, *et al*. Hesperidin: A therapeutic agent for obesity. *Drug Des Devel Ther*. 2019;13:3855-3866.

doi: 10.2147/DDDT.S227499

62. Musa AE, Omyan G, Esmaely F, Shabeeb D. Radioprotective effect of hesperidin: A systematic review. *Medicina (Kaunas)*. 2019;55(7):370.

doi: 10.3390/medicina55070370

63. Woessner JF Jr. The determination of hydroxyproline in

tissue and protein samples containing small proportions of this imino acid. *Arch Biochem Biophys*. 1961;93(2):440-447.

doi: 10.1016/0003-9861(61)90291-0

64. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J*. 1956;62(2):315-323.

doi: 10.1042/bj0620315

65. Hevel JM, Marletta MA. Nitric-oxide synthase assays. *Methods Enzymol*. 1994;233:250-258.

doi: 10.1016/S0076-6879(94)33028-X

66. Sazuka Y, Tanizawa H, Takino Y. Effect of adriamycin on the activities of superoxide dismutase, glutathione peroxidase and catalase in tissues of mice. *Jpn J Cancer Res*. 1989;80(1):89-94.

doi: 10.1111/J.1349-7006.1989.TB02250.X

67. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta*. 1979;582(1):67-78.

doi: 10.1016/0304-4165(79)90289-7

68. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol*. 1978;52:302-310.

doi: 10.1016/S0076-6879(78)52032-6

69. Waterborg JH, Matthews HR. The Lowry method for protein quantitation. *Methods Mol Biol*. 1994;32:1-4.

doi: 10.1385/0-89603-268-X:1

70. Biancotto S, Malizia A, Pinto M, Contessa GM, Coniglio A, D'Arienzo M. Analysis of a dirty bomb attack in a large metropolitan area: Simulate the dispersion of radioactive materials. *J Instrum*. 2020;15:P02019.

doi: 10.1088/1748-0221/15/02/P02019

71. Brambilla S, Nelson MA, Brown MJ. Dirty bomb source term characterization and downwind dispersion: Review of experimental evidence. *J Environ Radioact*. 2023;263:107166.

doi: 10.1016/J.JENVRAD.2023.107166

72. Niichel M, Brackett M, Purcell B, Gologorsky D, Werve B. Covert system for detecting nuclear dirty bombs in public venues. *Nucl Sci Technol*. 2024;2:2.

doi: 10.12688/NUCLSCITECHNOLOPENRES.17455.1

73. Abouarab MH, Salem IL, Degheidy MM, *et al*. Therapeutic options and postoperative wound complications after extremity soft tissue sarcoma resection and postoperative external beam radiotherapy. *Int Wound J*. 2018;15(1):148-158.

doi: 10.1111/IWJ.12851

74. Albano D, Benenati M, Bruno A, *et al*. Imaging side effects and complications of chemotherapy and radiation therapy: A pictorial review from head to toe. *Insights Imaging*. 2021;12(1):76.

doi: 10.1186/S13244-021-01017-2

75. Berger L, Grimm A, Sütterlin M, *et al*. Major complications after intraoperative radiotherapy with low-energy X-rays in early breast cancer. *Strahlenther Onkol*. 2024;200(4):276-286.

doi: 10.1007/S00066-023-02128-Z

76. Yan HK, Huang J, Yang ZH, *et al*. Comparison of the incidence of wound complications with preoperative and postoperative radiotherapy in patients with extremity soft tissue sarcoma resection: A meta-analysis. *Int Wound J*. 2024;21(2):e14441.

doi: 10.1111/IWJ.14441

77. Kaplani K, Koutsi S, Armenis V, *et al*. Wound healing related agents: Ongoing research and perspectives. *Adv Drug Deliv Rev*. 2018;129:242-253.

doi: 10.1016/j.addr.2018.02.007

78. Vabeiryureilai M, Lalrinzuali K, Jagetia GC. NF-κB and COX-2 repression with topical application of hesperidin and naringin hydrogels augments repair and regeneration of deep dermal wounds. *Burns*. 2022;48(1):132-145.

doi: 10.1016/j.burns.2021.04.016

79. Jagetia GC, Rajanikant GK. Role of curcumin, a naturally occurring phenolic compound of turmeric in accelerating the repair of excision wound, in mice whole-body exposed to various doses of gamma-radiation. *J Surg Res*. 2004;120(1):127-138.

doi: 10.1016/j.jss.2003.12.003

80. Jagetia GC, Rajanikant GK. Curcumin treatment enhances the repair and regeneration of wounds in mice exposed to hemibody γ-irradiation. *Plast Reconstr Surg*. 2005;115(2):515-528.

doi: 10.1097/01.PRS.0000148372.75342.D9

81. Jacobson LK, Johnson MB, Dedhia RD, Niknam-Bienia S, Wong AK. Impaired wound healing after radiation therapy: A systematic review of pathogenesis and treatment. *JPRAS Open*. 2017;13:92-105.

doi: 10.1016/J.JPRA.2017.04.001

82. Diaz C, Hayward CJ, Safoine M, *et al*. Ionizing radiation mediates dose dependent effects affecting the healing kinetics of wounds created on acute and late irradiated skin. *Surgeries*. 2021;2(1):35-57.

doi: 10.3390/SURGERIES2010004

83. Jagetia GC, Rao KVNM. Topical application of hesperidin, a citrus bioflavanone accelerates healing of full thickness dermal excision wounds in mice exposed to 6 Gy of whole body γ-radiation. *J Clin Res Dermatology*. 2017;4(3):1-8.

doi: 10.15226/2378-1726/4/3/00162

84. Jagetia GC, Rao KVNM. Acceleration in the repair and regenerative responses by different doses of hesperidin in

the deep full thickness cutaneous wound of mice whole body exposed to 6 Gy of γ-radiation. *Nurs Healthc Int J*. 2018;2(3):000149.

85. Jagetia GC, Rajanikant GK, Rao KVNM. Modulation of radiation-induced delay in the wound healing by ascorbic acid in mice exposed to different doses of hemi-body gamma radiation. *Wounds*. 2003;15(10):324-338.

86. Jagetia GC, Rajanikant GK, Baliga MS, Rao KVNM, Kumar P. Augmentation of wound healing by ascorbic acid treatment in mice exposed to gamma-radiation. *Int J Radiat Biol*. 2004;80(5):347-354.

doi: 10.1080/09553000410001692744

87. Jagetia GC, Rajanikant GK. Topical application of curcumin augments healing of deep dermal excision wound of mice exposed to whole-body gamma radiation. *J Nurs Healthc*. 2017;2(1):1-7.

88. Samadi A, Buro J, Dong X, *et al*. Topical α-Gal nanoparticles enhance wound healing in radiated skin. *Skin Pharmacol Physiol*. 2022;35(1):31-40.

doi: 10.1159/000518015

89. Antonio JDS, Jacenko O, Fertala A, Orgel JPRO. Collagen structure-function mapping informs applications for regenerative medicine. *Bioeng (Basel)*. 2020;8(1):3.

doi: 10.3390/BIOENGINEERING8010003

90. Xue M, Jackson CJ. Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. *Adv Wound Care (New Rochelle)*. 2015;4(3):119-136.

doi: 10.1089/wound.2013.0485

91. Gardeazabal L, Izeta A. Elastin and collagen fibres in cutaneous wound healing. *Exp Dermatol*. 2024;33(3):e15052.

doi: 10.1111/EXD.15052

92. Macarak EJ, Wermuth PJ, Rosenbloom J, Uitto J. Keloid disorder: Fibroblast differentiation and gene expression profile in fibrotic skin diseases. *Exp Dermatol*. 2021;30(1):132-145.

doi: 10.1111/EXD.14243

93. Hofmann E, Fink J, Pignet AL, *et al*. Human *in vitro* skin models for wound healing and wound healing disorders. *Biomedicines*. 2023;11(4):1056.

doi: 10.3390/BIOMEDICINES11041056

94. Mathew-Steiner S, Roy S, Sen C. Collagen in wound healing. *Bioeng (Basel)*. 2021;8:63.

doi: 10.3390/bioengineering8050063

95. Jagetia GC, Rajanikant GK, Rao KVNM. Ascorbic acid increases healing of excision wounds of mice whole body exposed to different doses of gamma-radiation. *Burns*. 2007;33(4):484-494.

doi: 10.1016/j.burns.2006.08.025

96. Calabrese V, Mancuso C, Calvani M, Rizzarelli E, Butterfield DA, Giuffrida Stella AM. Nitric oxide in the central nervous system: Neuroprotection versus neurotoxicity. *Nat Rev Neurosci*. 2007;8(10):766-775.

doi: 10.1038/NRN2214

97. Malone-Povolny MJ, Maloney SE, Schoenfisch MH. Nitric oxide therapy for diabetic wound healing. *Adv Healthc Mater*. 2019;8(12):e1801210.

doi: 10.1002/ADHM.201801210

98. Pinto RV, Carvalho S, Antunes F, Pires J, Pinto ML. Emerging nitric oxide and hydrogen sulfide releasing carriers for skin wound healing therapy. *ChemMedChem*. 2022;17(1):e202100429.

doi: 10.1002/CMDC.202100429

99. Lundberg JO, Weitzberg E. Nitric oxide signaling in health and disease. *Cell*. 2022;185(16):2853-2878.

doi: 10.1016/J.CELL.2022.06.010

100. Schäffer M, Weimer W, Wider S, *et al*. Differential expression of inflammatory mediators in radiation-impaired wound healing. *J Surg Res*. 2002;107(1):93-100.

doi: 10.1006/jsre.2002.6494

101. Doyle J, Li Y, Salloum A, FitzGerald TJ, Walton RL. The effects of radiation on neovascularization in a rat model. *Plast Reconstr Surg*. 1996;98(1):129-134.

doi: 10.1097/00006534-199607000-00020

102. Dahlberg WK, Little JB. Response of dermal fibroblast cultures from patients with unusually severe responses to radiotherapy and from ataxia telangiectasia heterozygotes to fractionated radiation. *Clin Cancer Res*. 1995;1(8):785-790.

103. Zelman D, Song IC, Porteous DD, Bromberg BE. The effect of total body irradiation on wound healing and the hematopoietic system in mice. *Bull N Y Acad Med*. 1969;45(3):293-300.

104. Shao L, Luo Y, Zhou D. Hematopoietic stem cell injury induced by ionizing radiation. *Antioxidants Redox Signal*. 2014;20(9):1447-1462.

doi: 10.1089/ARS.2013.5635

105. Lu Y, Hu M, Zhang Z, Qi Y, Wang J. The regulation of hematopoietic stem cell fate in the context of radiation. *Radiat Med Prot*. 2020;1(1):31-34.

doi: 10.1016/J.RADMP.2020.01.002

106. Stromberg LR, Woodward KT, Mahin DT, Donati RM. Altered wound healing in X-irradiated rats: The effect of bone marrow shielding. *Experientia*. 1967;23(12):1064-1065.

doi: 10.1007/BF02136454

107. Wang J, Boerma M, Fu Q, Hauer-Jensen M. Radiation responses in skin and connective tissues: Effect on wound healing and surgical outcome. *Hernia*. 2006;10(6):502-506.

doi: 10.1007/S10029-006-0150-Y

108. Schuster R, Younesi F, Ezzo M, Hinz B. The role of myofibroblasts in physiological and pathological tissue repair. *Cold Spring Harb Perspect Biol*. 2023;15(1):a041231.

doi: 10.1101/CSHPERSPECT.A041231

109. Younesi FS, Miller AE, Barker TH, Rossi FMV, Hinz B. Fibroblast and myofibroblast activation in normal tissue repair and fibrosis. *Nat Rev Mol Cell Biol*. 2024;25(8):617-638.

doi: 10.1038/S41580-024-00716-0

110. Bumann J, Santo-Holtje L, Loffler H, Bamberg M, Rodemann HP. Radiation-induced alterations of the proliferation dynamics of human skin fibroblasts after repeated irradiation in the subtherapeutic dose range. *Strahlenther Onkol*. 1995;171(1):35-41.

111. Cialdai F, Risaliti C, Monici M. Role of fibroblasts in wound healing and tissue remodeling on Earth and in space. *Front Bioeng Biotechnol*. 2022;10:958381.

doi: 10.3389/FBIOE.2022.958381

112. Bellini A, Mattoli S. The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. *Lab Invest*. 2007;87(9):858-870.

doi: 10.1038/LABINVEST.3700654

113. Correa-Gallegos D, Jiang D, Rinkevich Y. Fibroblasts as confederates of the immune system. *Immunol Rev*. 2021;302(1):147-162.

doi: 10.1111/IMR.12972

114. Comazzetto S, Shen B, Morrison SJ. Niches that regulate stem cells and hematopoiesis in adult bone marrow. *Develomental Cell*. 2021;56(13):1848-1860.

doi: 10.1016/j.devcel.2021.05.018

115. Jagetia GC, Rajanikant GK, Rao SK, Baliga M. Alteration in the glutathione, glutathione peroxidase, superoxide dismutase and lipid peroxidation by ascorbic acid in the skin of mice exposed to fractionated gamma radiation. *Clin Chim Acta*. 2003;332(1-2):111-121.

doi: 10.1016/S0009-8981(03)00132-3

116. Jagetia GC, Rajanikant GK. Curcumin stimulates the antioxidant mechanisms in mouse skin exposed to fractionated γ-irradiation. *Antioxidants (Basel)*. 2015;4(1):25-41.

doi: 10.3390/ANTIOX4010025

117. Jagetia GC, Ganapathi NG, Venkatesh P, Rao N, Baliga MS. Evaluation of the radioprotective effect of Liv 52 in mice. *Environ Mol Mutagen*. 2006;47(7):490-502.

doi: 10.1002/em.20218

118. Jagetia GC, Rao KVNM. Hesperidin, a citrus bioflavonoid reduces the oxidative stress in the skin of mouse exposed to partial body γ-radiation. *Transcriptomics*. 2015;3:2.

doi: 10.4172/2329-8936.1000111

119. Carvalho MTB, Araújo-Filho HG, Barreto AS, Quintans-Júnior LJ, Quintans JSS, Barreto RSS. Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. *Phytomedicine*. 2021;90:153636.

doi: 10.1016/J.PHYMED.2021.153636

120. Zulkefli N, Che Zahari CNM, Sayuti NH, *et al*. Flavonoids as potential wound-healing molecules: Emphasis on pathways perspective. *Int J Mol Sci*. 2023;24(5):4607.

doi: 10.3390/IJMS24054607

121. Choi SS, Lee SH, Lee KA. A comparative study of hesperetin, hesperidin and hesperidin glucoside: Antioxidant, anti-inflammatory, and antibacterial activities *in vitro*. *Antioxidants (Basel)*. 2022;11(8):1618.

doi: 10.3390/ANTIOX11081618

122. Kim HJ, Lee SH, Hong SK, Gil BI, Lee KA. *In vitro* biological activities of hesperidin-related compounds with different solubility. *Antioxidants (Basel)*. 2024;13(6):727.

doi: 10.3390/ANTIOX13060727

123. Ambrozova N, Ulrichova J, Galandakova A. Models for the study of skin wound healing. The role of Nrf2 and NF-κB. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2017;161(1):1-13.

doi: 10.5507/bp.2016.063

124. Lalrinzuali K, Vabeiryureilai M, Jagetia GC. Topical application of stem bark ethanol extract of Sonapatha, *Oroxylum indicum* (L.) Kurz accelerates healing of deep dermal excision wound in Swiss albino mice. *J Ethnopharmacol*. 2018;227:290-299.

doi: 10.1016/j.jep.2018.08.018

125. Tong J, Hu C, Wu Y, Liu Q, Sun D. Radiation-induced NF-κB activation is involved in cochlear damage in mice via promotion of a local inflammatory response. *J Radiat Res*. 2023;64(1):63-72.

