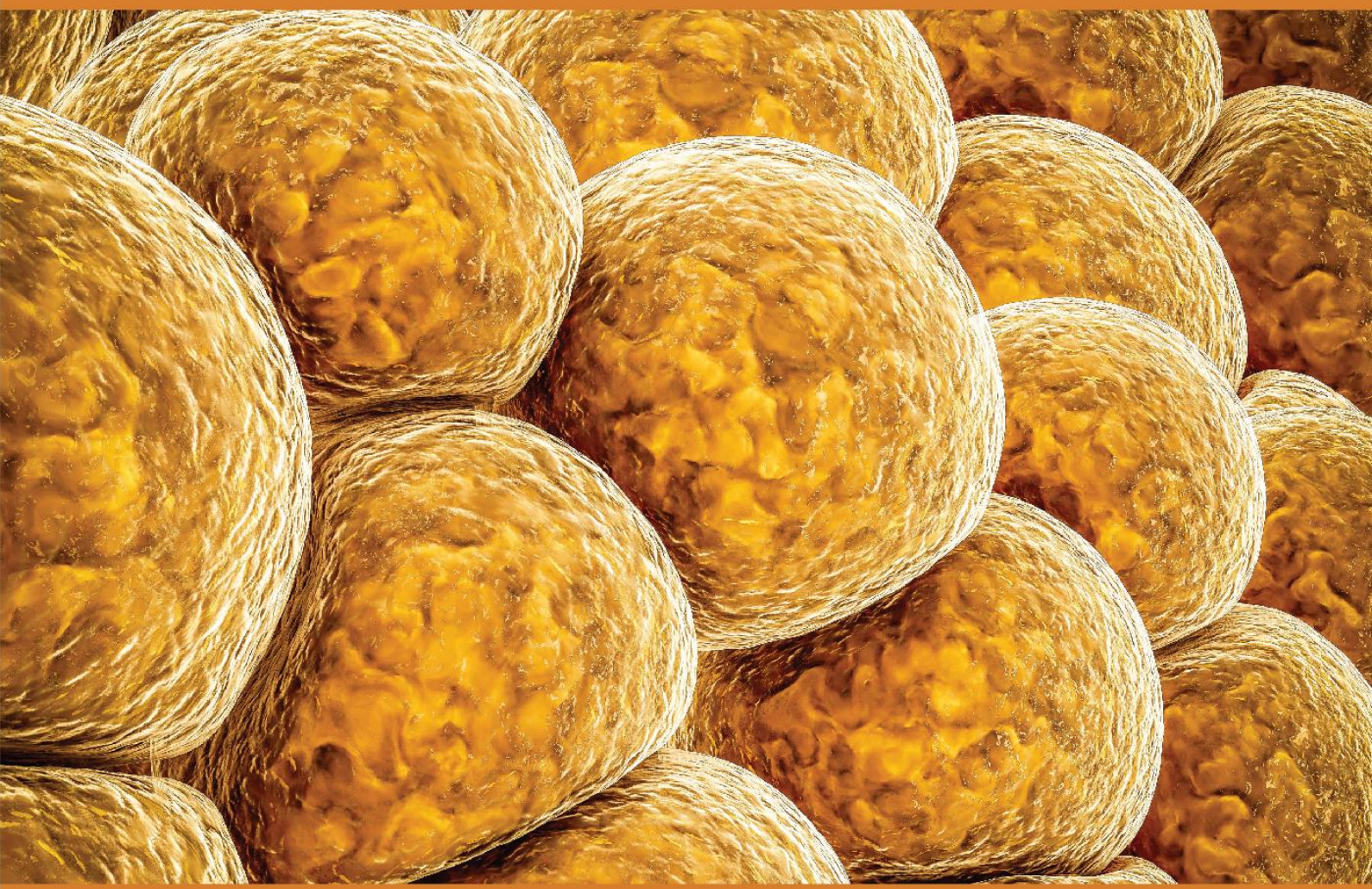
Journal of Clinical and Translational Research



Operating office:
APPLIVE BV, the Netherlands
www.jctres.com

ACCSCIENCE PUBLISHING
Volume 9 Issue 4, August 2023
ISSN: 2424-810X (online) ISSN: 2382-6533 (print)

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:

Internal medicine (all branches)
Vascular medicine and phlebology
Oncology
Cardiology
Intensive care medicine
Ophthalmology
Neurology and neurosciences
Anatomy, physiology, and embryology
Pathology
Clinical physics
Epidemiology
Medical devices
Pharmacology
Microbiology
Parasitology
Biomedical spectroscopy and spectrometry
Gastroenterology and hepatology
Surgery and transplantation
Hematology
Nephrology
Dermatology
Endocrinology and metabolism
Anesthesiology
Radiology and nuclear medicine
Clinical chemistry
Genetics and epigenetics
Global health
Nutrition
Immunology
Virology
Biomedical engineering

Key features

- Open access
- Reputable international editorial board
- Easy and fast submissions - no formatting rules (“your paper, your way”)
- No word count or reference restrictions
- Double blind review process to minimize bias
- Rapid online publication of articles upon acceptance
- Outlet for academic institutions and industry

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.).

Volume 9 Issue 4 • 2023
ISSN 2382-6533 (print) ISSN 2424-810X (online)

JOURNAL OF CLINICAL AND TRANSLATIONAL RESEARCH

ACCSCIENCE PUBLISHING

ACCSCIENCE PUBLISHING

2023 | Volume 9 | Issue 4 | Page 222 – 307 |

Journal of Clinical and Translational Research

https://www.jctres.com/en/home/

CONTENTS

CONTACTS

For general inquiries about the journal
y.liu@jctres.com (Dr. Yao Liu)

For ethical/legal inquiries
y.liu@jctres.com (Dr. Yao Liu)

Assistant editor
d.deklerk@jctres.com (Mr. Daniel de Klerk)

For translation and proofreading services (English)
y.liu@jctres.com (Dr. Yao Liu)

For professional illustration services
megan.reiniers@gmail.com (Ms. Megan Reiniers)
markvart@planet.nl (Ms. LibaMarkvart)

For manuscript production inquiries
production@jctres.com

For advertisement inquiries
y.liu@jctres.com (Dr. Yao Liu)

Journal development editors
felicia@accscience.com (Ms. Felicia Wang)

Other Journal Published by AccScience Publishing

International Journal of Bioprinting is an international journal covering the technology, science and clinical application of the broadly defined field of bioprinting. Bioprinting is defined as the use of 3D printing technology with materials that incorporate viable living cells or biological elements to produce tissue or biotechnological products.

We are interested in the scientific topics spanning all stages of bioprinting process from concept creation to fabrication and beyond. Knowledge generated in these researches must be related to bioprinting.

The journal publishes original research articles on basic and applied research as well as associated social implications of this research. The journal also publishes brief commentaries and reviews. Articles focusing on the practical applications of 3D-printed products are similarly welcome.

Journal of Clinical and Translational Research 2023; 9(4): 222-235

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

REVIEW ARTICLE

Safety and efficacy of intranasal insulin in patients with Alzheimer's disease: a systematic review and meta-analysis

Yosra Hussein AboEl-Azm[1], Mohamed El-Samahy[1,2], Nada Ibrahim Hendi[2,3], Amina Arar[2,4], Noha Samy Yasen[5], Shrouk Ramadan[2,3], Esraa M Zedan[2,6], Nada Mostafa Al-dardery[2,7], Abdulrhman Khaity[2,8]*

[1]Department of Medicine, Faculty of Medicine, Zagazig University, Zagazig, 44519, Egypt, [2]Department of Medicine, Medical Research Group of Egypt, Cairo 44523, Egypt, [3]Department of Medicine, Faculty of Medicine, Ain Shams University, Cairo 11566, Egypt, [4]Department of Medicine, Faculty of Medicine, Algiers University, Algeria, 16111, Algeria, [5]Department of Radiology, Faculty of Applied Medical Sciences, Misr University for Science and Technology, Cairo, 12566, Egypt, [6]Department of Medicine, Faculty of Medicine, Al-Azhar University, Cairo, 11651, Egypt, [7]Department of Medicine, Faculty of Medicine, Fayoum University, Fayoum, 63514, Egypt, [8]Department of Medicine, Faculty of Medicine, Elrazi University, Khartoum, 11115, Sudan

ARTICLE INFO

Article history:
Received: January 18, 2023
Revised: February 28, 2023
Accepted: March 23, 2023
Published online: July 12, 2023

Keywords:
Alzheimer's disease
intranasal insulin
mild cognitive impairment

**Corresponding authors:*
Abdulrhman Khaity
Faculty of Medicine, Elrazi University, 11115 Khartoum, Sudan.
Tel: +249999255031
Email: abdulrhman.marwan.khaity@gmail.com

ABSTRACT

Background and Aim: We performed this meta-analysis to evaluate the safety and efficacy of intranasal insulin in Alzheimer's disease (AD) patients.

Methods: A literature search was conducted for PubMed, Scopus, and Web of Science from inception until August 2022. Documents were screened for qualified articles, and all concerned outcomes were pooled as risk ratios or mean difference (MD) in the meta-analysis models using Review Manager (RevMan version 5.4).

Results: Our results from 12 studies favored intranasal insulin over placebo in terms of Alzheimer Disease's Assessment Scale-cognitive subscale (ADAS-cog) 20 IU, (MD = −0.13, 95% CI [−0.22, −0.05], $P = 0.003$). The overall effect did not favor either of the two groups for ADAS-cog 40 IU, memory composite 20 IU and 40 IU, and adverse events (MD = −0.08, 95% CI [−0.16, 0.01], $P = 0.08$), (MD = 0.65, 95% CI [−0.08, 1.39], $P = 0.08$), (MD = 0.25, 95% CI [−0.09, 0.6], $P = 0.15$), and (MD = 1.28, 95% CI [0.75, 2.21], $P = 0.36$), respectively.

Conclusion: Ultimately, this meta-analysis showed that intranasal insulin in small doses (20 IU) significantly affects patients with AD. Further studies are recommended on reliable insulin delivery devices to increase insulin in the central nervous system.

Relevance for Patients: Intranasal insulin has shown promising results in treating patients with AD. The lower doses (20 IU) can play a positive role in improving the disease. As research continues, it is likely that this treatment will become more widely accepted and utilized in clinical practice.

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative illness characterized by changes in behavior and personality, cognitive impairment, and memory loss [1]. Major neuropathological features of AD are thought to be the buildup of extracellular amyloid/senile plaques made of intracellular neurofibrillary tangles and amyloid-(A). It is worth mentioning that AD-sensitive brain areas exhibit substantial abnormalities in glucose metabolism and reduced neuron use of glucose as a result of disruptions in the insulin signaling pathway [2].

Several studies recently suggested that insulin may be essential in preserving the brain's mitochondrial balance and cerebral bioenergetics [3]. In addition, it could have an impact on the clearance of the significant factors in the path mechanism of AD, including

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013

amyloid peptide and tau protein phosphorylation [3]. Low insulin levels in the central nervous system (CNS) may be caused by impaired insulin transport through the blood-brain barrier (BBB). Therefore, raising brain insulin may stop the degenerative processes associated with AD [3]. Based on that, a wide range of pharmacological substances and delivery strategies has been developed and studied.

The olfactory bulb, cerebral cortex, hippocampus, hypothalamus, cerebellum, and choroid plexus have the highest insulin receptor density [4]. Accordingly, through the roof of the nose, insulin can cross through the BBB and systemic circulation, entering the brain through the olfactory, trigeminal, and nerve fiber pathways [5].

Binding insulin with its receptor will lead to autophosphorylation of the insulin receptor and induction of insulin receptor substrate (IRS). Activation of AKT, which is one of the signaling routes that insulin activates, through IRS phosphorylation has been linked to improvements in neuronal protection, learning, and memory functions among AD patients [6].

However, several previous studies have shown conflicting findings regarding the influence of intranasal insulin on dementia in AD patients. Therefore, in this study, we aimed to fill the gap in detecting the real effect of intranasal insulin on these patients.

2. Materials and Methods

This systematic review and meta-analysis were reported following the PRISMA declaration requirements [7]. The protocol of this study was registered on the PROSPERO (CRD42022355827).

2.1. Eligibility criteria

The following conditions were considered for the study:

(i) Population: Studies on patients who have AD or mild cognitive impairment.
(ii) Intervention: Studies where the exposed group was intranasal insulin.
(iii) Comparator: Studies where the control group received a placebo.
(iv) Outcome: Studies stated one or more of the following outcomes: Alzheimer Disease's Assessment Scale-cognitive subscale (ADAS-cog) either 40 IU or 20 IU, and adverse effects (headache, fall, and rhinitis/upper respiratory infection [URI]). In addition, memory composite (delayed story recall) 40 IU and 20 IU, dementia severity rating scale (DSRS), AD cooperative study-activities of daily living (ADCS-ADL), clinical dimension rating-sum of boxes (CDR-SOB), and cerebral spinal fluid (CSF) biomarkers of AD.
(v) Study design: Studies that were designated as randomized clinical trials (RCTs).
(vi) Studies excluded: Not published in the English language, comments, review articles, case reports, observational studies, abstracts, and letters to the editor.

2.2. Search strategy

Three electronic databases (PubMed, Scopus, and Web of Science) were searched from their inception until August 2022 using the following query: (Alzheimer OR [Senile Dementia] OR [Dementia Presenile]) AND [Insulin OR Novolin OR Iletin]).

2.3. Selection process

The titles and abstracts of all citations considered for inclusion were reviewed by three authors independently. Then, we extracted the full text of the selected studies to evaluate their applicability and validated them according to our systematic review and meta-analysis standards. Discrepancies were resolved by consensus.

2.4. Data extraction

Data were extracted from an online data extraction sheet by four independent authors. The extracted data included: (1) A summary of the included studies, (2) baseline characteristics for the included population, (3) risk of bias domains, and (4) outcome measures. Any disagreements were solved by a fifth author.

2.5. Quality appraisal

We used the Cochrane assessment tool 2 (ROB2) for randomized controlled trials [8]. Using that tool, each study was assessed for the possibility of bias in the following domains: (1) Random sequence generation, (2) allocation concealment, (3) blinding of participants, personnel, and outcome assessors, (4) incomplete outcome data, (5) selective outcome data reporting, and (6) other sources of bias. The degree of bias in the authors' conclusions is classified as "low risk," "some concerns," or "high risk."

2.6. Synthesis methods

Continuous were pooled as mean difference (MD) between the two groups from baseline to the endpoint in the meta-analysis models utilizing the inverse variance (IV) method. We assumed a fixed-effect model of the MD as the main analysis model. Nevertheless, relative risk (RR) was used to pool dichotomous data in a fixed-effect model using the Mantel-Haenszel (M-H) method. RevMan software (version 5.4 for Windows) was applied to run the statistical analysis. In addition, we used the Chi-square test (Cochrane Q test) to assess the statistical heterogeneity of the included studies. Significant heterogeneity was reflected by $I^2 > 50\%$ with $P < 0.1$.

3. Results

3.1. Results of study selection and characteristics

Our literature search process retrieved 9119 records. After removing duplicates, 6391 abstracts were evaluated, and 19 articles were eligible for full-text screening. Of them, 12 studies were included in this study. Due to the heterogeneity in some included studies, we conducted a meta-analysis of seven studies. The PRISMA flow diagram of the study selection process is shown in Figure 1.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013

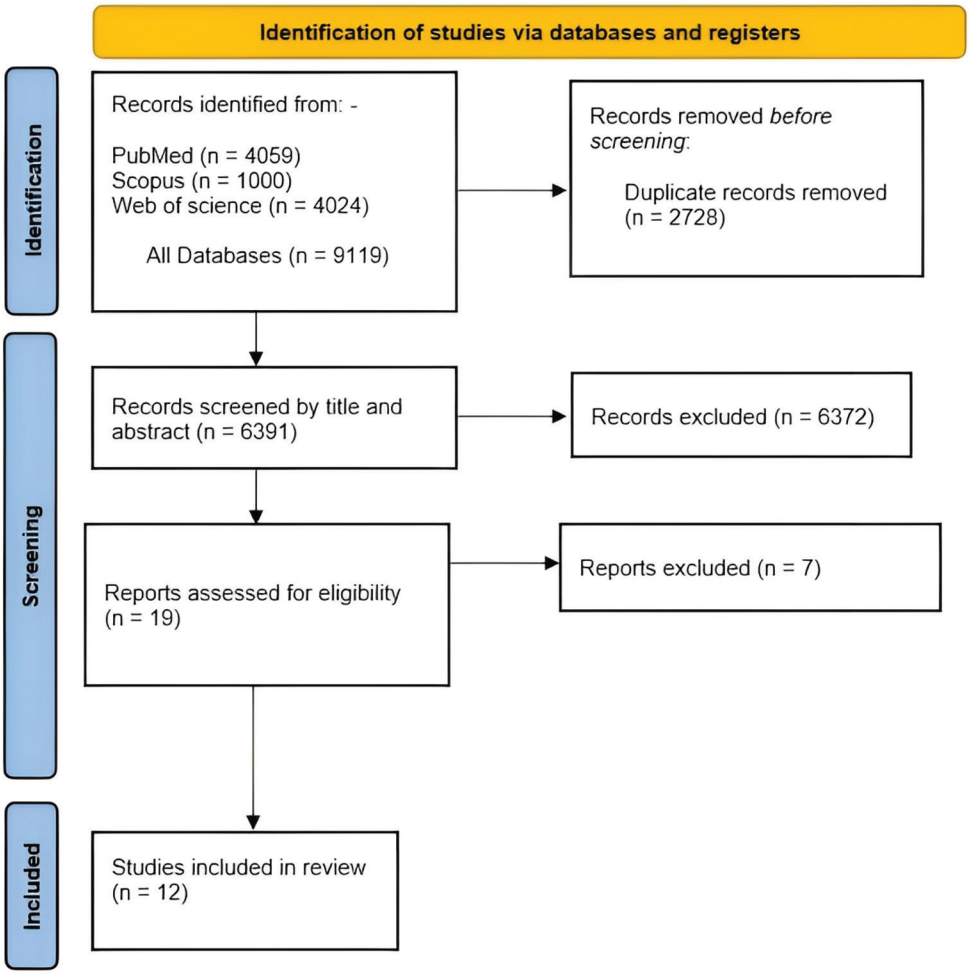


Figure 1. PRISMA flow diagram of studies' screening and selection.

The number of patients who were included in the meta-analysis was 620, including 382 who were treated with intranasal insulin and 238 who received a placebo. A summary of the eligible studies and the characteristics of their patients are presented in Tables 1 and 2.

3.2. Quality assessment

According to RoB2, we found four studies with an overall low risk of bias [9-12] and eight had some concerns. The reasons for some concerns are that four studies had some concerns in the randomization process [13-16] and four had some concerns in the selection of the reported results [17-20] (Figure 2**)**.

3.3. Outcomes

3.3.1. ADAS-cog 40 IU

Six studies were reported for ADAS-cog 40 IU with a total of 486 participants. The findings presented that there was no significant difference between the intranasal insulin and the placebo according to ADAS-cog 40 IU. The MD was −0.08 (95% CI: −0.16–0.01, $P = 0.08$). The pooled studies were homogenous ($I^2 = 0\%$, $P = 0.92$) (Figure 3A).

3.3.2. ADAS-cog 20 IU

Three studies represented ADAS-cog 20 IU with a total of 191 participants. Pooled studies favored the insulin effect over the placebo. The MD was −0.13 (95% CI: −0.22 – −0.05, $P = 0.003$). The results were homogenous ($I^2 = 0\%$, $P = 0.86$) (Figure 3B).

3.3.3. Memory composite (delayed story recall) 40 IU

We pooled the four studies that provided relevant data for memory composite 40 IU involving 400 participants. The overall effect did not favor either of the two groups in terms of memory composite 40 IU. The MD was 0.25 (95% CI: −0.09 – 0.6, $P = 0.15$). The results were homogenous ($I^2 = 0\%$, $P = 0.99$) (Figure 3C).

3.3.4. Memory composite (delayed story recall) 20 IU

Two studies reported relevant data for memory composite 20 IU involving a total of 132 participants. There was no significant difference between the intranasal insulin and the placebo. The MD was 0.65 (95% CI: −0.08 – 1.39, $P = 0.08$). The pooled studies were homogenous ($I^2 = 0\%$, $P = 0.51$) (Figure 3D).

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013

Table 1. Summary of the included studies

Study ID	Design	Country	Duration (months)	Patient's eligibility	Type of insulin	Outcomes measured	Main finding
Claxton *et al.* [9]	RCT	United States	48	• Patients with mild cognitive impairment or Alzheimer's disease	- Novolin R; Novo Nordisk, Princeton, New Jersey	- Primary: • Delayed story recall • DSRS - Secondary: • ADAS-Cog • ADCS-ADL	Intranasal insulin improves MCI and AD
Claxton *et al.* [15]	RCT	United States	24	• Patients with mild cognitive impairment or AD	- Levemir; Novo Nordisk, Princeton, New Jersey	- Primary • A verbal memory composite score - Secondary • Executive function • Visuospatial working memory • Caregiver-rated functional ability - Metabolic outcomes • Homeostatic model assessment for insulin resistance (HOMA-IR)	Intranasal insulin improves AD
Craft *et al.* [11]	RCT	United States	6	• Patients who scored between 0.5 and 1 on the Clinical Dementia Rating • Who >15 on the Mini-Mental State Examination	- Novolin R; Novo Nordisk, Princeton, New Jersey	Primary: • Delayed story recall • DSRS	Intranasal insulin improves MCI and AD
Craft *et al.* [14]	RCT	United States	4	• Patients who were probably AD • Who >15 on the Mini-Mental State Examination	- Humulin R U-100, Eli Lilly and Co. - Levemir®; Novo Nordisk, Princeton, New Jersey	Primary: • Delayed story recall Secondary: • ADAS-Cog • DSRS • MRI volume changes in AD-related regions of interest • Cerebrospinal fluid AD markers	Intranasal insulin improves AD
Craft *et al.* [10]	RCT	United States	48	• Adults between the ages of 55 and 85 • Patients with mild cognitive impairment or AD • MMSE scores of 20 or higher • Global clinical dementia ratings of 0.5 or 1.0 • Logical memory-delayed scores falling within a predetermined education-adjusted range.	- Humulin-RU-100; Lilly	Primary: • ADAS-Cog Secondary: • ADCS-ADL • CDR-SB • Immediate and delayed story recall.	Intranasal insulin does not improve MCI and AD

(Cont'd...)

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013

Table 1. *(Continued)*

Study ID	Design	Country	Duration (months)	Patient's eligibility	Type of insulin	Outcomes measured	Main finding
Rosenbloom *et al.* [16]	Randomized, cross-over	United States	6	• Mild-moderate AD • Who are >65 years old and 85 years old. • The Clinical Dementia Rating (CDR) for each individual ranged from 1 to 2 • Mini-Mental State Examination (MMSE) scores ranged from 18 to 26	- Rapid-acting insulin	• Peripheral glucose levels • Verbal memory, safety, and efficacy • Serum insulin levels acutely	Intranasal insulin does not improve MCI and AD
Rosenbloom *et al.* [12]	RCT	United States	6	• Patients with mild cognitive impairment or AD • Montreal Cognitive Assessment (MoCA) scores of 18–27	NR	Primary: • ADAS-Cog • CDR-SB Secondary: • (COWAT) • (WMS)-IV • Blood glucose and insulin level • Adverse effects (AEs) and severe adverse effects (SAEs)	Intranasal insulin does not improve MCI and AD
Mustapic *et al.* [13]	RCT	United States	4	• Patients aged 55 or greater • Patients with mild cognitive impairment or AD • Who is on stable doses of memantine (Namenda) or cholinesterase inhibit	NR	• ADAS-Cog • Mini-Mental State Examination • AD Assessment Scale for cognition and insulin signaling mediators as biomarkers, especially EV biomarkers of insulin resistance as (pS312-IRS-1 and pY-IRS-1)	Intranasal insulin improves MCI and AD
Kellar *et al.* [20]	RCT	United States	18	• Adults between the ages of 55 and 85 • Patients with mild cognitive impairment or AD • MMSE scores of 20 or higher • Global clinical dementia ratings of 0.5 or 1.0 • Logical memory-delayed scores falling within a predetermined education-adjusted range	- Humulin-R U100	• CSF macrophage-derived chemokine • CSF interferon-γ, CSF immune/inflammatory/vascular markers • Changes in cognition, brain volume, and amyloid and tau concentrations • CSF markers of inflammation, immune function, and vascular integrity and assessed their relationship with changes in cognition, brain volume, and CSF amyloid and tau concentrations, reduced interleukin-6, cerebral spinal fluid (CSF) biomarker profiles and slower symptom progression	Intranasal insulin improves MCI and AD
Reger *et al.* [18]	RCT	United States	NR	• Patients with mild cognitive impairment or AD	- Novolin R, Novo Nordisk, Princeton, NJ, USA	• Verbal memory • Verbal Memory • Plasma β-Amyloid • Plasma insulin and glucose levels	Intranasal insulin improves MCI and AD
Reger *et al.* [17]	RCT	United States	NR	• Patients with mild cognitive impairment or AD	- Novolin R, Novo Nordisk	Primary: • Intended to be verbal memory after a delay • Attention • Caregiver assessments of functional state Secondary: • Plasma levels of insulin, glucose, beta-amyloid, and cortisol	Intranasal insulin improves MCI and AD

(Cont'd...)

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013

Table 1. *(Continued)*

Study ID	Design	Country	Duration (months)	Patient's eligibility	Type of insulin	Outcomes measured	Main finding
Reger *et al.* [19]	RCT	United States	NR	• There were no neurological disorders (other than AD)	- Novolin R containing cresol, Novo Nordisk, Princeton, NJ, USA	Primary: • Cognitive data, such as (verbal memory for story recall) Secondary: • Plasma insulin • Blood glucose levels • Attention, • Working memory • Negative effects such as (nosebleed – nose soreness)	Intranasal insulin improves AD

Abbreviations: Apo e4: Apolipoprotein E4; DSRS: Dementia Severity Rating Scale; ADAS-cog: Alzheimer Disease's Assessment Scale-cognitive subscale; ADCS-ADL: Alzheimer's Disease Cooperative Study–activities of daily living; CDR-SOB: Clinical Dimension Rating–Sum of Boxes; MMSE: Mini-Mental State Examination; NR: Not reported; AD: Alzheimer's disease; MCI: Mild cognitive impairment; CSF: Cerebral spinal fluid; COWAT: Controlled Oral Word Association Test; WMS-IV: Wechsler Memory Scale

3.3.5. Memory composite (delayed story recall) long-acting

Two studies provided adequate data for memory composite long-acting involving 63 participants. We found no significant difference between the intranasal insulin and the placebo. The MD was 0.58 (95% CI: −0.04 – 1.19, $P = 0.07$). The results were homogenous ($I^2 = 0\%$, $P = 0.71$) (Figure 3E).

3.3.6. DSRS 40 IU

Three studies reported relevant data for DSRS 40 IU with a total of 160 participants. The overall effect did not favor either of the two groups in terms of DSRS 40 IU. The MD was −0.15 (95% CI: −0.88 – 0.57, $P = 0.68$). The findings of the studies were homogenous ($I^2 = 0\%$, $P = 0.6$) (Figure 3F).

3.3.7. DSRS 20 IU

Regarding DSRS 20 IU, we identified two relevant studies with a total of 132 participants. There was no significant difference between the two groups. The MD was −0.11 (95% CI: −0.82 – 0.6, $P = 0.76$). The pooled articles were homogenous ($I^2 = 0\%$, $P = 0.59$) (Figure 4A).

3.3.8. DSRS-LA

Two studies provided relevant data for DSRS-LA with a total of 63 participants. The overall effect did not favor either of the two groups in terms of DSRS-LA. The MD was 0.16 (95% CI: −3.98 – 4.29, $P = 0.94$). The results were homogenous ($I^2 = 0\%$, $P = 0.85$) (Figure 4B)

3.3.9. ADCS-ADL 40 IU

We identified three studies that reported relevant data for ADCS-ADL 40 IU involving a total of 376 participants. The overall effect did not favor either of the two groups in terms of ADCS-ADL 40 IU. The MD was 0.04 (95% CI: −0.07 – 0.15, $P = 0.49$). The pooled studies were homogenous ($I^2 = 0\%$, $P = 0.58$) (Figure 4C).

3.3.10. ADCS-ADL 20 IU

Two studies provided adequate data for ADCS-ADL 20 IU with a total of 132 participants. The overall effect did not favor either of the two groups in terms of ADCS-ADL 20 IU. The MD was 0.02 (95% CI: −0.09 – 0.13, $P = 0.72$). The results were homogenous ($I^2 = 0\%$, $P = 0.59$) (Figure 4D).

3.3.11. Clinical Dementia Rating-Sum of Boxes score

Two studies provided relevant data for clinical dimension rating – the sum of boxes involving 268 participants. We did not find a significant difference between the two groups. The MD was 0.36 (95% CI: −0.19 – 0.92, $P = 0.2$). The results were homogenous ($I^2 = 0\%$, $P = 0.54$) (Figure 4E).

3.3.12. CSF biomarkers of AD

We found a non-significance difference between the intranasal insulin and the placebo in the case of CSF biomarkers of AD. The MD was −3.23 (95% CI: −9.9 – 3.44, $P = 0.34$). In addition, the overall effect did not favor either of the two groups in terms of Abeta42, Tau, and Tau-P. More information is given in Figure 5.

3.3.13. Adverse effects

We categorized data into three subgroups (Headache, Rhinitis/URI, and Fall) involving 1001 participants. The findings of overall adverse events and the subgroups revealed no significant difference between the two groups. The RR of overall adverse events was 1.28 (95% CI: 0.75 – 2.21, $P = 0.36$). All details are in Figure 6.

4. Discussion

The introduction of effective medicine for several CNS-related disorders, including AD, by nose-to-brain drug administration, has been considered a revolutionary process [21]. Intranasal insulin is one of these treatments that have shown a beneficial impact on AD patients [11]. In the present study, 12 RCTs were included in the study. All the included studies retrieved from our literature search compared intranasal insulin with placebo in terms of safety and efficacy. The duration of treatment in the included studies ranged from 4 months to 4 years. The findings of our meta-analysis revealed that intranasal insulin might be significant in improving cognition in Alzheimer's disease patients measured by ADAS-cog with lower doses being more effective. There was

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013

Table 2. Baseline characteristics for the population of the included studies

Study ID	Groups	No of participants	Age, mean (SD)	Sex (m/f)	BMI, mean (SD)	Education, mean (SD)	Apo e4 status, N (%)	Diagnosis MCI/AD	DSRS, mean (SD)	ADAS-cog, mean (SD)	ADCS-AD, mean (SD)	Delayed story recall score, mean (SD)	MMSE, mean (SD)	CDR-SOB, mean (SD)
Craft *et al.* [11]	Placebo	30	74.9 (8.7)	17/13	27.4 (4.3)	15.3 (3.2)	13 (44.8)	21/9	1.64 (1.04)	1.93 (0.76)	3.75 (0.16)	2.25 (1.04)	NR	NR
	20 IU of Insulin	36	72.8 (9)	22/14	26.7 (4.8)	15.5 (3.0)	18 (50)	20/16	1.72 (0.96)	2.21 (0.72)	3.79 (0.18)	1.86 (1.02)	NR	NR
	40 IU of Insulin	38	69.9 (8.6)	20/18	26.9 (4.3)	16.2 (3.03)	16 (42.1)	23/15	1.78 (1.04)	2.26 (0.73)	3.77 (0.18)	1.99 (1.04)	NR	NR
Craft *et al.* [14]	Placebo	12	68.4 (8.9)	6|6	26.7 (3.3)	16.5 (2.0)	8 (66.66)	8/4	7.3 (6.9)	20 (11.7)	NR	NR	24.8 (4.2)	NR
	Regular insulin	12	70.5 (9.1)	7|5	28.8 (6.1)	15.6 (2.8)	8 (66.66)	7/5	7.7 (6.8)	19.8 (12.8)	NR	NR	26 (3.7)	NR
	detemir	12	67.3 (7.8)	6|6	29.4 (6.6)	14.8 (2.4)	6 (50)	7/5	8.7 (6.7)	21.6 (13.7)	NR	NR	25.2 (4.1)	NR
Craft *et al.* [10]	Placebo(blind)	119	71.1 (6.8)	61/58	NR	16.3 (2.9)	77 (64.7)	46/73	NR	24.73 (7.56)	NR	NR	24.84 (2.72)	3.35 (1.51)
	Placebo (open-label)	104	71 (7.0)	53/51	NR	16.4 (2.8)	65 (62.5)	41/63	NR	24.07 (7.3)	NR	NR	24.93 (2.73)	3.31 (1.53)
	Insulin (blind)	121	70.5 (7.4)	62/59	NR	16.1 (2.6)	79 (65.3)	41/80	NR	25.91 (8.28)	NR	NR	24.79 (2.75)	3.59 (1.51)
	Insulin (open-label)	106	70.3 (7.3)	57/49	NR	16.1 (2.7)	72 (67.9)	35/71	NR	25.34 (8.25)	NR	NR	24.95 (2.7)	3.56 (1.45)
Claxton *et al.* [9]	Placebo	30	74.84 (10.09)	17/13	27.39 (4.3)	15.26 (3.23)	13 (43.3)	64/40	7.17 (5.59)	2.05 (0.97)	43.7(6.8)	11.27 (7.6)	NR	NR
	20 IU of Insulin	36	72.8 (7.5)	22/14	26.7 (4.1)	15.5 (3.5)	18 (50)	64/40	7.16 (4.28)	2.21 (0.63)	44.17 (5.38)	9.3 (8.36)	NR	NR
	40 IU of Insulin	18	69.8 (9.12)	20/18	26.8 (4.9)	16.2 (2.8)	16 (42.1)	64/40	7.57 (5.36)	2.22 (0.65)	43.5 (6.61)	10.55 (8.23)	NR	NR
Claxton *et al.* [15]	Placebo	20	NR	NR	NR	NR	NR	39/21	NR	NR	NR	NR	NR	NR
	20 IU of Insulin	21	NR	NR	NR	NR	NR	39/21	NR	NR	NR	NR	NR	NR
	40 IU of Insulin	19	NR	NR	NR	NR	NR	39/21	NR	NR	NR	NR	NR	NR
Rosenbloom *et al.* [16]		12	72	9/3	NR	NR	NR		NR	NR	NR	NR	NR	NR
Rosenbloom *et al.* [12]	Insulin	19	68.4 (8.1)	10|9	25 (4.4)	NR	NR	14/21	NR	23.2 (5.4)	NR	NR	NR	NR
	Saline	16	74.4 (6.4)	10|6	24.5 (3.6)	NR	NR	9/10	NR	23.3 (6.1)	NR	NR	NR	NR
Mustapic *et al.* [13]	Placebo	26	76.2 (9.06)	11 |15	NR	NR	23 (50)	69/31	NR	1.93 (0.13)	NR	NR	85 (13.56)	NR
	20 IU Insulin	33	70.9 (6.83)	12/21	NR	NR	15 (45)	55/45	NR	2.21 (0.12)	NR	NR	83.64 (14.15)	NR
	40 IU Insulin	32	69.6 (9.13)	12 |20	NR	NR	14 (44)	63/38	NR	2.2 (0.12)	NR	NR	84.31 (15.44)	NR

(Cont'd...)

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013

Table 2. *(Continued)*

Study ID	Groups	No of participants	Age, mean (SD)	Sex (m/f)	BMI, mean (SD)	Education, mean (SD)	Apo e4 status, N (%)	Diagnosis MCI/AD	DSRS, mean (SD)	ADAS-cog, mean (SD)	ADCS-AD, mean (SD)	Delayed story recall score, mean (SD)	MMSE, mean (SD)	CDR-SOB, mean (SD)
Kellar et al, [20]	Placebo	20	71.58 (7.7)	13|7	NR	17.2 (2.48)	15 (75)	5/15	NR	24.13 (6.46)	43.25 (7.89)	NR	NR	2.69 (1.42)
	Insulin	18	69.94 (6.12)	10|8	NR	16.05 (2.87)	14 (78)	8/10	NR	25.11 (9.17)	38.89 (7.36)	NR	NR	3.25 (1.72)
Reger *et al.* [18]	Normal E4−	48	73.8 (1)		26.1 (0.6)	14.6 (0.4)	48 (52)	20/13	NR	NR	NR	NR	NR	NR
	Normal E4+	11	72.5 (2)		25.6 (1.2)	15.2 (0.8)	11 (12)	20/13	NR	NR	NR	NR	NR	NR
	Memory-impaired E4−	11	76.3 (2)		26.4 (1.2)	14.5 (0.8)	11 (12)	20/13	NR	NR	NR	NR	NR	NR
	Memory-impaired E4+	22	76.8 (1.4)		26.5 (0.8)	14.7 (0.6)	22 (24)	20/13	NR	NR	NR	NR	NR	NR
Reger *et al.* [19]	Normal E4−	27	75.4 (6.4)	12|15	25.6 (3)	15.3 (2.1)	NR	NR	NR	NR	NR	NR	NR	NR
	Normal E4+	8	73 (5.2)	3|5	25.9 (4.3)	15.4 (2.3)	NR	NR	NR	NR	NR	NR	NR	NR
	MCI E4−	8	76.8 (5.4)	4|4	24 (3.8)	14 (3.2)	NR	13/13	NR	NR	NR	NR	NR	NR
	MCI E4+	5	76.6 (3.7)	4|1	24.3 (2.3)	13.8 (3.6)	NR	13/13	NR	NR	NR	NR	NR	NR
	AD E4−	6	76.7 (7.4)	2|4	25.2 (1.7)	13.3 (4.0)	NR	13/13	NR	NR	NR	NR	NR	NR
	AD E4+	7	76.6 (5.3)	3|4	25.4 (2.8)	15.7 (2.1)	NR	13/13	NR	NR	NR	NR	NR	NR
Reger *et al.* [18]	Placebo	12	79.3 (1.7)		26 (1.3)	15.5 (0.9)	NR	14/11	NR	NR	NR	NR	NR	NR
	Insulin	13	77.1 (1.6)		26.9 (1.2)	14.9 (0.8)		14/11	NR	NR	NR	NR	NR	NR

Abbreviations: Apo e4: Apolipoprotein E4; DSRS: Dementia Severity Rating Scale; ADAS-cog: Alzheimer Disease's Assessment Scale-cognitive subscale; ADCS-ADL: Alzheimer's Disease Cooperative Study–activities of daily living; CDR-SOB: Clinical Dimension Rating–Sum of Boxes; MMSE: Mini-Mental State Examination; NR: Not reported; AD: Alzheimer's disease; MCI: Mild cognitive impairment

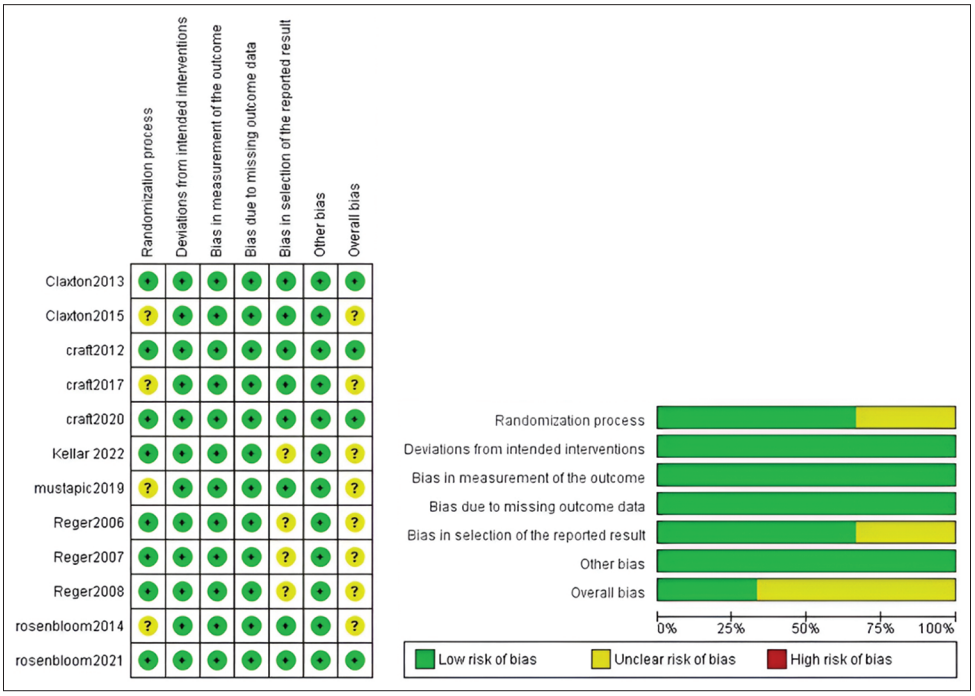


Figure 2. The risk of bias summary and risk of bias graph according to the Cochrane risk of bias assessment tool.

no difference in the incidence of adverse effects between both arms which suggests that short-term intranasal insulin could be safe in treating Alzheimer's disease patients. In addition, CSF biomarkers, clinical dementia rating score, dementia severity rating scale, and memory composite showed no significance in either dose of insulin compared to the placebo.

In general, ADAS-Cog is a reliable assessment tool for Alzheimer's disease. It contains items regarding language, memory, praxis, and orientation with higher scores representing greater impairment. These items are beneficial in not only diagnosing Alzheimer's disease patients from healthy people but also helping in determining the severity of the disease through the items in the orientation section [22]. Furthermore, we need to differentiate between MCI and dementia. MCI is a period between dementia and normal cognitive impairment associated with getting older. There are amnestic and non-amnestic types of it [23]. Dementia is a generic term for a deterioration in cognitive functions that makes it difficult to carry out daily tasks [24]. Once the patient is in the MCI stage, it is regarded as a major risk of being a dementia patient [23].

Our meta-analysis showed significantly less decline in ADAS-cog score from the baseline in the insulin 20 IU group when compared to the placebo. This is consistent with individual study results which showed that the insulin group had less decline in cognitive function over time when compared to the placebo [9,11,13].

The results did not reach a significant cutoff point when we compared insulin 40 IU and placebo regarding ADAS-cog scores. The findings of the individual studies involved in the analysis varied. Some studies supported our results that 40 IU insulin loses its effect on cognition. A possible explanation of this finding could be attributed to the small sample size, the short duration of the trials which make it difficult to detect significant differences, and the use of unreliable devices or insulin formulations that are not proven to be effective on memory [12,25,26]. Claxton 2013 [9] and Craft 2017 [14] showed a possible correlation between ApoE4 and the treatment response specific to the 40 IU insulin. In addition, Claxton 2013 [9] demonstrated a gender/ApoE4 interaction with a better improvement of cognitive function in ApoE4 negative males and more decline in ApoE4 negative females [9,14]. Given our p-value (0.08) and our confidence interval, most of it was in the direction of favoring 40 IU insulin. Further studies with larger sample sizes and longer durations are needed to confirm this association and the efficacy of using the 40 IU insulin in the treatment of Alzheimer's disease patients.

On the contrary, some studies showed that ADAS-cog score is significantly improved with the administration of 40 IU insulin compared to placebo. Craft 2012 [11] showed a significant difference in ADAS-cog score between 40 IU insulin and placebo [11]. Reger *et al*. demonstrated that the effect of insulin on cognition is dose-dependent and the curve shows a U-shaped pattern, meaning that enhanced cognition can be achieved by optimal dose while the extremes of doses will have less effect. The 40 IU might have exceeded the optimal dose of memory but not for other items of the ADAS-cog score and this explains the significant difference between the placebo in the ADAS-cog score and not in delayed story recall [18]. Another study showed that the insulin signaling pathway is better activated in smaller doses compared to higher doses which can cause insulin resistance and worsen the condition of already existing memory impairment [27]. Claxton's 2013 results support this theory as they showed similar results regarding delayed story recall and ADAS-cog score [9].

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013

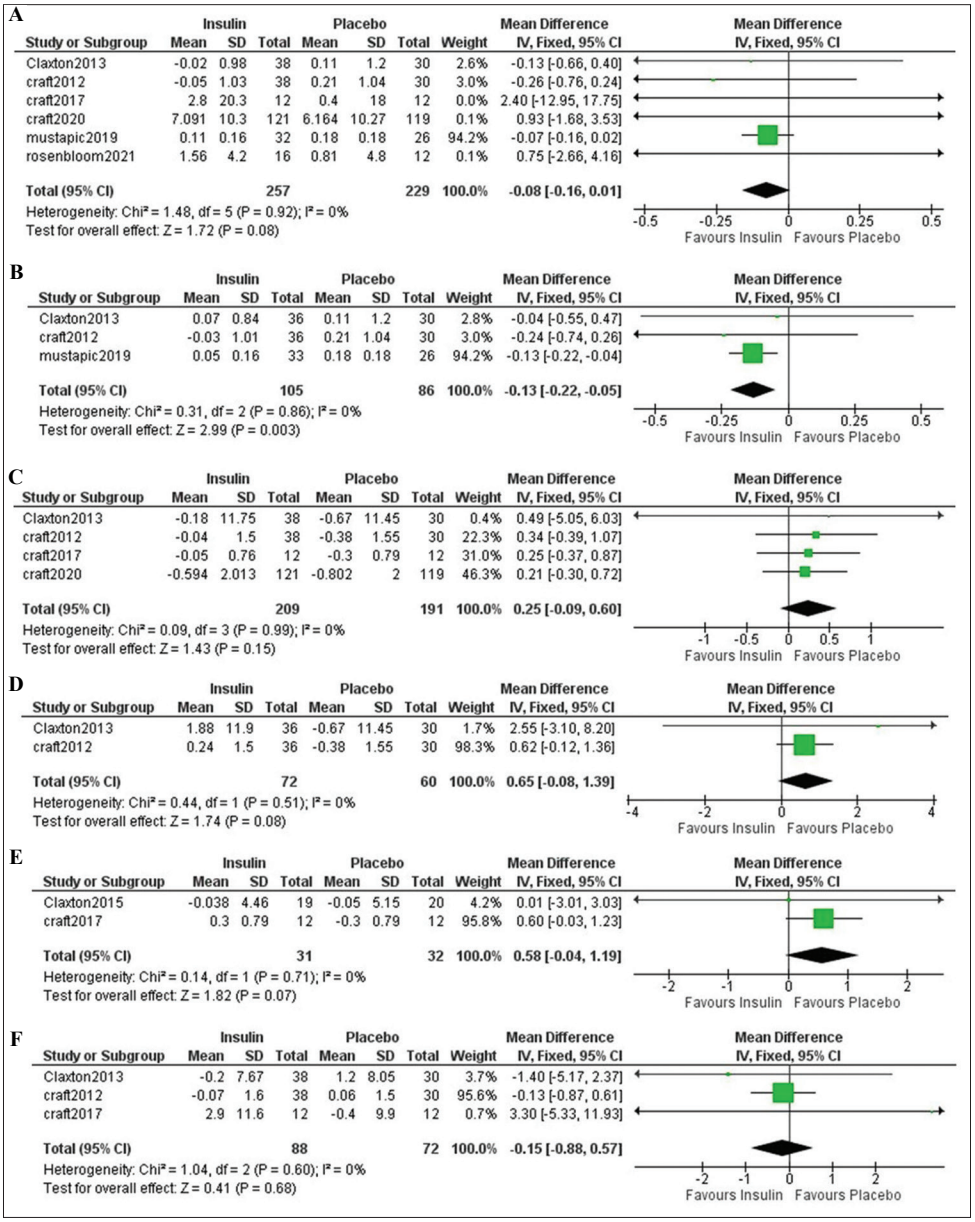


Figure 3. Forest plots of mean difference in (A) ADAS-cog 40 IU, (B) ADAS-cog 20 IU, (C) memory composite 40 IU, (D) memory composite 20 IU, (E) memory composite long-acting, and (F) DSRS 40 IU. Abbreviations: ADAS-cog: Alzheimer Disease's Assessment Scale-cognitive subscale; DSRS: Dementia Severity Rating Scale.

We found that there was a non-significant difference between 20 IU insulin and placebo regarding delayed story recall. Craft 2012 reported a significantly better story recall compared to a placebo [11]. This could be attributed to the imprecision and wide confidence interval observed in Claxton 2013 [9]. When we compared 40 IU insulin with a placebo, there was no significant difference in story recall between both groups. This is consistent with the results of individual studies and could be explained by the U-shaped dose-dependent theory that was mentioned above. This means that 40 IU insulin might have exceeded the optimal dose for memory composite.

The ADCS-ADL is a scale used to measure the capability of AD patients to perform daily activities with higher scores indicating better preservation of functional capacity [9]. Our results showed that there is no difference in ADCS-ADL between both insulin groups and the placebo. This is consistent with the findings of two of the included studies which reported this outcome. However, Claxton 2013 showed a difference in ADCS-ADL between males and females in favor of females [9]. Moreover, Craft 2012 showed that there is a significant difference between the insulin and placebo group for Alzheimer's disease but not for amnestic mild cognitive impairment (aMCI) [11].

The DSRS is a similar scale to ADCS-ADL determined by a questionnaire that contains questions about the cognitive, functional, and social status of the patient. Higher scores indicate greater impairment. Our results found no significant difference

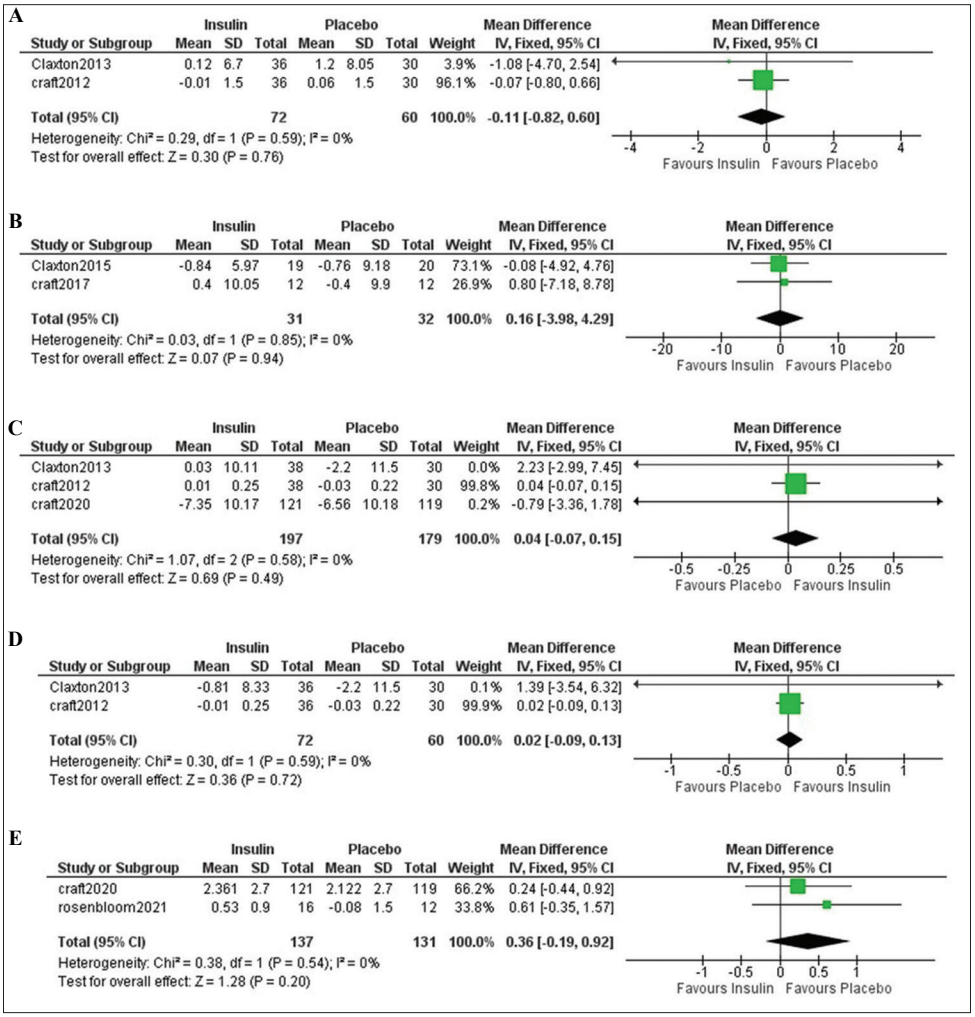


Figure 4. Forest plots of mean difference in (A) DSRS 20 IU, (B) DSRS-LA, (C) ADCS-ADL 40 IU, (D) ADCS-ADL 20 IU, and (E) Clinical Dementia Rating-Sum of Boxes score. Abbreviations: DSRS: Dementia Severity Rating Scale; ADCS-ADL: Alzheimer's disease Cooperative Study-activities of daily living.

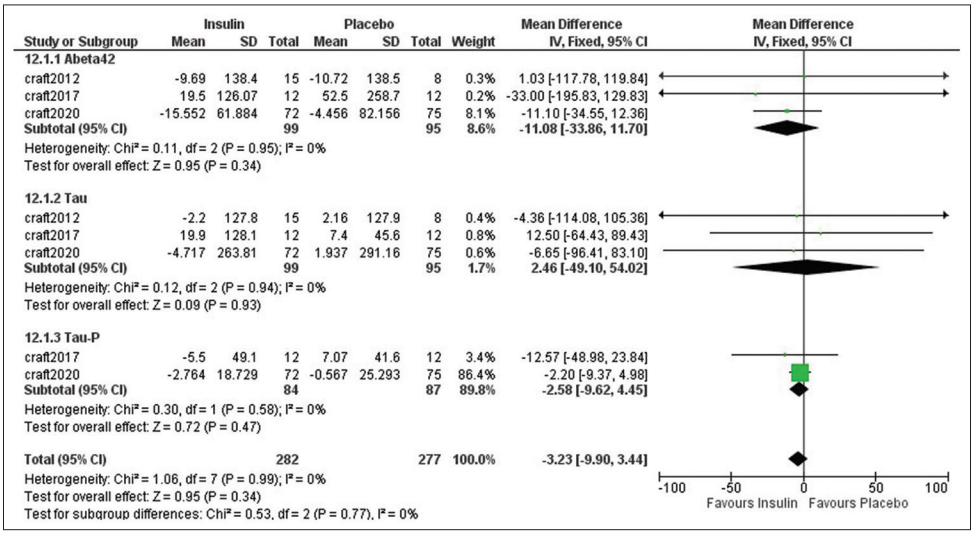


Figure 5. Forest plots of mean difference in CSF Biomarkers of AD. Abbreviations: CSF: Cerebral Spinal Fluid; AD: Alzheimer's disease.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013

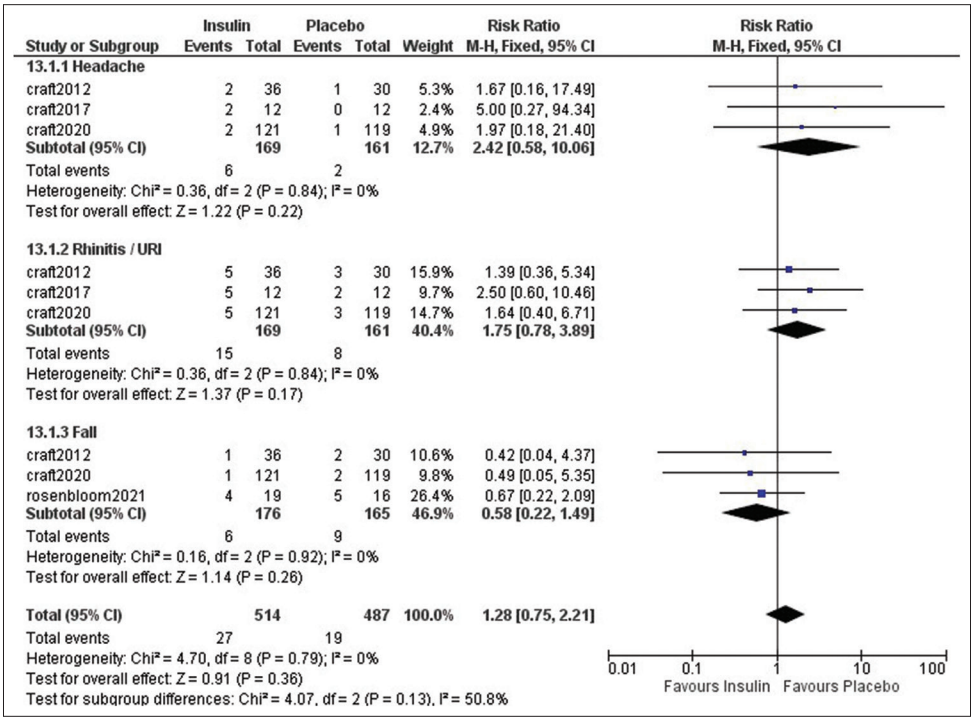


Figure 6. Forest plots of risk ration in adverse events (Headache, Rhinitis/URI, and Fall). Abbreviations: URI: Upper respiratory infection.

between the low dose of insulin and the placebo group. Craft 2012 reported similar results at 6 months of evaluation [11]. However, significant improvement in DSRS at 2 months was observed. Moreover, Claxton 2013 endpoint at 4 months revealed marginal significance in DSRS between placebo and 20 IU insulin [9]. This could be due to a time-dependent relation which suggests that 20 IU insulin might be beneficial in the short-term and relatively loses its benefits onward. Regarding 40 IU or long-acting insulin, our results as well as the results of the individual studies showed no difference with the placebo irrespective of the time of assessment.

In the context of the main pathophysiological changes in AD patients, Beta peptides, Tau protein, and Tau-p protein are known to play the main role in AD pathology. Insulin was thought to protect against amyloid-beta peptides and reduce tau phosphorylation [28-30]. However, the limited insulin transport across the BBB reduces this protective effect [31]. The intranasal administration of insulin was a new route to bypass the BBB as insulin travels along perivascular pathways following olfactory and trigeminal nerves [32].

In our study, we assessed the effect of intranasal insulin administration on the levels of the three biomarkers A beta, tau, and tau-p in the CSF. We found no significant difference in any of the three biomarkers between insulin and placebo. Although this is consistent with the individual study results, exploratory analysis of one study showed that increased levels of amyloid-beta concentration and decreased tau protein–to–AB 42 ratio were associated with improved delayed story recall and daily function. This association was only found in the insulin group. Thus, such results could not be attributed to disease progression status. Moreover, selection bias could have happened since not all participants underwent lumbar punctures [11]. However, additional studies with larger sample sizes are needed to further examine the effect of intranasal insulin on Alzheimer's disease pathology.

In the assessment of intranasal insulin safety, our meta-analysis showed no significant difference in the incidence of complications in the insulin group compared to the placebo group. The included studies reported no serious adverse effects, and the complications were limited to minor complications such as upper respiratory symptoms and rhinitis. Apart from a higher rate of nasal irritation reported in Rosenbloom and a higher total number of minor adverse events reported in Craft 2012, the overall results showed no significant difference in the incidence of complications between insulin and placebo groups [11,12]. Moreover, these studies reported good compliance which was not different between the two arms [11,12,14]. Thus, weighing the risk-benefit ratio of this treatment, intranasal insulin could be a safe therapy for Alzheimer's disease patients. Rosenbloom included non-insulin-dependent diabetic patients who reported that they well tolerated the treatment with no major adverse effects or hypoglycemia. However, these studies were done over a short duration, making their long-term safety and efficacy inconclusive. Thus, larger sample sizes and longer-duration clinical trials are needed to assess the long-term benefits of intranasal insulin and the correlation between patients' characteristics and their response to treatment.

In the latest network meta-analysis [33], the efficacy of six different antidiabetic drugs was evaluated, including intranasal insulin 40 IU and 20 IU. No discernable difference was found when assessing the acceptability of the agents (defined by all-

cause discontinuation). In addition, Cognitive assessment using ADAS-Cog showed no significant improvement in either dose (20 IU or 40 IU) compared with the placebo. Nonetheless, our study found that intranasal insulin delivered at 20 IU improved the ADAS-Cog, but not at 40 IU. This cognitive change in response to low-dosage intranasal insulin was related to neuronal extracellular vesicles (EV) biomarkers of insulin resistance (pS312-IRS-1, pY-IRS-1), suggesting activation of the insulin signaling cascade at the IRS-1 level.

4.1. Limitations and strengths of the study

The major limitation of this study included: The inability to perform a meta-analysis of five of the included studies due to several variations between these articles, such as reporting different outcomes utilizing various scores and some discrepancies in the duration of intervention. Future research is warranted to explore the efficacy of intranasal insulin in a larger sample with longer follow-ups, taking into consideration the apoE4 status and the progressive neurodegeneration that occurs over many years and needed longer duration studies.

Nevertheless, the strengths of our study are as follows: (1) Our meta-analysis represented the last updated evidence assessing the efficacy and safety of intranasal insulin in patients with AD, (2) we provided a more comprehensive analysis in an attempt to solve the previous conflicting findings, and (3) we complied the PRISMA checklist when representing this manuscript and conducted all steps as stated in the Cochrane Handbook in our review.

5. Conclusion

Ultimately, the current results of intranasal insulin are encouraging in terms of safety and efficacy. Our findings demonstrate that the administration of lower doses (20 IU) has distinctly more efficacy than higher doses (40 IU) as revealed by the ADAS-cog scale. To learn about the variations (sex, age, and ApoE4 carriage) in treatment responses and make the most of this intervention, further trials are required. In addition, a future investigation should require reliable insulin delivery devices with proven capacity to increase insulin in the CNS.

Acknowledgments

None.

Funding

None.

Conflicts of Interest

All authors have no conflicts of interest.

References

[1] Michailidis M, Tata DA, Moraitou D, Kavvadas D, Karachrysafi S, Papamitsou T, *et al.* Antidiabetic Drugs in the Treatment of Alzheimer's Disease. Int J Mol Sci 2022;23:4641.

[2] Kazkayasi I, Telli G, Nemutlu E, Uma S. Intranasal Metformin Treatment Ameliorates Cognitive Functions Via Insulin Signaling Pathway in ICV-STZ-induced Mice Model of Alzheimer's Disease. Life Sci 2022;299:120538.

[3] Akel H, Csóka I, Ambrus R, Bocsik A, Gróf I, Mészáros M, *et al. In Vitro* Comparative Study of Solid Lipid and PLGA Nanoparticles Designed to Facilitate Nose-to-brain Delivery of Insulin. Int J Mol Sci 2021;22:13258.

[4] Ghasemi R, Haeri A, Dargahi L, Mohamed Z, Ahmadiani A. Insulin in the Brain: Sources, Localization and Functions. Mol Neurobiol 2013;47:145-71.

[5] Maher MA, Kandeel WA, Hammam OA, Attia YM, Mahmoud S, Salah M. Histopathological Evaluation of Insulin-DMSO Formula Designed for Direct Nose-to-brain Delivery. Histol Histopathol 2021;37:431-9.

[6] Bazrgar M, Khodabakhsh P, Dargahi L, Mohagheghi F, Ahmadiani A. MicroRNA Modulation is a Potential Molecular Mechanism for Neuroprotective Effects of Intranasal Insulin Administration in Amyloid βeta Oligomer Induced Alzheimer's Like Rat Model. Exp Gerontol 2022;164:111812.

[7] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al.* The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews. BMJ 2021;372:n71.

[8] Sterne JA, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, *et al.* RoB 2: A Revised Tool for Assessing Risk of Bias in Randomised Trials. BMJ 2019;366:l4898.

[9] Claxton A, Baker LD, Wilkinson CW, Trittschuh EH, Chapman D, Watson GS, *et al.* Sex and ApoE Genotype Differences in Treatment Response to Two Doses of Intranasal Insulin in Adults with Mild Cognitive Impairment or Alzheimer's Disease. J Alzheimers Dis 2013;35:789-97.

[10] Craft S, Raman R, Chow TW, Rafii MS, Sun CK, Rissman RA, *et al.* Safety, Efficacy, and Feasibility of Intranasal Insulin for the Treatment of Mild Cognitive Impairment and Alzheimer Disease Dementia: A Randomized Clinical Trial. JAMA Neurol 2020;77:1099-109.

[11] Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, *et al.* Intranasal Insulin Therapy for Alzheimer Disease and Amnestic Mild Cognitive Impairment: A Pilot Clinical Trial. Arch Neurol 2012;69:29-38.

[12] Rosenbloom M, Barclay TR, Kashyap B, Hage L, O'Keefe LR, Svitak A, *et al.* A Phase II, Single-center, Randomized, Double-blind, Placebo-controlled Study of the Safety and Therapeutic Efficacy of Intranasal Glulisine in Amnestic Mild Cognitive Impairment and Probable Mild Alzheimer's Disease. Drugs Aging 2021;38:407-15.

[13] Mustapic M, Tran J, Craft S, Kapogiannis D. Extracellular Vesicle Biomarkers Track Cognitive Changes Following Intranasal Insulin in Alzheimer's Disease. J Alzheimers Dis 2019;69:489-98.

[14] Craft S, Claxton A, Baker LD, Hanson AJ, Cholerton B,

Trittschuh EH, *et al.* Effects of Regular and Long-acting Insulin on Cognition and Alzheimer's Disease Biomarkers: A Pilot Clinical Trial. J Alzheimers Dis 2017;57:1325-34.

[15] Claxton A, Baker LD, Hanson A, Trittschuh EH, Cholerton B, Morgan A, *et al.* Long-Acting Intranasal Insulin Detemir Improves Cognition for Adults with Mild Cognitive Impairment or Early-stage Alzheimer's Disease Dementia. J Alzheimers Dis 2015;44:897-906.

[16] Rosenbloom MH, Barclay TR, Pyle M, Owens BL, Cagan AB, Anderson CP, *et al.* A Single-dose Pilot Trial of Intranasal Rapid-acting Insulin in Apolipoprotein E4 Carriers with Mild-moderate Alzheimer's Disease. CNS Drugs 2014;28:1185-9.

[17] Reger MA, Watson GS, Green PS, Wilkinson CW, Baker LD, Cholerton B, *et al.* Intranasal Insulin Improves Cognition and Modulates Beta-amyloid in Early AD. Neurology 2008;70:440-8.

[18] Reger MA, Watson GS, Green PS, Baker LD, Cholerton B, Fishel MA, *et al.* Intranasal Insulin Administration Dose-dependently Modulates Verbal Memory and Plasma Amyloid-beta in Memory-impaired Older Adults. J Alzheimers Dis 2008;13:323-31.

[19] Reger MA, Watson GS, Frey WH 2nd, Baker LD, Cholerton B, Keeling ML, *et al.* Effects of Intranasal Insulin on Cognition in Memory-impaired Older Adults: Modulation by APOE Genotype. Neurobiol Aging 2006;27:451-8.

[20] Kellar D, Register T, Lockhart SN, Aisen P, Raman R, Rissman RA, *et al.* Intranasal Insulin Modulates Cerebrospinal Fluid Markers of Neuroinflammation in Mild Cognitive Impairment and Alzheimer's Disease: A Randomized Trial. Sci Rep 2022;12:1346.

[21] Prabakaran A, Agrawal M, Dethe MR, Ahmed H, Yadav A, Gupta U, *et al.* Nose-to-brain Drug Delivery for the Treatment of Alzheimer's Disease: Current Advancements and Challenges. Expert Opin Drug Deliv 2022;19:87-102.

[22] Anderson NH, Woodburn K. Old-age psychiatry. In: Companion to Psychiatric Studies. Amsterdam: Elsevier; 2010. p. 635-92.

[23] Chen YX, Liang N, Li XL, Yang SH, Wang YP, Shi NN. Diagnosis and Treatment for Mild Cognitive Impairment: A Systematic Review of Clinical Practice Guidelines and Consensus Statements. Front Neurol 2021;12:719849.

[24] Gale SA, Acar D, Daffner KR. Dementia. Am J Med 2018;131:1161-9.