doi: 10.1093/JRR/RRAC068

126. Leite CDS, Bonafé GA, Pires OC, *et al*. Dipotassium glycyrrhizininate improves skin wound healing by modulating inflammatory process. *Int J Mol Sci*. 2023;24(4):3839.

doi: 10.3390/IJMS24043839

127. Calabrese V, Cornelius C, Stella AMG, Calabrese EJ. Cellular stress responses, mitostress and carnitine insufficiencies as critical determinants in aging and neurodegenerative disorders: Role of hormesis and vitagenes. *Neurochem Res*. 2010;35(12):1880-1915.

doi: 10.1007/S11064-010-0307-Z

128. Calabrese V, Wenzel U, Piccoli T, *et al*. Investigating hormesis, aging, and neurodegeneration: From bench to clinics. *Open Med*. 2024;19(1):20240986.

doi: 10.1515/MED-2024-0986

129. Franco R, Navarro G, Martínez-Pinilla E. Plant-derived compounds, vitagens, vitagenes and mitochondrial function. *PharmaNutrition*. 2022;19:100287.

doi: 10.1016/J.PHANU.2021.100287

130. Miler M, Živanović J, Kovačević S, *et al*. Citrus flavanone effects on the Nrf2-Keap1/GSK3/NF-κB/NLRP3 regulation and corticotroph-stress hormone loop in the old pituitary. *Int J Mol Sci*. 2024;25(16):8918.

doi: 10.3390/IJMS25168918

131. Banerjee J, Lodhi N, Nguyen BN. The role of poly(ADP-ribose) polymerase-1 in cutaneous wound healing. *Adv Wound Care (New Rochelle)*. 2019;8(12):634-643.

doi: 10.1089/WOUND.2018.0821

132. Zhao G, Liu F, Liu Z, *et al*. MSC-derived exosomes attenuate cell death through suppressing AIF nucleus translocation and enhance cutaneous wound healing. *Stem Cell Res Ther*. 2020;11:174.

doi: 10.1186/s13287-020-01616-8

ACCSCIENCE PUBLISHING

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ORIGINAL ARTICLE

Prevalence and risk factors of osteoporosis in diabetic individuals above 50 years of age at a tertiary care hospital: An observational study

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Abstract

Background: Diabetes mellitus and osteoporosis coexist in the elderly population. However, there is no conclusive evidence as to whether diabetes affects the progression of osteoporosis in this population. In addition, diabetes-associated systemic complications may also contribute to an increased risk of fracture. **Aim:** To determine the prevalence and risk factors of osteoporosis in diabetic individuals above 50 years of age and to estimate the burden of the disease in males and females. **Methods:** A hospital-based cross-sectional, and observational study was conducted in the Department of General Medicine, All India Institute of Medical Sciences, Bhubaneswar (AIIMS). A total of 203 diabetic individuals above 50 years of age were recruited in the study, and their bone mineral density (BMD) was evaluated using a dual-energy X-ray absorptiometry scan. Other clinical and biochemical parameters were also assessed. A comparison was conducted among males and females and the three categories of BMD. **Results:** In our study setting, the overall prevalence of osteoporosis in diabetic individuals above 50 years of age was 40.89%. The prevalence was higher in females (56.5%) compared to males (26%). A higher proportion of individuals (51.6%) in the 71–80 years age group had osteoporosis. Increasing age and the duration of diabetes and menopause (in females) were the significant risk factors, along with serum creatinine and glycated hemoglobin. **Conclusion:** As there is a high prevalence of osteoporosis among diabetics, screening by assessing the BMD must be done in the elderly population to predict the fracture risk (FRAX score), implement appropriate preventive measures, and initiate treatment. **Relevance for patients:** Based on our findings, patients with diabetes mellitus are more prone to developing osteoporosis, which in turn increases their likelihood to fracture development. In order to prevent further morbidity, diabetics need special care for maintaining their health through the early detection and prevention of such events.

Keywords: Bone mineral density; T-score; FRAX score; Type 2 diabetes mellitus; Fracture risk

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a common metabolic disease, and its burden has been increasing with the aging population. It is estimated that by the year 2025, the number of diabetic cases in India will reach 69.9 million, with a vast majority still undiagnosed.[1] Among the many complications of T2DM, osteoporosis is also a commonly diagnosed metabolic disease in the Indian population over 50 years of age. Osteoporosis predisposes to fragility fractures, and the development of such fractures is associated with high rates of morbidity and mortality, especially in the elderly.[2] Globally, nearly 200 million people suffer from osteoporosis each year.[3] Risk factors associated with osteoporosis include age, female sex, ethnicity, family history of osteoporosis, smoking, Vitamin D deficiency, low calcium, high caffeine intake, immobilization, increased age at menarche, early menopause, and underweight.[4] The overall prevalence of osteoporosis among the adult population in India is 18.3%.[5] The coexistence of osteoporosis and T2DM is common among the elderly population, putting this group at higher risk of bone fracture. Bone turnover markers are altered in DM patients and may be associated with fractures.[6] In type 1 diabetes mellitus (T1DM), the low bone mineral density (BMD) is due to low circulating levels of insulin-like growth factor 1 (IGF-1) and insulin, and this usually occurs in young children before peak bone mass attainment. While T2DM is common in adults who have already attained peak bone mass, the effect of T2DM on BMD remains unclear, as it may increase, decrease, or remain constant.[7,8] Additional investigations are desirable to explore possible risk factors and predictors of osteoporosis among the elderly.

To provide optimal bone health care for the growing number of patients with DM, awareness of the epidemiology, careful clinical assessment, and appropriate prevention or treatment of skeletal diseases are pivotal. Due to the paucity of reports,[5,9,10] the present study was planned to investigate the prevalence of osteoporosis and the associated risk factors in male and female diabetic individuals above 50 years of age at our tertiary care hospital.

2. Methods

2.1. Study participants

Considering a population prevalence of 18%[5] and assuming a sample prevalence of 25%, the calculated sample size was 203. A cross-sectional and observational study was conducted in the Outpatient Department of General Medicine at All India Institute of Medical Sciences (AIIMS), Bhubaneswar, India, between June and August 2023. Approval from the Institutional Ethics Committee was obtained before starting the study (reference number: IEC/AIIMS BBSR/STS/2022-23/09). Informed written consent was obtained from the patients who were recruited into the study. A total of 203 patients above 50 years of age and diagnosed with T2DM were recruited by the convenient sampling for the study. The patients were excluded if they had any one of the following reasons: nonconsenting, nondiabetic, age below 50 years, having hyperthyroidism, hyperparathyroidism, connective tissue disorders, malignancy, Cushing's syndrome, on steroid therapy,[11] on anti-osteoporotic drugs, chronic kidney disease (CKD), or chronic infections (e.g., tuberculosis and human immunodeficiency virus [HIV]).

2.2. Assessment of BMD and diagnosis of osteoporosis

Measuring bone density has been the leading method for the early identification of individuals at high risk of fracture. Among several techniques used for measuring BMD, dual-energy X-ray absorptiometry (DEXA) is the gold standard in clinical practice for its accuracy, precision, stability, cost, subject radiation dose and compliance, freedom to select skeletal sites, speed, and ease of scanning. In the present study, DEXA was used to assess the BMD at the femoral neck (hip) and lumbar region (L1–L4 vertebrae). It was measured in the supine position with a Hologic Horizon A system (Hologic Inc., United States of America [USA]). Based on T-scores obtained, according to the World Health Organization (WHO) guidelines,[12] with standard reference mean estimated for young female adults, the subjects were classified as follows:

(i) Normal: T-score ≥ −1 standard deviation (SD)
(ii) Low bone mass (osteopenia): T-score < −1 SD and > −2.5 SD
(iii) Osteoporosis: T-score ≤ −2.5 SD.

2.3. Clinical evaluation

The recruited participants' demographic profile (age and sex), anthropometric assessment (body mass index [BMI] and waist-to-hip ratio [WHR]), menstrual history (for female participants), duration of diabetes, and relevant past, personal, and medication history were recorded in the preapproved case study pro forma. The duration of menopause was counted in years from the last menstrual period as recalled by the subject.

2.4. Biochemical analysis

After overnight fasting, 6 mL of venous blood samples were collected from all the patients. The serum urea, creatinine, and calcium were estimated by colorimetric method and HbA1C using the immunoturbidimetry method with ready-to-use reagents in the Beckman Coulter AU5800

autoanalyzer (Brea, CA, USA). Thyroid-stimulating hormone (TSH) and vitamin D levels were evaluated using a chemiluminescent immunoassay in a Cobas 800e system (Basel, Switzerland). Midstream, mid-morning urine samples were also collected, and urine albumin level was also evaluated using the immunoturbidimetry method in a Beckman Coulter 5800 autoanalyzer (Brea, CA, USA); the albumin-creatinine ratio (ACR) was calculated accordingly.

2.5. Estimating fracture risk using FRAX

The Fracture Risk Assessment Tool (FRAX®), released by the WHO in February 2008, is a web-based algorithm (https://frax.shef.ac.uk/FRAX/tool.aspx)[13] and was used to calculate the 10-year probability of hip fracture in men and women. FRAX uses seven readily available dichotomous clinical risk factors (inserted as yes or no into the calculator): prior fragility fracture, parental hip fracture, smoking, systemic glucocorticoid use, excess alcohol intake, rheumatoid arthritis, and other causes of secondary osteoporosis. Other factors included in FRAX are age, sex, and BMI. FRAX can calculate fracture probability with or without femoral neck BMD to accommodate situations where densitometric assessment is not available. In this study, the femoral fracture probability was calculated using the BMD.

2.6. Statistical analysis

SPSS 23.0 software was used for the statistical analysis. All continuous variables satisfying the normal distribution were expressed as mean and standard deviation (SD). The *t*-test and Mann–Whitney U-test were used to compare the parameters between two groups, i.e., males and females. The correlations between the various factors considered and BMD were performed using the Pearson correlation coefficient. The statistical significance was set at $P \leq 0.05$.

3. Results

In this study, data from 203 diabetic individuals above 50 years of age were evaluated. Table 1 describes the baseline characteristics of the participants. 51.2% were male and 48.8% were female. The average age of the participants was 63.13 years, with ages ranging from 50 to 80 years; the majority (47.78%) belonged to the 61–70 years' age group. The overall prevalence of osteoporosis was 40.9% and osteopenia was 32.5% (not shown). Among males and females, the prevalence of osteoporosis was 26% and 56.5%, respectively, and that of osteopenia was 37.5% and 27.3%, respectively. Females were significantly in higher proportion among low BMD as compared with males (Figure 1). Figure 2 displays the BMD among different age groups; 51.6% of osteoporosis participants were in the 71–80 years' age group.

Table 1. Comparison of general characters and laboratory results between the two genders

Parameter	Male (*n*=104; 51.2%)	Female (*n*=99; 48.8%)	*P*-value
BMI (kg/m²)	25.1 (4.3)	26.3 (5.1)	0.075
WHR	1.0 (0.1)	1.0 (0.1)	0.224
Age (years)	64.6 (5.9)	61.6 (7.8)	0.002*
Duration of diabetes (years)	9.7 (5.8)	8.2 (6.3)	0.079
HbA1C (%)	7.8 (1.8)	7.5 (1.4)	0.186
Serum levels (mg/dL)			
Calcium	9.6 (0.6)	9.6 (0.6)	0.632
Creatinine	1.1 (0.4)	0.8 (0.2)	0.000*
Urea	20.8 (11.9)	16.9 (9.7)	0.011*
Urine ACR (mg/g)	26.43 (17.04)	17.9 (11.08)	0.045a
TSH (mIU/L)	3.2 (1.5)	3.7 (1.7)	0.018*
Vit D (ng/mL)	33.2 (20.5)	37.4 (22.3)	0.555
FRAX score (%)			
Right hip	1.6 (1.5)	3.0 (4.6)	0.003*
Left hip	1.6 (1.6)	3.6 (5.4)	0.001*

Note: Data are presented as mean (standard deviation [SD]), except "Urine ACR." Data for "Urine ACR" is presented as median ± standard error (SE). a The *P*-value is derived from the Mann–Whitney U-test. *$P<0.05$.
Abbreviations: BMI: Body mass index; WHR: Waist-to-hip ratio; ACR: albumin-creatinine ratio; TSH: Thyroid-stimulating hormone; Vit D: Vitamin D.

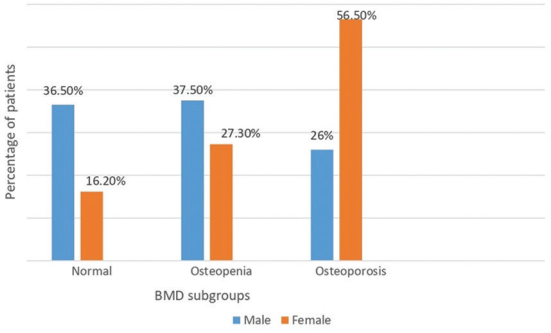

Figure 1. Distribution of bone mineral density subgroups between males and females

In the 50–60 years' age group, there were 42 women (56%) out of the total 99. Among them, 24 women (57.1%) had osteoporosis (not shown). The proportion of premenopausal women in them was found to be 33.3% (not shown).

We compared the general anthropometric measurements and lab reports among males (n = 104) and females (n = 99) using the *t*-test. There was a significant

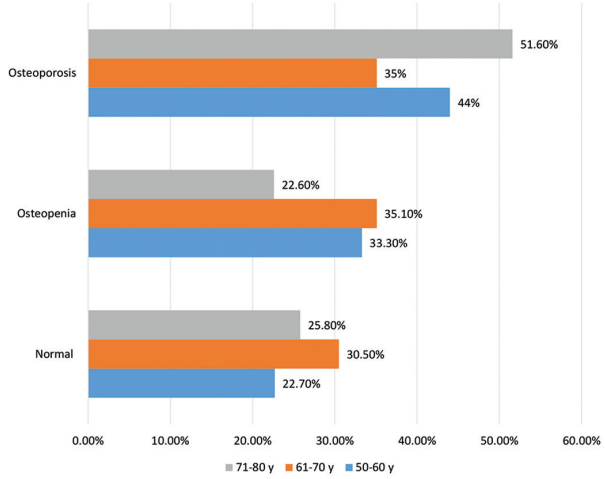

Figure 2. Distribution of bone mineral density among different age groups

difference in age (males > females; $P < 0.01$), as well as serum levels of urea ($P = 0.01$), creatinine ($P < 0.01$), and TSH ($P = 0.02$). There was no difference in the duration of diabetes, urine ACR, serum vitamin D, calcium, and HbA1c levels. The FRAX score was significantly higher in women in both left and right hips compared to men (Table 1).

Pearson correlation analysis (Table 2) was performed with all the probable risk factors for osteoporosis in the two groups, i.e., males and females. The results revealed that anthropometric measurements (BMI and WHR) and serum creatinine displayed a significant negative correlation ($P < 0.01$) with the T-score of lumbar vertebrae and a positive correlation with the T-score of the right and left femur. Age was negatively correlated with the T-scores of all three sites and statistically significant with the right femur. The serum urea, calcium, TSH, Vitamin D, and urine ACR did not correlate with the BMD. HbA1C and duration of menopause displayed a significant negative correlation with the T-scores of the lumbar vertebra and the femurs, respectively.

A total of 53 participants had an FRAX score (left hip), that is, a 10-year probability of left hip fracture >3%, and 41 participants had a right hip FRAX score greater than 3% (Table 3). Therefore, as many as 83 participants (40.89%) in our study require medical therapies to reduce the probability of suffering from a fracture in the next 10 years.