[25] Ito K, Hutmacher MM. Predicting the Time to Clinically Worsening in Mild Cognitive Impairment Patients and its Utility in Clinical Trial Design by Modeling a Longitudinal Clinical Dementia Rating Sum of Boxes from the ADNI Database. J Alzheimers Dis 2014;40:967-79.

[26] Evans S, McRae-McKee K, Wong MM, Hadjichrysanthou C, De Wolf F, Anderson R. The Importance of Endpoint Selection: How Effective does a Drug Need to be for Success in a Clinical Trial of a Possible Alzheimer's Disease Treatment? Eur J Epidemiol 2018;33:635-44.

[27] Sciacca L, Cassarino MF, Genua M, Pandini G, Le Moli R, Squatrito S, *et al.* Insulin Analogues Differently Activate Insulin Receptor Isoforms and Post-receptor Signalling. Diabetologia 2010;53:1743-53.

[28] Gasparini L, Gouras GK, Wang R, Gross RS, Beal MF, Greengard P, *et al.* Stimulation of Beta-amyloid Precursor Protein Trafficking by Insulin Reduces Intraneuronal Beta-amyloid and Requires Mitogen-activated Protein Kinase Signaling. J Neurosci 2001;21:2561-70.

[29] Lee CC, Kuo YM, Huang CC, Hsu KS. Insulin Rescues Amyloid Beta-induced Impairment of Hippocampal Long-term Potentiation. Neurobiol Aging 2009;30:377-87.

[30] De Felice FG, Vieira MN, Bomfim TR, Decker H, Velasco PT, Lambert MP, *et al.* Protection of Synapses Against Alzheimer's-linked Toxins: Insulin Signaling Prevents the Pathogenic Binding of Abeta Oligomers. Proc Natl Acad Sci U S A 2009;106:1971-6.

[31] Craft S, Peskind E, Schwartz MW, Schellenberg GD, Raskind M, Porte D Jr. Cerebrospinal Fluid and Plasma Insulin Levels in Alzheimer's Disease: Relationship to Severity of Dementia and Apolipoprotein E Genotype. Neurology 1998;50:164-8.

[32] Lochhead JJ, Wolak DJ, Pizzo ME, Thorne RG. Rapid Transport within Cerebral Perivascular Spaces Underlies Widespread Tracer Distribution in the Brain After Intranasal Administration. J Cereb Blood Flow Metab 2015;35:371-81.

[33] Cao B, Rosenblat JD, Brietzke E, Park C, Lee Y, Musial N, *et al.* Comparative Efficacy and Acceptability of Antidiabetic Agents for Alzheimer's Disease and Mild Cognitive Impairment: A Systematic Review and Network Meta-analysis. Diabetes Obes Metab 2018;20:2467-71.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00013

Journal of Clinical and Translational Research 2023; 9(4): 236-245

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

REVIEW ARTICLE

Effect of a 20% intravenous fat emulsion therapy on pregnancy outcomes in women with RPL or RIF undergoing IVF/ICSI: a systematic review and meta-analysis

Greg J. Marchand[1]*, Ahmed Taher Masoud[1,2], Hollie Ulibarri[1], Amanda Arroyo[1], Catherine Coriell[1], Sydnee Goetz[1], Carmen Moir[1], Atley Moberly[1], Daniela Gonzalez[1], Madison Blanco[1], Harry Randall Craig[3]

[1]Marchand Institute for Minimally Invasive Surgery, Mesa, Arizona, United States of America, [2]Fayoum University Faculty of Medicine, Fayoum, Egypt, [3]Fertility Treatment Center, Tempe, Arizona, United States of America

ARTICLE INFO

Article history:
Received: April 19, 2023
Revised: June 10, 2023
Accepted: June 11, 2023
Published online: July 12, 2023

Keywords:
Intralipid
Clinical pregnancy
Recurrent pregnancy loss
Recurrent implantation failure

**Corresponding author:*
Greg J. Marchand
Marchand Institute for Minimally Invasive Surgery, Mesa, Arizona, United States of America.
Tel: +1 4809990905; +1 4809990801
Email: gm@marchandinstitute.org

ABSTRACT

Background and Aim: The aim of this study was to evaluate the efficacy a 20% intravenous fat emulsion therapy in women suffering from recurrent pregnancy loss or recurrent implantation failure (RPL/RIF) who are undergoing *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI).
Materials and Methods: We searched Cochrane Library, ISI Web of Science, MEDLINE, ClinicalTrials.gov, PubMed, and Scopus using relevant keywords during February 2020 for randomized controlled trials (RCTs) comparing the therapy versus placebo or no intervention in women suffering from RPL/RIF and undergoing IVF/ICSI.
Results: We included five RCTs with 840 patients. The intravenous fat emulsion therapy was significantly effective in increasing clinical pregnancy rates compared to the control group (risk ratios [RR] = 1.48, 95% confidence intervals [CI] [1.23, 1.79], $P < 0.001$). Furthermore, ongoing pregnancy and live birth rates were significantly higher with 20% intravenous fat emulsion therapy RR = 1.71, 95% CI [1.27, 2.32], $P = 0.005$ and RR = 1.85, 95% CI [1.44, 2.38], $P < 0.001$. Despite the statistically significant differences, the quality of evidence was only considered moderate, and this was primarily due to high risk of bias in the included RCTs.
Conclusion: Our review provides a moderate level of evidence that intravenous fat emulsion therapy is effective in improving reproductive outcomes among women with RPL/RIF performing IVF/ICSI techniques. Further, investigation is required to ascertain optimal dosage and timing of administration.
Relevance for Patients: Women suffering from RPL or RIF may wish to consider discussing with their reproductive endocrinologist the addition of a 20% fat emulsion therapy to planned IVF or ICSI cycles, which may improve outcomes.

1. Introduction

Recurrent pregnancy loss (RPL) is the recurrent miscarriage of intrauterine pregnancies [1]. Exact definitions vary, but consensus statements by major US and European Obstetrical groups define RPL as the failure of two or more pregnancies, <12 weeks gestation, which do not necessarily have to be consecutive [1,2]. Some groups have included the loss of a single pregnancy in the second trimester to meet this criteria [3], while others require three first trimester miscarriages to meet their definition [4]. Natural spontaneous miscarriage occurs in at least 25% of human pregnancies, when pregnancy is detected early and regularly, as undetected miscarriages are also common [5,6]. Recurrent implantation failure (RIF) refers to the failure of pregnancy in spite of transferring a healthy embryo after three or more

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00060

in vitro fertilization (IVF) cycles [7]. Kumar *et al*. [8] established that the failure of implantation occurs in approximately 10% of IVF/intracytoplasmic sperm injection (ICSI) cycles.

Many factors can be responsible for RPL/RIF. These include abnormal embryonic karyotype (41%), uterine abnormalities (5%), endocrine dysfunction (6%), and antiphospholipid antibody (APA) syndrome (6%). Furthermore, it is estimated that the 25% of the time, the cause is unexplained or that 4% of the cases occur from mixed causes [9]. Different studies have proposed an immunological explanation for the RIF/RPL phenomenon, especially in those cases that lack an identified cause [6,10]. There is no consensus whether the most common cause is the failure of the apposition, adhesion, or invasion of the placenta into the uterine lining [11]. Immunological causes suspected to be responsible for RPL/RIF are extensive, and a full discussion is outside the realm of the present work. However, commonly suspected abnormalities include T-helper cells ratio imbalance, the deregulation of T-cells, the deregulation of dendritic cells, natural killer (NK) cells in the uterus, APA, and disorders of adaptive immunity and innate immunity, [12]. Various other biochemical factors have also been investigated, such as inflammatory mediators and human leukocyte antigens [13].

Accordingly, many immunomodulatory agents have been studied to reduce RPL/RIF rates. These therapies include low-molecular-weight heparin, aspirin, intravenous immunoglobulin (IVIG), corticosteroids, and 20% intravenous fat emulsion therapy [14-17]. Despite numerous studies, there is no clear consensus regarding the efficacy of these therapies in improving different pregnancy outcomes. This includes unclear efficacy in rates of achieving clinical pregnancy, maintaining ongoing pregnancies, and effect on live birth rates in patients with RPL and RIF [18].

Intralipid™ (20% intravenous fat emulsion) is a fat emulsion solution that consists of soybean oil, glycerin, egg phospholipid, water, and glycerol [15]. The medication is Food and Drug Administration approved for the administration of parenteral nutrition in patients with ingestion problems and is administered intravenously. It supplies the body with essential fatty acids, α-linolenic acid, and omega-3 fatty acids [19]. Interestingly, several studies have reported on benefits of intravenous fat emulsion therapy outside of the field of parenteral nutrition and the medications intended indication. These include a reduction in platelet aggregation, decline in interleukin-2 (IL-2) production, suppression of NK cell activity, and inhibition of TH1 cell activity [20]. Clearly, these properties spark an interest in the efficacy of this medication in the treatment of RPL and RIF.

While there is ample evidence that intravenous fat emulsion therapy is effective in reducing the production of proinflammatory cytokines and the production of NK cells [21-23], whether the medication actually improves outcomes in patients with RPL and RIF remains unclear. Before embarking on this review, we found conflicting bodies of evidence, with some studies reporting improved reproductive outcomes among patients with RPL/RIF undergoing IVF/ICSI when receiving the 20% intravenous fat emulsion therapy [24-26], and others failing to show any significant difference [27-30].

In light of this data, we found that there were sufficient RCTs performed on this topic to perform a meta-analysis, if the RCTs on RPL and RIF were combined (There was insufficient data separately.) Therefore, to see if a true difference exists, we aimed to design a meta-analysis to assess all of the available data regarding the effect of the 20% intravenous fat emulsion therapy on different pregnancy outcomes in women with RPL or RIF undergoing IVF/ICSI.

2. Materials and Methods

We performed this systematic review and meta-analysis accurately with the Cochrane Handbook for Systematic Reviews of Interventions [31]. We reported our findings according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [32].

We performed a computerized search in different databases (PubMed, Cochrane Library, Scopus and ISI Web of Science, MEDLINE, and ClinicalTrials.gov) during February of 2020. We used the following search strategy: (intralipid OR intralipid infusion OR soybean oil based lipid emulsion) AND (control OR comparator OR placebo OR saline OR no intervention) AND (pregnancy OR pregnant OR gestation OR recurrent pregnancy loss OR recurrent implantation failure OR RPL OR RIF) AND (clinical trial OR clinical trials OR trial OR random OR random allocation OR RCT OR randomized controlled trial OR controlled clinical trial). Two investigators performed the search strategy. There were no restrictions by language or year of publication.

Randomized controlled trials (RCTs) were included using this criteria:

(I) population: Women with RPL or RIF; (II) intervention: the 20% intravenous fat emulsion therapy; (III) comparator: placebo (normal saline) or no intervention; and (IV) study design: RCTs. We excluded studies for the following reasons: (1) reviews, (2) irrelevant studies, (3) letters to editors, and (4) studies whose data could not be extracted or entered into the analysis. Title and abstract screening and full-text screening were conducted by appropriate step by step analysis by the same two authors.

Our data were initially extracted by the two authors on a data extraction sheet. The extracted data included: list of authors, year of publication, sample size, and summary of included studies. In addition, we extracted our primary outcome (clinical pregnancy rate) and our secondary outcomes (ongoing pregnancy, miscarriage, live birth rates, and any adverse events of the 20% intravenous fat emulsion therapy). The category of adverse events was expanded to include reports of headache, dizziness, flushing, drowsiness, nausea, vomiting, and sweating.

Clinical pregnancies were defined as confirmation of fetal cardiac activity through sonography or Doppler, and this was found to be universally accepted in all included studies. Ongoing pregnancy was defined as a pregnancy that had reached more than or equal to 20 gestational weeks. Miscarriage was determined as spontaneous abortion or pregnancy loss prior to 20 weeks of gestation. Live birth was defined as the total number of deliveries

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00060

that resulted in a neonate who was born alive, and this ratio was calculated to 100 embryo transfers.

Two authors evaluated the methodological quality and the risk of bias in included studies using the Cochrane risk of bias assessment tool [33]. This tool involves six domains as the following: selection bias, performance bias (blinding of participant and personnel), detection bias (blinding of outcome assessment), attrition bias, reporting bias, and other potential sources of bias. The authors' judgment is categorized as "low risk," "high risk," and "unclear risk" of bias. Differences were discussed between the authors and consensus reached.

In addition, we assessed the methodologic quality of the evidence among the included studies using GRADEpro™ software. GRADEpro™ software provides fundamental details regarding the magnitude of the effect of the interventions examined, and the sum of available data on main outcomes in a summary of findings table produced by the software. This information is useful in examining the quality of evidence.

We pooled the dichotomous data as risk ratios (RR) with the corresponding 95% confidence intervals (CI) by the Mantel-Haenszel method. We used RevMan™ software to perform our statistical analysis. We assessed the statistical heterogeneity among included studies using I-squared (I^2) statistics, and values of ≥50% were indicative of high heterogeneity. We utilized a fixed-effect model, as heterogeneity was not significant in our selected outcomes.

We could not assess publication bias using the funnel plot method, and Egger's test is considered unreliable for less than ten included studies [34,35].

3. Results

3.1. Results of the literature search

Our search strategy resulted in 283 studies, and 23 articles were reliable for full-text screening after performing title and abstract screening. We excluded 18 full-text articles, and finally, five studies [25-29] matched our inclusion criteria and entered our final analysis. The PRISMA flow diagram for clarification is shown in Figure 1.

3.2. Characteristics of the included studies

Five RCTs [25-29] with 840 total patients were included. All included studies compared the intravenous 20% fat emulsion therapy versus placebo (normal saline) or no intervention in women with RIF/RPL undergoing IVF/ICSI. All women included in the studies were suffering from either primary or secondary infertility with RPL/RIF and intended to perform IVF/ICSI and embryo transfer technology. Controlled ovarian stimulation using different stimulation protocols was used in the included studies before the randomization process began. The summary of the included studies is shown in Table 1. All of the included RCTs used similar protocols for administration of the fat emulsion

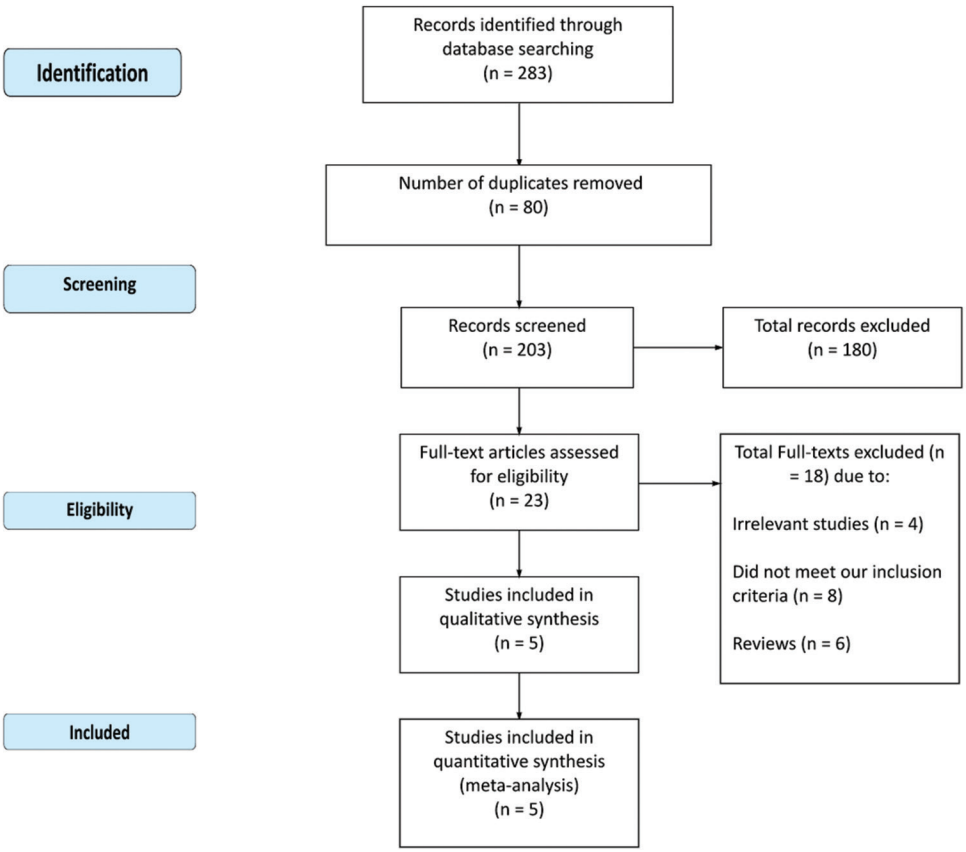


Figure 1. PRISMA flow diagram of selected studies.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00060

Table 1. Summary of the included studies

Study	Study arms	Country	Sample size	Age (year), mean±SD	Body mass index, mean±SD	GnRH analogue	GnRH protocol	Gn type	HCG dose	Number of oocytes retrieved, mean±SD	Number of embryos transferred, mean±SD	Infertility causes				Main findings
												Tubal	PCOS	Unexplained	Male	
Singh *et al.* (2019)[25]	Fat emulsion group (Two doses of 4 mL of 20% fat emulsion in normal saline of 250 mL; 1 dose at oocyte recovery, 1 dose on day of embryo transfer)	India	52	31.64±3.96	4.06±3.12	Leuprolide acetate/ Cetrorelix	Agonist/ Antagonist	rFSH	250 µg rHCG	5.96±3.45	2.09±1.1	13	6	19	13	Showed a significant increase in live birth rate and implantation rates in women who received the fat emulsion infusion after IVF/ICSI
	Control group (normal saline)		50	32.12±3.50	24.61±3.32					4.62±1.99	2.48±1.07	21	3	18	11	
Al-Zebeidi *et al.* (2019)[27]	Fat emulsion group (Two doses 20% 20% fat emulsion 100 mL diluted in 500 mL normal saline; 1 dose on day of embryo transfer, 1 dose on day of pregnancy test)	Saudi Arabia	71	35.32±4.23	28.30±4.66	Cetrorelix	Long antagonist	rFSH/ HMG	10,000 IU	NA	1.8±0.54	5	3	10	39	There was an improvement in the pregnancy rate among women with unexplained RIF who received the fat emulsion therapy; however, this improvement did not reach statistical significance.
	Control group (no intervention)		71	35.21±4.77	28.30±4.66					NA	1.9±0.51	2	5	10	45	
Dakhly *et al.* (2016) [29]	Fat emulsion group (Multiple doses of 2mL 20% fat emulsion diluted at 20% in 250 mL saline; infusions within 1 week of positive pregnancy test and every 2 weeks until end of first trimester)	Egypt	144	35.5±3.7	22.6±2.6	Triptorelin acetate	Long agonist	HMG	10,000 IU	8.5±2.8	2.5±0.5	NA	NA	NA	NA	Fat emulsion therapy did not increase chemical pregnancy rates. However, live birth and ongoing pregnancy rates were increased in the fat emulsion group.
	Control group (normal saline)		152	36±3.7	22.9±2.2					8.8±2.4	2.6±0.5	NA	NA	NA	NA	

(*Contd...*)

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00060

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00060

Table 1. (*Continued*)

El-Khayat and El Sadek (2015)[26]	Fat emulsion group (Two doses of 2 mL 20% fat emulsion in 250 mL normal saline; infusion between days 4–9 of ICSI and 1 dose within first week of positive pregnancy test)	Egypt	101	35.1±3.5	24.7±4.7	Triptorelin acetate	Long agonist	HMG	10,000 IU	5.1±1.4	1.94±0.31	NA	NA	NA	N	Fat emulsion infusion significantly improved clinical pregnancy rate, implantation rate, and the live birth rates in IVF/ICSI in women with RIF.
	Control group (normal saline)		102	35±3.6	24.1±4.3					4.9±1.7	1.93±0.33	NA	NA	NA	NA	
Gamaleldin *et al*. (2018)[28]	Fat emulsion group (Two doses of 2 mL 20% fat emulsion in 250 mL normal saline)	United Kingdom (UK)	48	35±3.4	NA	Triptorelin acetate	Long agonist	HMG	10,000 IU	NA	NA	NA	NA	NA	NA	Fat emulsion therapy did not significantly improve live birth rates in women with unexplained RIF undergoing IVF treatment.
	Control group (normal saline)		49	35.4±3.2	NA					NA	NA	NA	NA	NA	NA	

NA: Not available, IVF/ICSI: *In vitro* fertilization/intracytoplasmic sperm injection, RIF: Recurrent implantation failure, HMG: Human menopausal gonadotropin, HCG: Human chorionic gonadotropin, GnRH: Gonadotropin-releasing hormone, Gn: Gonadotropin, rFSH: Recombinant Follicle-stimulating hormone, rHCG: Recombinant human chorionic gonadotropin, IU: International unit, SD: Standard deviation

therapy, which included two or three doses of 2–4 mL of the 20% fat emulsion each diluted in saline, given in the time period surrounding embryo transfer [25-29]. While these protocols were similar in timing and dosage, they were not identical.

3.3. Risk of bias assessment

The risk of bias assessment for the included RCTs is shown in Figure 2. We performed the quality assessment of the included RCTs based on the Cochrane risk of bias assessment tool.

3.4. Outcomes

3.4.1. Clinical pregnancy rate

The intravenous fat emulsion therapy was effective in improving the clinical pregnancy rate when compared to the control group (RR = 1.48, 95% CI [1.23, 1.79], $P < 0.001$), as shown in Figure 3.

The pooled studies were homogeneous ($P = 0.13$, $I^2 = 45\%$). The quality of evidence was moderate, as shown in Figure 4.

3.4.2. Ongoing pregnancy rate

The intravenous fat emulsion therapy was beneficial in improving the ongoing pregnancy rate when compared to the control group (RR = 1.71, 95% CI [1.27, 2.32], $P = 0.005$), as shown in Figure 5. We found homogeneity among the pooled studies ($P = 0.50$, $I^2 = 0\%$). The quality of evidence was moderate, as shown in Figure 4.

3.4.3. Miscarriage rate

We found no significant difference between the intravenous fat emulsion group and the control group regarding miscarriage rate (RR = 0.78, 95% CI [0.50, 1.20], $P = 0.26$), as shown in Figure 6. We found homogeneity among the pooled studies ($P = 0.38$, $I^2 = 0\%$). The quality of evidence was moderate, as shown in Figure 4.

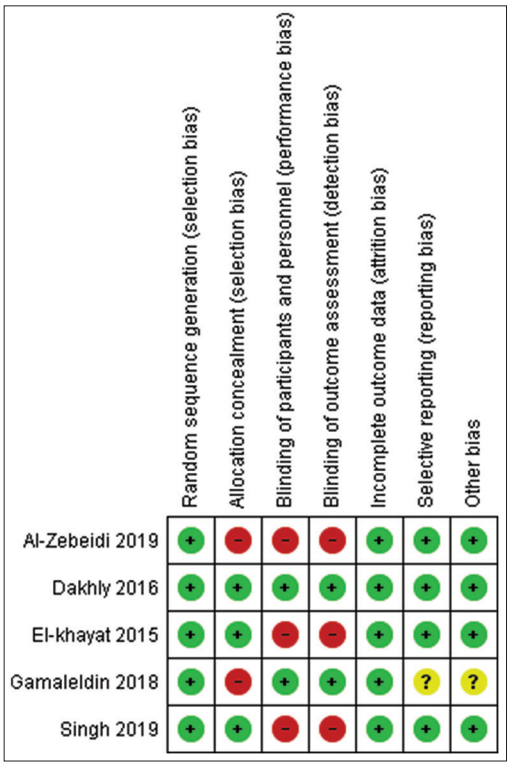


Figure 2. Risk of bias summary.

3.4.4. Live birth rate

The intravenous fat emulsion therapy significantly improved live birth rate over the control group (RR = 1.85, 95% CI [1.44, 2.38], $P < 0.001$), as shown in Figure 7. We found homogeneity among the pooled studies ($P = 0.55$, $I^2 = 0\%$). The quality of evidence was moderate, as shown in Figure 4.

3.4.5. Subgroups

As stated previously, there was insufficient data from the RCTs to perform a subgroup analysis for RPL and RIF separately.

3.4.6. Adverse events

There were no adverse events from the intravenous fat emulsion therapy administration reported by the included studies.

4. Discussion

Our study demonstrated a significant benefit on pregnancy outcomes in IVF/ICSI cycles of patients with a history of RIF or RPL with intravenous fat emulsion therapy. There was higher incidence of clinical pregnancy, ongoing pregnancy, and live birth rates in the fat emulsion therapy arm, which was statistically significant. However, the miscarriage rate did not show a significant difference between the fat emulsion therapy and control groups.

According to Moffett and Colucci, the prevailing theory for this difference stems from treatment of a hypothesized dysfunction of the immune system in the endometrium [36]. It is further theorized that this dysfunction, including a higher level of NK cell activity, may be one of the main causes of RPL and RIF. In addition, an elevated level of NK cell activity may actually be predictive of future pregnancy loss in subsequent pregnancies in patients who have RPL/RIF [37]. The theorized mechanisms by which the intravenous fat emulsion therapy produces its immune modulation effects include mitochondrial-dependent platelet aggregation reduction [20], decline in secretion of hepatic apolipoprotein M and insulin sensitivity amplification [38], alteration in the composition of the platelets (especially phospholipid membrane and consequently reduced platelets aggregation) [39], reduced secretion of IL-2, tumor necrosis factor-α, and IL-1β [21], and long-standing inhibition of the NK cells activity [23]. Singh *et al*. [25] demonstrated that intravenous fat emulsion therapy may also produce changes in the endometrium that favor the production of TH2 cytokines and may modify the NK cells to a phenotype more compatible with pregnancy [25].

Investigations have been performed in the roles of the uterine (endometrial) and peripheral measurements of NK cells as well in the treatment of RPL/RIG [16]. Studies performed by Seshadri and Sunkara [37] originally found a high percentage of NK cells in the periphery in women with RIF and RPL versus the control group [37]. However, such a significant difference was not

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00060

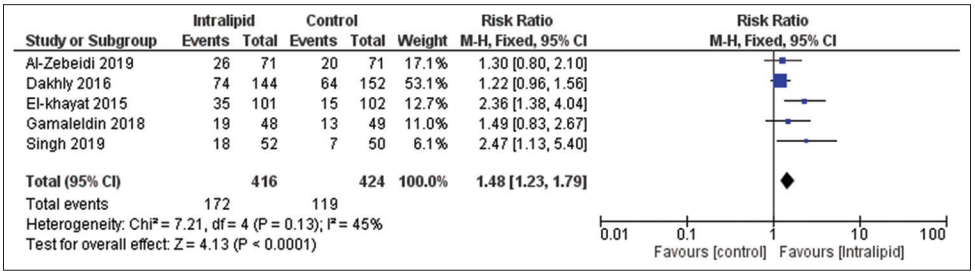
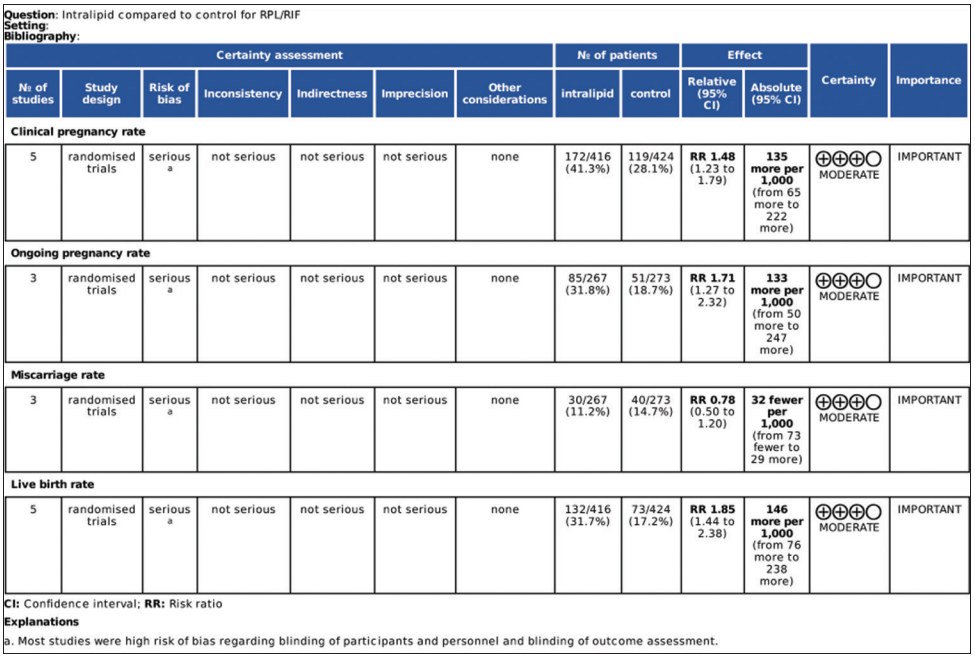


Figure 3. Forest plot of clinical pregnancy rate.



Question: Intralipid compared to control for RPL/RIF
Setting:
Bibliography:

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	intralipid	control	Relative (95% CI)	Absolute (95% CI)		
Clinical pregnancy rate												
5	randomised trials	serious [a]	not serious	not serious	not serious	none	172/416 (41.3%)	119/424 (28.1%)	**RR 1.48** (1.23 to 1.79)	**135 more per 1,000** (from 65 more to 222 more)	⨁⨁⨁◯ MODERATE	IMPORTANT
Ongoing pregnancy rate												
3	randomised trials	serious [a]	not serious	not serious	not serious	none	85/267 (31.8%)	51/273 (18.7%)	**RR 1.71** (1.27 to 2.32)	**133 more per 1,000** (from 50 more to 247 more)	⨁⨁⨁◯ MODERATE	IMPORTANT
Miscarriage rate												
3	randomised trials	serious [a]	not serious	not serious	not serious	none	30/267 (11.2%)	40/273 (14.7%)	**RR 0.78** (0.50 to 1.20)	**32 fewer per 1,000** (from 73 fewer to 29 more)	⨁⨁⨁◯ MODERATE	IMPORTANT
Live birth rate												
5	randomised trials	serious [a]	not serious	not serious	not serious	none	132/416 (31.7%)	73/424 (17.2%)	**RR 1.85** (1.44 to 2.38)	**146 more per 1,000** (from 76 more to 238 more)	⨁⨁⨁◯ MODERATE	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Most studies were high risk of bias regarding blinding of participants and personnel and blinding of outcome assessment.

Figure 4. GRADEpro™ assessment of methodologic quality of evidence.

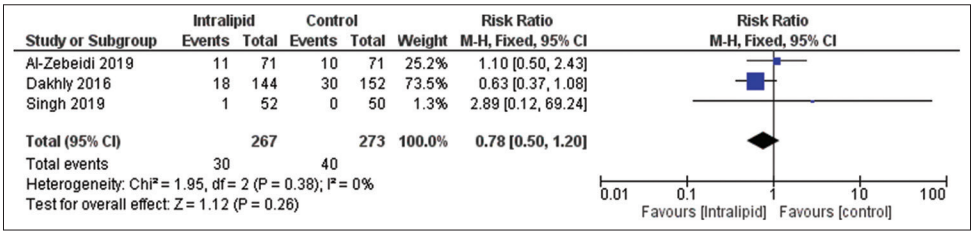


Figure 5. Forest plot of the ongoing pregnancy rate.

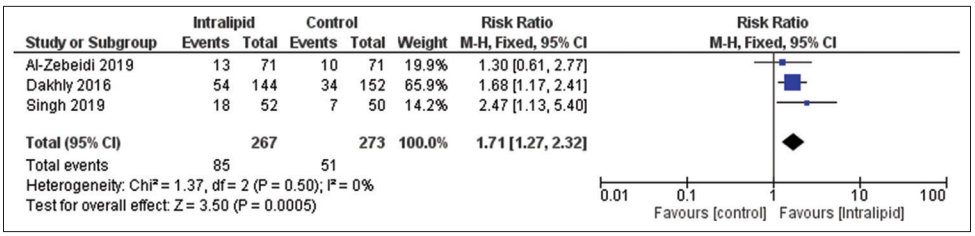


Figure 6. Forest plot of the miscarriage rate.

observed when NK cells were measured in uterine samples in the same study groups [39]. Evidence has been presented to show that uterine NK cells are detrimental to a newly invading placental trophoblast [40,41]. In addition, newer studies have found direct

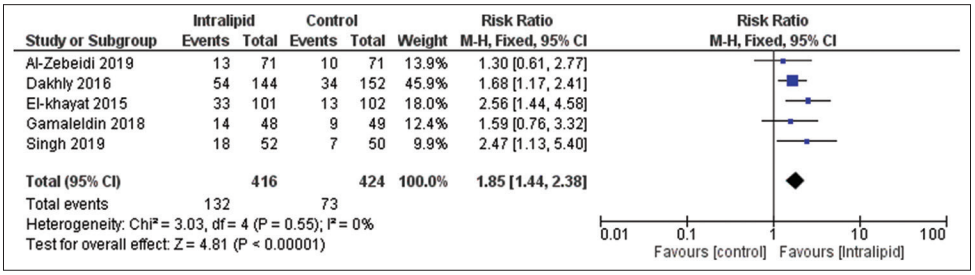


Figure 7. Forest plot of the live birth rate.

correlations between certain NK cell receptors and RPL [42].

At present, in the United States, intravenous 20% fat emulsion therapy is administered by many fertility clinics for patients with RPL/RIF, especially in the setting of empiric treatment of suspected fertility-related immunological dysfunction [43,44]. This may be secondary to the high cost of other investigations. IVIG therapy, for example, is extremely expensive, with a single course of therapy costing up to $14,000 [43]. In addition, the efficacy of IVIG for improving pregnancy outcomes (clinical pregnancy, ongoing pregnancy, and live birth rates) in RPL/RIF remains unproven. Risks with IVIG administration include the possibility of anaphylaxis and a low—but possible—risk for transmission of infections [44]. Thus, many clinicians feel that intravenous fat emulsion therapy may be safer, in addition, to being less expensive. There is, however, no universal consensus. Martini *et al*., [10] for example, failed to find that fat emulsion therapy was cost-effective despite an average cost of only $681.00 for the therapy in their case series. The lack of cost effectiveness was a result of finding very little efficacy versus their control [10].

Some of the most compelling evidence for fat emulsion therapy came from Singh *et al*., [25] which demonstrated a significant improvement in clinical pregnancy, ongoing pregnancy, and live birth rates in women with a history of RIF undergoing intravenous fat emulsion therapy. The adjusted odds ratio for clinical pregnancy in the fat emulsion therapy group, compared to placebo, was 3.1, 95% CI [1.02–9.70], $P = 0.046$. Another RCT, El-Khayat and El Sadek [26] agreed with these findings by concluding a significant improvement in clinical pregnancy, implantation, and live birth rates among women receiving the intravenous fat emulsion therapy [26]. In addition, Coulam and Acacio [24] proposed an estimated 61% increase in live birth rates after treatment with the intravenous fat emulsion therapy in cases of RPL/RIF with increased NK activity. Moreover, this effect was not different when compared against a cohort of IVIG.

Several of the analyzed RCTs had different conclusions. Al-Zebeidi *et al*. [27] failed to demonstrate a significant difference between the intravenous fat emulsion and control groups in terms of clinical pregnancy, miscarriage, and live birth rates. Similarly, Dakhly *et al*. [29] did not illustrate any significant difference in chemical pregnancy among women with RPL after intravenous fat emulsion therapy (58.3% vs. 50.0%, $P = 0.129$ for intravenous fat emulsion vs. control group, respectively). Furthermore, in a recent retrospective study, Lédée *et al*. [16] found that there was a benefit to fat emulsion therapy in RIF patients who exhibited an over-immune activation of uNK cells. They found an improvement to a 54% live birth rate with fat emulsion therapy in RIF patients [16]. Gamaleldin *et al*. [28] found that fat emulsion therapy did not create any significant improvement in live birth and clinical pregnancy rates in cases with RIF, and Cohen *et al*. [30] reported a similar lack of efficacy in patients aged 40–42 years with history of miscarriage [30].

To the best of our knowledge, this meta-analysis is the first to investigate the effect of intravenous fat emulsion therapy on different pregnancy outcomes in women suffering from recurrent miscarriage or implantation failure. Strengths of our study include that the characteristics of most of the included RCTs were extremely similar, with relatively few variables to control for. Other strengths include our strict adherence to the PRISMA guidelines and accepted principles of a systematic review.

Our main limitation was the heterogeneity of inclusion criteria in some of the included studies, although as stated above, we noted great similarity in the adhered protocols. Secondary to this increased heterogeneity, our strict grading of the studies led to a higher than expected risk of bias. This ultimately resulted in a moderate level of evidence. As noted in our quality of evidence assessment (Figure 4), this is almost entirely due to concerns of a lack of proper blinding of participants and personnel in four of the included studies. This led to an increased risk of bias and, thus, lowered the quality of our overall evidence. It is likely that if more well-designed RCTs are undertaken, this level of evidence would increase, especially if the researchers were able to specifically document that correct patient and personnel blinding procedures were followed.

Another limitation of this study was the necessity to combine the RPL and RIF groups to reach statistical significance among the included RCTs. A greater wealth of RCTs on this topic would allow for subgroup analysis or individual analysis to ascertain exactly which condition, if either, benefits more from fat emulsion therapies.

Furthermore, complicating our understanding of the usage of fat emulsion therapy, there is still no universal consensus as to the mechanism of action of intravenous fat emulsion therapy in patients with RPL and RIF, which may be limiting the interest in developing future RCTs. We would also recommend that future studies could focus on the cost-effectiveness of the fat emulsion therapy, as this is an important factor for many clinicians.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00060

5. Conclusion

Our findings show moderate-level evidence that intravenous 20% fat emulsion therapy is effective in improving clinical pregnancy, ongoing pregnancy, and live birth rates in IVF/ICSI procedures in women with RPL/RIF. Further RCTs are needed and improved methodologic evidence quality in those trials would greatly improve the quality of this recommendation.

Acknowledgments

The Marchand Institute for Minimally Invasive Surgery would like to acknowledge the efforts of all of the students, researchers, residents, and fellows at the institute who put their time and effort into these projects without compensation, only for the betterment of women's health. We firmly assure them that the future of medicine belongs to them.

Funding

There is no funding to report.

Conflicts of Interest

The authors have no conflicts of interest to declare.

References

[1] Practice Committee of the American Society for Reproductive Medicine. Definitions of Infertility and Recurrent Pregnancy Loss: A Committee Opinion. Fertil Steril 2013;99:63.

[2] ESHRE Guideline Group on RPL, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, *et al*. ESHRE Guideline: Recurrent Pregnancy Loss: An Update in 2022. Hum Reprod Open 2023;2023:hoad002.

[3] McBride KL, Beirne JP. Recurrent Miscarriage. InnovAiT 2014;7:25-34.

[4] Kutteh WH. Recurrent Pregnancy Loss. Semin Reprod Med 2006;24:54-66.

[5] Zinaman MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG. Estimates of Human Fertility and Pregnancy Loss. Fertil Steril 1996;65:503-9.

[6] Dimitriadis E, Menkhorst E, Saito S, Kutteh WH, Brosens JJ. Recurrent Pregnancy Loss. Nat Rev Dis Primers 2020;6:98.

[7] Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, *et al*. Recurrent Implantation Failure: Definition and Management. Reprod Biomed Online 2014;28:14-38.

[8] Kumar P, Mahajan S. Preimplantation and Postimplantation Therapy for the Treatment of Reproductive Failure. J Hum Reprod Sci 2013;6:88-92.

[9] Sugiura-Ogasawara M. Recurrent Pregnancy Loss and Obesity. Best Pract Res Clin Obstet Gynaecol 2015; 29:489-97.

[10] Martini AE, Jasulaitis S, Fogg LF, Uhler ML, Hirshfeld-Cytron JE. Evaluating the Utility of Intralipid Infusion to Improve Live Birth Rates in Patients with Recurrent Pregnancy Loss or Recurrent Implantation Failure. J Hum Reprod Sci 2018;11:261-8.

[11] Bashiri A, Halper KI, Orvieto R. Recurrent Implantation Failure-update Overview on Etiology, Diagnosis, Treatment and Future Directions. Reprod Biol Endocrinol 2018;16:121.

[12] Boomsma CM, Kamath MS, Keay SD, Macklon NS. Peri-implantation Glucocorticoid Administration for Assisted Reproductive Technology Cycles. Cochrane Database Syst Rev 2022;6:CD005996.

[13] Porter TF, Scott JR. Alloimmune Causes of Recurrent Pregnancy Loss. Semin Reprod Med 2000;18:393-400.

[14] Allahbadia GN. Low-Molecular-Weight Heparin (LMWH) in Women with Repeated Implantation Failure. J Obstet Gynaecol India 2012;62:381-3.

[15] Gelbaya TA, Kyrgiou M, Li TC, Stern C, Nardo LG. Low-dose Aspirin for *in Vitro* Fertilization: A Systematic Review and Meta-analysis. Hum Reprod Update 2007;13:357-64.

[16] Lédée N, Prat-Ellenberg L, Petitbarat M, Chevrier L, Simon C, Irani EE, *et al.* Impact of Prednisone in Patients with Repeated Embryo Implantation Failures: Beneficial or Deleterious? J Reprod Immunol 2018;127:11-5.

[17] Li J, Chen Y, Liu C, Hu Y, Li L. Intravenous Immunoglobulin Treatment for Repeated IVF/ICSI Failure and Unexplained Infertility: A Systematic Review and a Meta-Analysis. Am J Reprod Immunol 2013;70:434-47.

[18] Wong LF, Porter TF, Scott JR. Immunotherapy for Recurrent Miscarriage. Cochrane Database Systemat Rev 2014;2014:CD000112.

[19] Shreeve N, Sadek K. Intralipid Therapy for Recurrent Implantation Failure: New Hope or False Dawn? J Reprod Immunol 2012;93:38-40.

[20] Beaulieu LM, Vitseva O, Tanriverdi K, Kucukural A, Mick E, Hamburg N, *et al.* Platelet Functional and Transcriptional Changes Induced by Intralipid Infusion. Thromb Haemost 2016;115:1147-56.

[21] Granato D, Blum S, Rössle C, Le Boucher J, Malnoë A, Dutot G. Effects of Parenteral Lipid Emulsions with Different Fatty Acid Composition on Immune Cell Functions *in Vitro*. JPEN J Parenter Enteral Nutr 2000;24:113-8.

[22] Mayer K, Meyer S, Reinholz-Muhly M, Maus U, Merfels M, Lohmeyer J, *et al.* Short-time Infusion of Fish Oil-Based Lipid Emulsions, Approved for Parenteral Nutrition, Reduces Monocyte Proinflammatory Cytokine Generation and Adhesive Interaction with Endothelium in Humans. J Immunol 2003;171:4837-43.

[23] Roussev RG, Acacio B, Ng SC, Coulam CB. Duration of Intralipid's Suppressive Effect on NK Cell's Functional Activity. Am J Reprod Immunol 2008;60:258-63.

[24] Coulam CB, Acacio B. Does Immunotherapy for Treatment of Reproductive Failure Enhance Live Births? Am J Reprod Immunol 2012;67:296-304.

[25] Singh N, Davis AA, Kumar S, Kriplani A. The Effect of Administration of Intravenous Intralipid on Pregnancy Outcomes in Women with Implantation Failure After IVF/ICSI with Non-donor Oocytes: A Randomised Controlled Trial. Eur J Obstet Gynecol Reprod Biol 2019;240:45-51.

[26] El-Khayat W, El Sadek M. Intralipid for Repeated Implantation Failure (RIF): A Randomized Controlled Trial. Fertil Steril 2015;104:e26.

[27] Al-Zebeidi J, Agdi M, Lary S, Al-Obaid S, Salim G, Al-Jaroudi D. Effect of Empiric Intravenous Intralipid Therapy on Pregnancy Outcome in Women with Unexplained Recurrent Implantation Failure Undergoing Intracytoplasmic Sperm Injection-embryo Transfer Cycle: A Randomized Controlled Trial. Gynecol Endocrinol 2019;36:131-4.

[28] Gamaleldin I, Gomaa MF, Shafik A, Akande V. Intralipid Infusion does not Improve Live Birth Rates in Women with Unexplained Recurrent Implantation Failure and May Increase the Risk of Congenital Malformations, a Double-Blinded Randomised Controlled Trial. BJOG 2018;125:31-2.

[29] Dakhly DM, Bayoumi YA, Sharkawy M, Allah SH, Hassan MA, Gouda HM, *et al*. Intralipid Supplementation in Women with Recurrent Spontaneous Abortion and Elevated Levels of Natural Killer Cells. Int J Gynecol Obstet 2016;135:324-7.

[30] Cohen R, Check JH, Wilson C, Choe JK. Intravenous Intralipid Therapy is not Beneficial in Having a Live Delivery in Women Aged 40-42 with Previous History of Miscarriage Undergoing *in Vitro* Fertilization-Embryo Transfer. Fertil Steril 2009;92:S20-1.

[31] O'Connor D, Green S. Higgins JP Cochrane Handbook for Systematic Reviews of Interventions: Cochrane Book Series. Chichester (UK): John Wiley & Sons; 2008. p. 81-94.

[32] Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009;6:e1000097.

[33] Green S, Higgins JP, Alderson P, Clarke M, Mulrow DC. Cochrane handbook: Cochrane Reviews: Assessing risk of bias in included studies. In: Systematic Reviews of Interventions. Ch. 8. United States: John Wiley & Sons; 2011. p. 3-10.

[34] Egger M, Smith GD, Schneider M, Minder C. Bias in Meta-Analysis Detected by a Simple, Graphical Test. BMJ 2015;14:1-16.

[35] Terrin N, Schmid CH, Lau J, Olkin I. Adjusting for Publication Bias in the Presence of Heterogeneity. Stat Med 2003;22:2113-26.

[36] Moffett A, Colucci F. Uterine NK Cells: Active Regulators at the Maternal-Fetal Interface. J Clin Investig 2014;124:1872-9.

[37] Seshadri S, Sunkara SK. Natural Killer Cells in Female Infertility and Recurrent Miscarriage: A Systematic Review and Meta-Analysis. Hum Reprod Update 2014;20:429-38.

[38] Zheng L, Feng Y, Shi Y, Zhang J, Mu Q, Qin L, *et al*. Intralipid Decreases Apolipoprotein M Levels and Insulin Sensitivity in Rats. PLoS One 2014;9:e105681.

[39] Aviram M, Deckelbaum RJ. Intralipid Infusion into Humans Reduces *in Vitro* Platelet Aggregation and Alters Platelet Lipid Composition. Metabolism 1989;38:343-7.

[40] Moffett A, Shreeve N. First do no Harm: Uterine Natural Killer (NK) Cells in Assisted Reproduction. Hum Reprod 2015;30:1519-25.

[41] Aoki K, Kajiura S, Matsumoto Y, Ogasawara M, Okada S, Yagami Y, *et al*. Preconceptional Natural-Killer-Cell Activity as a Predictor of Miscarriage. Lancet 1995;345:1340-2.

[42] Habets DH, Schlütter A, van Kuijk SM, Spaanderman ME, Al-Nasiry S, Wieten L. Natural Killer Cell Profiles in Recurrent Pregnancy Loss: Increased Expression and Positive Associations with TACTILE and LILRB1. Am J Reprod Immunol 2022;88:e13612.

43] Practice Committee of the American Society for [Reproductive Medicine. Intravenous Immunoglobulin (IVIG) and Recurrent Spontaneous Pregnancy Loss. Fertil Steril 2006;86:S226-7.

[44] Katz U, Achiron A, Sherer Y, Shoenfeld Y. Safety of Intravenous Immunoglobulin (IVIG) Therapy. Autoimmun Rev 2007;6:257-9.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00060

Journal of Clinical and Translational Research 2023; 9(4): 246-252

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

Meta-analysis of clinical trials in the 2020s and beyond: a paradigm shift needed

Jonathan J. Shuster*

Department of Health Outcomes and Bioinformatics, College of Medicine, University of Florida, Gainesville, Florida 32605, United States of America

ARTICLE INFO

Article history:
Received: February 15, 2022
Revised: April 11, 2023
Accepted: June 10, 2023
Published online: July 12, 2023

Keywords:
clinical trial
meta-analysis
random effects

**Corresponding authors:*
Jonathan J. Shuster
Department of Health Outcomes and Bioinformatics, College of Medicine, University of Florida, 2026 NW 34 Ter, Gainesville, FL 32605, United States of America.
Tel: +1(352)682-0893
Email: shusterj@ufl.edu

ABSTRACT

Background: A peer-reviewed meta-analysis methods article mathematically proved that mainstream random-effects methods, "weights inversely proportional to the estimated variance," are flawed and can lead to faulty public health recommendations. Because the arguments causing this off-label (unproven) use of mainstream practices were subtle, changing these practices will require much clearer explanations that can be grasped by clinical and translational scientists. There are five assumptions underlying the mainstream's derivation of its statistical properties. This paper will demonstrate that if the first is true, it follows that the last two are false. Ratio estimation, borrowed from classical survey sampling, provides a rigorous alternative. Papers reporting results rarely fully disclose these assumptions. This is analogous to watching TV ads with the sound muted. You see high quality of life and do not hear about the complications. This article is a poster child for translational science, as it takes a theoretical discovery from the biostatistical world, translates it into language clinical scientists can understand, and thereby can change their research practice.

Aim: This article is aimed at future applications of meta-analysis of complete collections of randomized clinical trials. It leaves it to past authors as to whether to reanalyze their data. No blame for past use is assessed.

Methods: By treating the individual completed studies in the meta-analysis as a random sample from a conceptual universe of completed studies, we use ratio estimation to obtain estimates of relative risk (ratio of failure rates treatment: control) and mean differences, projecting our sample value to estimate the universe's value.

Results: Two examples demonstrate that the mainstream methods likely adversely impacted major treatment options. A third example shows that the key mainstream presumption of independence between the study weights and study estimates cannot be supported.

Conclusion: There is no rationale for ever using the mainstream for meta-analysis of randomized clinical trials.

Relevance for Patients: Future meta-analysis of clinical trials should never employ mainstream methods. Doing so could lead to potentially harmful public health policy recommendations. Clinical researchers need to play a primary role to assure good research practices in meta-analysis.

1. Introduction

As hard as this is to believe, the recent paper, Shuster [1], mathematically proved beyond any doubt that despite being in common use for over four decades, the mainstream methods of conducting random effects meta-analyses (true individual study-by-study effect sizes can differ) are unsound and are likely to produce misleading results that could be threats to public health. It is commendable if you, as a clinical investigator or reader, would be skeptical of this statement. Needless to say, because Shuster [1] was controversial, it was

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00019

one of the most heavily peer-reviewed biostatistical papers ever, with review material from nine of nine sources (including three world-renowned meta-analysts) agreeing with his conclusions. However, in this article, in a non-technical way, you will be shown clearly that the mainstream methods rely on five incompatible assumptions that underlie their validity. This makes the evidence basis of the mainstream no different than claiming an evidence basis for off-label usage of a drug. You will be shown that if the first assumption is true, the last two cannot be true. In one highly cited example, we show that the mainstream-based claim of efficacy for an invasive treatment has no scientific basis. In another, the mainstream methods failed to detect a highly significant outcome. Had these methods been available and used, the use of a cardiotoxic type II diabetes drug could have been discontinued at least 3 years earlier than what actually occurred. We provide free access to well-documented user-friendly Excel templates to conduct rigorous analyses of the main research questions. This paper places no blame on the well-intentioned researchers who developed these mainstream methods. However, if meta-analysis is to remain at the apex of "Evidence Pyramids," it is imperative that statistical practice should be changed. This paper is needed for two reasons. First, with the availability of user-friendly software for the mainstream methods, a high proportion of these analyses is done without input from biostatisticians or epidemiologists. Second, changes to statistical practice will not happen overnight. Readers should be concerned when they read papers using meta-analysis in the biomedical literature. In short, this is about proven science, not opinions.

2. Assumptions Underlying the Validity of Current Mainstream Methods

When methodologists derive analytic procedures, they make working distributional assumptions that enable them to complete their work. Every time the procedure is used in practice, these should be fully disclosed. In a specific application, if any of the assumptions are wrong, the evidentiary basis of the results is in jeopardy. Unfortunately, few analytic procedures have adequate diagnostic tests for their assumptions. In meta-analysis, there has been little vetting of the robustness of procedures when their assumptions fail.

"Weighting inversely proportional to the estimated variance estimation" (aka the mainstream method) is by far the most common method used in combining data from a complete set of randomized clinical trials of a research question. These methods were derived under five assumptions (A1-A5) below, which must be true up to strong approximations. These are rarely disclosed in full, and current software does not provide adequate warnings. Assumptions A1 and A3 are reasonable in most applications. Assumption A2 is questionable (no adequately powered diagnostic test exists for it). Unfortunately, even if Assumption A1 is true, it follows that Assumptions A4 and A5 are false. This leaves open the strong likelihood that past meta-analyses may have reached unsupportable conclusions, possibly contributing to inappropriate public health recommendations.

A1: The true primary effect sizes for each study are drawn independently from a single large "urn" of primary effect sizes. This assumption tells us we are targeting the unweighted mean of all studies in the urn.

A2: The true primary effect sizes in the urn follow a normal (bell-shaped) distribution whose unweighted mean is the target parameter of interest.

A3: The individual study provides an unbiased estimate of its study-specific true primary effect size and has an approximate normal distribution about its true primary effect size.

A4: Up to a strong approximation, the weights are "constants" rather than seriously random variables. In other words, if you repeat the total experiment under the same Assumptions A1-A3 and the same urn, this assumption presumes you obtain identical weights up to a strong approximation. This assumption is mandatory to use the formulas for the mean and standard error in the mainstream methods but will be shown to be false under Assumptions A1-A3. More on this below.

A5: There is no association between-study weight and study true effect size. For example, if big studies tend to have higher (lower) effect sizes than smaller studies, the method will tend to overestimate (underestimate), respectively, the overall effect size. This could lead to unacceptable bias.

3. How Mainstream Weighted Random Effects Methods Work

The "variance" for the estimates of effect size for each study consists of two components, the reasons why its individual study estimate of effect size differs from the true mean of the effect sizes in the urn: (a) Within-study variance, which is estimated under Assumption A3 and (b) Between-study variance, which is the variance of the true effect sizes in the urn, per Assumptions A1 and A2. The first component (a) depends on the accuracy of the within-study estimate and varies from study to study. The second component (b) is the same for all studies. The overall estimate is the weighted average of the individual study estimates with weights inversely proportional to the study's estimated variance, which is the sum of the estimated within-study variance and the estimated between-study variance. If all five assumptions were true, these weights would minimize the standard error of the estimate of the overall effect size, over all choices of weights that sum to one (a requirement for unbiasedness). Note that all other things being equal, larger between-study variance pushes the weights closer to equal weights and smaller between-study variance pushes the weights closer to fixed effects.

4. Why Assumption A4 is False

What this assumption requires is that if we repeat the experiment under Assumptions A1-A3, the resulting weights will be the same up to a strong approximation.

Imagine a meta-analysis where we independently generate the data twice under Assumptions A1-A3. Clearly, the true study effect sizes for these two repetitions are sure to differ. It follows that the diversity (sample variance) of these true effect sizes will

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00019

differ. All things being equal, the one with the greater sample variance in true effect sizes will have weights closer to equality than the other, thanks to a larger between-study variance. As a concrete example, when the number of studies combined is eight, there is a 61% probability that one sample variance for these true effect sizes will be at least 50% higher than the other. Assumption A4 requires these to be the same to a near certainty. The derivation of the 61% figure is in the Appendix for those with biostatistical expertise. The between-study variance is a major determinant of the weights and clearly differs between repetitions of obtaining the meta-analysis data under Assumptions A1-A3.

Support for the fact that weights are seriously random variables comes from an unlikely source, lead developer of perhaps the most popular software product for this subject, Comprehensive meta-analysis (CMA), Borenstein [2], who states this assumption in Section 7.4.3, "The studies that were performed are a random sample from the universe." This concedes the point that mainstream weights, which are functions of the studies, are seriously random variables, not constants. This potentially invalidates the claims of no bias in the overall effect size estimate and legitimacy of confidence intervals and P-values.

In short, the mainstream relies on theory that was never intended for this type of application and as such, the distribution theory is used off label.

5. Why Assumption A5 is False

This one should be clear from the fact that the weights are determined by the variances (diversity) of the effect sizes. The more diverse the true study-specific effect sizes are (Assumptions A1-A3), the closer the weights are to being equal. In short, the mainstream weights are in part determined by the effect size estimates rendering the claim of independence untrue.

6. Why Assumption A2 Should Not be Trusted

Assumption A2 presumes that the true effect size for each study is drawn from the same urn and has a normal distribution. This implies that on average, the true study-specific effect sizes are the same regardless of study design. There is no adequately powered diagnostic test that can prove with reasonable certainty that this is true. For example, as shown by Shuster [1], any non-zero correlation between weight and effect size will bias the overall estimate of effect size and invalidate its standard error formula. Further, there is no adequately powered diagnostic test that can prove with reasonable certainty that the individual true study-specific effect size follows a normal distribution.

7. How Ratio Estimation Works

Our inferential framework is identical to that of randomized clinical trials. The role of patient in the clinical trial is played by study in the meta-analysis. The following is a quotation from Shuster [1], "A meta-analysis (clinical trial) inference is based on the sample of studies (patients) in the meta-analysis (clinical trial) as a conceptual random sample of past, present, and future studies (patients), drawn from a large target population of studies (patients) with the same eligibility criteria. The inference is to this target population."

Our universe is a large conceptual population of completed studies and the actual studies are a conceptual random sample from this universe. Our inference is to the target parameter in the entire conceptual population. Our estimate is the corresponding value in the sample of studies in the analysis. The target metric simply projects what the relative risk (or difference in means or difference in proportions) would be if all patients received the experimental therapy versus that if all patients received the control therapy. This framework is different from the mainstream, and hence, it is important to note that the ratio method targets a different population parameter than the mainstream.

Note that this setup can accommodate any distribution of means or proportions for the two treatment arms, making it a model-free random effects framework for meta-analysis. The mainstream imposes severe restrictions through its five Assumptions A1-A5.

7.1. Illustration for relative risk (risk ratio)

For each study in the universe, if we had the number of failures on each treatment (experimental and control), we could project the number of "failures" that would occur if every subject was in the experimental group (control group), respectively. For each individual study, this would be the total sample size (treatment + control) for the study multiplied by the proportion failing in the experimental group (control group), respectively. For example, in the first study in Table 1, we see that the experimental group had two failures in 26 patients, while the control group had one failure in 26 patients. We project that if all 52 subjects had gotten the experimental treatment, we would project that we would have had 52(2/26) = 4 failures. Similarly, we would project that if all patients had received the control, we would project 52(1/26) = 2 failures. Note that projections need not be whole numbers. If, for each treatment, we added the projected number of failures for all studies in the universe and take the ratio that would yield the projected true relative risk: Projected # failing in the universe (experimental group) divided by Projected # failing in the universe (control group). The corresponding projected ratio in the actual conducted sample of completed studies gives us the estimate. Technical notes: The confidence intervals and P-values are derived using the natural logs of the ratio and back converting the confidence interval using natural antilogs. The Users' guide

Table 1. Neto *et al*.[4] example for relative risk

Study#	Deaths on RX	N (Rx)	Deaths on control	N (Control)
1	2	26	1	26
2	3	23	2	13
3	27	163	69	212
4	13	558	15	533
5	24	76	23	74
6	3	154	1	75
7	1	75	2	74
8	0	50	1	50
9	1	20	1	20

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00019

and Excel software do all calculations for you automatically if you enter the tabular data analogous to Table 1.

7.2. Illustration for a difference in means or proportions

For each study in the conceptual universe, we project the difference in its totals if all patients received treatment less than if all patients were controls as the difference in means (or proportions) multiplied by the total sample size (treatment + control). The target population projection adds these up for all studies in the universe and divides this total by the total number of patients in the universe of all conceptually completed studies. The estimate is simply the corresponding value in our sample. If you refer to the difference in means data from the second example in the users' guide, second study, you will note that the experimental group had a sample mean of −3.0 in 42 patients while the control group had a sample mean of −2.5 in 51 patients. This makes the projected mean difference of −0.5 (experimental minus control) in 93 patients for a projected total of −0.5*93 = −46.5. The Users' guide and Excel software do all calculations for you automatically if you enter the tabular data analogous to this example in the User's Guide.

8. How Equal Weighting Works

We do not advocate equal weighting, but it can give us important insight into the credibility of analyses that use mainstream weights. We use the same methods as the mainstream to calculate the estimate and standard error but use equal weights instead of mainstream weights.

9. How Statistical Inference is Done

For any form of meta-analysis, including the mainstream, to obtain point estimates, confidence intervals, and P-values, the following approximations are used: The standardized difference, the difference between the overall estimate of effect size and the true global mean effect size, divided by its standard error of the estimate is obtained.

a. The mainstream uses a standard normal approximation, although the package CMA now has an option to use a T-approximation with degrees of freedom equal to the number of studies being combined less one
b. The ratio estimation method uses a T-approximation with degrees of freedom equal to the number of studies being combined less two
c. The equally weighted method uses a T-approximation with degrees of freedom equal to the number of studies being combined less one.

More on these approximations will appear in the discussion.

10. Numerical Examples

We shall provide three illustrations, one for the primary published relative risk of an invasive intervention, one for the myocardial infarction data of Nissen and Wolski [3], and one from a submitted article that incorrectly reported one odds ratio. The correction did not affect within-study variance estimators, but dramatically impacted the weights, demonstrating that weights and effect size estimates, contrary to Assumption A5, cannot be presumed to be independent.

10.1. Example 1: Relative risk

Neto *et al*. [4] in a highly cited meta-analysis of randomized trials found a benefit in their invasive intervention over the control for their primary outcome, total mortality. Table 1 provides the published numerators and denominators for each of the contributing studies, while Table 2 provides the results (i) as published, (ii) doubling all numerators and denominators, (iii) equally weighted, and (iv) by the method of Shuster [1]. As of 11/2022, this Journal of the American Medical Association paper has been cited 877 times.

Table 2 yields surprising results. Intuitively, doubling all numerators and denominators which keep the study-by-study estimates (signals) the same, but would diminish the noise (standard errors) within each study by a factor of about 30%, should yield a more significant result. Why would the confidence interval for the overall estimate of effect size grow by 15% while losing the significant finding, with the *P*-value becoming 0.15 instead of 0.013? This is indeed a red flag that will be clarified in the discussion. Neither the equally weighted nor the Ratio estimate produces definitive results on efficacy. In this case, this published result affected public health policy based on an off-label use of statistical methodology.

10.2. Example 2: Rosiglitazone and increased myocardial infarction risk

In their publication, Nissen and Wolski [3] used a fixed-effects method, even though the combined trials were highly diverse in terms of control groups, eligibility, duration and dose of treatment, and duration of follow-up. They used odds ratios instead of relative risk, the preferred metric. When event rates are low, the distinction is minor. Table 3 contrasts the results of mainstream methods, the published result of Nissen and Wolski [3], with those of Shuster [1], for relative risk. The Nissen and Wolski published that confidence interval excludes the neutral value of 1.00 but includes clinically insignificant values close to 1.00. Had ratio methods been available, a full ban on rosiglitazone might have occurred in 2007, thanks to the fact that the confidence interval includes only clinically significant increased risk for rosiglitazone. Although sales dropped from over $2 billion in 2007 and beyond, a large volume of sales continued for years afterward. As late as 2010, annual sales totaled almost $700 million. Several other nations did not ban the drug until 2010 or 2011. To further confuse the situation in 2007, Diamond and Kaul [5] published a non-significant mainstream analysis which may have slowed the decline at the additional human cost of cardiac events. The Nissen and Wolski [3] New England Journal of Medicine publication is one of the most cited meta-analysis reports, with 5908 citations as of 11/2022.

10.3. Example 3: From a peer-review of a submission to a major medical journal

The crux of this six-study observational example is that a peer-reviewer discovered that the odds ratio estimate in one of the

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00019

Table 2. Results for data in Table 1

Method	Estimated relative risk RX: control (95% CI)	P-value: two-sided	Ratio of 95% confidence lengths method: Inv Var
Mainstream weights (Published)	0.71 (0.55, 0.93)	0.013	1.00
Double numerators and denominators	0.78 (0.56, 1.09)	0.15	1.15
Equally weighted	0.82 (0.54–1.26)	0.33	1.38
Ratio (Survey sampling)	0.70 (0.44, 1.11)	0.11	1.49

Table 3. Nissen and Wolski re-analysis for myocardial infarction relative risk for rosiglitazone

Method	Estimated relative risk RX: control (95% CI)	*P*-value: two-sided	Ratio of 95% confidence lengths method: Inv Var
Mainstream Weights (RR)	1.28 (0.94, 1.75)	0.12	1.00
Ratio (Survey Sampling) (RR)	1.41 (1.14, 1.75)	0.0026	0.82
Nissen and Wolski (OR)	1.43 (1.03–1.98)	0.032	1.03

studies was wrong, and the actual odds ratio was 1/reported odds ratio. The generic data are given in Table 4 below. The reported estimated odds ratio of Study 4 was 0.78 when in fact it was 1.28. This occurred in the largest study in the meta-analysis (62% of the subjects) and pushed its estimated odds ratio from near the center of the original meta-analysis to close to being the largest estimated odds ratio. This resulted in a substantial increase in the between-study variance estimate. According to Assumption A1, this came from a single "draw" from the urn that affected the between-study variance estimation. Contrary to Assumption A5, the impact of the effect size change upon the weights was dramatic: Under the original scenario, the weight for this study was 23.3%. Under the corrected data, it dropped to 19.7%, and weights for the other five studies also changed. Note that equal weighting would assign 16.7% weight to each of the six studies. The change of one effect size estimate altered its weight by 3.6% or about half of the way from its original weight to equal weights. Therefore, the value of the study mean effect sizes drawn from the urn (A1) impacts the between-study variance estimate, and hence, Assumption A5 cannot be trusted. Note also that sample size weights can be vastly different from mainstream weights (study 4 had 62% of patients, but 19.7% weight for the mainstream).

Table 4. Generic data from submitted article to a major journal

Study	Group A events #Yes/#No (Odds)	Group B events #Yes/#No (Odds)	Estimated odds ratio (calculated)
1	14/225 (0.062)	245/1599 (0.153)	0.41
2	46/489 (0.094)	453/2570 (0.176)	0.53
3	90/551 (0.163)	625/2355 (0.265)	0.62
4	594/2204 (0.270)	3198/15218 (0.210)	1.28
5	42/342 (0.123)	97/806 (0.120)	1.02
6	22/277 (0.079)	107/1872 (0.057)	1.38

11. Discussion

Despite 48 years of practice, the mainstream method for weighted random effects meta-analysis should not be used in the future. "Bayes" methods also have some of the same issues (sample sizes are random variables not constants, and associations between sample size and effect size will produce bias).

11.1. Assumptions underlying inferences for the three methods

(a) For the standardized difference, mainstream methods rely on a "normal distribution" that in addition to Assumptions A1-A5, presumes that the number of studies is large enough to utilize the standard normal distribution. (b) The ratio method relies on the single assumption that the number of studies is large enough to apply its large sample T-distribution, with degrees of freedom equal to the number of studies less two, to its standardized difference. Within-study approximations are not relevant. Studies with zero events on one or both arms are included. Continuity corrections are unnecessary and never made. (c) The equally weighted method relies on the single assumption that the number of studies is large enough to apply its T-approximation, with degrees of freedom equal to the number of studies less one, to its standardized difference. Within-study approximations are not relevant.

11.2. Assumptions behind the ratio method

There are no assumptions except for (b) above. Shuster *et al*. [6] vetted the approximation for relative risks, when the number of studies ranged from 5 to 20, with nearly 40,000 diverse scenarios, each replicated 100,000 times. The coverage of the 95% confidence intervals was consistently close to 95%. However, the corresponding coverage using the less conservative normal approximation was generally well below 95%. This should be a warning that the mainstream coverage of their purported 95% confidence intervals is suspect when the number of studies being combined is in the 5–20 range. The vetting of differences in means and proportions is more difficult and needs independent funding with supercomputers to properly vet. For these studies, a limitation is needed in any paper with fewer than 20 studies.