The BMD results, classified as normal, osteopenia, and osteoporosis, were compared for each risk variable in females and males (Table 4). There was a significant difference in BMI and WHR among the subgroups in both males and females. The *post hoc* test (Table 4) revealed a significant difference in BMI between the osteoporosis group with osteopenia and normal DEXA in both sexes. The differences in WHR were inconsistent and did not exhibit a trend in the male and female groups.

The anthropometric and biochemical parameters usually advised during the follow-up of T2DM patients were used as the dependent variables to predict the T-score of the lumbar vertebra in all patients (Tables 5 and 6). The overall multiple linear regression was statistically significant ($R^2 = 0.307$; $F = 3.402$; regression degrees of freedom [$df_{regression}$] = 6; residual degrees of freedom [$df_{residual}$] = 196; $P = 0.003$). The T-score was obtained using a constant of −9.531 and the respective values of BMI (0.139), WHR (0.185), age (0.081), duration of T2DM (−0.068), HbA1c (0.053), urine ACR (0.06), and duration of menopause in women (−0.186) (Table 7):

T-score of lumbar vertebra = −9.531 + BMI + WHR + HbA1c + duration of T2DM (years) + age (years) + urine ACR + duration of menopause in women (I)

Where the duration of menopause will only apply to women who have attained menopause; its value will be zero for men and women in the reproductive period.

The individual variables were not statistically significant, but the combined prediction was significant, with the *P*-values indicated in Table 7. The observed and predicted T-scores were well-correlated (Figure 3).

4. Discussion

In the present study, the overall prevalence of osteoporosis in diabetic individuals above 50 years of age was found to be 40.9%, which is greater than the 33% reported by Aleti *et al.*[14] On the contrary, the prevalence of osteopenia in our study was 32.5%, which is lower than 40% as reported by the same study.[14] Osteoporosis being a widely prevalent metabolic bone disease is aggravated in DM and further increases the disease burden.[15]

Increasing age and female gender were identified as risk factors for osteoporosis, which was consistent with previous studies.[14,16-18] This might be due to the decreased physical activity with age, decreased calcium absorption in the gut, and decreased synthesis of 1,25-dihydroxyvitamin D3 in the kidneys.[19] However, 25-hydroxyvitamin D3 levels did not have a significant association with BMD in our study, similar to a previous report,[11] suggesting that altered 25-hydroxyvitamin D3 levels are not a major reason for bone loss.

Menopausal duration was found to be a significant risk factor for low BMD, as bone absorption may be affected by the depletion of ovarian follicles in postmenopausal women and the decline in levels of sex hormones.

Table 2. Correlation of various factors with the T-scores

Parameter	T-scores					
	Lumbar vertebra		Right femur		Left femur	
	r	*P*	r	*P*	r	*P*
BMI (kg/m^2)	−0.271**	0	0.244**	0	0.154*	0.028
WHR	−0.275**	0	0.249**	0	0.180*	0.01
Duration of T2DM (years)	−0.031	0.662	−0.035	0.619	−0.043	0.538
Age (years)	−0.004	0.952	−0.141*	0.044	−0.129	0.066
HbA1C (%)	−0.168*	0.017	0.151*	0.031	0.137	0.051
Serum calcium (mg/dL)	−0.034	0.631	0.066	0.352	0.084	0.234
Serum creatinine (mg/dL)	−0.142*	0.044	−0.147*	0.036	−0.181**	0.01
Serum urea (mg/dL)	−0.051	0.467	0.084	0.233	0.121	0.085
Urine ACR (mg/g)	−0.089	0.205	−0.002	0.982	0.045	0.52
TSH (mIU/L)	0.046	0.514	−0.018	0.801	−0.043	0.545
Menopause duration (years)	0.148	0.145	−0.305**	0.002	−0.315**	0.002
Vitamin D (g/mL)	0.054	0.751	0.131	0.44	0.078	0.646
FRAX score						
Right hip (%)	0.395**	0	−0.665**	0	−0.614**	0
Left hip (%)	0.389**	0	−0.593**	0	−0.675**	0

Note: ** Correlation is significant at the 0.01 level (two-tailed); *correlation is significant at the 0.05 level (two-tailed); *r* denotes Pearson correlation; *p* denotes significance.
Abbreviations: BMI: Body mass index; HER: Waist-to-hip ratio; T2DM: Type 2 diabetes mellitus; ACR: Albumin-creatinine ratio; TSH: Thyroid-stimulating hormone.

Table 3. Risk assessment with FRAX

FRAX	Females		Males		Total	OR	95% Confidence interval	
	n	%	*n*	%			Lower	Upper
Right hip								
>3	25	25.25	16	15.38	41	1.86	0.923	3.740
<3	74	74.74	88	84.61	-	-	-	-
Left hip								
>3	36	36.36	17	16.34	53	2.9	1.442	5.433
<3	63	63.63	87	83.65	-	-	-	-
Total	99	-	104	-	-	-	-	-

Abbreviation: OR: Odds ratio.

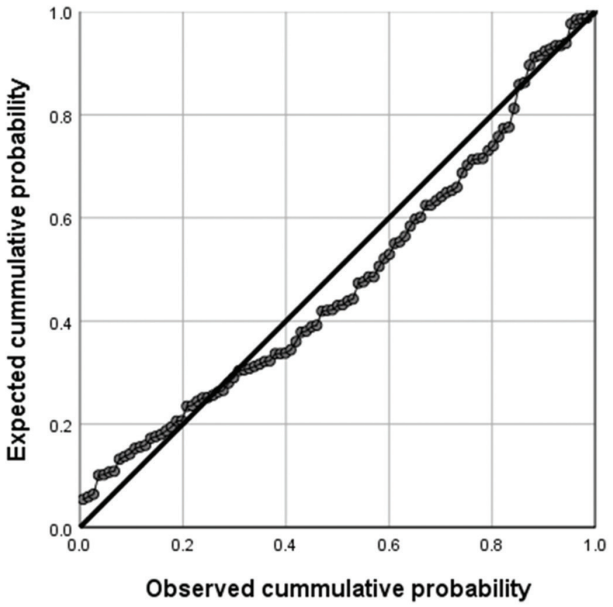

Figure 3. Normal P-P Plot of regression-standardized residual based on the T-score of the lumbar vertebrae as the dependent variable.

Increased microRNA-151a-3p levels in postmenopausal women promote osteoclast differentiation and affect BMD by targeting the suppressor of cytokine signaling 5 and activating the JAK2/STAT3 signaling pathway.[20,21]

Around 14.2% of women with osteoporosis in our study were pre-menopausal, indicating that other factors apart from menopause also play a role in disease progression. Since patients with secondary osteoporosis were excluded from our study, one possible cause of osteoporosis in pre-menopausal women could be idiopathic low BMD. In a study by Cohen *et al.*,[22] osteoporosis in pre-menopausal women could be due to osteoblast dysfunction with resistance to IGF-1. Low and high-normal IGF-1 levels are both related to

Table 4. Comparison of BMD status with biochemical and anthropometric findings in the two groups

Parameter	Female				Male			
	Normal (*n*=16)	Osteopenia (*n*=27)	Osteoporosis (*n*=56)	*P*	Normal (*n*=38)	Osteopenia (*n*=39)	Osteoporosis (*n*=27)	*P*
BMI (kg/m^2)	28.10 (6.27)	28.51 (5.19)	24.64(3.99)	0.038[b,c]	26.32 (3.97)	25.73 (4.37)	22.41 (3.50)	0.00[b,c]
WHR	0.97 (0.06)	0.98 (0.06)	0.94 (0.06)	0.01[c]	0.99 (0.06)	0.96 (0.06)	0.95 (0.0)	0.02[b]
Duration of diabetes (years)	7.06 (4.84)	9.07 6.18)	8.14 (6.83)	0.60	10.34 (5.26)	8.69 (6.49)	10.37 (5.42)	0.37
Age (years)	59.88 (8.45)	60.89 (6.02)	62.41 (8.35)	0.45	64.84 (5.65)	64.15 (5.87)	64.93 (6.30)	0.83
HbA1C (%)	7.29 (1.01)	7.92 (1.47)	7.41 (1.36)	0.21	8.38 (2.08)	7.63 (1.79)	7.35 (1.23)	0.05
Serum level (mg/dL)								
Calcium	9.69 (0.58)	9.75 (0.52)	9.50 (0.55)	0.13	9.48 (0.77)	9.64 (0.60)	9.54 (0.53)	0.55
Creatinine	0.86 (0.20)	0.82 (0.17)	0.82 (0.25)	0.85	1.15 (0.50)	1.05 (0.33)	1.15 (0.44)	0.51
Urea	15.04 (6.92)	15.68 (10.37)	17.98 (10.0)	0.43	23.02 (10.56)	18.58 (11.37)	20.87 (14.24)	0.27
TSH (mIU/L)	3.90 (1.93)	3.80 (1.91)	3.58 (1.49)	0.74	3.09 (1.64)	3.12 (1.42)	3.35 (1.32)	0.75
Vitamin D (ng/mL)	32.72 (25.34)	38.50 (22.13)	37.90 (24.07)	0.96	31.03 (20.97)	39.32 (19.97)	25.59 (22.29)	0.53
Urine ACR (mg/g)	81.20 (148.11)	40.66 (77.32)	65.56 (111.88)	0.47	110.33 (202.88)	109.75 (159.17)	75.20 (152.25)	0.67
T-score								
Lumbar vertebrae	−0.18 (0.91)	−1.84 (0.41)	−3.49 (0.71)	0.00[a,b,c]	0.38 (1.55)	−1.66 (0.35)	−3.14 (0.57)	0.00[a,b,c]
Right femur	−0.85 (0.88)	−1.63 (0.82)	−2.46 (0.93)	0.00	−0.34 (1.13)	−1.49 (0.69)	−2.09 (0.64)	0.00
Left femur	−0.98 (0.88)	−1.78 (0.78)	−2.61 (0.93)	0.00[a,b,c]	−0.35 (1.11)	−1.39 (0.85)	−2.14 (0.63)	0.00[a,b,c]
BMD (g/cm^2)								
Right femur	0.77 (0.11)	0.68 (0.09)	0.58 (0.10)	0.00[a,b,c]	0.88 (0.15)	0.74 (0.12)	0.64 (0.06)	0.00[a,b,c]
Left femur	0.76 (0.12)	0.67 (0.09)	0.57 (0.10)	0.00[a,b,c]	0.88 (0.14)	0.75 (0.13)	0.64 (0.08)	0.00[a,b,c]
Menopause duration (years)	8.25 (7.57)	8.78 (5.94)	10.98 (8.59)	0.31				
FRAX score (%)								
Right hip	0.76 (1.0)	1.26 (0.97)	4.55 (5.61)	0.00[a,b,c]	0.76 (0.77)	1.57 (1.37)	2.82 (1.61)	0.00[b,c]
Left hip	0.89 (1.21)	1.48 (1.28)	5.34 (6.61)	0.00[b,c]	0.80 (0.98)	1.55 (1.51)	2.94 (1.68)	0.00[b,c]

Note: *P* is derived from analysis of variance (ANOVA); data is presented as the mean (standard deviation [SD]); *post-hoc* Bonferroni test was used; the differences between the subgroups were: [a]Normal versus Osteopenia; [b]Normal versus Osteoporosis; and [c]Osteopenia versus Osteoporosis.
Abbreviations: Abbreviations: BMI: Body mass index; HER: Waist-to-hip ratio; T2DM: Type 2 diabetes mellitus; ACR: Albumin-creatinine ratio; TSH: Thyroid stimulating hormone; BMD: Bone mineral density.

Table 5. Multiple regression analysis (regression statistics) to predict the T-score of the lumbar vertebra from independent variables

Parameter	Value
Modelb	1
R	0.307[a]
R^2	0.094
Adjusted R^2	0.067
Standard error of the estimate	1.6307
Observations	202

Note: [a]Predictors: Constant, urine ACR (mg/g), BMI (kg/m^2), HbA1C (%), age (years), duration of T2DM (years), and WHR; b Dependent variable: T-score of the lumbar vertebrae.
Abbreviations: ACR: Albumin-creatinine ratio; BMI: Body mass index; T2DM: Type 2 diabetes mellitus; WHR: Waist-hip ratio.

Table 6. Multiple regression analysis (analysis of variance [ANOVA]) to predict the T-score of the lumbar vertebra from independent variables

Parameter	Sum of squares	Degree of freedom (df)	Mean square	*F*	Significance
Regression	54.277	6	9.046	3.402	0.003[a]
Residual	521.215	196	2.659	-	-
Total	575.492	202	-	-	-

Note: [a]Dependent variable: T-score of the lumbar vertebrae.

insulin resistance.[23] The increased bone loss and fragility are also attributed to the microvascular changes in diabetes.[24,25] However, though the biological mechanism of this complex phenomenon is yet to be elucidated, osteoporosis can be considered a complication of DM.

Table 7. Multiple regression analysis (coefficient constants) to predict the T-score of the lumbar vertebra from independent variables

Parameter	*b*	*β*	*P*	95% confidence interval for *b*	
				Lower bound	Upper bound
Constant	−9.531	-	0.00	−13.934	−5.127
BMI (kg/m^2)	0.05	0.139	0.12	−0.013	0.113
WHR	5.01	0.185	0.04	0.248	9.771
HbA1C (%)	0.055	0.053	0.46	−0.092	0.202
Duration of diabetes (years)	−0.019	−0.068	0.40	−0.063	0.025
Age (years)	0.019	0.081	0.31	−0.018	0.057
Urine ACR (mg/g)	0.001	0.06	0.40	−0.001	0.002
Duration of menopause (years)	−0.033	−0.186	0.113	−0.74	0.008

Abbreviations: BMI: Body mass index; WHR: Waist-hip ratio; ACR: Albumin-creatinine ratio.

In the present study, it was identified that BMI correlated negatively with BMD, i.e., increased osteoporosis in overweight or obese DM individuals. Several studies[26,27] suggest that BMD decreases in both underweight and obese women, which correlates with higher risk for fracture.[28] People with high BMI have higher body fat content, which may be converted into fat-related hormones (Vitamin D, estrogen, androgen, etc.) through the secretion of adipokines (leptin, adiponectin, and tumor necrosis factor), thereby affecting the bone metabolism by stimulating inflammatory factors that increase bone resorption.[26]

Similarly, increased WHR was associated with decreased BMD of the spine and increased BMD in the neck of the femur in our study. This was similar to the finding in a previous study on osteoporosis.[28] This can be explained by the fact that greater fat mass is associated with increased mechanical loading on the bone, which in turn may stimulate bone formation to increase bone density.[29] The risk for fractures in relation to BMI is site-specific; low BMI is protective for lower limb fractures and has a higher risk for spine and upper arm fractures.[30] In addition, the effect of fat on bone is likely to be involved in a web of interrelated regulatory pathways, including estrogen, leptin, adiponectin, resistin, and interleukin-6.[31-33]

In our study, HbA1c levels displayed a positive correlation with the femur and a negative correlation with spine BMD. However, previous studies have reported conflicting results regarding the influence of glycemic control on BMD.[34,35] Therefore, the clinical significance of these differences remains to be determined.

Raised serum creatinine levels were associated with osteoporosis in our study. In DM patients with CKD, a higher serum creatinine level was associated with increased fracture risk in previous studies.[36] However, in our study, patients with CKD were excluded. Creatinine is a marker for muscle mass and physical activity and is regarded as a stable indicator of human muscle metabolism.[37,38] Consequently, higher serum creatinine levels correspond to greater muscle mass, which protects against osteoporosis in a normal population. A similar positive correlation between serum creatinine and BMD was found in a study by Chen *et al*.[39] The combined effect of insulin resistance and insufficiency with diabetic nephropathy increases the development of osteoporosis.[10]

We did not identify any significant correlation between BMD and the other factors, e.g., duration of diabetes, serum urea, urine ACR, and TSH levels. This could be due to the sample population being limited to a single tertiary care center. The FRAX score significantly correlated negatively with the spine and positively with the hip BMD. Several reports suggest that the FRAX score can be used without incorporating BMD.[40,41]

The strength of our study is that we presented the analysis results of related risk factors for male and female patients, respectively. We tried to include several factors in our study and assessed their correlation for identifying potential risk factors. Moreover, this is the first study to evaluate the burden of osteoporosis in diabetic individuals conducted in this region of the country. In places where the facility for DEXA is not available, T-score prediction can be made using routine clinical and biochemical findings.

There were several limitations in our study. Firstly, it was a cross-sectional study with no follow-up and fracture incidence data. We did not measure the serum estrogen levels of female participants, as estrogen levels have a direct bearing on the BMD. The inclusion of serum lipid profiles, fasting insulin levels, and other bone turnover markers in our study could have widened the scope of risk assessment. However, the lack of a control group and diet records prevented us from making a fair comparison.

We have demonstrated a high prevalence of osteoporosis and osteopenia in patients with T2DM. The clinical and biochemical evaluation of BMD will aid in diagnosing T2DM patients at risk of developing fractures. Management of such patients will improve the quality of life and decrease morbidity.

5. Conclusion

From our findings, the overall prevalence of osteoporosis in diabetic individuals above 50 years of age in our study

setting is 40.89%. The prevalence is higher in females (56.5%) compared to males (26%). The proportion of pre-menopausal women with osteoporosis was found to be 14.2%. A higher proportion of individuals (51.6%) in the 71–80 years' age group had osteoporosis. Anthropometric measurements (BMI and WHR), HbA1C, and serum creatinine displayed a significantly positive correlation with T-scores. Age and menopausal duration (in females) exhibited a significantly negative correlation with the T-scores; increasing age and menopausal duration (in females) are potential risk factors for osteoporosis in our study population. Other factors (i.e., duration of diabetes, serum urea, serum calcium, Vitamin D, urine ACR, and TSH levels) do not have any significant association with the T-scores. Similar to the normal population, increasing age and menopause are significant risk factors in diabetic individuals as well. Moreover, the 10-year fracture probability was significantly high (>3%) in 53 individuals (26.1% of the participants). United States (US) Food and Drug Administration-approved medical therapies are recommended in almost 41% of the participants to prevent the formation of fractures. Thus, we recommend that all patients with T2DM aged 50 years and above, especially females, should be screened for osteopenia and osteoporosis, and offered preventive, therapeutic, and rehabilitative management.

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Conflict of interest

The authors declare that there is no financial or non-financial interest in the present study.

Author contributions

Conceptualization: Sujata Devi, Deenadayalan Subhiksha
Investigation: All authors
Writing - original draft: Sujata Devi, Suchanda Sahu, Deenadayalan Subhiksha
Writing - review & editing: Kishore Kumar Behera, Nibedita Priyadarsini, Anupam Dey, Debananda Sahoo, Arpita Dash

Ethics approval and consent to participate

The present study was conducted after obtaining approval from the Institution Ethics Committee (approval number: IEC/AIIMS BBSR/STS/2022-23/09, dated November 23, 2023) and informed written consent from each study participant.

Consent for publication

Informed written consent was obtained from the patients who were recruited into the study.

Data availability

Data prepared from the patients' medical and laboratory records are not available publicly. However, on reasonable request, it can be provided.

References

1. Misra A, Gopalan H, Jayawardena R, *et al*. Diabetes in developing countries. J Diabetes. 2019;11(7):522-539.
doi: 10.1111/1753-0407.12803
2. Kanis J. *Assessment of Osteoporosis at the Primary Health Care Level. Summary Report of a WHO Scientific Group*. *World Health Organization Collaborating Centre for Metabolic Bone Diseases*. Report No.: Technical Report. United Kingdom: University of Sheffield; 2007. Available from: https://frax.shef.ac.uk/frax/pdfs/who_technical_report.pdf [Last accessed on 2024 Sep 01].
3. Sözen T, Özışık L, Başaran NÇ. An overview and management of osteoporosis. Eur J Rheumatol. 2017;4(1):46-56.
doi: 10.5152/eurjrheum.2016.16011
4. Abushaikha L, Omran S. A survey of osteoporosis risk factors and practices among Jordanian women. J Int Womens Stud. 2010;11(4):152-161.
doi: 10.1136/jirs.11.4.152
5. Babhulkar S, Seth S. Prevalence of osteoporosis in India: An observation of 31238 adults. Int J Res Orthop. 2021;7(2):362-368.
doi: 10.18203/issn.2455-4510
6. Chinese Medical Association. Guideline for primary care of primary osteoporosis (2019). *Chin J Gen Pr*. 2020;19(4):304-315.
doi: 10.3760/cma.j.issn.1674-6397.2020.04.001
7. Wongdee K, Charoenphandhu N. Osteoporosis in diabetes mellitus: Possible cellular and molecular mechanisms. *World J Diabetes*. 2011;2(3):41-48.
doi: 10.4239/wjd.v2.i3.41
8. Adil C, Aydın T, Taşpınar Ö, *et al*. Bone mineral density evaluation of patients with type 2 diabetes mellitus. *J Phys Ther Sci*. 2015;27(1):179-182.
doi: 10.1589/jpts.27.179
9. Xu L, Cheng M, Liu X, Shan P, Gao H. Bone mineral density

and its related factors in elderly male Chinese patients with type 2 diabetes. *Arch Med Res*. 2007;38(2):259-264.

doi: 10.1016/j.arcmed.2006.09.012

10. Xia J, Zhong Y, Huang G, Chen Y, Shi H, Zhang Z. The relationship between insulin resistance and osteoporosis in elderly male type 2 diabetes mellitus and diabetic nephropathy. *Ann Endocrinol* (*Pairs*). 2012;73(6):546-551.

doi: 10.1016/j.ando.2012.05.004

11. Stein E, Shane E. Secondary osteoporosis. Endocrinol Metab Clin North Am. 2003;32(1):115-134, 7.

doi: 10.1016/S0889-8529(02)00098-0

12. Imran M, Singh A, Bhardwaj A, Agrawal D. Prevalence of osteoporosis and associated risk factors among postmenopausal women: A cross-sectional study from Northern India. J Midlife Health. 2022;13(3):206-212.

doi: 10.47713/jlh.v13i3.189

13. Kanis JA. *Fracture Risk Assessment Tool, FRAX*. Available from: https://frax.shef.ac.uk/frax/tool.aspx?country=9 [Last accessed on 2024 Sep 01].

14. Aleti S, Pal R, Dutta P, *et al*. Prevalence and predictors of osteopenia and osteoporosis in patients with type 2 diabetes mellitus: A cross-sectional study from a tertiary care institute in North India. Int J Diabetes Dev Ctries. 2020;40(2): 262-268.

doi: 10.1007/s13410-020-00822-9

15. Kao WH, Kammerer CM, Schneider JL, Bauer RL, Mitchell BD. Type 2 diabetes is associated with increased bone mineral density in Mexican-American women. Arch Med Res. 2003;34(5):399-406.

doi: 10.1016/S0187-5509(03)00088-1

16. Palermo A, Tuccinardi D, Defeudis G, *et al*. BMI and BMD: The potential interplay between obesity and bone fragility. Int J Environ Res Public Health. 2016;13(6):544.

doi: 10.3390/ijerph13060544

17. Chen P, Li Z, Hu Y. Prevalence of osteoporosis in China: A meta-analysis and systematic review. BMC Public Health. 2016;16(1):1039.

doi: 10.1186/s12889-016-3701-1

18. Tian L, Yang R, Wei L, *et al*. Prevalence of osteoporosis and related lifestyle and metabolic factors of postmenopausal women and elderly men: A cross-sectional study in Gansu province, Northwestern of China. Medicine (Baltimore). 2017;96(43):e8294.

doi: 10.1097/MD.0000000000008294

19. Cui L, Chen L, Xia W, *et al*. Vertebral fracture in postmenopausal Chinese women: A population-based study. Osteoporos Int. 2017;28(9):2583-2590.

doi: 10.1007/s00198-017-4094-2

20. Yamaguchi T, Sugimoto T. Bone metabolism and fracture risk in type 2 diabetes mellitus [Review]. Endocr J. 2011;58(8):613-624.

doi: 10.1507/endocrj.EJ11-0147

21. Fu Y, Xu Y, Chen S, Ouyang Y, Sun G. MiR-151a-3p promotes postmenopausal osteoporosis by targeting SOCS5 and activating JAK2/STAT3 signaling. Rejuvenation Res. 2020;23(4):313-323.

doi: 10.1089/rej.2019.2294

22. Cohen A, Dempster DW, Recker RR, *et al*. Abnormal bone microarchitecture and evidence of osteoblast dysfunction in premenopausal women with idiopathic osteoporosis. J Clin Endocrinol Metab. 2011;96(10):3095-3105.

doi: 10.1210/jc.2011-1542

23. Friedrich N, Thuesen B, Jørgensen T, *et al*. The association between IGF-I and insulin resistance. Diabetes Care. 2012;35(4):768-773.

doi: 10.2337/dc11-1652

24. Abdulameer SA, Sulaiman SAS, Hassali MAA, Subramaniam K, Sahib MN. Osteoporosis and type 2 diabetes mellitus: What do we know, and what we can do? Patient Prefer Adherence. 2012;6:435-448.

doi: 10.2147/PPA.S30204

25. Vogt MT, Cauley JA, Kuller LH, Nevitt MC. Bone mineral density and blood flow to the lower extremities: The study of osteoporotic fractures. J Bone Miner Res. 1997;12(2): 283-289.

doi: 10.1359/jbmr.1997.12.2.283

26. He Y, Chen D, Guo Q, Shi P, You C, Feng Y. MicroRNA-151a-3p functions in the regulation of osteoclast differentiation: Significance to postmenopausal osteoporosis. Clin Interv Aging. 2021;16:1357-1366.

doi: 10.2147/CIA.S327287

27. Lee JH, Kim JH, Hong AR, Kim SW, Shin CS. Optimal body mass index for minimizing the risk for osteoporosis and type 2 diabetes. Korean J Intern Med. 2020;35(6):1432-1442.

doi: 10.3904/kjim.2020.036

28. Głogowska-Szeląg J, Kos-Kudła B, Marek B, Nowak M, Siemińska L. Assessment of selected adipocytokines in obese women with postmenopausal osteoporosis. Endokrynol Pol. 2019;70(6):478-483.

doi: 10.5603/EP.a2019.0048

29. Etherington J, Harris PA, Nandra D, *et al*. The effect of weight-bearing exercise on bone mineral density: A study of female ex-elite athletes and the general population. J Bone Miner Res. 1996;11(9):1333-1338.

doi: 10.1002/jbmr.5650110912

30. Johansson H, Kanis JA, Odén A, *et al*. A meta-analysis of the

association of fracture risk and body mass index in women. J Bone Miner Res. 2014;29(1):223-233.

doi: 10.1002/jbmr.20443

31. Zernicke R, MacKay C, Lorincz C. Mechanisms of bone remodeling during weight-bearing exercise. Appl Physiol Nutr Metab. 2006;31(6):655-660.

doi: 10.1139/h06-090

32. Franchimont N, Wertz S, Malaise M. Interleukin-6: An osteotropic factor influencing bone formation? Bone. 2005;37(5):601-606.

doi: 10.1016/j.bone.2005.06.019

33. Lenchik L, Register TC, Hsu FC, *et al*. Adiponectin as a novel determinant of bone mineral density and visceral fat. Bone. 2003;33(4):646-651.

doi: 10.1016/S8756-3282(03)00208-5

34. Oh KW, Lee WY, Rhee EJ, *et al*. The relationship between serum resistin, leptin, adiponectin, ghrelin levels and bone mineral density in middle-aged men. Clin Endocrinol (Oxf). 2005;63(2):131-138.

doi: 10.1111/j.1365-2265.2005.02272.x

35. Cutrim DM, Pereira FA, Paula FJ, Foss MC. Lack of relationship between glycemic control and bone mineral density in type 2 diabetes mellitus. Braz J Med Biol Res. 2007;40(2):221-227.

doi: 10.1590/S0100-879X2007000200006

36. Gregorio F, Cristallini S, Santeusanio F, Filipponi P, Fumelli P. Osteopenia associated with non-insulin-dependent diabetes mellitus: What are the causes? Diabetes Res Clin Pract. 1994;23(1):43-54.

doi: 10.1016/0168-8227(94)90059-5

37. Myong JP, Kim HR, Koo JW, Park CY. Relationship between bone mineral density and moderate to severe chronic kidney disease among general population in Korea. J Korean Med Sci. 2013;28(4):569-574.

doi: 10.3346/jkms.2013.28.4.569

38. Schutte JE, Longhurst JC, Gaffney FA, Bastian BC, Blomqvist CG. Total plasma creatinine: An accurate measure of total striated muscle mass. J Appl Physiol. 1981;51(3):762-766.

doi: 10.1152/jappl.1981.51.3.762

39. Chen S, Zhou S, Chen Y, Liu R. Association between serum creatinine and osteoporosis in early postmenopausal women: A cross-sectional study. Clin Exp Obstet Gynecol. 2024;51(2):46.

doi: 10.31083/j.ceog5102046

40. Favarato MHS, Almeida MFD, Lichtenstein A, Martins MDA, Junior MF. Risk of osteoporotic fracture in women using the FRAX tool with and without bone mineral density score in patients followed at a tertiary outpatient clinic-an observational study. Clinics (*Sao Paulo)*. 2022;77:100015.

doi: 10.6061/clinics/2022/e100015

41. Simpkins RC, Downs TN, Lane MT. FRAX prediction with and without bone mineral density testing. Fed Pract. 2017;34(5):40-43.

doi: 10.1002/ehf2.12468

ACCSCIENCE PUBLISHING

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ORIGINAL ARTICLE

Two simple methods to predict Pediatric Dose of Antituberculosis Medicines: Application of Allometry and Salisbury Rule

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Abstract

Background: In drug development, selecting the first-in-human dose is crucial. Similarly, in pediatric drug development, determining the first-in-pediatric dose is of paramount importance. Given that the pharmacokinetic, safety, and efficacy of a product are generally well established in adults, this information can be used to select an appropriate first-in-pediatric dose for pediatric clinical trials. **Methods:** Two simple methods – Salisbury Rule and allometric scaling – were evaluated for predicting the first-in-pediatric dose to initiate a clinical trial for antituberculosis medicines. To assess the predictive performance of these methods, the predicted doses were compared with the observed doses recommended by the World Health Organization (WHO) or the United States Food and Drug Administration (US FDA). **Results:** This study included seven antituberculosis drugs with 62 observations across different body weight groups. The predictive accuracy of both methods was excellent, with over 80% of the observations falling within a 30% prediction error. **Conclusion:** The predicted pediatric doses of antituberculosis drugs using the two proposed methods reconciled well with the recommended human doses from WHO or the US FDA. The methods are simple and can easily be calculated on a spreadsheet or calculator in a short amount of time. **Relevance for patients:** These two approaches are helpful for optimizing the selection of appropriate antituberculosis medication dosages in pediatric patients with tuberculosis, ensuring effective treatment, and minimizing potential risks.

Keywords: Antituberculosis medicines; Allometry; Body weight; Dose; Pediatric; Salisbury rule

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1. Introduction

Tuberculosis (TB) is a prevalent disease in Africa and many South Asian countries.[1-3] Considering the life-threatening nature of this disease, over the years, many antituberculosis medicines have been developed to prevent, manage, or treat TB. On a global scale, the World Health Organization (WHO) is heavily involved in TB control programs. The main objective of WHO is to provide guidance for early diagnosis of TB, timely treatment with appropriate antituberculosis medicines with appropriate therapeutic dosing.