The first two numerical examples demonstrate the dangers of relying upon the mainstream methods. The first is counterintuitive while the second illustrates that estimation bias in the mainstream is a real threat to getting a conclusive result.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00019

Shuster [1] reported on a small sample of 32 highly cited past meta-analyses that used mainstream methods for relative risk and found major disparities in eight (25%). It is fortunate that this is not higher, but this is not good enough for trust in mainstream methods.

An analysis of the 31 of these studies reported in Shuster *et al*. [6] and Shuster and Walker [7] (the 32nd study's reanalysis had a few studies added but trended as the 31 we analyzed) also dispel the one remaining scientific as opposed to traditional reasons for using the mainstream: The mainstream might produce on average narrower confidence intervals. If you analyze the natural logs of the ratios of the lengths of the confidence intervals (the traditional way to analyze non-negative ratios) and treat the studies as a random sample of highly cited meta-analyses, we obtain an estimate of the population ratio of widths (Mainstream: Shuster) of 1.10 (Mainstream wider) with 95% confidence interval from 0.93 to 1.29. The mainstream plausible mean in the total population of such potential reanalysis ranges from slightly shorter to substantially longer.

Further, due to mainstream proponents' concerns about its normal approximation, newer versions of CMA have added a t-option (degrees of freedom number of studies less one) that can be used instead of the normal approximation. When we replaced the normal with the t, the new mainstream methods were significantly less accurate than the survey-based method. For our sample of 31 meta-analyses, in the log scale, the mainstream averaged 30% wider than the survey-based methods, with 95% confidence interval from 9% wider to 54% wider. This is yet another strong reason not to use the mainstream.

Note that diagnostic test information, such as Cochran's Q, I^2, and Egger's test for selection bias, as described in Borenstein *et al*. [8] is not relevant to the validity of Shuster [1].

Shuster *et al*. [6], with a substantial number of simulations, found that when the target population relative risks in the two universes were the same (ratio and equal weighting), the ratio method had an average confidence interval length reduction of about 10% compared to equal weighting.

11.3. The mainstream's moving target

Suppose we have a sequence of meta-analyses where the urns described for obtaining the true study-specific effect sizes (Assumption A1) are identical, but each member of the sequence has within-study variances of 90% of the previous member of the sequence. Under the mainstream model, all of these meta-analyses have the same true effect sizes, namely the unweighted mean effect size in the urn. The true mainstream variance of the global effect size estimate is the sum of its between-study variance (Same for all members in the urn) and the within-study variance (which will shrink toward zero as you get later into the sequence). Thus, the mainstream estimates will become closer and closer to the unweighted estimator as we get further into the sequence. You therefore cannot rule out an artifact of where you might be in the sequence for any meta-analysis where the qualitative conclusions of the mainstream differ from the unweighted (one significant and one not). The Neto *et al*. [4] example is one case of this, but this is a very common occurrence. Note that if Assumption A4 is false, every weighted combination is estimating a different overall target population mean, and the mainstream analysis of the sequence will push the overall estimate toward targeting the unweighted mean in the urn. The key question when looking at the difference between the mainstream point estimate and the unweighted point estimate is whether it is simply sampling error or is it bias induced by failure of Assumption A5 (that the presumption that weights and effect sizes are uncorrelated is false). There is no way to be sufficiently certain, as there is no adequately powered statistical test that can prove the lack of such a correlation.

Note that the phenomenon of seeing two sets of data with the same signals but noise level of the second reduced by a common factor and turning the result from significant to not significant cannot occur in the common statistical methods: t-tests, analysis of variance or covariance, regression, logistic regression, frequency tables, or Cox regression (survival analysis).

11.4. Recommendations for meta-analysis of clinical trials with tabular data

(1) Use random effects rather than fixed effects; (2) With fewer than five studies, do a Systematic Review, not a formal meta-analysis, since the large sample distribution of the estimates should not be trusted; (3) with 5-20 studies, issue a limitation that the number of studies is small with a caveat on successful vetting for relative risk; (4) use Shuster [1] until new methods become available; (5) if you have individual patient data, note that the off-label implications for the mainstream for tabular data may or may not apply to individual patient data. Shuster [1] can still work if any of Assumptions A2, A4, and A5 are used in the individual patient analysis, potentially endangering its evidence base; (6) a biostatistics group with access to supercomputers should conduct large simulations along the lines of Shuster *et al*. [6] for the robustness of the T-approximation of the survey sampling method for differences in means or proportions; (7) a parallel width of confidence interval comparison on mainstream versus survey sampling should be done, and (8) if you have published a meta-analysis that had a substantive influence on public health policy, consider conducting an equal weighted analysis on the log of the relative risks or differences in means or proportions to see if this new analysis supports your original conclusions. If they do not, consider writing a report, using the recommended methods of Shuster [1].

12. Conclusion

Based on a reasonable fear gleaned from examples 1 and 2, the continued use of the mainstream methods is threatening to public health interests. In example 1, despite the published mainstream inference, there is no evidence that a widely used invasive intervention is effective. Example 2 had the survey sampling method been available and utilized, and the use of rosiglitazone in Type II diabetes would likely have been eliminated far earlier than what occurred, saving a substantial number of cardiac events from happening. Other similar misjudgments stemming from the mainstream methods are all but certain to occur in the future.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00019

Biostatisticians accept the fact that an unlucky dataset can yield misleading results, but they cannot accept misleading results caused by the use of off-label statistical methodology.

Acknowledgments

The author wishes to thank Editor, Dr. Michal Heger for his critically important assistance in the write-up.

Funding

This paper was entirely self-funded. The author has personal licenses for all software used.

Conflicts of Interest

None.

References

[1] Shuster JJ. Meta-Analysis 2020: A Dire Alert and a Fix. Biostat Biom Open Access J 2021;10:73-8.

[2] Borenstein M. Common Mistakes in Meta-Analysis and How to Avoid Them. Englewood, NJ: Biostat Inc.; 2019.

[3] Nissen SE, Wolski K. Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes. N Engl J Med 2007;356:2457-71.

[4] Neto AS, Cardosa SO, Manetta JA, Pereira VG, Esposito DC, de Oliveira Prado Pasqualucci M, *et al*. Association Between Use of Lung-Protective Ventilation with Lower Tidal Volumes and Clinical Outcomes Among Patients Without Acute Respiratory Distress Syndrome: A Meta-Analysis. JAMA 2012;308:1651-9.

[5] Diamond GA, Kaul S. Rosiglitazone and Cardiovascular Risk. N Engl J Med 2007;357:938-9.

[6] Shuster JJ, Guo JD, Skyler JS. Meta-Analysis of Safety for Low Event-Rate Binomial Trials. Res Synth Methods 2012;3:30-50.

[7] Shuster JJ, Walker MA. Low-Event-Rate Meta-Analyses of Clinical Trials: Implementing Good Practices. Stat Med 2016;35:2467-78.

[8] Borenstein M, Hedges LV, Rothstein HR, Higgins JP. Introduction to Meta-Analysis. New York, NY: John Wiley and Sons; 2009.

[9] Shuster JJ. Nonparametric Optimality of the Sample Mean and Sample Variance. Am Stat 1982;36:176-8.

APPENDIX

(A) How to obtain free software:

To obtain free quality assured software to help you analyze a collection of randomized clinical trials, download, and save the three files from the Website

https://biostat.ufl.edu/research/faculty-developed-software/

There is a user-friendly User's Guide and two Excel templates, one for relative risk and one for differences in means and proportions. There are two real worked examples that guide you through the data input and interpretation of the results.

(B) This part of the appendix is for readers with some statistical training:

Quantification of the randomness of the between-study variance, a key component of the weights

Suppose we look at the true between-study variance in the urn (Assumption A1) denoted by σ^2 and suppose "an informer" had the true study-specific effect sizes for the completed studies in the meta-analysis. Suppose she denotes the unweighted sample variance of two independent repetitions of these true study-specific effect sizes by S_j^2 (j = 1,2) and provides us only with this value. Absent additional extraneous information, S_j^2 (j = 1,2) are each optimal estimates of its between-study variance (minimum variance unbiased) per Shuster [9]. It is superior to (less random) than the mainstream estimate of σ^2. It follows from Assumptions A1 and A2 that (M-1) S_j^2/σ^2 are independent and have Chi-square distributions with degrees of freedom (M-1) where M is the number of studies being combined. The ratio $F = S_2^2/S_1^2$ has a central F-distribution with degrees of freedom M-1 for both the numerator and denominator.

A meta-analysis of eight studies would have a 61% chance that the larger of the two sample variances would be at least 50% larger than the smaller. The between-study variance is therefore a seriously random variable making the mainstream weights, which rely heavily on the between-study variance, seriously random variables. The mainstream reliance on the weights being near constants is not supportable.

Why the ratio estimates are expected to perform well in their target population

The relative risk and difference of means and proportions are calculated as the ratio of sample means. Both the numerator and denominator are optimal (i.e., nonparametric minimum variance unbiased estimators per Shuster [9]) for their corresponding population parameter.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00019

Journal of Clinical and Translational Research 2023; 9(4): 253-260

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

Association between carotid and coronary atherosclerotic plaque morphology: a virtual histology intravascular ultrasound study

Dace Sondore[1], Kārlis Trušinskis[1,2], Matīss Linde[2]*, Ieva Briede[1,3], Inga Narbute[1,3], Sanda Jēgere[1,3], Kārlis Griķis[1,3], Kārlis Štrenge[1,3], Andrejs Ērglis[1,3]

[1]Latvian Centre of Cardiology, Pauls Stradins Clinical University Hospital, Riga, Latvia, [2]Department of Internal Diseases, Faculty of Medicine, Riga Stradins University, Riga, Latvia, [3]Department of Cardiology and Cardiosurgery, Faculty of Medicine, University of Latvia, Riga, Latvia.

ARTICLE INFO

Article history:
Received: February 14, 2023
Revised: April 22, 2023
Accepted: May 22, 2023
Published online: July 12, 2023

Keywords:
Atherosclerosis
Virtual histology
Percutaneous coronary intervention
Carotid artery stenting

**Corresponding authors:*
Matīss Linde
Faculty of Medicine, Riga Stradins University, Riga, Latvia.
Tel: +371 28141749
Email: matiss.linde@rsu.lv

ABSTRACT

Background and Aim: Atherosclerosis is considered to be a systemic disease; however, evidence exists on the heterogeneous nature of atherosclerotic disease. To date, continuous research seeks to determine the morphological differences between carotid and coronary artery disease. This study aimed to evaluate the relationship of morphological characteristics assessed by virtual histology intravascular ultrasound (VH-IVUS) between carotid and coronary plaque composition among patients with and without a history of cerebrovascular events.

Methods: This study was a single-center prospective study (n = 100; age 69.6 ± 8.4). All patients were scheduled for carotid or coronary artery stenting and underwent VH-IVUS examination of the carotid and coronary arteries before intervention.

Results: There was a modest, but statistically significant correlation between the carotid and coronary necrotic core ([NC] r = 0.46, P < 0.01), fibrofatty ([FF] r = 0.38, P < 0.01), dense calcium (r = 0.56, P < 0.01), and fibrous (r = 0.42, P < 0.01) plaque composition. The high amount of NC was detected in both arteries of the carotid artery stenting (CAS) group with higher proportion in the coronary artery (20.2% ± 9.4% vs. 22.7% ± 6.8%, P = 0.02). More fibrolipid content was observed in carotid plaque compared to coronary (19.6% ± 9.9% vs. 12.2% ± 8.1%, P < 0.01). Patients with a history of cerebrovascular events had a numerically greater proportion of necrotic tissue in the carotid artery compared to asymptomatic and symptomatic CAS group patients (23.5% ± 10.7% vs. 18.9% ± 8.2% and 18.7% ± 9.5%, P = 0.11).

Conclusion: The percentage of all analyzed plaque components was moderately correlated between coronary and carotid artery plaques. Nevertheless, the proportion of NC plaque tissue was greater in the coronary arteries, while the carotid arteries showed more %FF atherosclerotic lesions. CAS group patients with a history of cerebrovascular events had a tendency of greater proportion of necrotic tissue in analyzed carotid plaques compared to others in the CAS group.

Relevance for Patients: In this study, we found that patients with a history of cerebrovascular event had a tendency of increased NC content in culprit lesion of carotid artery. Complementary use of non-invasive and invasive imaging modalities allows to detect high-risk atherosclerotic plaques and adjust treatment strategy.

1. Introduction

Atherosclerosis is a chronic systemic inflammatory disease affecting the arterial wall throughout the human body. Common clinical manifestations are ischemic cardiovascular events, such as cerebrovascular accidents and myocardial infarctions, which are the results of atherosclerotic changes in the extracranial carotid and coronary arteries. Associations

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00030

between carotid atherosclerotic changes and the extent and severity of coronary artery disease (CAD) are well-known [1-4]. Virtual histology (VH) is an established technique that allows for the in vivo assessment of plaque composition [5]. As such, VH intravascular ultrasound (VH-IVUS) provides information about plaque features, such as necrotic core (NC) tissue, which among others characterize the so-called vulnerable plaque [6]. VH-IVUS allows to assess coronary plaque composition and to detect thin cap fibroatheroma which, along with the degree of plaque burden, is predictors of adverse outcome [7,8].

The aim of the present study was to evaluate the relationship of morphological characteristics assessed by VH-IVUS between carotid and coronary plaque composition among patients with a history of cerebrovascular accidents and subjects without a prior adverse event.

2. Study Design

This was a single-center and cross-sectional study performed at the Latvian Center of Cardiology, Pauls Stradins Clinical University Hospital. Study participants were consecutive patients referred to the center with symptoms of ischemia (cerebral or cardiac) for invasive diagnosis of artery disease. After coronary/carotid artery angiography, patients were scheduled for percutaneous coronary intervention (PCI) or carotid artery stenting (CAS). Based on this qualification, all patients were divided into two groups: The CAS and PCI group. Indications for this procedure in the CAS group were stenosis ≥60% in the ipsilateral carotid artery in patients with current symptoms, a history of cerebrovascular event, or hemodynamically significant stenosis (≥75%) in asymptomatic patients. In the PCI group, patients with hemodynamically significant coronary artery narrowing were scheduled for PCI. All patients had atherosclerotic lesion with <50% narrowing in other vascular bed (non-culprit) and no other lesions requiring revascularization. Both, the culprit and non-culprit lesion, were selected for VH-IVUS analysis. The study included 78 patients who underwent CAS and 22 patients who underwent PCI. Patients in the CAS group were categorized in asymptomatic, symptomatic, and history of cerebrovascular event subgroups. PCI group was divided into stable angina and asymptomatic patients. The study was approved by the Local Ethics Committee, and all subjects provided informed written consent.

2.1. VH

All patients underwent VH-IVUS examination of coronary and carotid plaques. Under fluoroscopy, an IVUS catheter (Eagle Eye™; *Volcano Therapeutics Inc.*; *CA*, *USA*) was positioned in the carotid artery and then in the coronary artery. The IVUS catheter was pulled back at a continuous speed of 0.5 mm/s from the distal part of the carotid or coronary artery. The length of the pullback segment varied according to plaque length. The pullback was initiated 10–20 mm distal to the plaque and terminated 10–20 mm proximal to the plaque. In the carotid artery, if a cerebral protection device was used, the IVUS pullback catheter was positioned on the cerebral protection device wire. For CAS procedures, but not for carotid artery imaging with IVUS in the absence of intervention [9], distal protection devices (Spider, EV3; Filter wire EZ, Boston Scientific; Emboshield, Abbott) were used.

2.2. Statistical analysis

Continuous data are represented as mean ± standard deviation, whereas categorical data were expressed as numbers or frequencies of occurrence. For the categorical data analysis, we used a Chi-square test. For the continuous data, we first assessed normality using visual inspection of a normal probability plot and a formal test, the Shapiro–Wilk test. Differences between carotid and coronary VH-IVUS findings were analyzed using a non-parametric related samples test, the Wilcoxon signed-rank test, and a parametric paired samples *t*-test. Pearson's correlations were used to assess the relation between carotid and coronary atherosclerotic plaque components. Two-way analysis of variance test was used to test for the main effects of each independent variable, as well as the interaction effect between them. All statistical analyses were performed using the IBM SPSS software package (IBM SPSS Statistics 22.0, Chicago, IL, USA). $P < 0.01$ was considered to indicate statistical significance.

3. Results

A total of 100 patients were enrolled in this study, and VH-IVUS examination was performed on the carotid and coronary arteries. In the CAS group, the mean age between asymptomatic, symptomatic, and with a history of cerebrovascular events patients varied from 67 to 69 years, and predominantly more men were present in each group. Furthermore, the SYNTAX score for each CAS subgroup is shown in Table 1. Overall, baseline clinical characteristics of the study population are shown in Tables 1 and 2.

Table 3 summarizes the VH-IVUS characteristics of the analyzed carotid and coronary artery plaques in the CAS group. Carotid arteries were larger according to the analyzed VH-IVUS parameters and had a higher plaque burden and necrotic tissue percentage in analyzed arteries compared to coronary arteries. The analyzed segment length did not differ between the coronary and carotid arteries (17.1 ± 9.9 mm and 15.8 ± 8.3 mm, $P = 0.26$).

Procedural and VH-IVUS characteristics of the PCI group are shown in Table 4.

VH comparison between carotid and coronary arteries is shown in Table 5. The analyzed segment of the carotid artery had a lower percentage of necrotic tissue and calcium, but a significantly higher percentage of fibrolipids. The percentage of fibrotic tissue did not differ among the analyzed vascular beds.

The analyzed segments showed moderately positive, statistically significant correlations among the analyzed vascular beds – NC (r = 0.46, $P < 0.01$), fibrotic tissue (r = 0.42, $P < 0.01$), fibro-fatty tissue ([FF] r = 0.37, $P < 0.01$), and dense calcium tissue ([DC] r = 0.56, $P < 0.01$). The correlation between carotid and coronary plaque composition of all analyzed lesions is displayed in Figure 1.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00030

Table 1. Baseline characteristics of CAS group

Characteristic	Asymptomatic, *n*=31	Symptomatic, *n*=32	H/O cerebrovascular event, *n*=15	*P*-value
Age, years	69.26±8.36	69.63±8.64	67.27±9.15	0.17
Body mass index, kg/m^2	27.05±3.61	27.55±5.64	28.37±3.81	0.44
Gender				
Male, *n* (%)	23 (74.2)	18 (56.3)	10 (66.7)	0.32
Female, *n* (%)	8 (25.8)	14 (43.8)	5 (33.3)	0.32
Analyzed artery				
Left internal carotid artery, *n* (%)	15 (48.4)	17 (53.1)	9 (60.0)	0.76
Right internal carotid artery, *n* (%)	16 (51.6)	15 (46.1)	6 (40.0)	0.76
Left anterior descending coronary artery, *n* (%)	16 (51.6)	13 (40.6)	8 (53.3)	0.60
Left circumflex coronary artery, *n* (%)	8 (25.8)	6 (18.8)	2 (13.3)	0.59
Right coronary artery, *n* (%)	7 (22.6)	13 (40.6)	5 (33.3)	0.31
SYNTAX score	17.66±4.42	16.32±5.42	19.51±3.68	0.78
Smoking				
Active smoker, *n* (%)	2 (6.5)	3 (9.4)	4 (26.7)	0.12
Former smoker, *n* (%)	9 (29.0)	8 (25.0)	4 (26.7)	0.94
Non-smokers, *n* (%)	11 (35.5)	17 (53.1)	5 (33.3)	0.27
Diabetes mellitus, *n* (%)	7 (22.6)	9 (28.1)	5 (33.3)	0.73
Dyslipidemia, *n* (%)	30 (96.8)	31 (96.9)	15 (100)	0.78
Arterial hypertension, *n* (%)	26 (83.9)	27 (84.4)	15 (100)	0.25
Myocardial infarction, *n* (%)	12 (38.7)	11 (34.4)	6 (40.0)	0.91
PCI in history, *n* (%)	12 (38.7)	12 (37.5)	4 (26.7)	0.71
Peripheral arterial disease, *n* (%)	15 (48.4)	8 (25.0)	7 (46.7)	0.12
Familial history of cardiovascular disease, *n* (%)	7 (22.6)	3 (9.4)	5 (33.3)	0.15
Atrial fibrillation				
Permanent, (%)	6 (19.4)	6 (18.8)	3 (20.0)	0.99
Paroxysmal, (%)	1 (3.2)	4 (12.5)	4 (26.7)	0.06
None, (%)	23 (74.2)	22 (68.8)	8 (53.3)	0.36

CAS: carotid artery stenting, PCI: Percutaneous coronary intervention

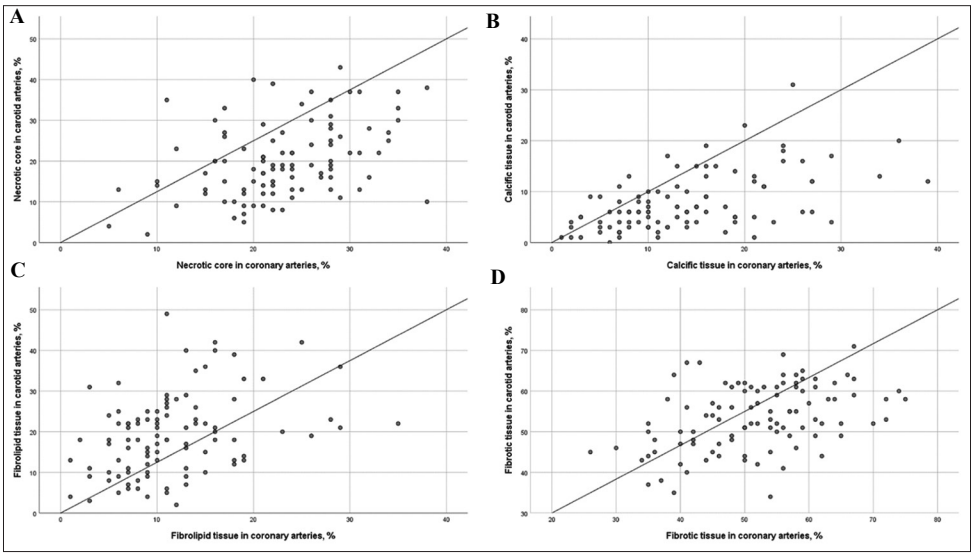


Figure 1. Correlation between carotid and coronary plaque composition in study patients. Correlation of: A) necrotic cores, B) calcified tissue, C) fibrolipidic tissue, and D) fibrotic tissue among carotid and coronary arteries.

Patients with history of cerebrovascular event had a tendency toward a higher percentage of necrotic tissue and a lower percentage of fibrolipids in carotid artery plaques. Fibrotic tissue and calcium in the carotid artery did not differ between patients

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00030

Table 2. Baseline characteristics of PCI group

Characteristic	Stable angina (*n*=16)	Asymptomatic (*n*=6)	*P*-value
Age, years	69.47±8.31	67.27±8.60	0.42
Body mass index, kg/m^2	27.59±3.83	28.13±3.95	0.50
Gender			
Male, *n* (%)	8 (50.0)	1 (16.7)	0.21
Female, *n* (%)	8 (50.0)	5 (83.3)	0.21
Analyzed artery			
Left internal carotid artery, *n* (%)	12 (75.0)	3 (50.0)	0.31
Right internal carotid artery, *n* (%)	3 (18.8)	3 (50.0)	0.35
Left anterior descending coronary artery, *n* (%)	5 (31.3)	2 (33.3)	0.40
Left circumflex coronary artery, *n* (%)	3 (18.8)	1 (16.7)	0.88
Right coronary artery, *n* (%)	7 (43.8)	3 (50.0)	0.64
Smoking			
Active smoker, *n* (%)	3 (18.8)	0 (0.0)	0.25
Former smoker, *n* (%)	1 (6.3)	1 (16.7)	0.45
Non-smokers, *n* (%)	12 (75.0)	4 (66.7)	16
Diabetes mellitus, *n* (%)	2 (12.5)	2 (33.3)	0.26
Dyslipidemia, *n* (%)	16 (100)	6 (100)	n/a
Arterial hypertension, *n* (%)	15 (93.8)	6 (100)	0.53
Acute cerebral event in history, *n* (%)	7 (43.8)	1 (16.7)	0.24
Myocardial infarction, *n* (%)	4 (25.0)	3 (50.0)	0.26
PCI in history, *n* (%)	3 (18.8)	3 (50.0)	0.14
Peripheral arterial disease, *n* (%)	7 (43.8)	2 (33.3)	0.66
Familial history of cardiovascular disease, *n* (%)	5 (31.3)	1 (16.7)	0.48
Atrial fibrillation			
Permanent, (%)	2 (12.5)	0 (0.0)	0.36
None, (%)	14 (100)	6 (100)	0.36

PCI: Percutaneous coronary intervention

Table 3. Procedural and VH-IVUS characteristics of CAS group

Characteristic	Carotid artery (*n*=78)	Coronary artery (*n*=78)	*P*-value
CAS artery			
Left internal carotid artery, *n* (%)	41 (52.6)	-	-
Right internal carotid artery, *n* (%)	37 (47.4)	-	-
Analyzed artery			
Left anterior descending coronary artery, *n* (%)	-	37 (47.4)	-
Left circumflex coronary artery, *n* (%)	-	16 (20.5)	-
Right coronary artery, *n* (%)	-	25 (32.1)	-
Minimal lumen diameter, mm, mean±SD	2.5±0.6	2.1±0.4	<0.01
Mean lumen diameter, mm, mean±SD	4.3±0.8	3.0±0.5	<0.01
Minimal lumen area, mm, mean±SD	7.9±4.0	4.8±1.7	<0.01
Minimal vessel diameter, mm, mean±SD	5.8±0.7	3.8±0.7	<0.01
Mean vessel diameter, mm, mean±SD	7.2±0.7	4.6±0.7	0.36
Vessel volume, mm^3, mean±SD	715.2±476.6	258.8±159.4	<0.01
Plaque volume, mm^3, mean±SD	439.8±306.4	153.2±105.7	0.22
Plaque burden, %, mean±SD	61.8±10.4	56.4±8.5	<0.01
Segment length, mm, mean±SD	17.1±9.9	15.8±8.3	0.26
Necrotic tissue, %, mean±SD	20.2±9.4	22.7±6.8	0.02
Fibrotic tissue, %, mean±SD	52.5±7.7	51.7±10.3	0.37
Fibrolipids, %, mean±SD	19.6±9.9	12.2±8.1	<0.01
Calcium, %, mean±SD	8.24±5.9	13.7±6.8	<0.01

CAS: carotid artery stenting, VH-IVUS: Virtual histology intravascular ultrasound, SD: Standard deviation

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00030

Table 4. Procedural and VH-IVUS characteristics of PCI group

Characteristic	Carotid artery (*n*=22)	Coronary artery (*n*=22)	*P*-value
PCI artery			
Left anterior descending coronary artery, *n* (%)	-	8 (36.4)	-
Left circumflex coronary artery, *n* (%)	-	4 (18.2)	-
Right coronary artery, *n* (%)	-	10 (45.5)	-
Analyzed artery			
Left internal carotid artery, *n* (%)	6 (27.3)	-	-
Right internal carotid artery, *n* (%)	16 (72.7)	-	-
Minimal lumen diameter, mm, mean±SD	3.6±0.6	2.0±0.7	<0.01
Mean lumen diameter, mm, mean±SD	5.0±0.5	2.9±0.1	<0.01
Minimal lumen area, mm, mean±SD	13.8±3.9	4.2±0.3	<0.01
Minimal vessel diameter, mm, mean±SD	6.3±1.0	3.8±0.1	<0.01
Mean vessel diameter, mm, mean±SD	7.4±0.8	4.5±0.2	<0.01
Vessel volume, mm^3, mean±SD	716.7±447.9	309.6±37.7	<0.01
Plaque volume, mm^3, mean±SD	390.3±268.5	182.3±24.0	<0.01
Plaque burden, %, mean±SD	53.4±7.7	57.8±5.8	0.03
Segment length, mm, mean±SD	15.4±8.3	18.1±1.5	0.17
Necrotic tissue, %, mean±SD	18.6±9.4	22.5±1.9	0.05
Fibrotic tissue, %, mean±SD	56.5±8.0	50.6±2.1	0.07
Fibrolipids, %, mean±SD	19.3±10.2	13.9±2.7	0.36
Calcium, %, mean±SD	5.6±4.4	13.6±1.8	<0.01

VH-IVUS: Virtual histology intravascular ultrasound, PCI: Percutaneous coronary intervention, SD: Standard deviation

Table 5. Virtual histology comparison between carotid and coronary arteries

Characteristic	Carotid artery (*n*=100)	Coronary artery (*n*=100)	*P*-value
Necrotic tissue, %, mean±SD	19.8±9.4	22.6±7.3	<0.01
Fibrotic tissue, %, mean±SD	53.4±8.0	51.7±10.3	0.09
Fibrolipids, %, mean±SD	19.6±9.6	12.5±9.1	<0.01
Calcium, %, mean±SD	7.7±5.6	13.6±8.2	<0.01

SD: Standard deviation

with and without a history of cerebrovascular event. In contrast, the percentage of all analyzed tissue types in the coronary arteries did not differ among patients with and without cerebrovascular event histories (Table 6).

Two patients had post-procedural acute ipsilateral cerebrovascular events. Their carotid plaque composition was similar to others in the CAS group. Non-ipsilateral post-procedural intrahospital cerebrovascular events were not observed in our study population.

Representative VH-IVUS case analysis is shown in Figure 2, in which increased necrotic tissue amount in carotid and coronary artery of 74.0% and 48.8%, respectively, was identified by VH-IVUS analysis of culprit lesion in the left internal carotid artery and non-culprit lesion in the mid-third of the right coronary artery. VH-IVUS data revealed higher amount of DC tissue in the analyzed coronary segment compared to carotid plaque (13.7% vs. 9.0%).

4. Discussion

This study investigated the association between carotid and coronary plaque types as assessed by VH-IVUS in patients with multiple risk factors who were scheduled for carotid or coronary artery stenting. The findings of the present study were as follows: (1) the carotid plaque composition was significantly correlated with coronary plaque phenotype; (2) the percentage of NC and calcium tissue was significantly higher in the coronary arteries, whereas the amount of FF plaque component was greater in the carotid arteries; and (3) patients with a history of cerebrovascular events had a tendency of greater proportion of NCs in their carotid arteries compared to others in CAS group.

Inflammation in atherosclerotic plaque produces systemic effects, thus, active, ongoing inflammation at one vascular bed could enhance inflammation in another. A NC in VH corresponds to tissue areas in which the extracellular matrix is lacking (total loss of supporting collagen) and has been replaced by dead cells and lipid-rich cellular debris [10,11], thus, tissue characterized as the NC is the most vulnerable part of atherosclerotic lesions with ongoing inflammation. We observed that all analyzed tissue types by VH-IVUS, including NCs, were correlated between carotid and coronary vascular beds. Together with knowledge from previous studies, VH-IVUS analysis confirms that patients with more vulnerable plaque patterns at one site are at higher risk of having more vulnerable plaques at another site. However, different coronary and carotid impacts of shear stress, artery size, and static force are of great importance for the pattern of atherosclerosis in different vascular beds. Despite the correlation of the percentage of all analyzed tissue types in coronary and carotid artery plaques, we observed that the percentage of NCs was higher in the coronary artery (Table 3). Moreover, we observed that coronary arteries were more calcified, but carotid arteries

Table 6. Virtual histology analysis of CAS group

Characteristic	Asymptomatic (*n*=31)	Symptomatic (*n*=32)	H/O cerebrovascular event (*n*=15)	*P*-value
Carotid artery				
Necrotic tissue, %, mean±SD	18.9±8.2	18.7±9.5	23.5±10.7	0.11
Fibrotic tissue, %, mean±SD	54.4±7.3	52.1±8.4	53.3±8.6	0.47
Fibrolipids, %, mean±SD	20.3±9.4	20.9±10.5	15.9±9.7	0.15
Calcium, %, mean±SD	7.3±4.9	8.4±7.4	7.4±3.9	0.69
Coronary artery				
Necrotic tissue, %, mean±SD	22.8±7.9	21.8±6.8	23.1±6.9	0.74
Fibrotic tissue, %, mean±SD	50.4±10.3	52.6±11.2	53.0±9.2	0.53
Fibrolipids, %, mean±SD	12.8±9.8	12.8±9.4	11.6±7.3	0.87
Calcium, %, mean±SD	14.5±8.4	12.9±8.5	12.8±7.2	0.62

CAS: carotid artery stenting, SD: Standard deviation

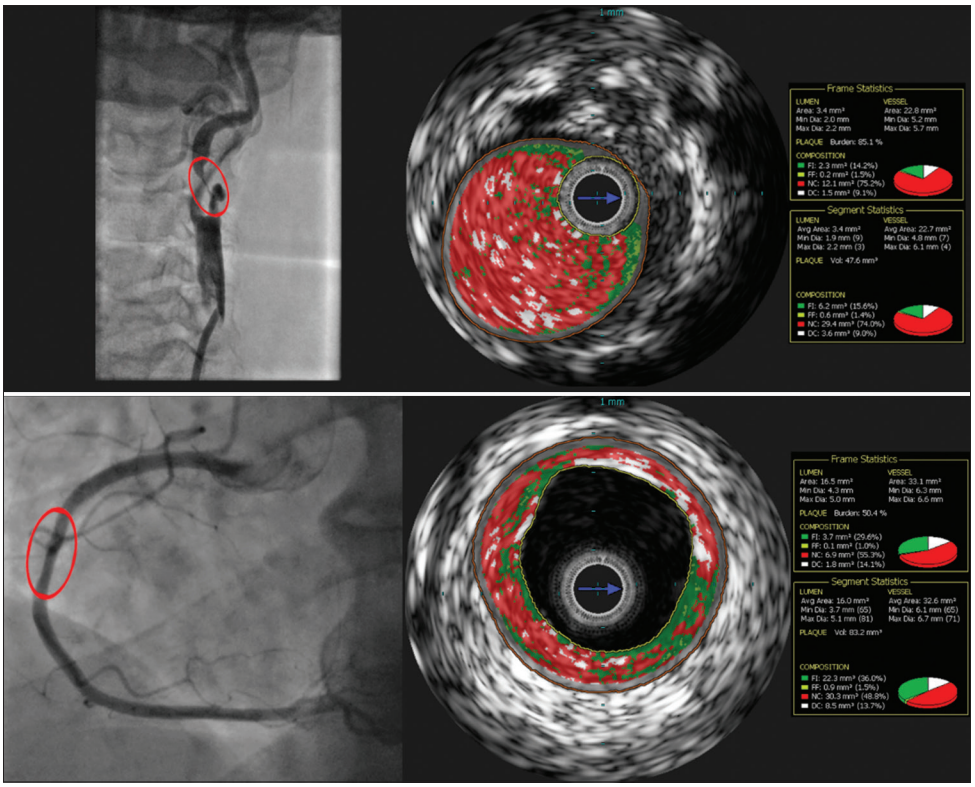


Figure 2. Representative case example of VH-IVUS analysis in the CAS patient.

contained more fibrolipids. A study by Samady *et al*. showed that wall shear stress alters the progression and composition of coronary atherosclerotic plaques [12]. Similarly, Eshtehardi *et al*. showed that low wall shear stress was associated with a NC and calcium coronary plaque tissue, emphasizing the impact of local hemodynamic conditions on plaque phenotype and atherosclerotic changes in the vessel wall [13]. As such, although atherosclerosis is considered a systemic inflammatory disease, its manifestations are heterogeneous within the same individual due to differences in arterial geometry, shear stress, and static forces.