TB is caused by a bacterium called Mycobacterium tuberculosis. The main organ of bacterial infection in TB is the lungs but other organs such as kidneys, spine and brain can

also be infected by TB bacteria. TB disease can be divided into two types: latent TB infection and active TB disease. If not treated, latent TB infection can progress to TB disease. Although, TB is treatable but without proper treatment TB patients can die.[1] TB bacteria spread through the air from one person to another. Drug-resistant TB occurs when the bacteria become resistant to TB medicines.[1]

Like adults, children are also susceptible to TB. In 2020, it was found that 11% children aged <15 years of the estimated 10 million cases of TB were affected and 16% children died of TB disease (230,000 of 1.4 million).[2,3] Children <5 years of age, children with human immunodeficiency virus (HIV), and malnourished children are at high risk for TB.[4]

Over the years, WHO has revised the pediatric dosing for TB and in 2014 pediatric dosing was adjusted based on the pharmacokinetic (PK) studies as well as safety.[5,6] The FDA and the European Medicines Agency (EMA) recommended that pediatric optimal dose should be based on the child-adult exposure matching.[7,8] The notion behind child-exposure matching is that if the exposures are comparable between children and adults then a similar treatment effect is expected in children as of adults.[7,8] PK studies for first-line antituberculosis medicines such as isoniazid, rifampicin, pyrazinamide, and ethambutol have indicated that the exposures in children are lower than adults[9-11] at the recommended doses by WHO and as such may be suboptimum.

There are physiological and biochemical differences between children and adults hence, dosing of medicines in children require proper understanding of these processes. Besides, pediatric diseases may differ from adults in terms of mechanisms, etiology, and the course of disease. All these factors impact the PK and pharmacodynamics (PD) of medicines and can be different in children than adults.[12-14]

In pediatric drug development, the first-in-children dose is of practical significance. Before conducting a pediatric clinical trials, generally, the PK information and a safe and efficacious dose of a medicine in adults are well known. Such information plays an important role and provide insight for the selection of first-in-pediatric dose.

Over the years, several methods in terms of modeling have been proposed to predict first-in-pediatric dose.[12] Models such as allometric scaling, modeling and simulation using adult data, population pharmacokinetics, and physiologically-based pharmacokinetic (PBPK) models (whole body or minimal) have been suggested to predict the first-in-children dose.[15-27]

Over the years, many investigators have attempted to device simple mathematical rules and these rules are available in the literature.[12,28,29] These rules are: Clark's rule (2-17 years), Clark's surface area rule, Young's rule, Webster's rule, Fried's rule, and Shirkey's body surface area (BSA) recommendation.[12,28] However, the predictive power of these rules are not robust and lack precision. In a study, Munzenberger and McKercher[29] evaluated the performance of several pediatric dosing rules (Clark's weight rule, Clark's surface area rule, Young's age rule, and Shirkey's dosing recommendations) by comparing the predicted doses with the actual doses given to pediatric patients. The authors concluded that these pediatric dosing rules although, simple but were not reliable.

A simple method known as 'Salisbury Rule' to predict pediatric dose was developed by Lack and Stuart.[30] This method is based on body weight and is very useful for pediatric dosing for small molecules.[30] Salisbury Rule, along with allometric scaling, has been successfully used to predict pediatric doses for small molecules as well as therapeutic proteins.[26,31] The predicted doses of these medicines by Salisbury Rule or allometry reconciled very well with the recommended pediatric dose. A study[26] involving small molecules compared the Salisbury Rule and allometry with whole-body PBPK modeling. The results indicated that the predicted pediatric doses predicted by the Salisbury Rule and allometry with whole-body PBPK modeling. The results indicated that the predicted pediatric doses by Salisbury Rule or allometry were comparable with whole body PBPK. In a recent study, Mahmood[32] used Salisbury Rule and allometry to predict pediatric dose for antimalarial medicines and the predicted doses reconciled vary well with the administered doses.

Since the Salisbury Rule and allometric scaling for the prediction of pediatric dose is simple and robust, the objective of this study was to evaluate the predictive performance of the Salisbury Rule and the allometric scaling to predict pediatric doses of antituberculosis medicines and compare the predicted doses with the recommended clinical doses of antituberculosis medicines.

2. Methods

There were 7 antituberculosis medicines in this study. These medicines were approved by the FDA and EMA for adult and pediatric use. At least four (Isoniazid, pyrazinamide, ethambutol, rifampicin) out of seven medicines are considered first-line antituberculosis medicines. Three other medicines used in this study are levofloxacine, rifapentene, and streptomycin.

The recommended therapeutic doses of the aforementioned antituberculosis medicines in pediatrics were obtained from WHO guidelines for the treatment of TB and the FDA package inserts. The recommended

clinical dose(s) of these medicines were used as the observed dose(s) to compare with the predicted dose(s) by allometry or Salisbury Rule. The allometric method used in this study was previously developed and validated using external data.[12,15,26,32]

2.1. Method 1: Salisbury rule

The following two methods known as Salisbury Rule were used for the prediction of antituberculosis medicines proposed by Lack and Stuart-Taylor[30]:

For children weighing less than 30 kilograms:

2 × Weight in kilograms = % of adult dose (1)

For children weighing greater than ≥30 kilograms but less than 70 kg:

Weight in kilograms + 30 = % of adult dose (2)

2.2. Method 2: Allometric scaling

Generally, pediatric dose is recommended based on per kg body weight (derived from adult dose and body weight). This approach assumes that there is a linear relationship (exponent 1.0) between body weight and dose, irrespective of age. Considering that, body weight based dosing across the age groups may not be a linear process, a theoretical exponent 0.75 has been proposed.[12] This exponent, though useful, is not universally applicable, particularly for younger children (typically those aged 2 years or younger).[12] Therefore, in this study, a middle ground strategy was taken to choose an allometric exponent to predict pediatric dose across all ages. The mid-point between 0.75 and 1.0 is 0.87 and it was rounded to 0.90. Hence, exponent 0.90 was used to predict pediatric dosse using body weight in equation 3. This approach was taken in other studies for pediatric dose prediction.[12,15,26,32] The pediatric doses of antituberculosis medicines across different age groups were predicted by equation 3:

Pediatric Dose = Adult Dose × (weight of the child/weight of the adults) $^{0.9}$ (3)

Where, the 'adult dose' is the adult dose of a given drug. Generally, in equation 3, an adult body weight of 70 kg is used. However, it was noted that the recommended antituberculosis dose in most instances is equal to an adult dose starting as low as 34 kg body weight. Therefore, an adult body weight was varied according to the lowest starting dose for adults. For example, if the recommended dose in adults starts from 30 kg body weight then the adult body weight used in equation 3 was 50 kg (a midpoint between 30 to 70 kg). Similarly, an adult body weight of 60 kg as a mid-point was used between 50 and 70 kg in equation 3. For children, the recommended dose was based on body weight range or band therefore, a midpoint of the body weight range was used. For example, for body weight ranging from 10 to 20 kg, a mid-point of 15 kg was used in equation 3.

2.3. Statistical analysis

In the literature, a 2-fold prediction error (0.5 – 2-fold) is considered acceptable. However, a 2-fold prediction error appears to be too high and may be of little practical value even for the first-time-pediatric dose selection. Therefore, in this study, a prediction error of 0.5 – 1.5 (a 50% prediction error on either side of 1) in place of 0.5-2-fold error was considered acceptable. A more stringent criteria in terms of 0.7-1.3 (a 30% prediction error on either side of 1) was also used. The fold-error between predicted and observed was calculated as follows:

Fold error = predicted dose/observed dose (4)

The predicted dose in this study was a single value and was compared with a single recommended dose. In clinical practice, due to the differences in response across the patient population, the recommended dose used in this study may differ from the administered dose.

3. Results

The results of this study are summarized below and in Table 1 and in the supplementary Tables S1-S7. There were 7 antituberculosis medicines with 62 observations (different weight groups for each drug) for allometry or the Salisbury Rule.

For allometric scaling, 96.8%, 90.3%, and 88.7% observations were within 0.5 – 2-fold, 0.5 – 1.5-fold, and 0.7 – 1.3-fold prediction error, respectively. For the Salisbury Rule, 100%, 98.4%, and 80.6% observations were within 0.5 – 2-fold, 0.5 – 1.5-fold, and 0.7 – 1.3-fold prediction error, respectively (Table 1). Overall, predicted dose of antituberculosis medicines in children by the two proposed methods reconciled very well with the observed or recommended clinical dose. The robustness of the methods can be gauged from the fact that more than 80%

Table 1. Prediction of pediatric antituberculosis dose based on the Salisbury rule or allometry

Fold error	Allometry (*n*=62)		Salisbury Rule (*n*=62)	
	Number (%)	Range	Number (%)	Range
0.5–2.0	60 (96.8)	0.70 – 1.80	62 (100)	0.55 – 1.68
0.5–1.5	56 (90.3)	0.70 – 1.44	61 (98.4)	0.55 – 1.38
0.7–1.3	55 (88.7)	0.70 – 1.27	50 (80.6)	0.72 – 1.28
>2	2 (3.2)	2.28 – 2.40	0	0
<0.5	0	0	0	0

Note: The analysis included seven antituberculosis medicines, with 62 total observations (different weight groups for each drug).

of the observations were predicted within a 30% prediction error.

4. Discussion

Generally, pediatric dose is extrapolated from adults (especially, in neonates) based on the body weight basis (per kg), mainly due to the difficulties in conducting pediatric clinical trials. Although, this approach is simple but not satisfactory. Besides, some other simple methods as described previously in the introduction section were not found reliable.[12,28,29]

Lack and Stuart-Taylor[30] developed a simple method known as the "Salisbury Rule" to predict pediatric doses.[30] The method is based on body weight but is not linearly related with body weight (per kg). Despite its simplicity and fairly accurate prediction of pediatric doses,[26,31,32] Salisbury Rule has not been widely used for pediatric dose prediction. Mahmood used Salisbury Rule to predict pediatric dose for small molecules as well as macromolecules26, 31, 32. Mahmood applied the Salisbury Rule to predict pediatric doses for both small and large molecules.[26,31,32] For small molecules, the Salisbury Rule was comparable to the whole-body PBPK model for pediatric dose selection.[26] The predictive performance of the Salisbury Rule for pediatric dosing reconciled very well with the recommended human clinical dose. In a recent study,[32] Salisbury Rule was used to predict pediatric doses of antimalarial medicines with excellent results (n = 88; 100% observations within 50% prediction error, 97.7% within 30% prediction error, and 93.2% within 20% prediction error).

Both the Salisbury Rule and allometric methods provide a basis to initiate a pediatric clinical trial or to be used in a clinical setting with a very simple but robust approach to predict pediatric doses not only for antituberculosis medicines but for other classes of medicines.[26,31,32]

Although, the WHO emphasizes that the doses of antituberculosis medicines both in adults and children be based on PK studies,[6] the methodology used by the WHO for the recommendation of pediatric doses for antituberculosis medicines is not clear. It is unknown whether these recommended doses are based on clinical trials, pediatric PK studies, or extrapolated from adult doses on a per kg body weight basis. In addition, the pediatric recommended doses by the WHO may also be influenced by the unavailability of appropriate pediatric formulations, which limits the ability to administer more precise doses in children.

Through PK, one can estimate optimal or near optimal dose of a drug in adults and pediatrics. The most important PK parameter is CL which is inverse of exposure (CL = Dose/area under the curve (AUC)). It is widely believed that the pediatric doses should be selected based on the exposure or CL of a medicine rather than per kg body weight basis extrapolated from adults.[12-14]

A general tendency for PK-based pediatric dose selection is by matching the adult exposure of a medicine to pediatrics.[6,13,14] This concept may and may not be always applicable. There are different manifestations of tuberculosis, for example, severity of tuberculosis by age or nutritional status, ethnicity, the region (South East Asia or Africa), and co-infection with other diseases such as HIV. These are important factors that should be considered before pediatric doses are selected for antituberculosis medicines. Therefore, from a clinical perspective, the predicted pediatric dose of antituberculosis medicines should be based on pediatric PK studies not on body weight. However, the results of this study indicate that body weight can provide reasonable dose of antituberculosis medicines provided the body weight is used as described in this manuscript (not on a per kg body weight basis). It should be noted that titration of dose is an integral part of clinical practice due to a patient's response to the dose. Therefore, a dose can vary from one patient to another.

From a dosing perspective, isoniazid offers a complex problem. There are slow, intermediate, and fast acetylators, and the clearance of isoniazid in these three groups considerably vary. Population PK studies[33] indicated that the clearance of isoniazid not only differs among the three groups of acetylators but within the group there is a high variability.

In adults, the FDA and WHO recommend 5 mg/kg up to 300 mg daily dose of isoniazid as a single dose or 15 mg/kg up to 900 mg/day, two or three times/week. In children, the recommended dose is 10 mg/kg to 15 mg/kg up to 300 mg daily as a single dose, or 20 mg/kg to 40 mg/kg up to 900 mg/day, two or three times/week. In both cases, there is no mention about dose adjustment across the three groups of acetylators.

Based on various population PK studies,[34] the opinion of this author is that one can use a median clearance value of 13 L/hr, 20 L/hr, and 30 L/hr for slow, intermediate, and fast acetylators, respectively. In this study, where pediatric data were available, in order to predict pediatric dose, an adult dose of 300 mg/day, 450 mg/day, and 600 mg/day was used for slow, intermediate, and fast acetylators (Table S1). Based on a population PK study, Jing *et al.*[33] suggested an isoniazid dose of 300 mg/day, 500-600 mg/day and 700-900 mg/day, for slow, intermediate, and fast acetylators, respectively.

Both the allometric and Salisbury Rule approaches are not based on a linear system per kg body weight basis. In

this study, both methods provided an excellent prediction of pediatric doses for antituberculosis medicines, as recommended by WHO and the FDA and this may be an indication that the recommended pediatric doses of antituberculosis by WHO and FDA are not based on per kg body weight but still requires optimization.

Neonates or very young children can also be infected by TB through adults or other unknown sources. Preterm and term neonates, as well as neonates with extremely low birth weight or very low birth weight, may have entirely different exposure of antituberculosis medicines than the older children. Since TB is common to all ages, clinical trials should include very young children after safety and efficacy of an antituberculosis medicines is established in adults. All models are erratic and uncertainty remains in the predictive power of these models[35] therefore, dedicated clinical studies at least in terms of PK should be conducted in the neonates and toddlers.

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Population PK studies can be conducted with sparse sampling across the age groups. Many such population PK studies for antituberculosis medicines have been conducted with a claim that allometric scaling was used in these studies. In fact, in these studies, theoretical exponents 0.75 and 1.0 for clearance and volume distribution, respectively, were used. Allometry is not defined by some fixed theoretical exponents. Over the years, dozens of manuscripts by the experts in the field have identified the limitations of the theoretical exponents.[36-38] It is incomprehensible that the slope or exponent of a regression line will never change rather will remain same irrespective of the data. The exponents of allometry are data dependent and are not fixed. There are enough data and wide body weight range in a population PK study to determine the exponents for both clearance and volume of distribution. Therefore, the exponents of allometry in population PK studies should be determined rather than fixed because there will be enough data points and wider body weight range (children to adults) to determine the allometric exponent.

The WHO and FDA recommend the dosing for antituberculosis medicines based on weight bands. The weight bands should be revaluated especially, in the younger children since some weight bands are very wide and there may be concerns with safety and efficacy.

It is important to recognize that the physiology of adults differs significantly from that of younger children (e.g., premature neonates, neonates, and toddlers). These physiological differences lead to variations in the PK of medicines and, consequently, dosing strategies. In this study, there were 12 observations in children aged ≤12 months, and both methods predicted the dose with the same level of accuracy in this age group as in older children. However, the sample size in this study is too small to draw definitive conclusions about the impact of age on the accuracy of dose predictions for antituberculosis medicines using these two models.

5. Conclusion

Robust PK studies are needed to determine the 'right dose' for antituberculosis medicines both in adults and children. Isoniazid dosing both in adults and children require re-evaluation considering the PK characteristics of different acetylator categories.

In this report, two simple methods to predict first-in-pediatric dose to initiate a pediatric clinical trial for antituberculosis medicines were evaluated. The predictive power of both methods are robust and accurate since more than 80% observations were within 30% prediction error. Considering the accuracy of these two methods, they could be used in clinical settings in emergency situations when clinical trial-based pediatric doses are unavailable. Both methods are straightforward to implement, demonstrating that simple approaches can be as accurate as more complex and elaborate methods. Complexity does not necessarily guarantee greater accuracy than simplicity.

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Conflict of interest

The author declares no conflicts of interest.

Author contributions

This is a single-authored article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

References for the data used in this study are provided within the article.

References

1. WHO. *Operational Handbook on Tuberculosis Module 5: Management of Tuberculosis in Children and Adolescents*; 2022. Available from: https://www.who.int/publications/i/item/9789240046832 [Last accessed on 2024 Aug 19].

2. World Health Organization. *WHO Operational Handbook on Tuberculosis. Module 4: Treatment: Drug-susceptible Tuberculosis Treatment*; 2022. Available from: https://apps.who.int/iris/handle/10665/354548 [Last accessed on 2024 Aug 19].

3. World Health Organization. *Global Tuberculosis Report*; 2020. Available from: https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf [Last accessed on 2024 Aug 19].

4. Drobac PC, Shin SS, Huamani P, *et al*. Risk factors for in-hospital mortality among children with tuberculosis: The 25-year experience in Peru. *Pediatrics*. 2012;130(2):e373-e379.

   doi: 10.1542/peds.2011-3048

5. World Health Organization. *Implementing the End TB Strategy: The Essentials*; 2022. Available from: https://www.who.int/publications/i/item/9789240065093 [Last accessed on 2024 Aug 19].

6. World Health Organization. *Technical Report on the Pharmacokinetics and Pharmacodynamics (PK/PD) of Medicines Used in the Treatment of Drug-resistant Tuberculosis*. World Health Organization; 2018. Available from: https://apps.who.int/iris/handle/10665/260440 [Last accessed on 2024 Aug 20].

7. European Medicines Agency. *Joint Evaluation of Regulation (EC) No 1901/2006 of the European Parliament and of the Council of 12 December 2006 on Medicinal Products for Paediatric Use and Regulation (EC) No 141/2000 of the European Parliament and of the Council of 16 December 1999 on Orphan Medicinal Products*. Available from: https://health.ec.europa.eu/system/files/2020-08/orphan-regulation_eval_swd_2020-163_part-1_0.pdf [Last accessed on 2024 Aug 20].

8. US Food and Drug Administration. *General Clinical Pharmacology Considerations for Pediatric Studies for Medicines and Biological Products. Draft Guidance*. Available from: https://www.fda.gov/downloads/medicines/guidancecomplianceregulatoryinformation/guidances/ucm425885.pdf [Last accessed on 2024 Aug 20].

9. Antwi S, Yang H, Enimil A, *et al*. Pharmacokinetics of the first-line antituberculosis drugs in Ghanaian children with tuberculosis with or without HIV coinfection. *Antimicrob Agents Chemother*. 2017;61:e01701-e01716.

   doi: 10.1128/AAC.01701-16

10. Bekker A, Schaaf HS, Draper HR, *et al*. Pharmacokinetics of rifampin, isoniazid, pyrazinamide, and ethambutol in infants dosed according to revised WHO-recommended treatment guidelines. *Antimicrob Agents Chemother*. 2016;60:2171-2179.

    doi: 10.1128/AAC.02600-15

11. Justine M, Yeconia A, Nicodemu I, *et al*. Pharmacokinetics of first-line drugs among children with tuberculosis in rural Tanzania. *J Pediatr Infect Dis Soc*. 2020;9(1):14-20.

    doi: 10.1093/jpids/piy106

12. Mahmood I. Dosing in children: A critical review of the pharmacokinetic allometric scaling and modelling approaches in paediatric drug development and clinical settings. *Clin Pharmacokinet*. 2014;53:327-346.

    doi: 10.1007/s40262-014-0134-5

13. Green DJ, Zineh I, Burckart GJ. Pediatric drug development: Outlook for science-based innovation. *Clin Pharmacol Ther*. 2018;103:376-378.

    doi: 10.1002/cpt.1001

14. Abernethy DR, Burckart GJ. Pediatric dose selection. *Clin Pharmacol Ther*. 2010;87:270-271.

    doi: 10.1038/clpt.2009.292

15. Mahmood I. Dose selection in children. In: *Pharmacokinetic Allometric Scaling in Pediatric Drug Development*. Rockville: Pine House Publishers; 2013. p. 151-60.

16. Rowland M, Peck C, Tucker G. Physiologically-based pharmacokinetics in drug development and regulatory science. *Annu Rev Pharmacol Toxicol*. 2011;51:45-73.

    doi: 10.1146/annurev-pharmtox-010510-100540

17. Zhou W, Johnson TN, Xu H, *et al*. Predictive performance of physiologically based pharmacokinetic and population pharmacokinetic modeling of renally cleared drugs in children. *CPT Pharmacometrics Syst Pharmacol*. 2016;5:475-483.

    doi: 10.1002/psp4.12101

18. Edginton AN, Theil FP, Schmitt W, Willmann S. Whole body physiologically-based pharmacokinetic models: Their use in clinical drug development. *Expert Opin Drug Metab Toxicol*. 2008;4:1143-1152.

    doi: 10.1517/17425255.4.9.1143

19. Mahmood I. Prediction of drug clearance in premature and mature neonates, infants, and children ≤2 years of age: A comparison of the predictive performance of 4 allometric models. *J Clin Pharmacol*. 2015;56:733-739.

    doi: 10.1002/jcph.652

20. Cao Y, Balthasar JP, Jusko WJ. Second-generation minimal physiologically-based pharmacokinetic model for monoclonal antibodies. *J Pharmacokinet Pharmacodyn*.

2013;40:597-607.

doi: 10.1007/s10928-013-9332-2

21. Cao Y, Jusko WJ. Applications of minimal physiologically-based pharmacokinetic models. *J Pharmacokinet Pharmacodyn*. 2012;39:711-723.

doi: 10.1007/s10928-012-9280-2

22. Björkman S. Reduction and lumping of physiologically based pharmacokinetic models: Prediction of the disposition of fentanyl and pethidine in humans by successively simplified models. *J Pharmacokinet Pharmacodyn*. 2003;30:285-307.

doi: 10.1023/a:1026194618660

23. Björkman S. Prediction of drug disposition in infants and children by means of physiologically based pharmacokinetic (PBPK) modelling: Theophylline and midazolam as model drugs. *Br J Clin Pharmacol*. 2004;59:691-704.

doi: 10.1111/j.1365-2125.2004.02225.x

24. Mahmood I, Tegenge MA. A comparative study between allometric scaling and physiologically based pharmacokinetic modeling for the prediction of drug clearance from neonates to adolescents. *J Clin Pharmacol*. 2019;59:189-197.

doi: 10.1002/jcph.1310

25. Mahmood I, Tegenge MA. Spreadsheet-based minimal physiological models for the prediction of clearance of therapeutic proteins in pediatric patients. *J Clin Pharmacol*. 2021;61:S108-S116.

doi: 10.1002/jcph.1846

26. Mahmood I. A comparison of different methods for the first-in-pediatric dose selection. *J Clin Transl Res*. 2022; 8:369-81.

27. Mahmood I, Ahmad T, Mansoor N, Sharib SM. Prediction of clearance in neonates and infants (≤ 3 months of age) for drugs that are glucuronidated: A comparative study between allometric scaling and physiologically based pharmacokinetic modeling. *J Clin Pharmacol*. 2017;57:476-483.

doi: 10.1002/jcph.837

28. Elias GP, Antoniali C, Mariano RC. Comparative study of rules employed for calculation of pediatric drug dosage. *J Appl Oral Sci*. 2005;13:114-119.

doi: 10.1590/s1678-77572005000200004

29. Munzenberger PJ, McKercher P. Pediatric dosing--the pharmacist's dilemma. *Contemp Pharm Pract*. 1980;3:11-4.

30. Lack JA, Stuart-Taylor ME. Calculation of drug dosage and body surface area of children. *Br J Anaesth*. 1997;78(5):601-605.

doi: 10.1093/bja/78.5.601

31. Mahmood I. A simple method for the prediction of therapeutic proteins (monoclonal and polyclonal antibodies and non-antibody proteins) for first-in-pediatric dose selection: Application of salisbury rule. *Antibodies* (*Basel*). 2022;11:66.

doi: 10.3390/antib11040066

32. Mahmood I. Application of allometric scaling and salisbury rule for the prediction of antimalarial drugs for first-in-pediatric dose selection. *Eur J Drug Metab Pharmacokinet*. 2023;48:587-594.

doi: 10.1007/s13318-023-00848-2

33. Jing W, Zong Z, Tang B. Population pharmacokinetic analysis of isoniazid among pulmonary tuberculosis patients from China. *Antimicrob Agents Chemother*. 2020;64:e01736-19.

doi: 10.1128/AAC.01736-19

34. Thomas L, Raju A, Sekhar C, *et al*. Influence of N-acetyltransferase 2 (NAT2) genotype/single nucleotide polymorphisms on clearance of isoniazid in tuberculosis patients: A systematic review of population pharmacokinetic models. *Eur J Clin Pharmacol*. 2022;78:1535-1553.

doi: 10.1007/s00228-022-03362-7

35. Box GE. Science and Statistics. *J Am Stat Assoc*. 1976;71:791-799.

doi: 10.2307/2286841

36. Mahmood I. Misconceptions and issues regarding allometric scaling during the drug development process. *Expert Opin Drug Metab Toxicol*. 2018;14:843-854.

doi: 10.1080/17425255.2018.1499725

37. Glazier DS. Variable metabolic scaling breaks the law: From 'Newtonian' to 'Darwinian' approaches. *Proc Biol Sci*. 2022;289:20221605.

doi: 10.1098/rspb.2022.1605

38. Valkengoed D, Krekels E, Knibbe C. All you need to know about allometric scaling: An integrative review on the theoretical basis, empirical evidence, and application in human pharmacology. *Clin Pharmacokinet*. 2024;64:173-192.

doi: 10.1007/s40262-024-01444-6

ACCSCIENCE PUBLISHING

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CASE REPORT

The importance of clinical and radiological follow-up after conservative treatment of iatrogenic type A aortic dissection: A case report

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Abstract

Background: Iatrogenic type A dissections can be managed conservatively, but clear guidelines for treatment are lacking. **Case presentation:** This case illustrates a borderline situation that necessitated a change in treatment approach. A patient underwent endo-prosthetic treatment for an aneurysm. During the insertion of a guiding catheter through the axillary artery, the ascending aorta was dissected, with contrast injected into the vascular wall. Due to the mechanism of injury and the absence of an intra-aortic intimal tear (as evidenced by contrast stagnation in the false lumen), conservative management was initially chosen. A follow-up computed tomography (CT) scan showed complete resolution after 1 month. However, 10 days later, the patient was readmitted with acute chest pain, and a new CT scan revealed an acute dissection, with a free-moving intimal flap in the ascending aorta, requiring emergency surgery. Current guidelines recommend surgery for iatrogenic type A dissections that extend or propagate several centimeters into the ascending aorta. However, these guidelines do not account for the mechanism of injury, the localization, or the size of the intimal tear. **Conclusion:** In this case, the assumption of spontaneous healing with an intact intima was initially supported. However, further developments highlight the importance of clinical and radiological follow-up, regardless of the injury's mechanism. **Relevance for patients:** Iatrogenic aortic dissection is a highly complex condition that requires close monitoring and, if necessary, a reassessment of the chosen treatment strategy.

Keywords: Intravascular catheterization; Intimal tear; Iatrogenic type A aortic dissection; Mechanism of injury; Conservative management; Clinical follow-up; Radiological follow-up; Treatment guidelines

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1. Background

Iatrogenic type A aortic dissections due to intravascular catheterization are often managed conservatively, in contrast to iatrogenic dissections caused directly by open-heart surgery or transcatheter aortic valve implantation. However, clear treatment guidelines for this clinical context, which could guide the decision to pursue or avoid a conservative approach, are lacking. The present case illustrates a borderline situation that ultimately led to a change in the treatment strategy.

2. Case presentation

A 79-year-old patient, who had undergone exclusion of a degenerative abdominal aortic aneurysm 5 years earlier using a bifurcated bi-iliac endovascular prosthesis, was admitted for elective complementary endovascular treatment of a progressively developing aneurysm in the distal right iliac artery (Figure 1). After the successful retrograde advancement of a catheter through the right femoral artery, several attempts to insert a percutaneous guiding catheter antegrade through the left axillary artery failed to reach the descending thoracic aorta. Subsequent contrast injection revealed stagnation of the contrast medium at the aortic arch and ascending aorta (Figure 2A). A computed tomography (CT) angiography of the aorta showed a large false lumen filled with the previously used contrast medium, extending throughout the ascending aorta and into the distal aortic arch (Figures 2B and 2D). The contrast-delayed phase showed a lack of contrast enhancement in this region, confirming an iatrogenic type A dissection (Figure 2C and 2E).

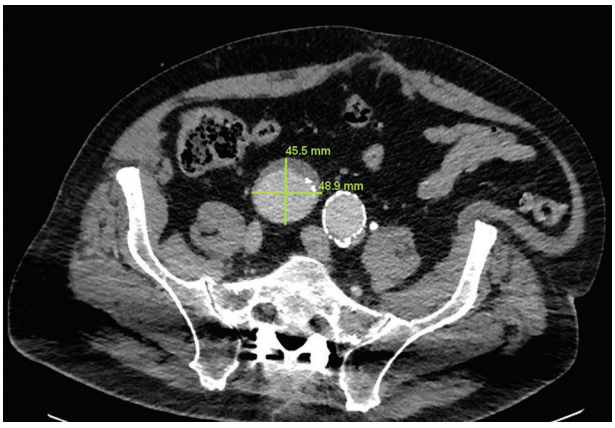

Figure 1. Aneurysm of the distal right iliac artery

Given the mechanism of injury and the likely absence of an intra-aortic intimal tear (evidenced by contrast stagnation in the false lumen), conservative treatment was initially chosen. Antihypertensive treatment with metoprolol (75 mg/day) and amlodipine (10 mg/day) was initiated.

A follow-up CT scan 48 h later showed near-complete regression of the false lumen. The patient was extubated and transferred to rehabilitation 14 days after admission, with an unremarkable clinical course (Figure 3A). A subsequent CT scan after 1 month confirmed the near-complete regression of the false lumen (Figure 3B).

Ten days later, the patient was readmitted due to acute, oppressive chest pain. The CT scan now revealed the classic features of an acute aortic dissection, including a free-moving intimal flap in the ascending aorta with persistent blood flow in both lumens (simultaneous contrast enhancement) and an enlarged aortic diameter of approximately 50 mm (Figure 4).

Emergency surgical replacement of the dissected aorta with a Dacron vascular prosthesis (ascending aorta and hemiarch) was performed under moderate hypothermia, with continuous selective antegrade cerebral perfusion through the brachiocephalic trunk. The patient was extubated on the 1st post-operative day without further complications.