Analyzing atherosclerotic plaques among patients with and without cerebrovascular events, we observed a tendency toward a higher NC in carotid plaques in patients with previous cerebrovascular events, but no difference between symptomatic and asymptomatic patients. Interestingly, two animal studies found no correlation between the VH-IVUS size of the NC and histological findings [14,15]. However, in the CAPITAL study, 15 patients underwent VH-IVUS examination of carotid plaque immediately followed by carotid endarterectomy. The results showed a strong correlation between VH-IVUS carotid plaque characterization and the true histological examination of the plaque, particularly in "vulnerable" plaque types [16]. These differences can be explained by different tissue types in animal and human atherosclerotic lesions. In the clinical setting, a series of 25 patients undergoing CAS reported a strong association between total plaque volume and FF volume on VH IVUS and

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00030

the quantity of atherosclerotic debris obtained on retrieval of the distal embolic protection device [17]. Winston *et al*. showed an association between periprocedural cerebrovascular events and plaque composition by VH-IVUS [18]. We compared patients with and without cerebrovascular complications (cerebral infarction and transitory ischemic attack <24 h) after CAS and found no difference in carotid tissue composition by VH-IVUS. However, our study was not designed and does not have sufficient power to draw conclusions regarding post-procedural cerebrovascular complications. Similar carotid plaque composition in symptomatic and asymptomatic patients by VH-IVUS analysis highlights a need for further research to detect high-risk plaques combining invasive and non-invasive imaging modalities.

In our study, we found a higher percentage of calcium in the coronary compared to carotid arteries. A global registry of more than 3000 patients undergoing coronary VH IVUS showed that NC and DC content increases with age, were more common in men than in women, and were positively associated with serum low-density lipoprotein cholesterol, diabetes, and hypertension [19]. Pooled data from two clinical trials showed coronary calcium increase in patients receiving long-term high-dose statin therapy without impact on adverse cardiovascular events suggesting plaque stabilization [20]. All patients in our study received guideline-directed lipid-lowering therapy which could explain higher amount of DC in analyzed coronary arteries. In line with published evidence, our data confirm the systemic characteristics of atherosclerosis and the similar carotid and coronary plaque composition, but differences in calcium distribution between arterial territories.

The main limitation of our study was lack of blinded core laboratory VH-IVUS analysis. Furthermore, patients included in the CAS group were in greater proportion compared to the PCI group.

5. Conclusion

The percentage of all analyzed plaque components was correlated among coronary and carotid artery plaques. Nevertheless, coronary arteries contained more NCs and calcium, while carotid arteries had a higher percentage of fibrolipidic tissue. Patients with cerebrovascular events had a tendency toward a higher percentage of NCs in the carotid plaques.

Acknowledgments

None.

Funding

None.

Conflicts of Interest

None.

References

[1] Shenouda R, Vancheri S, Bassi EM, Nicoll R, Sobhi M, El Sharkawy E, *et al*. The Relationship between Carotid and Coronary Calcification in Patients with Coronary Artery Disease. Clin Physiol Funct Imaging 2021;41:271-80.

[2] Cohen GI, Aboufakher R, Bess R, Frank J, Othman M, Doan D, *et al*. Relationship between Carotid Disease on Ultrasound and Coronary Disease on CT Angiography. JACC Cardiovasc Imaging 2013;6:1160-7.

[3] Den Ruijter HM, Peters SA, Anderson TJ, Britton AR, Dekker JM, Eijkemans MJ, *et al*. Common Carotid Intima-Media Thickness Measurements in Cardiovascular Risk Prediction: A Meta-Analysis. JAMA 2012;308:796-803.

[4] Amato M, Montorsi P, Ravani A, Oldani E, Galli S, Ravagnani PM, *et al*. Carotid Intima-Media Thickness by B-Mode Ultrasound as Surrogate of Coronary Atherosclerosis: Correlation with Quantitative Coronary Angiography and Coronary Intravascular Ultrasound Findings. Eur Heart J 2007;28:2094-101.

[5] Nasu K, Tsuchikane E, Katoh O, Vince DG, Virmani R, Surmely JF, *et al*. Accuracy of *In Vivo* Coronary Plaque Morphology Assessment: A Validation Study of *In Vivo* Virtual Histology Compared with *In Vitro* Histopathology. J Am Coll Cardiol 2006;47:2405-12.

[6] Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the Vulnerable Plaque. J Am Coll Cardiol 2006;47(8 Suppl):C13-8.

[7] Stone GW, Maehara A, Lansky AJ, de Bruyne B, Cristea E, Mintz GS, *et al*. A Prospective Natural-History Study of Coronary Atherosclerosis. N Engl J Med 2011;364:226-35.

[8] Cheng JM, Garcia-Garcia HM, de Boer SP, Kardys I, Heo JH, Akkerhuis KM, *et al*. *In Vivo* Detection of High-Risk Coronary Plaques by Radiofrequency Intravascular Ultrasound and Cardiovascular Outcome: Results of the ATHEROREMO-IVUS Study. Eur Heart J 2014;35:639-47.

[9] Musialek P, Pieniazek P, Tracz W, Tekieli L, Przewlocki T, Kablak-Ziembicka A, *et al*. Safety of Embolic Protection Device-Assisted and Unprotected Intravascular Ultrasound in Evaluating Carotid Artery Atherosclerotic Lesions. Med Sci Monit 2012;18:MT7-18.

[10] Thim T, Hagensen MK, Bentzon JF, Falk E. From Vulnerable Plaque to Atherothrombosis. J Intern Med 2008;263:506-16.

[11] Stefanadis C, Antoniou CK, Tsiachris D, Pietri P. Coronary Atherosclerotic Vulnerable Plaque: Current Perspectives. J Am Heart Assoc 2017;6:e005543

[12] Samady H, Eshtehardi P, McDaniel MC, Suo J, Dhawan SS, Maynard C, *et al*. Coronary Artery Wall Shear Stress is Associated with Progression and Transformation of Atherosclerotic Plaque and Arterial Remodeling in Patients with Coronary Artery Disease. Circulation 2011;124:779-88.

[13] Eshtehardi P, McDaniel MC, Suo J, Dhawan SS, Timmins LH, Binongo JN, *et al*. Association of Coronary Wall Shear Stress with Atherosclerotic Plaque Burden, Composition, and Distribution in Patients with Coronary Artery Disease. J Am Heart Assoc 2012;1:e002543.

[14] Granada JF, Wallace-Bradley D, Win HK, Alviar CL, Builes A, Lev EI, *et al*. *In Vivo* Plaque Characterization using Intravascular Ultrasound-Virtual Histology in a Porcine Model of Complex Coronary Lesions. Arterioscler Thromb Vasc Biol 2007;27:387-93.

[15] Thim T, Hagensen MK, Wallace-Bradley D, Granada JF, Kaluza GL, Drouet L, *et al*. Unreliable Assessment of Necrotic Core by Virtual Histology Intravascular Ultrasound in Porcine Coronary Artery Disease. Circ Cardiovasc Imaging 2010;3:384-91.

[16] Diethrich EB, Margolis MP, Reid DB, Burke A, Ramaiah V, Rodriguez-Lopez JA, *et al*. Virtual Histology Intravascular Ultrasound Assessment of Carotid Artery Disease: The Carotid Artery Plaque Virtual Histology Evaluation (CAPITAL) Study. J Endovasc Ther 2007;14:676-86.

[17] Matsumoto S, Nakahara I, Higashi T, Iwamuro Y, Watanabe Y, Takezawa M, *et al*. Fibro-Fatty Volume of Culprit Lesions in Virtual Histology Intravascular Ultrasound is Associated with the Amount of Debris during Carotid Artery Stenting. Cerebrovasc Dis 2010;29: 468-75.

[18] Winston B, Siewiorek GM, Finol EA, Wholey M. A Case Series of Virtual Histology Intravascular Ultrasound in Carotid Artery Stenting. Vasc Dis Manage 2011;8:E144-50.

[19] Philipp S, Böse D, Wijns W, Marso SP, SchwartzRS, König A, *et al*. Do Systemic Risk Factors Impact Invasive Findings from Virtual Histology? Insights from the International Virtual Histology Registry. Eur Heart J 2010;31:196-202.

[20] Henein M, Granåsen G, Wiklund U, Schmermund A, Guerci A, Erbel R, *et al*. High Dose and Long-Term Statin Therapy Accelerate Coronary Artery Calcification. Int J Cardiol 2015;184:581-6.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00030

Journal of Clinical and Translational Research 2023; 9(4): 261-264

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

CASE REPORT

A rare case of asymptomatic Paget's disease of the skull in a 60-year-old Asian female

Muhammad Ali[1]*, Omama Farooq[1], Zahra Rafique[1], Hajrah Farooq[1], Fazeelat Iftikhar[1], Muqadsa Malik[2]

[1]Department of Internal Medicine, Islamabad Medical Complex, NESCOM Hospital, Islamabad, Pakistan, [2]Department of Internal Medicine, Fauji Foundation Medical College, Rawalpindi, Pakistan

ARTICLE INFO

Article history:
Received: November 05, 2022
Revised: January 22, 2023
Accepted: May 30, 2023
Published online: July 26, 2023

Keywords:
Alkaline phosphatase
Paget's disease of bone
Osteitis deformans
Tc-99m-methylene diphosphonate
Bisphosphonates

**Corresponding authors:*
Muhammad Ali
Department of Internal Medicine, Islamabad Medical Complex, Nescom Hospital, Islamabad, Pakistan.
Tel: +92 345 5240908
Email: muhammad.ali.janjua93@gmail.com

ABSTRACT

Background and Aim: Paget's disease of the bone refers to a chronic cumulative disorder characterized by enhanced osteoclastic function followed by a secondary surge in osteoblastic activity. The condition can manifest as a polyostotic or monostotic bone disease with most patients having an asymptomatic presentation, although some may complain of pain localized to the affected bone while others express symptoms of nerve compression. A pagetic bone is predisposed to develop pathological fractures, bony deformities, and a rare yet detrimental transformation into osteosarcoma. Detection is often accidental when performing radiographic tests for other indications or when elevated blood levels of alkaline phosphatase (ALP) are detected. Treatment with third-generation bisphosphonates is helpful in preventing further bone resorption and, additionally, reduces bony pains that are believed to be caused by excessive metabolic activity. Here, we present a case of a middle-aged asymptomatic female with elevated serum ALP levels up to 1537 IU/L (reference range 40–150 U/L) during her pre-operative evaluation for elective cholecystectomy. ^{99m}Tc-methylene diphosphonate bone scintigraphy revealed diffuse uptake in the skull and, hence, was diagnosed as a case of isolated Paget's disease of the skull.

Relevance for Patients: The rarity of this disease in Southeast-Asians, its uncontrived detection, and the isolated skull involvement, imparts high clinical relevance on this case. Early detection and management of this disease can help prevent the development of life-threatening complications in affected patients, hence decreasing the morbidity.

1. Introduction

Paget's disease of bone (PDB), also known as osteitis deformans, is a chronic skeletal bone disorder characterized by focal areas of excessive disorganized bone remodeling, targeting any bone throughout the skeleton. This disease primarily affects the axial skeleton, where pelvis is the most common site of involvement, followed by femur, lumbar spine, skull, and tibia [1]. Most patients are asymptomatic and often present with an incidental finding of high serum alkaline phosphatase (ALP) levels during routine blood examination [2]. Diagnostic modalities commonly utilized include X-ray, biochemical analysis of serum total ALP, computed tomography (CT) scan, bone scintigraphy, and magnetic resonance imaging [3].

2. Case Presentation

A 60-year-old Asian female with a known case of hypertension and treated hepatitis C virus infection presented to the surgical outpatient department for her scheduled pre-operative

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00186

visit for elective cholecystectomy. She was referred to the medical department for further workup after she was found to have isolated elevated ALP levels of up to 1537 IU/L (reference range 40–150 IU/L). According to the patient she only had a history of mild, non-radiating, non-pulsatile, bilateral headache that occurred on and off several times during the day and that receded without intervention. The patient had no history of deafness, tinnitus, dental malocclusion, fractures, fatigue, or generalized body weakness. She gave no associated history of any abnormal bone enlargement, including her head, with no increase in hat size in recent years.

On inquiring further, she reported a history of dark-colored urine for the past 20 days with no history of pruritis, clay-colored stool, or weight loss. Examination revealed mild frontal bossing, whereas the rest of her general physical and systemic examination were within normal limits. Biochemical tests revealed an ALP 1537 IU/L with normal total bilirubin, aspartate transaminase, and alanine transaminase levels. Ultrasound of the abdomen showed multiple calculi in the lumen of the gallbladder with a normal caliber of the common bile duct. On further investigations, serum calcium and phosphorus levels were within normal limits.

Gamma GT was within normal limits. Urine for Bence–Jones protein came back negative and serum protein electrophoresis was within normal limits, hence ruling out multiple myeloma. X-ray of the skull revealed widening of diploid space and diffusely scattered multiple sclerotic lesions in skull vault, giving a cotton wool appearance (Figure 1). The patient was then referred to the rheumatology outpatient department for further investigations and treatment plan. A CT scan of the brain without contrast showed expanded bones of the skull with multiple sclerotic and lytic lesions (Figure 2).

Bone scintigraphy confirmed the diagnosis of PDB involving the skull bone only, showing diffusely increased uptake in the skull bone (Figure 3). The patient was commenced on third-generation intravenous bisphosphonate (15-min infusion of 5 mg zoledronic acid). The patient was advised to follow-up after 3 months at the rheumatology outpatient department to determine ALP levels. On follow-up after 3 months, repeat ALP test showed markedly reduced levels down to 250 IU/L. The patient was also advised a repeat bone scintigraphy scan on the same visit but was lost to follow-up for subsequent visits.

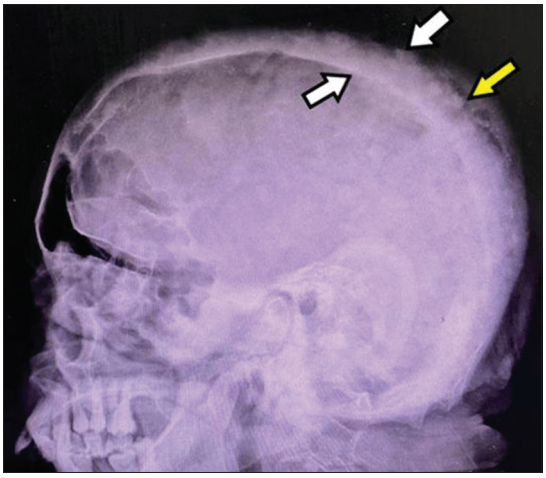


Figure 1. X-ray (lateral view) of the skull showing widening of diploid space (white arrows) and diffusely scattered multiple sclerotic lesions in skull vault (yellow arrow), yielding a cotton wool appearance.

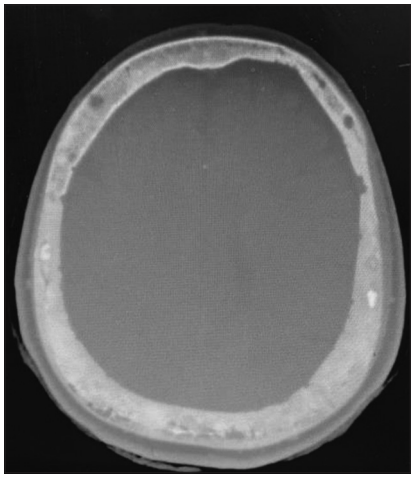


Figure 2. Computed tomography scan of the brain without contrast, showing expanded bones of the skull with multiple sclerotic and lytic lesions.

3. Discussion

PDB is ranked second to osteoporosis as the most common bone disorder, exhibiting higher prevalence throughout Western Europe, America, and Australia, but very low incidence among Asians and Africans. Diagnosis before the age of 40 is rare. The key abnormality in this disease points to aberrant osteoclastic bone resorption accompanied by marrow fibrosis, leading to increased bone vascularity and enhanced osteoblastic activity. A mosaic pattern of bone and lamellar tissue with enlarged osteoclast-containing distinct nuclear inclusion bodies is revealed histologically [4].

The etiology of PDB remains unclear, whereas multiple studies suggest an association with genetic mutations, environmental factors, and paramyxoviral infection [5]. Clinically, the usual presentation is asymptomatic but may present with bone pains, nerve entrapment, bony deformities, and increased propensity to develop fractures. Clinical symptoms differ with the disease location; skull involvement usually causes increase in the hat size, along with headaches or deafness, whereas when base of the skull is affected, it can lead to hydrocephalus, platybasia, and basilar invagination [3,6]. High-output cardiac failure, although very uncommon, is often precipitated due to enhanced vascularity of the affected bone, whereas osteosarcoma, although rare, is usually the feared complication in a pre-existing pagetic bone [7].

According to the US Endocrine Society clinical practice guidelines, effective management protocols have been formulated for patients suspected of PDB, where the first-line investigation remains to be plain X-ray of the affected regions, followed by ^{99m}Tc-methylene diphosphonate (MDP) bone scan, after the

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00186

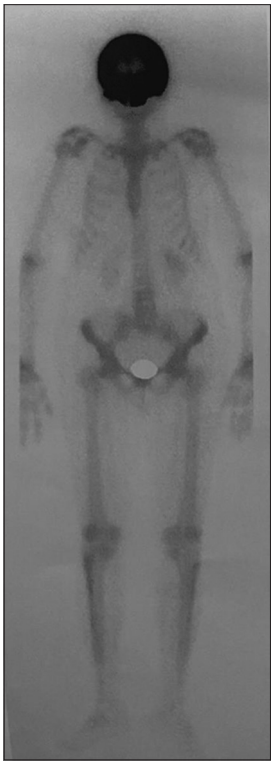


Figure 3. Anterior view of the bone scan showing diffuse uptake of the technetium-99 m in the skull bones.

confirmation of diagnosis through plain X-ray. Biochemical test such as an elevated serum ALP levels aid in evaluating the activity and progression of PDB and indicate the response to treatment. Furthermore, recent studies have estimated a significant rise in serum osteopontin levels as a marker for active PDB [8]. X-ray of the skull may manifest radiolucent areas around the suture lines typically in the occipital and frontal bones, an enlarged diploic space, and focal osteosclerosis giving cotton wool appearance [3,8,9]. Nonetheless, the putative imaging modality is radionuclide bone scintigraphy (MDP), exhibiting substantially increased uptake in the affected bone. Cranial CT scans generally show evidence of cranial vault thickening [10]. Isolated skull involvement may include neurological complications such as hearing loss, headache, bone pains, tinnitus, basilar impression, and rarely cranial nerve deficits [9].

Pagetic bone pains are generally the key indicator for treatment and may recede or disappear with medical therapy. The mainstay of treatment is the use of intravenous third-generation bisphosphonates such as zoledronic acid for active PDB which works by impeding bone resorption through osteoclastic activity inhibition, expending an anti-cancerous effect by enhancing the proliferation of gamma delta T cells [11], and alleviates bone pains that are believed to be caused by excessive metabolic activity. Zoledronate (zoledronic acid) is administered as 5 mg given as a single infusion over 15 min. Retreatment is mostly not required as a single infusion balances the bone turnover for about 5 years [8,9,12]. Serum ALP levels are monitored to assess the disease status and are measured at an interval of 3 months for the first 6 months after initial treatment, and then after 6 months subsequently [11].

4. Conclusion

In conclusion, isolated raised serum alkaline phosphatase levels should always be further investigated to rule out asymptomatic patients. Management includes treatment with third-generation bisphosphonates and pain control. Effective and time-sensitive management can impart a huge difference in the outcome of Paget's disease.

Acknowledgments

None.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Conflicts of Interest

The authors have no conflicts of interest associated with the material presented in this paper.

Ethics Approval and Consent to Participate

Informed consent was obtained from the patient by the authors.

Consent to Participation

Consent was given by the patient to publish her data and images in this paper.

References

[1] Corral-Gudino L, Tan AJ, Del Pino-Montes J, Ralston SH. Bisphosphonates for Paget's Disease of Bone in Adults. Cochrane Database Syst Rev 2017;12:CD004956.

[2] Rianon NJ, Des Bordes JK. Paget Disease of Bone for Primary Care. Am Fam Physician 2020;102:224-8.

[3] Theodorou DJ, Theodorou SJ, Kakitsubata Y. Imaging of Paget Disease of Bone and Its Musculoskeletal Complications: Review. AJR Am J Roentgenol 2011;196:S64-75.

[4] Caicedo AI, Escobar VE, Coy Urrea VA, Charry JS, Loaiza JH. Sporadic Paget's Disease of the Bone. Case Series and Literature Review. Rev Colomb Reumatol (English Ed) 2020;27:103-11.

[5] Karunakaran K, Murugesan P, Rajeshwar G, Babu S. Paget's Disease of the Mandible. J Oral Maxillofac Pathol 2012;16:107-9.

[6] Bone HG. Nonmalignant Complications of Paget's Disease. J Bone Miner Res 2006;21:P64-8.

[7] Shankar YU, Misra SR, Vineet DA, Baskaran P. Paget Disease of Bone: A Classic Case Report. Contemp Clin

Dent 2013;4:227-30.

[8] Singer FR, Bone HG 3rd, Hosking DJ, Lyles KW, Murad MH, Reid IR, *et al*. Lyles, Paget's Disease of Bone: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab2014;99:4408-22.

[9] Aransiola CO, Ipadeola A. Asymptomatic Paget's Disease of Bone in a 62-Year-Old Nigerian Man: Three Years Post-Alendronate Therapy. Endocrinol Diabetes Metab Case Rep 2016;2016:160005.

[10] Michou L, Brown JP. Emerging Strategies and Therapies for Treatment of Paget's Disease of Bone. Drug Des Devel Ther 2011;5:225-39.

[11] Durgia H, Sahoo J, Kamalanathan S, Palui R, Kumar R, Halanaik D, *et al*. Response to Zoledronic Acid in Patients with Active Paget's Disease of Bone: A Retrospective Study. Indian J Endocrinol Metab 2019;23:117-21.

[12] De Castro GR, Buss ZD, Rosa JS, Facchin BM, Fröde TS. Evaluation of Bone Metabolism Biomarkers in Paget's Disease of Bone. Cureus 2019;11:e4791.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00186

Journal of Clinical and Translational Research 2023; 9(4): 265-271

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

Quality of life assessment in the first episode of acute coronary syndrome

Smitha Pernaje Seetharam[1], Vinutha Shankar[2]*, Kaviraja Udupa[3], Raveesha Anjanappa[4], Niranjan Reddy[5]

[1]Department of Physiology, Sapthagiri Institute of Medical Sciences and Research Centre, Bengaluru, Karnataka, India, [2]Department of Physiology, Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar, Karnataka, India, [3]Department of Neurophysiology, NIMHANS, Bengaluru, Karnataka, India, [4]Department of General Medicine, R. L. Jalappa Hospital, Sri Devaraj Urs Academy of Higher Education and Research Kolar, Karnataka, India, [5]Department of Cardiology, R. L. Jalappa Hospital, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India

ARTICLE INFO

Article history:
Received: March 10, 2023
Revised: April 19, 2023
Accepted: June 26, 2023
Published: July 26, 2023

Keywords:
Myocardial infarction
Sudden death
Quality of life
Acute coronary syndrome
Health survey

**Corresponding authors:*
Vinutha Shankar
Department of Physiology, Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar - 563 103, Karnataka, India.
Tel: +91-9845065374/+91-08152 243003.
Fax: +91 (8152) 243008.
Email: vinutha.shankar@gmail.com

Abstract

Background: Assessment of health-related quality of life (HRQoL) is an important measure of a patient's recovery after an illness. However, HRQoL among acute coronary syndrome (ACS) survivors has not been extensively studied following cardiac management.
Aim: The purpose of this study was to assess the quality of life (QoL) among ACS patients who have undergone percutaneous coronary intervention (PCI).
Methods: This cohort study included 145 consecutive male ACS patients between March 2021 and May 2022. Of these patients, 138 (mean age 54.3 ± 10.7 years) completed the QoL assessment using the short form-12 (SF-12) health survey questionnaire. Seventy (51%) of them presented with ST-segment elevation myocardial infarction (STEMI), 18 (13%) had non-STEMI, 39 (28%) had evolved MI, and 11 (8%) had unstable angina. Recruited patients' QoL data were assessed at various time points post-PCI.
Results: At the end of the 12 months of follow-up, major clinical events (MCE) defined as death, sudden death, or re-acute myocardial infarction occurred in 54.9% of patients. Out of 7 MCE, four deaths and three re-AMIs had occurred. SF-12 physical component score was found to be significantly improved when compared to the mental component score, which seems to improve without reaching statistical significance over time. Among event-free ACS patients, we found a significant positive correlation between left ventricular ejection fraction and HRQoL.
Conclusion: Improvement in HRQoL (physical component) was seen among ACS patients post-PCI.
Relevance for Patients: QoL assessment outcomes should be considered in clinical settings, practice guidelines, and treatment modality post-PCI to improve QoL in post-ACS survivors.

1. Introduction

Acute coronary syndrome (ACS) is a term used to describe one of two conditions: A heart attack (myocardial infarction) or when a person suffering from severe chest pain (unstable angina). Myocardial infarction is further classified as ST-segment elevation myocardial infarction (STEMI) and non-STEMI (NSTEMI). India has the world's highest ACS burden as shown by a prospective registry study (CREATE) which generated data from ten regions and 50 cities in 89 centers [1]. Even though the advent of therapies has increased survival in ACS, the rates of event-free survival at 1 year and 2 years were 88 ± 60.3% for males and 83% for females, respectively ($P = 0.58$) [2]. In addition, persons who have ACS report significant physical and mental discomfort related to the condition and subsequent clinical management. Hence, there is a need to understand the effects of

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00049

ACS impact on the physical and mental health status of ACS survivors.

A self-administered generic tool short-form health survey (SF-36) has been used in angina, acute myocardial infarction (AMI), and heart failure. The SF-36 has been demonstrated to be a sensitive measure for identifying improvements in HRQoL following active intervention in individuals with recent AMI. The SF-12 and SF-8 are condensed forms of the questionnaire which are accessible for use and are quicker to complete [3].

Multiple studies have shown that QoL is better after PCI than it was before. Complete angina resolution was better with PCI, according to a meta-analysis of 14 randomized controlled trials comparing PCI to medical therapy in 7818 patients enrolled from 1987 to 2005. Furthermore, it has been observed that the SF-12, or condensed version of the SF-36, correlates favorably with the SF-36 summary scores in a variety of illnesses, including angina [4].

The reason for using the SF-12 health survey questionnaire was with only 12 items, health status could be assessed aptly when compared to the 36-item SF-36 [5]. The SF-12 questionnaire has been proven to be accurate in a variety of medical conditions as well as in the general population [6,7].

Further, not many studies have looked into the effect of comorbidities on the quality of life (QoL) measured post-PCI. Therefore, this study aimed to evaluate the QoL among ACS patients post-PCI using the SF-12 health survey questionnaire and to explore the effect of comorbidities on QoL.

2. Materials and Methods

2.1. Study setting

This cohort study was conducted at the Cardiology outpatient department, R.L. Jalappa Hospital, in association with the Department of Physiology, attached to Sri Devaraj Urs Medical College, Kolar, Karnataka, India.

2.2. Ethical consideration

Central Ethics Committee clearance was obtained (CEC No. SDUAHER/KLR/R & I/91/2021-22). Each participant provided written informed consent to participate in the study.

The sample size was calculated [8] using nMaster 2.0 software and was estimated with 0.9 as the statistical power and <0.05 as the significant *P*-value (Figure 1).

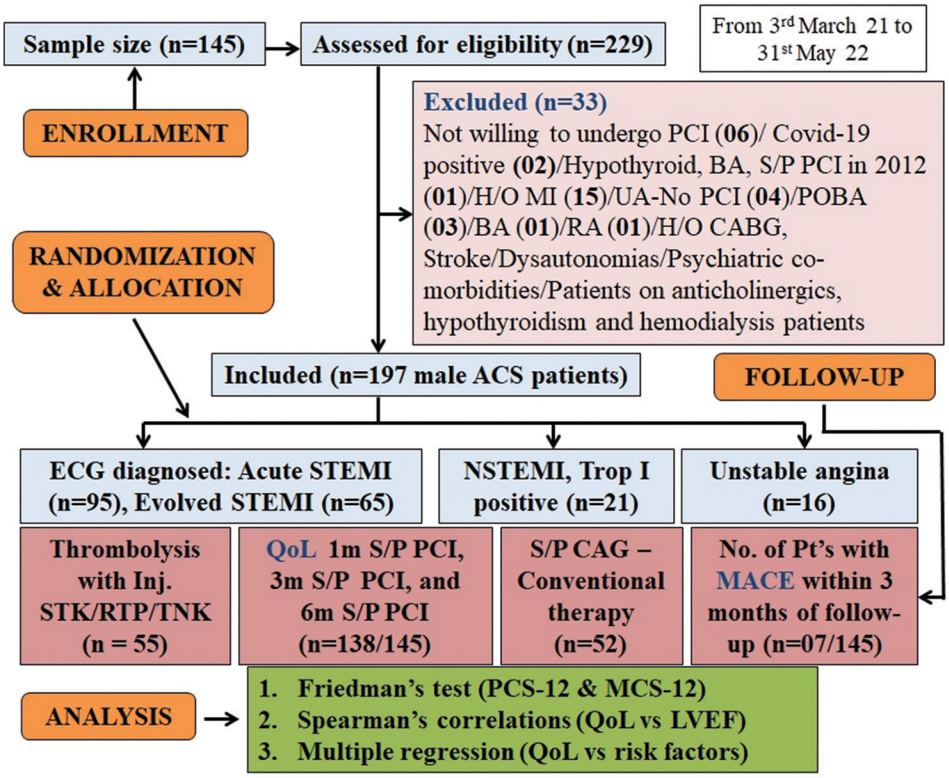


Figure 1. Study flow before and after PCI.
Abbreviations: PCI: Percutaneous coronary intervention; BA: Bronchial asthma; S/P PCI: Status post-PCI; MI: Myocardial infarction; UA: Unstable angina; POBA: Percutaneous old balloon angioplasty; RA: Rheumatoid arthritis; CABG: Coronary artery bypass graft; IHD: Ischemic heart disease; STEMI: ST elevation myocardial infarction; NSTEMI: Non-ST elevation MI; STK: Streptokinase; RTP: Reteplase; TNK: Tenecteplase; CAG: Coronary angiogram; MACE: Major adverse cardiac event; PCS: Physical component score; MCS: Mental component score; QoL: Quality of life; LVEF: Left ventricular ejection fraction.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00049

2.3. Study population

2.3.1. Study design

The study participants were chosen using a systematic random sampling technique.

2.3.2. Inclusion and exclusion criteria

One forty-five ACS (AHA/ACC/ESC classification) [9,10] male patients who have undergone PCI between March 2021 and May 2022 were included in the study. Among the enrolled, 62 patients had a first AMI (STEMI), 18 patients had NSTEMI, 44 had evolved MI, and 11 patients exhibited unstable angina. Of the 145 participants, seven had major clinical events (termed as death or re-AMI), providing a final sample of 138 individuals (95%) for the health survey. Smokers (cigarette/beedi) [11,12], tobacco chewers, and alcoholics [13] were also included in this study. Among the recruited, 26% of them were diabetics and 37% of them were hypertensive. Individuals were excluded if they were critically ill/mentally challenged. All the subjects went through reperfusion therapy using percutaneous coronary intervention (Primary/Elective PCI). Included participants were COVID-19 negative. Heart failure patients with preserved/mid-range EF (HFpEF or HFmrEF) [14] were considered.

2.4. Study design

We prospectively studied consecutive patients admitted to our intensive coronary care unit with the first episode of ACS, who underwent primary PCI of the culprit coronary vessel within 3–24 h of symptom onset in acute STEMI patients. Non-STEMI patients went through elective PCI within 24 h of symptom onset. Patients were also included if they had earlier failed thrombolysis (rescue PCI), as pointed out by the persistent ST-segment elevation. In case of evolved MI/unstable angina patients, symptom-to-door timings were 2 h to a month duration.