3. Discussion

According to the European registry of iatrogenic type A aortic dissections following cardiovascular procedures, peripheral endovascular procedures account for only 1% of all dissections reported. The registry comprises 18 centers across eight European countries and includes 3902 patients

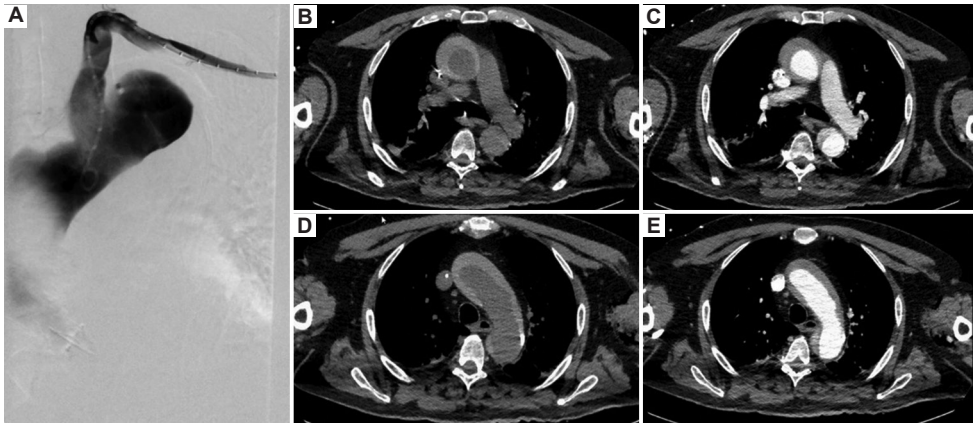

Figure 2. Computed tomography angiography of the aorta. (A) Stagnation of contrast medium in the ascending aorta and the aortic arch after direct injection in the left axillary artery catheter. (B and D) Native computed tomography scan shows a large false lumen extending throughout the ascending aorta (B) and into the distal aortic arch (D), filled with the previously the previously injected contrast medium. (C and E) After intravenous contrast medium injection, confirmation of an iatrogenic type A dissection.

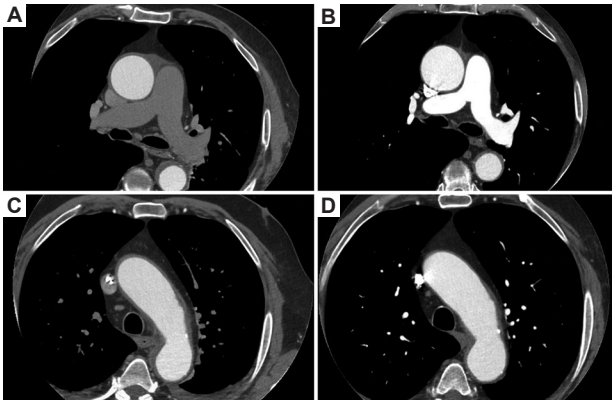

Figure 3. Follow-up computed tomography scan, 48 h later showed near-complete regression of the false lumen. (A and C) Early phase of contrast injection; (B and D) Delayed phase of contrast injection.

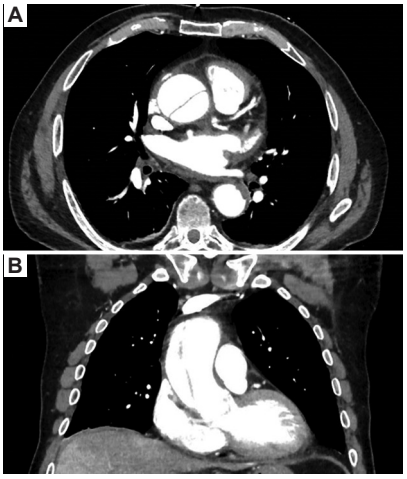

Figure 4. Follow-up computed tomography scan at 10 days shows classic features of an acute aortic dissection. (A) Axial view and (B) frontal view.

over approximately 15 years (Figure 3). Other common etiologies include previous cardiac surgery (37.8%), percutaneous coronary intervention (36.9 %), diagnostic coronary angiography (13.6%), and transcatheter aortic valve replacement (10.7%).[1-3]

In previous case series, conservative treatment has been suggested based on the extent of the iatrogenic dissection, particularly in cases involving limited intimal tears near or at the coronary ostia as a complication of cardiac catheterization.[4,5] In most of these instances, immediate intra-ostial stenting is used to stabilize the intimal tear and prevent further progression of the aortic dissection.

The optimal treatment strategy for iatrogenic type A dissections following endovascular procedures remains unclear, as the treatment concepts discussed in the literature are based on only a few cases.[6,7] While surgical aortic replacement is most commonly performed, the mechanisms of injury (such as direct intimal damage to the aorta caused by retrograde transaortic advancement of guiding catheters or endoprostheses) differ from the case presented here.[6,7] In our case, the patient experienced a two-stage clinical evolution. First, only a contrast-enhancing aortic hematoma (without intimal tear) was observed, for which conservative treatment was administered without stenting of the entry point. This treatment was initially successful, with complete regression of the periaortic hematoma at 1 month. However, in the second phase, an intimal tear developed despite well-managed anti-hypertensive treatment, necessitating emergency surgery for ascending aorta replacement. To the best of our knowledge, no similar case has been reported in the literature.

In these situations, regardless of etiology, the guidelines vaguely recommend that "…dissections extending over several centimeters into the ascending aorta or further propagating require emergency cardiac surgery…."[8,p.2901] However, the role of the causative mechanism is not clearly defined, and in this context, the term "iatrogenic" refers to a variety of possible causes.

In the present case, the initial treatment decision was influenced by the underlying mechanism of injury. While acute surgical treatment typically aims to stabilize the ascending aorta by removing the intimal tear and replacing the damaged segment with a vascular prosthesis, it is likely that, at least initially, there was no corresponding tear in the ascending aorta. Instead, the dissection was provoked by the forcible retrograde advancement of a catheter via the subclavian artery, followed by the injection of contrast medium under pressure between the layers of the aortic wall. The absence of contrast leakage into the true aortic lumen, along with the rapid (observed on a follow-up CT scan at 48 h) and complete (as exhibited by a follow-up CT scan at 1 month) regression of the false lumen, seemed to support this hypothesis. However, this initial conservative strategy did not lead to the desired healing, and it is possible that the patient suffered a new lesion in the already damaged aorta due to an acute event.

4. Conclusion

This case represents a specific form of the iatrogenic type A dissection in terms of its pathogenesis, the variable two-step clinical course, and the resulting treatment approach. It emphasizes the importance of close and long-term follow-up care.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Lars Niclauss, Filip Dulguerov, Matthias Kirsch
Data curation: Lars Niclauss, Filip Dulguerov
Methodology: Lars Niclauss, Filip Dulguerov, Matthias Kirsch
Writing – original draft: Lars Niclauss, Filip Dulguerov
Writing – review & editing: All authors

Ethics approval and consent to participate

Ethics approval and consent to participate are not required for this case report.

Consent for publication

The patient's consent has been obtained to publish data.

Availability of data

The data on which this article is based are available in the article.

References

1. Biancari M, Pettinari M, Mariscalco G, *et al*. Outcome after surgery in iatrogenic acute type A aortic dissection. *J Clin Med.* 2022;11:6729.

   doi: 10.3390/jcm11226729

2. Nunez-Gil I, Bautista D, Cerrato E, *et al*. Incidence, management, and immediate- and long-term outcomes after iatrogenic aortic dissection during diagnostic or interventional coronary procedures. *Circulation.* 2015;131:2114-2119.

   doi: 10.1161/CIRCULATIONAHA.115.015334

3. Safdar A, Young L, Khatri J. Management of iatrogenic aortic dissection after percutaneous intervention of chronic total occlusion. *Catheter Cardiovasc Interv.* 2024;104:1442-1446.

   doi: 10.1002/ccd.31262

4. Elefteriades JA, Zafar MA, Ziganshin BA. Iatrogenic aortic dissection: Review of the literature. *Aorta (Stamford).* 2016;4:240-243.

   doi: 10.12945/j.aorta.2016.16.067

5. Ramasamy A, Bajaj R, Jones DA, *et al*. Iatrogenic catheter-induced ostial coronary artery dissections: Prevalence, management, and mortality from a cohort of 55,968 patients over 10 years. *Catheter Cardiovasc Interv.* 2021;98:649-55.

   doi: 10.1002/ccd.29382

6. Piffaretti G, Mariscalco G, Tozzi M, Bruno VD, Sala A, Castelli P. Acute iatrogenic type A aortic dissection following thoracic aortic endografting. *J Vasc Surg.* 2010;51:993-9.

   doi: 10.1016/j.jvs.2009.10.105

7. Wolfers MP, Yu Q, Montorfano L, Bordes SJ, King T. Type A thoracic aortic dissection following endovascular repair of a common iliac artery aneurysm. *Cureus.* 2021;13(3):e13971.

   doi: 10.7759/cureus.13971

8. Erbel R, Aboyans V, Boileau C, *et al*. 2014 ESC guidelines on the diagnosis and treatment of aortic diseases: Document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The task force for the diagnosis and treatment of aortic diseases of the European Society of Cardiology (ESC). *Eur Heart J.* 2014;35:2873-926.

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ACCSCIENCE PUBLISHING

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CASE REPORT

Meropenem-induced cholestasis in a pyelonephritis patient: A case report and evaluation using the updated RUCAM scale

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Abstract

Background: Meropenem, a broad-spectrum carbapenem antibiotic, is widely used to treat severe infections but has been associated with rare cases of drug-induced liver injury, including cholestasis. Recognizing and managing this potential adverse effect is essential for ensuring patient safety, particularly in the context of prolonged antibiotic use. **Case presentation:** We report a case of meropenem-induced cholestasis in a 57-year-old female patient with a history of recurrent urinary tract infections. The patient developed elevated liver enzymes and jaundice after the initiation of meropenem therapy. This report describes the clinical presentation, diagnostic workup, management, and outcome. The case was assessed using the updated Roussel Uclaf Causality Assessment Method scale, yielding a score of 9, indicating a "highly probable" causality. This case highlights the importance of vigilant liver function monitoring in patients receiving meropenem and other potentially hepatotoxic medications. **Conclusion:** As antibiotic stewardship becomes increasingly crucial in mitigating antimicrobial resistance, recognizing and understanding the potential side effects of these agents is essential for safer prescribing practices. **Relevance for patients:** This case highlights the importance of vigilant liver function monitoring in patients on meropenem, reinforcing early recognition, and discontinuation to enhance patient safety and support personalized antibiotic therapy.

Keywords: Meropenem; Cholestasis; Drug-induced liver injury; Updated Roussel Uclaf Causality Assessment Method; Roussel Uclaf Causality Assessment Method

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1. Introduction

Meropenem is a broad-spectrum carbapenem antibiotic widely used in clinical practice to treat severe and life-threatening infections caused by a variety of Gram-positive and Gram-negative bacteria. Its mechanism of action involves inhibiting bacterial cell wall synthesis, making it effective against resistant strains, including those producing beta-lactamases. Given its potent efficacy, meropenem is often reserved for serious infections such as pneumonia, meningitis, sepsis, and complicated urinary tract infections.[1,2]

While generally well-tolerated, meropenem is not without potential adverse effects. Drug-induced liver injury is a rare but clinically significant complication associated

with meropenem therapy. The spectrum of liver injuries ranges from mild transaminase elevations to severe cholestasis, hepatic failure, and, in extreme cases, death.[3] Cholestasis, characterized by impaired bile flow, can manifest as jaundice, pruritus, and elevated liver enzymes, particularly alkaline phosphatase (ALP) and bilirubin. The pathophysiology of meropenem-induced cholestasis remains poorly understood, with proposed mechanisms including direct hepatocellular toxicity, idiosyncratic reactions, and hypersensitivity. In addition, genetic predisposition, underlying liver disease, and concomitant use of other hepatotoxic medications may increase the risk.[3]

In the context of rising antibiotic resistance and the continued reliance on broad-spectrum agents such as meropenem, recognizing and managing potential drug-induced liver injury is critical.[4] Timely identification and discontinuation of the offending agent can prevent further liver damage and improve clinical outcomes. This case report presents a detailed account of a patient who developed cholestasis following meropenem therapy, emphasizing the importance of liver function monitoring in patients receiving this antibiotic. By raising awareness of this potential adverse effect, we aim to enhance clinical vigilance and improve patient safety in antibiotic therapy.

2. Case presentation

A 57-year-old female with a history of recurrent urinary tract infections, hypertension, and mild chronic kidney disease was admitted with a diagnosis of pyelonephritis. The patient reported a fever of 38.9°C and severe right-sided flank pain, rated 8/10 in intensity, exacerbated by movement. She also experienced dysuria, characterized by a burning sensation. Mild nausea was noted, but there was no vomiting or diarrhea. Her medication history included lisinopril, metformin, and prior treatment with nitrofurantoin. There was no recent history of hypotension, shock, or ischemia.

On admission, meropenem 1 g IV every 8 h was initiated. On day 4 of therapy, she developed pruritus and noticed yellowing of her skin and eyes. Clinical and physical examination revealed stable vital signs, mild jaundice, and no abdominal tenderness or signs of ascites. Laboratory findings showed abnormal liver function tests, with elevated aspartate aminotransferase (300 U/L [normal: <40 U/L]), alanine aminotransferase (ALT; 280 U/L [normal: <40 U/L]), total bilirubin (4.5 mg/dL [normal: <1.2 mg/dL]), (ALP; 250 U/L [normal: <120 U/L]), along with a serum creatinine of 1.2 mg/dL (baseline). Abdominal ultrasound showed no biliary obstruction and a normal-sized liver. The differential diagnosis included acute viral hepatitis, drug-induced liver injury, and autoimmune hepatitis. Laboratory investigations for hepatitis B virus, hepatitis C virus, acute cytomegalovirus infection, Epstein-Barr virus, and herpes simplex virus were negative. Given the temporal association between meropenem administration and symptom onset, along with the exclusion of other potential causes, a diagnosis of meropenem-induced cholestasis was established.

Meropenam was discontinued immediately on day 5. The patient was managed with antihistamines for pruritus and monitored closely for liver function recovery. Liver function tests were repeated weekly. One week after discontinuation of meropenem, liver enzymes began to decline (Table 1). The patient reported resolution of pruritus and jaundice by the 2nd week. For her ongoing infection, ceftriaxone was initiated as an alternative antibiotic.

The pharmacovigilance assessment classified the reaction as "moderate" according to the Hartwig-Seigel scale.[5] Preventability assessment using the Schumock-Thornton scale[6] categorized the reaction as "not preventable." The pattern of liver injury was determined based on the R-value, calculated as the ratio of ALT/upper limit of normal (ULN): ALP/ULN. An R-value of ≥5 indicates hepatocellular injury, ≤2 indicates cholestatic injury, and values ranging 2 – 5 indicate mixed-type injury. In this case, the calculated R-value was 3.36, suggesting mixed-type injury.

Causality was further assessed using the updated Roussel Uclaf Causality Assessment Method (RUCAM),[7] a validated tool for assessing drug-induced liver injury. The total RUCAM score was 9, grading the case as "highly probable." (Table 2) The case was subsequently reported to the national pharmacovigilance program.

Table 1. Temporal trends in liver function and clinical markers

Days of therapy	AST (U/L)	ALT (U/L)	Total bilirubin (mg/dL)	Alkaline phosphatase (U/L)	Creatinine (mg/dL)
Day 0	27	24	0.4	80	1.1
Day 4	300	280	4.5	250	1.2
Day 11	150	120	2.0	163	1.2
Day 18	50	45	1.0	110	1.0

Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Table 2. Updated Roussel Uclaf Causality Assessment Method (RUCAM) scoring for this case

Variables	Case details	Score
1. Time of onset		
From the beginning of the drug	<5 days	+1
From the cessation of the drug	≤15 days	+1
2. Course		
Change in ALP between the peak value and ULN	Decrease ≥50% within 180 days	+2
3. Risk factors		
Alcohol/Pregnancy	Absent	+0
Age	Age of the patient ≥55 years	+1
4. Concomitant drugs	None or no information or concomitant drug with incompatible time to onset	0
5. Exclusion of other causes: Group I (7 causes): • Acute viral hepatitis due to HAV (IgM anti-HAV), or • HBV (HBsAg and/or IgM anti-HBc), or • HCV (anti-HCV and/or HCV RNA with appropriate clinical history) • HEV (Anti-HEV-IgM, anti-HEV-IgG, HEV-RNA) • Biliary obstruction (Through imaging) • Alcoholism (History of excessive intake and AST/ALT ≥2) • Recent history of hypotension, shock, or ischemia (within 2 weeks of onset) Group II (2 categories of causes): • Complications of underlying disease (s) such as autoimmune hepatitis, sepsis, chronic hepatitis B or C, primary biliary cirrhosis or sclerosing cholangitis; or • Clinical features or serologic and virologic tests indicating acute CMV, EBV, or HSV.	All causes in Group I and II were ruled out	+2
6. Previous information on the hepatotoxicity of the drug: Reaction labeled in the product characteristics		+2
7. Response to re-administration:	Not done	0
Total RUCAM score		9

Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; HAV: Hepatitis A virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HEV: Hepatitis E virus; HSV: Herpes simplex virus; ULN: Upper limit normal.

3. Discussion

Meropenem is an important therapeutic option for managing serious infections, particularly in hospitalized patients and those with multidrug-resistant organisms. However, as illustrated in this case, meropenem can induce cholestasis, a potentially serious side effect that warrants increased awareness among healthcare providers.[8] The exact mechanisms by which meropenem induces cholestasis remain unclear, but several hypotheses exist. Some studies suggest that certain antibiotics can directly damage hepatocytes or cholangiocytes, leading to bile duct obstruction or impaired bile formation. This can result in a cholestatic pattern of liver injury, characterized by elevated ALP and bilirubin levels. Drug-induced liver injury is often idiosyncratic, meaning it is unpredictable and not necessarily dose-dependent.[9] Genetic variations in drug metabolism may pre-dispose certain individuals to cholestasis following meropenem exposure, as variability in hepatic enzyme activity can alter drug metabolism and increase the risk of hepatotoxicity.[10] In some cases, an immune response may contribute to liver injury. The immune system may mistakenly target hepatocytes after drug exposure, triggering inflammation and subsequent cholestasis. This immune response can be particularly pronounced in patients with underlying autoimmune conditions or those on multiple medications. Patients receiving meropenem often have complex medical histories and may be on polypharmacy regimens, increasing the potential for drug-drug interactions that affect liver function. For example, non-steroidal anti-inflammatory drugs, other antibiotics, or medications for chronic conditions may interact with meropenem, further increasing the risk of liver injury.[11]

Early identification of cholestasis is critical, as prompt discontinuation of the offending agent can lead

to rapid liver function recovery. In this case, cessation of meropenem led to significant improvement in liver function within weeks, highlighting the reversible nature of drug-induced liver injury when detected early. Patients receiving meropenem should undergo regular liver function monitoring, especially those with pre-existing liver disease or those receiving concomitant hepatoxic medications. A baseline liver enzyme assessment should be performed before therapy initiation, with regular follow-up testing throughout treatment.[12]

Clinicians should maintain a high index of suspicion for drug-induced liver injury in patients presenting with jaundice or elevated liver enzymes, particularly when recent antibiotic therapy is involved. Other potential causes, such as viral hepatitis, autoimmune liver disease, or biliary obstruction, must be ruled out through appropriate investigations. In addition, patient education is essential. Patients should be informed about the early signs of liver dysfunction, including jaundice and pruritus, and should be advised to report these symptoms promptly. Interdisciplinary collaboration among pharmacists, nurses, and physicians can enhance monitoring efforts and improve overall patient safety during antibiotic therapy.

4. Conclusion

Meropenem is a critical agent in the management of serious infections; however, its potential to induce cholestasis warrants careful consideration. This case underscores the necessity for routine liver function monitoring in patients receiving meropenem and highlights the importance of early recognition and intervention to prevent significant liver injury. Further research is needed to elucidate the underlying mechanisms of drug-induced cholestasis and to identify patient-specific risk factors that could inform safer prescribing practices in the future.

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The author certifies that he/she has obtained all appropriate patient consent forms.

Consent for publication

In the patient consent form, the patient(s) has/have given his/her/their consent for his/her/their clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Availability of data

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References

1. Aurilio C, Sansone P, Barbarisi M, *et al*. Mechanisms of action of carbapenem resistance. *Antibiotics*. 2022;11(3):421.
   doi: 10.3390/antibiotics11030421
2. Armstrong T, Fenn SJ, Hardie KR. JMM profile: Carbapenems: A broad-spectrum antibiotic. *J Med Microbiol*. 2021;70(12):001462.
   doi: 10.1099/jmm.0.001462
3. Tattersall T, Wright H, Redmond A. Meropenem-induced liver injury and beta-lactam cross-reactivity. *BMJ Case Rep*. 2018;11(1):e227124.
   doi: 10.1136/bcr-2018-227124
4. Shiraishi C, Kato H, Ogura T, Iwamoto T. An investigation of broad-spectrum antibiotic-induced liver injury based on the FDA adverse event reporting system and retrospective observational study. *Sci Rep*. 2024;14(1):18221.
   doi: 10.1038/s41598-024-69279-6
5. Hartwig SC, Siegel J, Schneider PJ. Preventability and severity assessment in reporting adverse drug reactions. *Am J Hosp Pharm*. 1992;49(9):2229-2232.
6. Schumock GT, Thornton JP. Focusing on the preventability of adverse drug reactions. *Hosp Pharm*. 1992;27(6):538.
7. Danan G, Teschke R. RUCAM in drug and herb induced liver injury: The update. *Int J Mol Sci*. 2016;17(1):14.
   doi: 10.3390/ijms17010014
8. Fish DN. Meropenem in the treatment of complicated skin and soft tissue infections. *Ther Clin Risk Manag*. 2006;2(4):401-15.
   doi: 10.2147/tcrm.2006.2.4.401
9. Kullak-Ublick GA. Drug-induced cholestatic liver disease. In: *Madame Curie Bioscience Database*. Austin, TX: Landes Bioscience; 2000-2013. Available from: https://www.ncbi.

nlm.nih.gov/books/NBK6102 [Last accessed on 2024 Oct 20].

10. Sundaram V, Björnsson ES. Drug-induced cholestasis. *Hepatol Commun*. 2017;1(8):726-735.
doi: 10.1002/hep4.1088

11. Adams DH, Ju C, Ramaiah SK, Uetrecht J, Jaeschke H. Mechanisms of immune-mediated liver injury. *Toxicol Sci*. 2010;115(2):307-321.
doi: 10.1093/toxsci/kfq009

12. Cheung S, Bulovic J, Pillai A, Manoj T, Neeraj K. A case of meropenem-induced liver injury and jaundice. *J Community Hosp Intern Med Perspect*. 2021;11(1):143-144.
doi: 10.1080/20009666.2020.1845929

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COMMENTARY

Reprogramming of lipid droplets by host cells as a defense mechanism against viral infection

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Abstract

A recent study revealed the crucial roles of host cells in reprogramming lipid droplet (LD) synthesis to resist viral infection. Understanding the molecular mechanisms of host resistance to viral infection is necessary to develop safe and effective strategies to control viral infection. This study provides a thorough examination of the interplay between the transcription factor YY1 and LD synthesis in the context of viral infection, with potential implications for both fundamental research and practical applications in the swine industry.

Keywords: Viral infection; Lipid droplet; Host cell reprogramming; Antiviral mechanism

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Porcine reproductive and respiratory syndrome virus (PRRSV) has been circulating for more than 30 years since it was first identified in the late 1980s. Due to the biological characteristics of PRRSV, such as high variability, strain diversity, immunosuppression, and immune escape, it has not been effectively controlled, resulting in increasing harm to the global pig industry.[1] Porcine alveolar macrophages (PAMs), the primary host cell targets for PRRSV infection, play a crucial role in immune defense. During infection, PRRSV hijacks various intracellular systems for its replication and evades host immune surveillance.[2-4] Host cells, in turn, respond to viral infection by inhibiting viral replication and clearing the virus.[5,6] Exploring strategies for host resistance to viral infection is important for identifying new targets to control PRRSV. Lipid droplets (LDs), organelles rich in neutral lipids, are involved in lipid storage and maintaining lipid homeostasis in cells; they are also important sites for intracellular energy storage and release.[7] PRRSV hijacks LDs to acquire the energy necessary for replication and serve as assembly sites for progeny virions.[8] Yin yang 1 (YY1) is widely reported to be overexpressed in cancer tissues, such as lung cancer, breast cancer, prostate cancer, and cervical cancer tissues; it is directly or indirectly involved in the development of various cancers through its transcriptional regulatory activity.[9] However, this study provides a new perspective on how the transcription factor YY1 reprograms LDs to promote resistance to viral infection.

A study reported a high level of YY1 expression in PAMs and the lungs of PRRSV-infected piglets and focused on determining the mechanism by which YY1 affects the proliferation of PRRSV.[10] YY1 was shown to be a limiting factor for PRRSV proliferation both *in vitro* and *in vivo*, suggesting that the upregulation of YY1 following viral infection serves as an effective mechanism for host cell resistance to viral infection. The study reported that the number of LDs in cells increased after viral infection and

that YY1 played an active role in this process. Notably, the PRRSV virions, nucleocapsid proteins, dsRNA, and replication and transcription complex were found not to colocalize with intracellular LDs, indicating that the virus does not rely on LDs for the assembly of progeny virions. In addition, exogenously induced LD synthesis inhibited PRRSV replication, suggesting that increased LD synthesis observed after viral infection is likely a host cell strategy to resist viral infection. This highlights that there is an interaction between PRRSV and LDs, encompassing both viral utilization and host resistance. Next, the researchers explored the mechanism by which YY1 reprogrammed LD synthesis. They identified two lipid metabolism proteins, fatty acid synthase (FASN) and peroxisome proliferator-activated receptor gamma (PPARγ), whose gene expression was regulated by YY1 and reported that YY1 exerts antiviral effects by negatively regulating the FASN-mediated fatty acid synthesis pathway and positively regulating the PPARγ-mediated LD synthesis pathway.

The study investigated a previously unexplored aspect of virus-host interactions: host cell reprogramming of LD synthesis to resist viral infection. It provides new insights into the interaction of PRRSV with LDs and, for the 1st time, elucidates how host cells reprogram LD synthesis through YY1 in response to viral infection. This novel insight not only expands our understanding of the dynamic mechanism of LD synthesis but also complements the existing theories of the antiviral activity of LDs. By elucidating the antiviral function of LD synthesis and the role of YY1 in this context, this study provides a foundation for the development of treatment strategies and innovative prevention strategies for viral infection. For example, this study designed rigorous *in vitro* and *in vivo* experiments on YY1 and identified the antiviral function of YY1 and its potential therapeutic target through comprehensive analysis. In addition, this study provides a possible explanation for puzzling phenomena, such as the increase in LD synthesis after viral infection. Previous studies have shown that PRRSV induces lipophagy and releases fatty acids for viral replication.[11] If the increase in LDs results from active induction by PRRSV, the virus could directly hijack the fatty acids synthesized by LDs for its replication, bypassing the need for inducing synthesis followed by lipolysis. Increasing LD synthesis may serve as a host defense mechanism against viral infection, but the virus may evolve countermeasures to induce LDs lipolysis into fatty acids to supply energy for viral replication. This dynamic reflects the coexistence of resistance and utilization between LDs and viruses. At present, most studies are interested in viruses hijacking and utilizing LDs and exploring the underlying mechanism from different perspectives. However, few studies have clarified the antiviral function of LDs. Following Zika virus and herpes simplex virus type 1 infection, the host protein epidermal growth factor receptor-driven LD synthesis has been shown to activate the expression of type □ and type III interferons, thereby exerting antiviral effects.[12]

Moreover, researchers have reported that the LD surface protein, viperin, interacts with the non-structural protein 5A of the hepatitis C virus to inhibit viral genome replication.[13] Hundreds of functional proteins are located on the surface of mature LDs, and their potential roles in virus-LD interactions remain to be further elucidated. Further exploration of the mechanisms underlying increased LD synthesis after virus infection and the corresponding antiviral responses from the perspective of the host is essential to understand the complex dynamics of virus-LD dynamic interactions fully.

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References

1. Cui Z, Liu J, Xie C, *et al*. High-throughput screening unveils nitazoxanide as a potent PRRSV inhibitor by targeting NMRAL1. *Nat Commun*. 2024;15(1):4813.

   doi: 10.1038/s41467-024-48807-y

2. Gao P, Liu Y, Wang H, *et al*. Viral evasion of PKR restriction by reprogramming cellular stress granules. *Proc Natl Acad Sci U S A*. 2022;119(29):e2201169119.

   doi: 10.1073/pnas.2201169119

3. Ke W, Zhou Y, Lai Y, Long S, Fang L, Xiao S. Porcine reproductive and respiratory syndrome virus nsp4 positively

regulates cellular cholesterol to inhibit type I interferon production. *Redox Biol*. 2022;49:102207.

doi: 10.1016/j.redox.2021.102207

4. Li Y, Fang L, Zhou Y, Tao R, Wang D, Xiao S. Porcine reproductive and respiratory syndrome virus infection induces both eIF2α phosphorylation-dependent and-independent host translation shutoff. *J Virol*. 2018;92(16):e00600-18.

doi: 10.1128/jvi.00600-18

5. Zhang A, Duan H, Zhao H, *et al*. Interferon-induced transmembrane protein 3 is a virus-associated protein which suppresses porcine reproductive and respiratory syndrome virus replication by blocking viral membrane fusion. *J Virol*. 2020;94(24):e01350-20.

doi: 10.1128/jvi.01350-20

6. Zhang X, Dong W, Wang X, *et al*. Exostosin glycosyltransferase 1 reduces porcine reproductive and respiratory syndrome virus infection through proteasomal degradation of nsp3 and nsp5. *J Biol Chem*. 2022;298(2):101548.

doi: 10.1016/j.jbc.2021.101548

7. Olzmann JA, Carvalho P. Dynamics and functions of lipid droplets. *Nat Rev Mol Cell Biol*. 2019;20(3):137-155.

doi: 10.1038/s41580-018-0085-z

8. Zhang J, Lan Y, Li MY, *et al*. Flaviviruses exploit the lipid droplet protein AUP1 to trigger lipophagy and drive virus production. *Cell Host Microbe*. 2018;23(6):819.e5-831.e5.

doi: 10.1016/j.chom.2018.05.005

9. Meliala ITS, Hosea R, Kasim V, Wu S. The biological implications of Yin Yang 1 in the hallmarks of cancer. *Theranostics*. 2020;10(9):4183-4200.

doi: 10.7150/thno.43481

10. Zheng Z, Ling X, Li Y, *et al*. Host cells reprogram lipid droplet synthesis through YY1 to resist PRRSV infection. *mBio*. 2024;15:e0154924.

doi: 10.1128/mbio.01549-24

11. Wang J, Liu JY, Shao KY, *et al*. Porcine reproductive and respiratory syndrome virus activates lipophagy to facilitate viral replication through downregulation of NDRG1 expression. *J Virol*. 2019;93(17):e00526-19.

doi: 10.1128/jvi.00526-19

12. Monson EA, Crosse KM, Duan M, *et al*. Intracellular lipid droplet accumulation occurs early following viral infection and is required for an efficient interferon response. *Nat Commun*. 2021;12(1):4303.

doi: 10.1038/s41467-021-24632-5

13. Helbig KJ, Eyre NS, Yip E, *et al*. The antiviral protein viperin inhibits hepatitis C virus replication via interaction with nonstructural protein 5A. *Hepatology*. 2011;54(5):1506-1517.

doi: 10.1002/hep.24542

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