Patients with typical chest pain lasting ≥30 min who also had ST-segment elevation of ≥0.1 mV in ≥2 adjacent leads on the admission electrocardiogram (ECG) were diagnosed to have acute STEMI. Non-STEMI was associated with ST-segment depression of ≥0.1 mV in ≥2 contiguous leads on admission ECG, T wave inversion, with the troponin-I positive, and a typical chest pain lasting ≥30 min. Evolved MI was detected with the on and off symptoms from a few hours to a week, from hyperacute T waves to ST-segment elevation, and T wave inversion in ≥2 neighboring leads on the admission ECG. Unstable angina was identified with the on and off symptoms from a few hours to a month duration.

The demographic, anthropometric, clinical, and laboratory parameters obtained for each patient were: age, height, weight, several cardiovascular risk factors, infarction location, symptom-to-door timings, culprit coronary vessels (defined as the presence of diameter stenosis more than 50%), thrombolytic therapy, thrombolysis in myocardial infarction coronary flow grade, peak values of cardiac biomarkers (creatine kinase-myocardial band or troponin-I), and drug therapy.

Left ventricular ejection fraction was measured on admission, 1-month post-PCI/post-phase 2 cardiac rehabilitation, 3-month post-PCI, and 6-month post-PCI by 2-D echocardiography based on biplane Simpson's method.

2.5. Health-related QoL assessment

The patient's QoL was investigated using the SF-12 health survey questionnaire [6,8,15,16]. The SF-12 consists of eight health concepts representing physical functioning; role-limitations due to physical health problems, bodily pain, general health, energy/fatigue, social functioning, role-limitations due to emotional problems, and mental health (psychological distress or psychological well-being). The 12 questions in this instrument assessed health-related QoL (HRQoL) in the past 4 weeks, producing two different 0-100 scores, namely, physical component (PCS-12) and mental component (MCS-12) scores. The results of the SF-12 with a higher score indicated a better QoL. Thus, face-to-face interviews were conducted at cardiology OPD at various time points. Consequently, PCS-12 and MCS-12 scores were measured 1-month, 3-month, and 6-month post-PCI.

2.6. Data analysis

Descriptive statistics were calculated and expressed as percentages, mean, and standard deviation. Data were tested for normal distribution using the Kolmogorov–Smirnov test. Non-parametric tests were performed because the data did not show Gaussian distribution. Friedman's test was used to measure all the quantitative variables (PCS-12 and MCS-12) and was stated as the median. Furthermore, Spearman's correlation analysis was performed to ascertain the relationship between QoL scores and LVEF%. Then, multiple regression analysis was performed to explore the effect of confounding variables (such as smoking, tobacco chewing, alcohol consumption, Killip class, comorbidities, and drugs) on QoL. All the data were analyzed using IBM SPSS software for Windows (version 22.0; SPSS, Chicago, IL, USA).

3. Results

The study population consisted of 145 ACS patients (aged 54.3 ± 10.7 years) with LVEF of 44.8 ± 9.6%. Patients were on antiplatelets (100%); 96% of patients received antianginal therapy; 35% were treated with β-blockers; 98% of them were on anticoagulants; 9% were on vasodilators; 99% were on statins; 56% were on diuretics; 27% were on oral hypoglycemic agents/insulin; 7% were on angiotensin receptor blockers; 2% were on ACE inhibitors; and 7% were on calcium-channel blockers. The baseline characteristics of the study population (n = 138) are presented in Table 1.

QoL assessment was done among post-ACS survivors at various time points using Friedman's test. Since P = 0.011, we concluded that there was a significant improvement in the QoL scores during the follow-up (Table 2).

3.1. Correlation analysis

Spearman's correlation analysis was done to identify the likely correlation between QoL and left ventricular ejection fraction (%). All QoL parameters had significant correlations with the LVEF

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00049

Table 1. Baseline characteristics of the study population (n=138)

Characteristics	Mean±SD
Mean age (years)	54.3±10.7
BMI (Kg/m^2)	25.2±4.0
Killip classification	
Class I	118
Class II	01
Class III	05
Class IV	03
Clinical data	
Systolic blood pressure (mmHg)	128±24
Diastolic blood pressure (mmHg)	80±11
Heart rate (bpm)	82±13
Left ventricular ejection fraction (%)	45±10
Cardiovascular risk factors	
Diabetes mellitus, *n* (%)	36 (26)
Hypertension, *n* (%)	50 (37)
Smoking, *n* (%)	42 (31)
Alcohol consumption, *n* (%)	21 (15)
Tobacco chewing, *n* (%)	05 (4)
Coronary angiography and PCI data (*n*)	
Primary PCI	79
Rescue PCI	01
Elective PCI	58
TIMI flow grade III (post-PCI)	138

TIMI: Thrombolysis in myocardial infarction; PCI: Percutaneous coronary intervention

Table 2. Quality of life scores after percutaneous coronary intervention using Friedman's test

QoL scores	1-month post-PCI	3-month post-PCI	6-month post-PCI	Chi-square value	*P*-value
PCS-12	53.07	54.00	54.00	8.97	0.011*
MCS-12	55.96	57.00	56.00	3.43	0.180^{NS}

Values expressed as median; *Significance $P \leq 0.05$; NS: Not significant; QoL: Quality of life; PCS-12: Physical component score; MCS-12: Mental component score.

except the correlation between MCS-12 (3 months) and LVEF (3 months) with P = 0.068 showing suggestive significance, as shown in Table 3.

Spearman's correlation analysis was done to identify the likely correlation between QoL scores and age, and body mass index (BMI). One-month post-PCI PCS-12 and MCS-12 QoL scores had significant negative correlations with age suggesting a decline in QoL on aging. Besides, a positive correlation was observed between MCS-12 (1 month) and BMI with P = 0.022, as shown in Table 4.

3.2. Multivariate tests

Multivariate tests were executed to know the likely role of smoking, tobacco chewing, alcohol consumption, and Killip class on QoL parameters (Table 5). Mentioned predictors had no significant association with the QoL scores.

Multivariate tests were accomplished to know whether diabetes mellitus (DM) and hypertension (HTN) had got any effect on QoL parameters (Table 5). Both DM and HTN had no statistically significant association with any of the QoL scores.

Table 3. Spearman's correlation of QoL scores (PCS-12 and MCS-12) with left ventricular ejection fraction

QoL scores	Spearman's rho	LVEF (%) (1 month)	LVEF (%) (3 months)	LVEF (%) (6 months)
PCS 12 (1 month)	Correlation coefficient	0.57		
	P-value	0.000**		
PCS 12 (3 months)	Correlation coefficient		0.35	
	P-value		0.000**	
PCS 12 (6 months)	Correlation coefficient			0.31
	P-value			0.000**
MCS 12 (1 month)	Correlation coefficient	0.31		
	P-value	0.000**		
MCS 12 (3 months)	Correlation coefficient		0.16	
	P-value		0.068	
MCS 12 (6 months)	Correlation coefficient			0.25
	P-value			0.004**

*Moderately significant $P \leq 0.05$; **Strongly significant $P \leq 0.01$; NS: Not significant; QoL: Quality of life; PCS: Physical component score; MCS: Mental component score

Table 4. Spearman's correlation of QoL scores with age and body mass index

QoL scores	Spearman's rho	Age (years)	BMI (kg/m^2)
PCS 12 (1 month)	Correlation coefficient	−0.19	0.06
	P-value	0.029*	0.502^{NS}
MCS 12 (1 month)	Correlation coefficient	−0.19	0.20
	P-value	0.024*	0.022*

*Significance $P \leq 0.05$; NS: Not significant; BMI: Body mass index; QoL: Quality of life; PCS: Physical component score; MCS: Mental component score.

Table 5. Multivariate tests between smoking, tobacco chewing, alcohol consumption, Killip classification, hypertension, diabetes mellitus, and quality of life scores

Effect	Value	F	Hypothesis df	Error df	Sig.
Intercept	0.019	2074.35^b	3.00	122.00	0.000
Smoking	0.997	0.13^b	3.00	122.00	0.940^{NS}
Tobacco chewing	0.985	0.62^b	3.00	122.00	0.605^{NS}
Alcohol consumption	0.995	0.23^b	3.00	122.00	0.879^{NS}
Killip classification	0.899	1.10	12.00	323.07	0.357^{NS}
Intercept	0.003	14633.67^b	3.00	132.00	0.000
Diabetes mellitus	0.970	1.350^b	3.00	132.00	0.261^{NS}
Hypertension	0.983	0.742^b	3.00	132.00	0.529^{NS}

*Significance $P \leq 0.05$; ^{NS}Not significant; ^{b}Exact statistic

Multivariate tests were performed to know whether drugs have got any effect on QoL measures (Table 6). Various drugs had no statistically significant effect on any of the QoL scores. While, with the corrected model, β-blockers had a significant effect on the PCS-12 component of QoL scores with P = 0.042.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00049

Table 6. Multivariate tests between various medications and quality of life scores

Effect	Value	F	Hypothesis df	Error df	Sig.
Intercept	0.142	374.36[b]	2.00	124.00	0.000
Antianginal drugs	0.997	0.17[b]	2.00	124.00	0.846[NS]
Anticoagulants	0.992	0.49[b]	2.00	124.00	0.614[NS]
Thrombolytics	0.981	1.19[b]	2.00	124.00	0.308[NS]
β-blockers	0.967	2.09[b]	2.00	124.00	0.128[NS]
Vasodilators	0.978	1.40[b]	2.00	124.00	0.249[NS]
CCBs	0.996	0.26[b]	2.00	124.00	0.768[NS]
ACE inhibitors	0.986	0.89[b]	2.00	124.00	0.414[NS]
OHAs	0.992	0.49[b]	2.00	124.00	0.612[NS]
Statins	0.984	1.03[b]	2.00	124.00	0.360[NS]
Diuretics	0.996	0.24[b]	2.00	124.00	0.784[NS]

*Significance $P \leq 0.05$; [NS]Not significant; [b]Exact statistic; CCBs: Calcium channel blockers; ACE: Angiotensin-converting enzyme; OHA: Oral hypoglycemic agent

4. Discussion

The major finding of the present study is the significant increase in the PCS-12 component of QoL scores from 1 to 6 months post-PCI among ACS survivors. Results suggest that patients' QoL might have improved on successful revascularization with PCI in the physical domain when compared to their mental component scores.

A prospective cohort study by Seto *et al.* [17] included 1445 PCI patients. Wherein QoL was measured using SF-36 and the Seattle angina questionnaire (SAQ). QoL improved in 58 – 75% of PCI patients for different domains at 6 months. Another prospective cohort study by Wong *et al.* [18] included 78 PCI patients. QoL was assessed using SF-36 and SAQ. Statistically significant improvements in six out of eight SF-36 and five out of five SAQ domains at 1 and 3 months were observed in PCI patients. Yet, another prospective cohort study by Melberg *et al.* [19] enrolled 609 PCI patients, with significant improvement in QoL (measured using SF-36) in PCI patients at 6 months. The present study findings were in line with the literature, wherein QoL in the physical domain (PCS-12) improved significantly from 1-, 3-, and 6-month post-PCI among ACS patients.

Anchah *et al.* [20] recruited 112 patients with newly diagnosed ACS. The SF-36 questionnaire was used to obtain QoL data. Their physical and mental health summaries showed poorer results at baseline. Yet, these improved gradually and significantly over time. Our study enrolled 1-month post-PCI/post-phase 2 cardiac rehabilitation ACS patients, where we also observed a significant improvement in the physical component of QoL when compared to the non-significant improvement in the mental component of QoL over time.

In addition, in the present study, we found a strong positive correlation between LVEF with QoL scores (both in physical and mental domains) except for the association between MCS-12 at 3 months post-PCI and LVEF 3 months post-PCI. In contrast, in a study by Juenger *et al.* [21], among 205 patients with congestive heart failure and systolic dysfunction LVEF, duration of disease, and age showed no association with QoL.

In the present study, age had a significant negative correlation with the physical and mental components of QoL scores. Our study findings suggest that advanced age could have had a detrimental effect on QoL. Whereas, patients' age was not associated with any of the World Health Organization QoL-BREF domains scores [22]. In addition, advanced age, always drinking alcohol, a high-fat diet, and HTN affected the various domain-specific European QoL Five Dimension (EQ-5D) Five-level scale scores in coronary heart disease patients [23].

QoL in HFpEF was observed to be the poorest in patients who are young, obese, and have diabetes [24]. Our study included 138 heart failure patients with mid-range to preserved ejection fraction wherein QoL was the poorest on admission to hospital set-up which later improved after successful revascularization with PCI. Besides, BMI (Obese: ≥30 Kg/m^2; n = 10/138 ACS patients) had a significant positive correlation with the mental component of the QoL measure.

According to a review by Goldenberg *et al.* [25], a negative relationship between smoking and QoL exists even with secondhand smoke. Further, a Chinese study found that smoking had a negative correlation with the QoL. Smokers had an 11.65% lower average chance of having a higher QoL than non-smokers [26]. Conversely, predictors such as smoking, tobacco chewing, alcohol consumption, and Killip class had no significant association with the QoL scores in our study.

The present study results showed that both diabetes mellitus (DM) and HTN had no significant effect on any of the QoL scores. While in a study with 364 diabetic patients, the physical domain score was negatively associated with the duration of DM [22]. Besides, higher rates of DM significantly decreased EQ-5D index and visual analog scale scores [23].

Regarding outcomes (serious adverse events and major adverse cardiovascular events), the long-term risk of reinfarction during follow-up, QoL, and angina, additional information is needed to approve or reject the clinical effects of β-blockers on the outcomes in patients with or suspected of acute MI [27]. However, on regression analysis, β-blockers had a significant effect on the physical component of QoL in our study. Denoting the beneficial effect of β-blockers in improving the physical well-being of ACS patients post-PCI in the long run. In a meta-analysis, it was evident that β-blocker therapy does not alter QoL. Accordingly, clinicians could add β-blockers to traditional treatment without apprehensions of harming QoL in patients with congestive heart failure [28].

4.1. Study limitations

The nutritional status [29] of the ACS patients on admission and follow-up was not addressed in the present investigation. Since the nutrition status of the patients might have had a significant effect on QoL in the long run, this issue needs to be considered as the future direction of this study. In addition, the present study was a single-center cohort study with small sample size. Hence, multi-centric large-scale studies will be required for additional validation of the usefulness of QoL measurement post-PCI as

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00049

one of the prognostic evaluators for QoL among ACS survivors. Further, we analyzed the overall effect of various confounding factors on QoL post-PCI in patients with and without MCE. Since no significant association between confounding factors and QoL was observed, future studies could analyze the effect of risk factors (especially comorbidities such as HTN and diabetes mellitus) on QoL among patients who had MCE. The study population was heterogeneous in terms of clinical presentation, comorbidities, and pathophysiology. In accordance, QoL and ejection fraction would be different between patients with STEMI and unstable angina patients. Besides, if an unstable angina patient undergoes PCI for the culprit lesion, the patient would be relieved of any symptoms, and hence, their ejection fraction would be normal. Hence, future studies should focus further research on QoL post-acute MI/STEMI patients only.

5. Conclusions

The present study results revealed a significant increase in the physical component of QoL from 1 to 6 months post-PCI among ACS survivors. QoL scores correlated well with the echocardiographic measure of LV ejection fraction. Further, age had a significant negative effect on QoL. Therefore, QoL assessment outcomes should be considered in routine clinical practice and treatment modality post-PCI to improve QoL.

Acknowledgments

The authors appreciate the assistance of all the investigators who worked on this study and all the volunteers who offered their time to take part.

Funding

None.

Conflicts of Interest

The authors claim to have no conflicts of interest.

References

[1] Xavier D, Pais P, Devereaux PJ, Xie C, Prabhakaran D, Reddy KS, *et al.* CREATE Registry Investigators. Treatment and Outcomes of Acute Coronary Syndromes in India (CREATE): A Prospective Analysis of Registry Data. Lancet 2008;371:1435-42.

[2] Bounhoure JP, Farah B, Fajadet J, Marco J. Prognosis After Acute Coronary Syndrome. Lack of Difference According to Sex. Bull Acad Natl Med 2004;188:383-97; discussion 397-9.

[3] Coelho R, Prata J. Quality of life measures in acute coronary syndromes: The Evaluation of predictors in this field of research. In: Preedy VR, Watson RR, editors. Handbook of Disease Burdens and Quality of Life Measures. New York, NY: Springer; 2010. p. 3015-32.

[4] Blankenship JC, Marshall JJ, Pinto DS, Lange RA, Bates ER, Holper EM, *et al*; Society for Cardiovascular Angiography and Interventions. Effect of Percutaneous Coronary Intervention on Quality of Life: A Consensus Statement from the Society for Cardiovascular Angiography and Interventions. Catheter Cardiovasc Interv 2013;81:243-59.

[5] Ware JE Jr., Sherbourne CD. The MOS 36-Item Short-form Health Survey (SF-36). I. Conceptual Framework and Item Selection. Med Care 1992;30:473-83.

[6] Failde I, Medina P, Ramírez C, Arana R. Assessing Health-Related Quality of Life among Coronary Patients: SF-36 vs SF-12. Public Health 2009;123:615-7.

[7] Gandek B, Ware JE, Aaronson NK, Apolone G, Bjorner JB, Brazier JE, *et al.* Cross-Validation of Item Selection and Scoring for the SF-12 Health Survey in Nine Countries: Results from the IQOLA Project. J Clin Epidemiol 1998;51:1171-8.

[8] Abrootan S, Yazdankhah S, Payami B, Alasti M. Changes in Heart Rate Variability Parameters After Elective Percutaneous Coronary Intervention. J The Univ Heart Ctr 2015;10:80-4.

[9] Gulati M, Levy PD, Mukherjee D, Amsterdam E, Bhatt DL, Birtcher KK, *et al.* 2021 AHA/ACC/ASE/CHEST/SAEM/SCCT/SCMR Guideline for the Evaluation and Diagnosis of Chest Pain: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation 2021;144:e368-454.

[10] Collet JP, Thiele H, Barbato E, Barthe´le´my O, Bauersachs J, Bhatt DL, *et al.* 2020 ESC Guidelines for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation. The Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J 2021;42:1289-367.

[11] U.S. Department of Health and Human Services. The Health Consequences of Smoking-50 Years of Progress: A Report of the Surgeon General. Atlanta: U.S: Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014.

[12] Hackshaw A, Morris JK, Boniface S, Tang JL, Milenković D. Low Cigarette Consumption and Risk of Coronary Heart Disease and Stroke: Meta-Analysis of 141 Cohort Studies in 55 Study Reports. BMJ 2018;363:k5035.

[13] Roerecke M, Rehm J. Chronic Heavy Drinking and Ischaemic Heart Disease: A Systematic Review and Meta-Analysis. Open Heart 2014;1:e000135.

[14] Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland John GF, Coats Andrew JS, *et al.* 2016 ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure of the European

Society of Cardiology (ESC) Developed with the Special Contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016;37:2129-200.

[15] Melville MR, Lari MA, Brown N, Young T, Gray D. Quality of Life Assessment using the Short Form 12 Questionnaire is as Reliable and Sensitive as the Short Form 36 in Distinguishing Symptom Severity in Myocardial Infarction Survivors. Heart 2003;89:1445-6.

[16] Ware J Jr., Kosinski M, Keller SD. A 12-Item Short-Form Health Survey (SF-12): Construction of Scales and Preliminary Tests of Reliability and Validity. Med Care 1996;32:220-33.

[17] Seto TB, Taira DA, Berezin R, Chauhan MS, Cutlip DE, Ho KK, *et al.* Percutaneous Coronary Revascularization in Elderly Patients: Impact on Functional Status and Quality of Life. Ann Intern Med 2000;132:955-8.

[18] Wong MS, Chair SY. Changes in Health-Related Quality of Life Following Percutaneous Coronary Intervention: A Longitudinal Study. Int J Nurs Stud 2007;44:1334-42.

[19] Melberg T, Nordrehaug JE, Nilsen DW. A Comparison of the Health Status After Percutaneous Coronary Intervention at a Hospital with and Without On-Site Cardiac Surgical Backup: A Randomized Trial in Nonemergent Patients. Euro J Cardiovasc Prevent Rehabil 2010;17:235-43.

[20] Anchah L, Hassali MA, Lim MS, Ibrahim MI, Sim KH, Ong TK. Health Related Quality of Life Assessment in Acute Coronary Syndrome Patients: The Effectiveness of Early Phase I Cardiac Rehabilitation. Health Qual Life Outcomes 2017;15:10.

[21] Juenger J, Schellberg D, Kraemer S, Haunstetter A, Zugck C, Herzog W, *et al.* Health Related Quality of Life in Patients with Congestive Heart Failure: Comparison with other Chronic Diseases and Relation to Functional Variables. Heart 2002;87:235-41.

[22] AlRuthia Y, Sales I, Almalag H, Alwhaibi M, Almosabhi L, Albassam AA, *et al.* The Relationship between Health-Related Quality of Life and Trust in Primary Care Physicians among Patients with Diabetes. Clin Epidemiol 2020;12:143-51.

[23] Mei YX, Wu H, Zhang HY, Hou J, Zhang ZX, Liao W, *et al.* Health-Related Quality of Life and its Related Factors in Coronary Heart Disease Patients: Results from the Henan Rural Cohort Study. Sci Rep 2021;11:5011.

[24] Reddy YN, Rikhi A, Obokata M, Shah SJ, Lewis GD, AbouEzzedine OF, *et al.* Quality of Life in Heart Failure with Preserved Ejection Fraction: Importance of Obesity, Functional Capacity, and Physical Inactivity. Eur J Heart Fail 2020;22:1009-18.

[25] Goldenberg M, Danovitch I, IsHak WW. Quality of Life and Smoking. Am J Addict 2014;23:540-62.

[26] Cheng X, Jin C. The Association between Smoking and Health-Related Quality of Life among Chinese Individuals Aged 40 Years and Older: A Cross-Sectional Study. Front. Public Health 2022;10:779789.

[27] Safi S, Sethi NJ, Nielsen EE, Feinberg J, Jakobsen JC, Gluud C. Beta-Blockers for Suspected or Diagnosed Acute Myocardial Infarction. Cochrane Database Syst Rev 2019;12:CD012484.

[28] Dobre D, van Jaarsveld CH, de Jongste MJ, Haaijer Ruskamp FM, Ranchor AV. The Effect of Beta-Blocker Therapy on Quality of Life in Heart Failure Patients: A Systematic Review and Meta-Analysis. Pharmacoepidemiol Drug Saf 2007;16:152-9.

[29] Casas R, Castro-Barquero S, Estruch R, Sacanella E. Nutrition and Cardiovascular Health. Int J Mol Sci 2018;19:3988.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00049

Journal of Clinical and Translational Research 2023; 9(4): 272-281

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

PD-L1 expression and prognosis in definitive radiotherapy patients with neuroendocrine cervical carcinoma

Huiling Li[1]*, Xiuhua Li[1], Meichun Yang[1], Huiyan Su[1], Jianqiu Zhang[1], Chunmiao Hu[2], Yingming Sun[3], Dan Hu[4], Li Chen[5]

[1]Department of Gynecology, Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, Fuzhou, Fujian, China, [2]Department of Radiology, Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, Fuzhou, Fujian, China, [3]Department of Radiotherapy, Affiliated Sanming First Hospital, Fujian Medical University, Sanming, Fujian, China, [4]Department of Pathology, Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, Fuzhou, Fujian, China, [5]School of Arts and Sciences, Fujian Medical University, Fuzhou, Fujian, China

ARTICLE INFO

Article history:
Received: May 15, 2023
Revised: June 10, 2023
Accepted: July 11, 2023
Published online: July 28, 2023

Keywords:
Cervical carcinoma
Neuroendocrine carcinoma
Small-cell neuroendocrine carcinoma
Chemotherapy
Chemoradiotherapy

**Corresponding authors:*
Huiling Li
Department of Gynecology, Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, Fuzhou, Fujian, China.
E-mail: huilin888999@126.com

ABSTRACT

Background: Neuroendocrine carcinoma of the cervix (NECC) is more prone to lymphatic infiltration, lymph node involvement, local recurrence, and distant metastasis. Using concurrent chemoradiotherapy (CCRT) with or without adjuvant chemotherapy as the standard treatment for locally advanced NECCs and CCRT for patients with early lesions confined to the cervix. However, the prognosis of NECC patients treated with definitive radiotherapy (RT) is unknown. Immune checkpoint inhibitors are a promising therapeutic strategy for locally advanced cervical cancer. Some reports suggest that the expression of PD-L1 in solid tumors correlates with prognosis.

Aim: This study investigates prognostic factors for survival in patients with neuroendocrine cervical carcinoma (NECC) treated with definitive RT and the relationship between PD-L1 expression and prognosis in these patients.

Methods: This retrospective study included 66 patients with histologically confirmed NECC who received RT with or without chemotherapy. From January 2015 to December 2020, patients received routine extended-field irradiation (EFI), and PD-L1 expression was assessed by immunohistochemistry. The most commonly used chemotherapy agents were etoposide-platinum and paclitaxel-platinum.

Results: PD-L1 expression was positive in 17 of 45 (37.8%) patients. There were 52 cases of pure NECC and 14 cases of mixed carcinoma. Sixty stage IB-III patients received definitive RT. The 3- and 5-year progression-free survival (PFS) was 39.8% and 34.1%, and 3- and 5-year overall survival (OS) was 48.0% and 40.2%, respectively. There was no significant difference in 3 and 5-year PFS and 3 and 5-year OS between patients with pure and mixed carcinoma. Positive PD-L1 expression was associated with higher 3-year PFS in patients with mixed histology. Univariate analysis showed that lymph node metastasis (LNM) and the International Federation of Gynecology and Obstetrics stages predicted 3- and 5-year PFS in patients who received definitive RT. The median OS in patients receiving less than four cycles and at least four cycles of chemotherapy (CT) was 26.0 and 44.0 months, respectively ($P = 0.038$); moreover, 3- and 5-year PFS was 34.1% and 25.7% in the former and 46.4% and 40.4% in the latter. There were no significant differences in OS and PFS between pelvic irradiation and prophylactic EFI in patients treated with definitive RT. There were no significant differences in para-aortic failure rate after concurrent chemoradiotherapy between patients who underwent pelvic irradiation or prophylactic EFI ($P = 0.147$).

Conclusion: In patients with mixed NECC, positive PD-L1 expression is correlated with higher 3-year PFS. Chemoradiotherapy was effective for NECCs. The LNM and stage predicted PFS. Four or more cycles of chemotherapy improve prognosis. Prophylactic EFI did not significantly improve PFS and OS.

Relevance for Patients: This study is relevant to patients as it confirms that chemoradiotherapy is effective for both early and locally advanced NECC and that four or more cycles of chemotherapy improved prognosis. The regimen should be carefully evaluated to ensure that patients receive the most effective radiation therapy for the prophylactic of para-aortic LNM. Potential risk factors for the recurrence of radical radiotherapy should be fully understood to minimize these risks. This study observed that PD-L1 expression positive in patients with mixed NECC types is correlated with higher 3-year PFS.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

1. Introduction

Neuroendocrine carcinoma of the cervix (NECC) is a rare histologic type of cervical cancer, accounting for 0.9 – 1.5% of cervical cancer cases [1-3]. Unlike squamous cell carcinoma and adenocarcinoma, NECC is more prone to lymphatic infiltration, lymph node involvement, local recurrence, and distant metastasis (DM) [4].

Small-cell NECC (SCNEC) is the most common type of NECC, accounting for approximately 80% of NECC cases. Large-cell NECC (LCNEC) and other histological types represent approximately 12% and 8% of NECC cases, respectively. Common markers of NECC include chromogranin A (CgA), synaptophysin (Syn), and CD56.

Adjuvant chemoradiotherapy after radical hysterectomy is feasible for early-stage cervical cancer, and concurrent chemoradiotherapy (CCRT) or chemotherapy alone is feasible for locally advanced and metastatic disease [5-7]. The first-line chemotherapy for NECC is etoposide or paclitaxel combined with a platinum agent (cisplatin or carboplatin). The National Comprehensive Cancer Network (2022) recommends using CCRT with or without adjuvant chemotherapy as the standard treatment for stage IB3-IVA NECC and CCRT for patients with early lesions confined to the cervix. Prognostic factors for cervical cancer include race, age, tumor stage and grade, histological type, tumor volume, lymph node involvement and location, performance status, and type of treatment [8]. However, the prognosis of NECC patients treated with radical radiotherapy (RT) is unknown.

Immune checkpoint inhibitors are a promising therapeutic strategy for locally advanced cervical cancer (LACC) [9]. A clinical trial found that ipilimumab combined with nivolumab achieved satisfactory results in three patients with recurrent NECC, including two with positive PD-L1 expression [10]. PD-L1 expression in solid tumors correlates with prognosis. For instance, PD-L1 expression is a good prognostic biomarker in human papillomavirus (HPV)-associated head and neck cancer. Conversely, PD-L1 expression is associated with poor prognosis in patients with renal cancer [11-13].

This study assessed the efficacy of radiation therapy for NECC, prognostic factors for NECC, and the relationship between PD-L1 expression and patient survival.

2. Methods

2.1. Patients

The study included patients with histologically confirmed NECC who received RT with or without chemotherapy at our cancer center between January 2009 and December 2020. Patients gave written informed consent before therapy. The diagnosis was based on the morphological and immunohistochemical characteristics of tumors. The inclusion criteria were patients with no history of previous treatment or malignancies, patients who completed a treatment course, patients with a follow-up of at least 3 months, and patients whose imaging data allowed tumor staging based on the 2018 International Federation of Gynecology and Obstetrics (FIGO) cervical cancer staging system.

2.2. Immunohistochemistry

Immunohistochemistry was performed on 3-5-μm-thick sections. The sections were incubated with antibodies against CgA, Syn, CD56, Ki-67, and PD-L1. PD-L1 immunostaining was performed using clone 28-8 as an anti-PD-L1 antibody (Dako, Carpentaria, CA, USA). PD-L1 expression was scored by counting the total number of PD-L1-positive cells, including tumor cells, lymphocytes, and macrophages, and dividing by the total number of living tumor cells ×100 [14]. PD-L1 expression in tissues (or assays) with a score of ≥1 was considered positive.

2.3. Treatment

2.3.1. RT

The standard protocol included external beam RT (EBRT) and high-dose-rate brachytherapy (HDR-BT). From January 2009 to December 2014, EFI was performed in the pelvis and para-aortic lymph nodes (PALNs) if PALN metastasis was detected at the initial diagnosis. From January 2015 to December 2020, patients received EFI routinely. The patients were planned using 3D conformal RT or intensity-modulated RT. EBRT was performed using either 40.0 – 46.0 Gy in 20 – 23 fractions or 45.0 – 50.4 Gy in 1.8 Gy fractions. HDR-BT was performed during or after EBRT at a dose of 6.0 – 7.0 Gy for each fraction once or twice a week, with a median total dose of 28.0 Gy (range, 21.0 – 35.0 Gy). Palliative RT included EBRT with or without brachytherapy.

2.3.2. Chemotherapy

Patients with no contraindications to platinum received chemotherapy. Therapies included CCRT with etoposide-platinum (EP) or paclitaxel-platinum (TP), followed by adjuvant chemotherapy with EP or TP. In addition, a few patients were treated with chemoradiotherapy involving a single platinum agent concurrent radiotherapy (CRT) followed by adjuvant chemotherapy with EP or TP.

2.3.3. Observation and follow-up

The patients were followed up every 3 months for the first 2 years, every 6 months for the next 3 years, and every 12 months after the 5th year. A physical examination, Papanicolaou smear, and routine blood tests were performed during the follow-up. Radiographic examinations were performed if disease recurrence was suspected. Patient survival with or without recurrence or metastasis was measured.

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL). Median overall survival (OS) and median progression-free survival (PFS) were estimated using the Kaplan–Meier method and compared between groups using the log-rank test. Prognostic factors were analyzed by Cox regression analysis. $P < 0.05$ were considered statistically significant.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

2.5. Ethnical statements

2.5.1. Ethical approval

This study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital (Review Number K2022-208-01).

2.5.2. Consent to participate

Informed consent was obtained from all individual participants included in the study.

2.5.3. Consent to publish

The authors affirm that human research participants provided informed consent for the publication of the images in Figures 1 and 2, Tables 1-6.

3. Results

3.1. Patients and tumor characteristics

A total of 188 patients with newly diagnosed NECCs were treated at our center. Of these, 66 patients treated with RT were included in the study (age: 31 – 86 years; median: 50 years). Fifty-two (78.8%) patients presented pure NECCs, including 50 with SCNEC, one with LCNEC, and one with SCNEC + LCNEC. Fourteen cases (21.2%) of NECCs were associated with other malignancies, including adenocarcinoma (11 cases), squamous cell carcinoma (two cases), and adenosquamous carcinoma (one case). Sixty patients with stage IB-III received definitive RT, and six patients with stage IVB received palliative RT. Treatments included RT alone (four patients), CRT (four patients), and CCRT (58 patients).

Disease stages and the respective number of cases were as follows: IB (1), IIA (7), IIB (14), IIIA (2), IIIB (5), IIIC1 (22), IIIC2 (9), and IVB (6). The clinicopathologic features and treatment modalities are summarized in Table 1.

3.2. Immunohistochemistry

Ki-67 protein expression levels were measured in 59 patients. The number of Ki-67-positive cells in each patient ranged from 20% to 100%, with a median of 75%. Immunohistochemistry showed that 92.2% (59/64), 41.9% (26/62), and 67.2% (41/61) patients were positive for Syn, CgA, and CD56, respectively.PD-L1 expression was positive in 17 (37.8%) patients.

3.3. OS

The follow-up period ranged from 13 to 156 months, with a median of 33 months. The 3- and 5-year OS was 41.7% and 35.2%, and 3- and 5-year PFS was 35.6% and 30.6%, respectively. The 5-year OS and PFS were 60.0% and 56.3% in patients with stage I-IIA and 42.3% and 32.7% in patients with stage IIB-IIIc.

The 3-year OS in patients with true and mixed carcinoma was 50.7% and 37.3%, respectively ($P = 0.633$). Five-year OS in these groups was 40.0% and 24.9%, respectively ($P = 0.400$); 3-year PFS was 42.8% and 27.7% ($P = 0.248$), and 5-year PFS was 35.3% and 13.8% ($P = 0.178$).

3.4. PD-L1 expression and patient survival

For patients with mixed histology, positive PD-L1 expression was associated with higher 3-year PFS compared with negative PD-L1 expression (66.7% vs. 16.7%, $P = 0.042$). There were no significant differences in survival between the two pathological types (Table 2).

Among the 60 patients who received definitive EBRT, whole pelvis irradiation, EFI, and prophylactic EFI were performed in 27, 9, and 24 patients, respectively. Thirty-six (54.55%) patients experienced tumor persistence, recurrence, metastasis, or progression. Distal metastases were more common in supraclavicular, mediastinum, and hilum lymph nodes. The most common hematogenous metastasis was pulmonary in 16 cases (16/30), hepatic in 10 cases (10/30), bone in 12 cases (12/30), and pancreatic in four cases (4/30). Brain metastasis occurred in one case. The survival status of patients is shown in Table 3. The

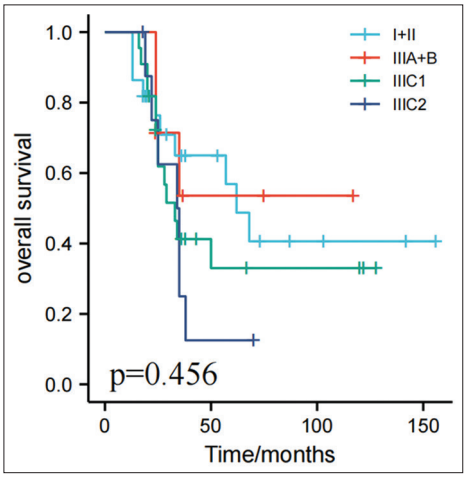


Figure 1. Overall survival by International Federation of Gynecology and Obstetrics stage.

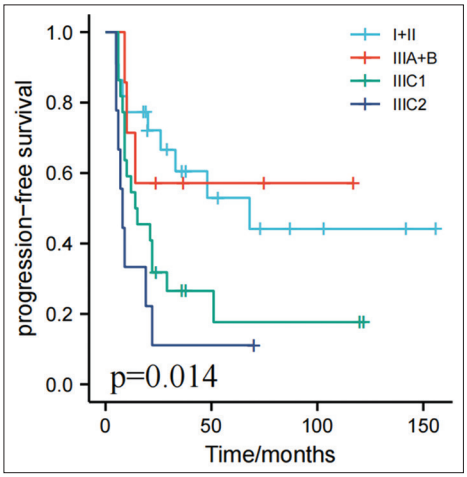


Figure 2. Progression-free survival by International Federation of Gynecology and Obstetrics stage.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

Table 1. Patients, tumor characteristics, and treatment modalities.

Characteristics	Number of patients	Percentage
Age (years)		
<60	50	75.8 (50/66)
≥60	16	24.2 (16/66)
Histology		
Pure	52	
Small-cell neuroendocrine carcinoma	50	75.8 (50/66)
Large-cell neuroendocrine adenocarcinoma	1	1.5 (1/66)
Small-cell+large-cell	1	1.5 (1/66)
Mixed	14	
Small-cell neuroendocrine carcinoma and adenocarcinoma	11	16.7 (11/66)
Small-cell neuroendocrine carcinoma and squamous cell carcinoma	1	3.0 (1/66)
Small-cell neuroendocrine carcinoma and adenosquamous carcinoma	2	3.0 (2/66)
FIGO stage		
IB	1	1.5 (1/66)
IIA	7	10.6 (7/66)
IIB	14	21.2 (14/66)
IIIA	2	3.0 (2/66)
IIIB	5	7.6 (5/66)
IIIC1	22	33.3 (22/66)
IIIC2	9	13.6 (9/66)
IVB	6	9.1 (6/66)
Immunohistochemistry		
Syn-positive	59	92.2 (59/64)
CgA-positive	26	41.9 (26/62)
CD56-positive	41	67.2 (41/61)
Tumor size (cm)		
<4	10	15.2 (10/66)
≥4	56	84.9 (56/66)
Lymph node involvement		
Pelvic	22	33.3 (22/66)
Pelvic and para-aortic	9	13.6 (9/66)
Radiotherapy		
3DCRT	21	31.8 (21/66)
IMRT	45	68.2 (45/66)
Definitive	60	90.9 (60/66)
Palliative	6	16.7% (6/66)
Chemotherapy		
TP	28	42.4 (28/66)
EP	31	46.9 (31/66)
Treatment		
CCRT	58	45.5 (58/66)
Radiotherapy	4	6.1 (4/66)
Platinum + radiotherapy	4	6.1 (4/66)
Number of chemotherapy cycles (EP or TP)		
1 – 3	23	34.9 (23/66)
4 – 7	36	57.6 (36/66)
Definitive external beam radiotherapy		
Pelvic irradiation	27	45.0 (27/60)
Extended-field irradiation	33	55.0 (33/60)

(*Contd...*)

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

Table 1. (*Continued*)

Characteristics	Number of patients	Percentage
PD-L1-positive		
≥1	17	37.8 (17/45)
<1	20	44.4 (20/45)
0	8	17.8 (8/45)
Ki-67-positive		
<75	14	23.7 (14/59)
≥75	45	76.3 (45/59)

3DCRT: 3D conformal radiotherapy; IMRT: Intensity-modulated radiotherapy; CCRT: Concurrent chemo radiotherapy; EP: Etoposide-platinum; TP: Paclitaxel-platinum; CgA: Chromogranin A, Syn: Synaptophysin

Table 2. Progression-free survival and overall survival based on PD-L1 expression in 45 patients with neuroendocrine cervical carcinoma.

Factors	Cases	3-year PFS			5-year PFS			3-year OS			5-year OS		
		PD-L1 Positive	PD-L1 Negative	*P*	PD-L1 Positive	PD-L1 Negative	*P*	PD-L1 Positive	PD-L1 Negative	*P*	PD-L1 Positive	PD-L1 Negative	*P*
Total	45	45.4	34	0.559	15.1	34	0.897	39.8	41.3	0.685	39.8	26.6	0.733
Age (years)													
≤50	22	37.5	36.9	0.933	0	36.9	0.594	33.8	42.3	0.402	33.8	31.7	0.386
>50	23	57.1	31.5	0.428	57.1	31.5	0.428	51.4	40.4	0.721	51.4	20.2	0.721
Histology													
Pure	36	41.7	38.4	0.897	20.8	38.4	0.689	37.3	40.9	0.553	37.7	30.7	0.407
Mixed	9	66.7	16.7	0.042	0	16.7	0.194	50	41.7	0.735	50	0	0.441
FIGO stage													
I+II	15	83.3	48.6	0.349	41.7	48.6	0.663	0	51.9	0.405	83.3	34.6	0.708
III	26	33.3	26.5	0.705	0	26.5	0.902	33.3	40.1	0.395	33.3	30.1	0.527
IV	4	0	0	0.808	0	0	0.808	0	0	0.695	0	0	0.695
Lymph node involvement													
No	17	71.4	52.5	0.624	35.7	52.5	0.927	64.3	52.1	0.867	64.3	34.7	0.875
Pelvic or PALN	28	37.5	19	0.421	0	19	0.601	37.5	38.1	0.72	37.5	28.6	0.881
Number of chemotherapy cycles													
≤3	18	33.3	34.1	0.892	0	34.1	0.515	33.3	35.1	0.703	33.3	0	0.368
4–6	27	51.3	36.3	0.741	25.6	36.3	0.890	45.7	62.9	0.332	45.7	25.2	0.636

PALN: Para-aortic lymph nodes; PFS: Progression-free survival; OS: Overall survival

median PFS and OS were 22.0 and 35.0 months, respectively. In addition, 3- and 5-year PFS was 39.8% and 34.1%, and 3- and 5-year OS was 48.0% and 40.2%, respectively.

The 3-year OS in patients with stages I+II, IIIA+B, IIIC1, and IIIC2 was 60.5%, 57.1%, 26.5%, and 11.1%,respectively; 5-year OS in these groups was 53.0%, 57.1%, 17.7%, and 10.3%. The disease stage increased as survival rates decreased (Figure 1). Advanced-stage NECC (P = 0.011), lymph node metastasis (LNM), and the number of chemotherapy cycles predicted PFS. The 3- and 5-year PFS was 60.5% and 53.0% in stage I+II, 57.1% and 57.1% in stage IIIA+B, 26.5% and 17.7% in stage IIIC1, and 11.1% and 11.1% in stage IIIC2 (Figure 2). The 3- and 5-year PFS was 39.7% and 27.6% in patients with LNM and 48.7% and 30.1% in patients without LNM, respectively (P = 0.029). The median OS in patients receiving less than four cycles and at least four cycles of CT was 26.0 and 44.0 months, respectively (P = 0.038); moreover, 3-year and 5-year PFS was 34.1% and 25.7% in the former and 46.4% and 40.4% in the latter (Table 3).

Univariate analysis showed that LNM and FIGO stages predicted 3- and 5-year PFS in patients who received definitive RT (Table 4). Multivariate Cox regression analysis demonstrated that FIGO stages were independent factors affecting PFS (Table 5).

There were no significant differences in OS and PFS between pelvic irradiation and EFI. Furthermore, there was no significant difference in the incidence of para-aortic failure after CCRT or CRT between patients treated with pelvic irradiation or prophylactic EFI (P = 0.147) (Table 6).

4. Discussion

NECC is strongly associated with HPV infections [15], providing a rationale for studying the molecular characteristics of NECC. Since the efficacy of CRT for advanced diseases is low, it is critical to identify biomarkers associated with survival, local control, and DM. PD-L1 is highly expressed in NECC [16,17] and is thus a potential therapeutic target. PD-L1 expression was positive in more than 50% of patients

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

Table 3. Progression-free survival and overall survival based on the clinical characteristics of 60 patients with neuroendocrine cervical carcinoma treated with definitive radiotherapy.

Factors	Cases	Median PFS 95% CI	*P*-value	3-year PFS	5-year PFS	Median OS 95% CI	*P*-value	3-year OS	5-year OS
Total	60	22.0 (14.0, 68.0)		0.398 (0.288, 0.549)	0.341 (0.231, 0.502)	35.0 (33.0, NR)		0.480 (0.362, 0.637)	0.402 (0.284, 0.569)
Median age (years)			0.527				0.894		
≤50	32	19.0 (9.0, NR)		0.427 (0.283, 0.643)	0.332 (0.194, 0.569)	35.0 (33.0, NR)		0.475 (0.322, 0.701)	0.435 (0.284, 0.666)
>50	28	22.0 (14.0, NR)		0.367 (0.220, 0.611)	----	35.0 (28.0, NR)		0.489 (0.326,0.733)	0.335 (0.172, 0.655)
Histology			0.178				0.393		
Pure	47	26.0 (14.0, NR)		0.428 (0.304, 0.602)	0.392 (0.267, 0.575)	50.0 (33.0, NR)		0.508 (0.377, 0.685)	0.438 (0.304, 0.630)
Mixed	13	15.0 (6.0, NR)		0.277 (0.110, 0.699)	0.138 (0.026, 0.733)	33.0 (24.0, NR)		0.373 (0.173, 0.806)	0.249 (0.082, 0.755)
FIGO stage			0.011				0.456		
I+II	22	68.0 (26.0, NR)		0.605 (0.422, 0.867)	0.530 (0.339, 0.826)	62.0 (33.0, NR)		0.650 (0.468, 0.902)	0.569 (0.374, 0.865)
IIIA+B	7	NR (10.0, NR)		0.571 (0.301, 1.000)	0.571 (0.301, 1000)	NR (24.0, NR)		0.536 (0.257, 1.000)	0.536 (0.257, 1.000)
IIIC1	22	14.5 (9.0, NR)		0.265 (0.131, 0.539)	0.177 (0.061, 0.515)	33.0 (25.0, NR)		0.413 (0.244, 0.697)	0.330 (0.167, 0.654)
IIIC2	9	8.0 (6.0, NR)		0.111 (0.017, 0.705)	0.111 (0.017, 0.705)	34.5 (25.0, NR)		0.250 (0.075, 0.830)	0.125 (0.020, 0.782)
Tumor size (cm)			0.113				0.333		
≤4	18	NR (10.0, NR)		0.530 (0.335, 0.839)	0.530 (0.335, 0.839)	57.0 (33.0, NR)		0.635 (0.437, 0.921)	0.463 (0.259, 0.826)
>4	42	20.0 (10.0, 48.0)		0.343 (0.153, 0.464)	0.267 (0.153, 0.464)	35.0 (26.0, NR)		0.419 (0.287, 0.613)	0.381 (0.250, 0.582)
Lymph node involvement			0.029				0.191		
No	29	48.0 (26.0, NR)		0.487 (0.436, 0.818)	0.397 (0.389, 0.683)	40.0 (33.0, NR)		0.581 (0.452, 0.838)	0.524 (0.382, 0.803)
Pelvic or PALN	31	18.0 (9.0, 22.0)		0.301 (0.113, 0.432)	0.276 (0.369, 0.398)	32.9 (25.0, NR)		0.365 (0.244, 0.596)	0.321 (0.141, 0.518)
Number of chemotherapy cycles			0.023				0.038		
1 – 3	21	15.0 (9.0, 22.0)		0.341 (0.064, 0.459)	0.257 (0.209, 0.474)	26.0 (24.0,50.0)		0.316 (0.091, 0.513)	0.252 (0.058, 0.455)
4 – 6	32	33.0 (29.0, NR)		0.464 (0.450, 0.809)	0.404 (0.450, 0.809)	44.0 (27.0, NR)		0.576 (0.599, 0.930)	0.486 (0.464, 0.872)
EBRT			0.434				0.073		
Pelvic irradiation	27	26.0 (11.0, 41.0)		0.310 (0.189, 0.509)	0.186 (0.078, 0.445)	53.0 (39.0,75.0)		0.329 (0.197, 0.549)	0.211 (0.093, 0.481)
Extended-field irradiation	33	14.0 (7.0, 28.0)		0.310 (0.189, 0.509)	0.186 (0.078, 0.445)	33.0 (28.0,42.0)		0.414 (0.312, 0.660)	0.338 (0.241, 0.595)
PD-L1 expression			0.687				0.723		
Positive	15	48.0 (10.0, NR)		0.333 (0.189, 0.589)	0.333 (0.189, 0.589)	33.0 (25.0, NR)		0.463 (0.254, 0.845)	0.463 (0.254, 0.845)
Negative	27	20.0 (9.0, NR)		0.389 (0.218, 0.694)	0.389 (0.218, 0.694)	35.0 (33.0, NR)		0.473 (0.304, 0.736)	0.331 (0.169, 0.651)

PFS: Progression-free survival; OS: Overall survival; NR: Not reached

Table 4. Univariate analysis of progression-free survival and overall survival.

Factors	3-year PFS		5-year PFS		3-year OS		5-year OS	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Median age (years)								
≤50	1		1		1		1	
>50	0.860 (0.438 – 1.689)	0.662	0.797 (0.412 – 1.542)	0.501	1.024 (0.487 – 2.153)	0.950	1.047 (0.526 – 2.083)	0.896
Histology								
Pure	1		1		1		1	
Mixed	1.567 (0.730 – 3.363)	0.249	1.699 (0.817 – 3.530)	0.156	1.361 (0.578 – 3.206)	0.480	1.406 (0.632 – 3.128)	0.404
FIGO stage								
I+II	1		1		1		1	
IIIA+B	1.530 (0.395 – 5.982)	0.538	1.401 (0.371 – 5.295)	0.619	1.340 (0.346 – 5.195)	0.672	1.077 (0.294 – 3.940)	0.911
IIIC1	2.263 (0.929 – 5.511)	0.072	2.191 (0.942 – 5.094)	0.069	1.419 (0.558 – 3.607)	0.462	1.180 (0.515 – 2.702)	0.696
IIIC2	4.769 (1.714 – 13.275)	0.003	4.330 (1.609 – 11.649)	0.004	1.915 (0.643 – 5.702)	0.243	1.832 (0.691 – 4.858)	0.224
Lymph node metastasis								
No	1		1		1		1	
Pelvic or PALN	2.447 (1.168 – 5.129)	0.018	2.389 (1.172 – 4.871)	0.017	1.435 (0.662 – 3.110)	0.360	1.323 (0.656 – 2.669)	0.434
Tumor size (cm)								
≤4	1		1		1		1	
>4	1.740 (0.877 – 3.450)	0.113	1.873 (0.956 – 3.668)	0.067	1.421 (0.672 – 3.007)	0.358	1.227 (0.618 – 2.439)	0.559
Number of chemotherapy cycles								
1 – 3	1		1		1		1	
4 – 6	0.688 (0.331 – 1.434)	0.391	0.694 (0.341 – 1.412)	0.314	0.616 (0.267 – 1.422)	0.256	0.687 (0.322 – 1.463)	0.330
PD-L1 expression								
Positive	1		1		1		1	
Negative	1.519 (0.624 – 3.696)	0.357	1.201 (0.530 – 2.722)	0.661	0.895 (0.351 – 2.281)	0.816	0.926 (0.387 – 2.215)	0.863
Ki-67								
<75%	1		1		1		1	
≥75%	0.985 (0.453 – 2.144)	0.971	0.931 (0.442 – 1.960)	0.851	1.173 (0.492 – 2.794)	0.718	1.082 (0.497 – 2.353)	0.843

PALN: Para-aortic lymph nodes

with SCNEC [18,19]. In turn, Carroll *et al.* [20] examined 40 specimens from patients with NECC, including SCNEC (23 cases), LCNEC (five cases), undifferentiated NECC (three cases), and mixed NECC (nine cases), and showed that only two (8%) of 25 patients with pure NECC and three (50%) of six patients with mixed NECC were PD-L1-positive, and all 28 (100%) samples were microsatellite stable. Another study found that PD-L1 expression was positive in 10% of patients with NECC [21]. In our cohort, PD-L1 expression was positive in 37.8% (17/45) of the patients.

The prognostic value of PD-L1 for cervical cancer is debatable [22,23]. Kim *et al*. [24] observed that PD-L1 positivity was associated with lower OS in patients with gastroenteropancreatic neuroendocrine tumors. Chen *et al.* [18] evaluated 46 patients with SCNEC and found that recurrence and mortality in PD-L1-positive patients were lower than in PD-L1-negative patients (P = 0.048 and 0.033, respectively). Another study involving 48 cases of SCNEC showed that PD-L1 positivity was correlated with high survival in SCNEC (P = 0.039) [19]. In patients with mixed histology, we found that positive PD-L1 expression was associated with higher 3-year PFS compared with negative PD-L1 expression (66.7% vs. 16.7% , P = 0.042).

Although NECC patients treated with chemoradiotherapy had satisfactory outcomes, few studies assessed the efficacy of this type of therapy in NECC patients. NECC has a worse prognosis than other types of cervical cancer because of the high rates of early LNM and DM [25,26]. Moreover, prognostic factors of definitive RT and chemotherapy in locally advanced NECC patients with stage IB3, IIA2, or IIB-IIIC have not been identified.

There is controversy regarding the effectiveness of radiation therapy in early-stage NECC [7,27]. Chen *et al.* [28] reported that the curative effect of radical surgery was slightly better than that of RT for stage I-II patients. However, Ruiz *et al.* [29] and Hou *et al*. [26] observed that RT was as effective as surgery for patients with early-stage NECC. Patients with late-stage NECC are successfully treated with RT and chemotherapy [30,31]. A study based on the SEER database showed that 5-year OS for AJCC stage III was 28% [25]. In our cohort, 5-year OS was 35.2%, higher than previously reported (30%) [32]. In addition, 5-year OS in patients with stage I-IIA and stage IIB-IIIc2 (LACC) was 56.3% and 42.3%.

LNM is a prognostic factor for carcinoma of the uterine cervix. Chen *et al.* have reported that initial LNM is a poor prognostic factor for LACC [33]. Yamashita *et al.* [34] found that PLN and

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

Table 5. Multivariate analysis of progression-free survival and overall survival.

Factors	3-year PFS		5-year PFS		3-year OS		5-year OS	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Median age (years)								
≤50	1		1		1		1	
>50	1.479 (0.497 – 4.405)	0.482	1.389 (0.490 – 3.938)	0.537	1.173 (0.360 – 3.822)	0.792	1.368 (0.438 – 4.275)	0.590
Histology								
Pure	1		1		1		1	
Mixed	1.342 (0.400 – 4.501)	0.633	2.250 (0.733 – 6.907)	0.156	0.495 (0.102 – 2.405)	0.383	0.612 (0.163 – 2.295)	0.467
FIGO stage								
I+II	1		1		1		1	
IIIA+B	1.386 (0.227 – 8.462)	0.724	1.212 (0.211 – 6.948)	0.829	1.826 (0.252 – 13.240)	0.551	1.713 (0.259 – 11.340)	0.577
IIIC1	3.948 (1.103 – 14.130)	0.035	3.412 (1.044 – 11.152)	0.042	3.446 (0.814 – 14.589)	0.093	2.265 (0.635 – 8.079)	0.208
IIIC2	6.427 (1.116 – 36.997)	0.037	5.231 (1.044 – 26.218)	0.044	2.832 (0.323 – 24.837)	0.347	2.400 (0.384 – 14.989)	0.349
Tumor size (cm)								
≤4	1		1		1		1	
>4	0.927 (0.287 – 2.997)	0.899	1.162 (0.382 – 3.532)	0.792	0.739 (0.206 – 2.653)	0.643	0.573 (0.173 – 1.901)	0.363
Number of chemotherapy cycles								
1–3	1		1		1		1	
4–6	0.639 (0.205 – 1.994)	0.441	0.614 (0.198 – 1.902)	0.398	0.258 (0.057 – 1.172)	0.079	0.376 (0.093 – 1.526)	0.171
PD-L1 expression								
Positive	1		1		1		1	
Negative	1.061 (0.344 – 3.268)	0.918	0.878 (0.299 – 2.578)	0.813	0.430 (0.121 – 1.532)	0.193	0.453 (0.136 – 1.501)	0.195
Ki-67								
<75%	1		1		1		1	
≥75%	1.131 (0.323 – 3.968)	0.847	0.819 (0.253 – 2.653)	0.740	0.897 (0.208 – 3.876)	0.938	1.330 (0.241 – 3.653)	0.926

HR: Hazard ratio; CI: Confidence interval

Table 6. Para-aortic failure after pelvic irradiation and prophylactic extended-field irradiation.

Pelvic lymph node	Pelvic irradiation			Prophylactic extended-field irradiation			*P*-value
	Cases	Para-aortic failure	Failure rate	Cases	Para-aortic failure	Failure rate	
Yes	14	6	22.2% (6/27)	10	1	4.2% (1/24)	0.172
No	13	1	3.7% (1/27)	14	1	4.2% (1/24)	1.000
Total	27	7	25.9% (7/27)	24	2	8.3% (2/24)	0.147

PALN status significantly affected survival, and PALN metastasis was the most important prognostic factor for LACC. Similarly, for neuroendocrine tumors of the uterine cervix, PALN metastasis was associated with poor survival [35]. In our cohort, univariate analysis showed that LNM and FIGO stages predicted 3-and 5-year PFS, and multivariate Cox regression analysis demonstrated that FIGO stages predicted 3-and 5-year PFS in patients treated with definitive RT.

Pelvic RT combined with prophylactic EFI can reduce the incidence of para-aortic failure in patients without positive PALN on imaging. However, whether prophylactic EFI can reduce para-aortic failure in patients with cervical cancer is unknown [36]. Hoskins *et al.* [30] analyzed 31 cases of SCNEC, including 17 patients treated with CCRT and EBRT (PLN plus or minus PALN) and 14 treated with CCRT combined with the routine irradiation of PALNs. The outcomes of the two irradiation methods were similar: 3-year OS and failure-free survival were 60% and 57%, respectively. In our cohort, metastasis to PALNs alone after treatment occurred in one case, and metastasis to PALNs associated with LN metastasis in other sites or hematogenous metastasis occurred in nine cases. Prophylactic EFI did not significantly improve PFS and OS, irrespective of PLN metastasis. Nonetheless, larger clinical trials are needed to assess the efficacy of prophylactic EFI in NECC.

Zivanovic *et al.* [37] support the use of chemotherapy for distant control and radiation therapy for the local control of SCNEC. In chemoradiotherapy for patients with stage IIB-IVB SCNEC, at least five cycles of primary chemotherapy with etoposide and platinum were associated with significantly higher 5-year disease-free survival (42.9% vs. 11.8%, $P = 0.041$) and OS (45.6% vs. 17.1%, $P = 0.035$) than fewer cycles. In addition, more than five cycles of CCRT and EP therapy were associated with higher 5-year disease-free survival (62.5% vs. 13.1%, $P = 0.025$) and OS (75.0% vs. 16.9%, $P = 0.016$) [38]. Ishikawa *et al.* [35] found

that less than four cycles of chemotherapy were associated with lower OS in patients with cervical neuroendocrine tumors. In our cohort, compared with less than four cycles of chemotherapy, four or more cycles were associated with significantly higher 3-year PFS (46.4% vs. 34.1%) and 5-year PFS (40.4% vs. 25.7%) and significantly higher 3-year OS (57.6% vs. 31.6%) and 5-year OS (48.6% vs. 25.2%).

This study has limitations. First, the small number of cases with a complete follow-up, the single-center design, and changes in the treatment plan and FIGO staging during the study period (2009 – 2020) may have caused bias in selection, implementation, and measurements. Second, the retrospective design did not allow assessing the clinical effects of anti-PD-L1 therapies in NECC. Third, immunohistochemistry has a limited ability to detect PD-L1 because of the heterogeneity of PD-L1 expression in tumor specimens.

5. Conclusion

Positive PD-L1 expression was associated with higher 3-year PFS in patients with mixed histology. RT for patients with early NECC has the same effect as surgery and is effective for treating locally advanced disease. Four or more cycles of chemotherapy are more effective than a smaller number of courses. Prophylactic EFI did not significantly improve PFS and OS. Nonetheless, the effects of prophylactic EFI should be further studied.

Acknowledgments

None.

Funding

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

References

[1] Gadducci A, Carinelli S, Aletti G. Neuroendrocrine Tumors of the Uterine Cervix: A Therapeutic Challenge for Gynecologic Oncologists. Gynecol Oncol 2017;144:637-46.

[2] Burzawa J, Gonzales N, Frumovitz M. Challenges in the Diagnosis and Management of Cervical Neuroendocrine Carcinoma. Expert Rev Anticancer Ther 2015;15:805-10.

[3] Salvo G, Martin AG, Gonzales NR, Frumovitz M. Updates and Management Algorithm for Neuroendocrine Tumors of the Uterine Cervix. Int J Gynecol Cancer 2019;29:986-95.

[4] Kitajima K, Kihara T, Kawanaka Y, Kido A, Yoshida K, Mizumoto Y, *et al.* Neuroendocrine Carcinoma of Uterine Cervix Findings Shown by MRI for Staging and Survival Analysis-Japan Multicenter Study. Oncotarget 2020;11:3675-86.

[5] Atienza-Amores M, Guerini-Rocco E, Soslow RA, Park KJ, Weigelt B. Small Cell Carcinoma of the Gynecologic Tract: A Multifaceted Spectrum of Lesions. Gynecol Oncol 2014;134:410-8.

[6] Gardner GJ, Reidy-Lagunes D, Gehrig PA. Neuroendocrine Tumors of the Gynecologic Tract: A Society of Gynecologic Oncology (SGO) Clinical Document. Gynecol Oncol 2011;122:190-8.

[7] Zhang Y, Li L, Wang Z, Huang Y, Luo S, Peng Y, *et al.* Preferred Method of Therapy for Patients with Early-stage High-grade Neuroendocrine Carcinoma of the Cervix. Am J Cancer Res 2021;11:4595-606.

[8] Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical Cancer. Lancet 2019;393:169-82.

[9] De Felice F, Marchetti C, Palaia I, Ostuni R, Muzii L, Tombolini V, *et al.* Immune Check-point in Cervical Cancer. Crit Rev Oncol Hematol 2018;129:40-3.

[10] Towner M, Novak K, Chae YK, Matei D. Ipilimumab and Nivolumab for Recurrent Neuroendocrine Cervical Carcinoma. Gynecol Oncol Rep 2022;42:101039.

[11] Badoual C, Hans S, Merillon N, Van Ryswick C, Ravel P, Benhamouda N, *et al.* PD-1-expressing Tumor-infiltrating T Cells are a Favorable Prognostic Biomarker in HPV-associated Head and Neck Cancer. Cancer Res 2013;73:128-38.

[12] Droeser RA, Hirt C, Viehl CT, Frey DM, Nebiker C, Huber X, *et al.* Clinical Impact of Programmed Cell Death Ligand 1 Expression in Colorectal Cancer. Eur J Cancer 2013;49:2233-42.

[13] Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC, *et al.* PD-1 is Expressed by Tumor-infiltrating Immune Cells and is Associated with Poor Outcome for Patients with Renal Cell Carcinoma. Clin Cancer Res 2007;13:1757-61.

[14] Loharamtaweethong K, Puripat N, Praditphol N, Thammasiri J, Tangitgamol S. PD-L1 Protein Expression and Copy Number Gains in HIV-positive Locally Advanced Cervical Cancer. Ther Adv Med Oncol 2020;12:1-15.

[15] Alejo M, Alemany L, Clavero O, Quiros B, Vighi S, Seoud M, *et al.* Contribution of Human Papillomavirus in Neuroendocrine Tumors from a Series of 10,575 Invasive Cervical Cancer Cases. Papillomavirus Res 2018;5:134-42.

[16] Morgan S, Slodkowska E, Parra-Herran C, Mirkovic J. PD-L1, RB1 and Mismatch Repair Protein Immunohistochemical Expression in Neuroendocrine Carcinoma, Small Cell Type, of the Uterine Cervix. Histopathology 2019;74:997-1004.

[17] Ji X, Sui L, Song K, Lv T, Zhao H, Yao Q. PD-L1, PARP1, and MMRS as Potential Therapeutic Biomarkers for Neuroendocrine Cervical Cancer. Cancer Med 2021;10:4743-51.

[18] Chen L, Yang F, Feng T, Wu S, Li K, Pang J, *et al.* PD-L1, Mismatch Repair Protein, and NTRK Immunohistochemical Expression in Cervical Small Cell Neuroendocrine Carcinoma. Front Oncol 2021;11:752453.

[19] Sun X, Liu L, Wan T, Huang Q, Chen J, Luo R, *et al.* The Prognostic Impact of the Immune Microenvironment in Small-cell Neuroendocrine Carcinoma of the Uterine Cervix: PD-l1 and Immune Cell Subtypes. Cancer Cell Int 2022;22:348.

[20] Carroll MR, Ramalingam P, Salvo G, Fujimoto, Soto LMS, Phoolcharoen N, *et al*. Evaluation of PARP and PDL-1 as potential therapeutic targets for women with high-grade neuroendocrine carcinomas of the cervix. Int J Gynecol Cance 2020;30:1303-7.

[21] Cimic A, Vranic S, Arguello D, Contreras E, Gatalica Z, Swensen J. Molecular Profiling Reveals Limited Targetable Biomarkers in Neuroendocrine Carcinoma of the Cervix. Appl Immunohistochem Mol Morphol 2021;29:299-304.

[22] Gu X, Dong M, Liu Z, Mi Y, Yang J, Zhang Z, *et al.* Elevated PD-L1 Expression Predicts Poor Survival Outcomes in Patients with Cervical Cancer. Cancer Cell Int 2019;19:146.

[23] Enwere EK, Kornaga EN, Dean M, Koulis TA, Phan T, Kalantarian M, *et al.* Expression of PD-L1 and Presence of CD8-positive T Cells in Pre-treatment Specimens of Locally Advanced Cervical Cancer. Mod Pathol 2017;30:577-86.

[24] Kim ST, Ha SY, Lee S, Ahn S, Lee J, Park SH, et alThe impact of PD-L1 expression in patients with metastatic GEP-NETs. J Cancer 2016;7:484–9.

[25] Pei X, Xiang L, Chen W, Jiang W, Yin L, Shen X, *et al.* The Next Generation Sequencing of Cancer-related Genes in Small Cell Neuroendocrine Carcinoma of the Cervix. Gynecol Oncol 2021;161:779-86.

[26] Hou WH, Schultheiss TE, Wong JY, Wakabayashi MT, Chen YJ. Surgery Versus Radiation Treatment for High-grade Neuroendocrine Cancer of Uterine Cervix: A Surveillance Epidemiology and End Results Database Analysis. Int J Gynecol Cancer 2018;28:188-93.

[27] Winer I, Kim C, Gehrig P. Neuroendocrine Tumors of the Gynecologic Tract Update. Gynecol Oncol 2021;162:210-9.

[28] Chen J, Sun Y, Chen L, Zang L, Lin C, Lu Y, *et al.* Prognostic Factors and Treatment of Neuroendocrine Tumors of the Uterine Cervix Based on the FIGO 2018 Staging System: A Single-institution Study of 172 Patients. PeerJ 2021;9:e11563.

[29] Ruiz MP, Dziadek OL, Algren SD. Nonsurgical Management of Neuroendocrine Cancer of the Cervix: Brief Report. Int J Gynecol Cancer 2016;26:1290-2.

[30] Hoskins PJ, Swenerton KD, Pike JA, Lim P, Aquino-Parsons C, Wong F, *et al.* Small-cell Carcinoma of the Cervix: Fourteen Years of Experience at a Single Institution Using a Combined-modality Regimen of Involved-field Irradiation and Platinum-based Combination Chemotherapy. J Clin Oncol 2003;21:3495-501.

[31] Bajaj A, Gopalakrishnan M, Harkenrider MM, Lurain JR, Small W Jr. Advanced Small Cell Carcinoma of the Cervix-successful Treatment with Concurrent Etoposide and Cisplatin Chemotherapy and Extended Field Radiation: A Case Report and Discussion. Gynecol Oncol Rep 2018;23:4-6.

[32] Cohen JG, Kapp DS, Shin JY, Urban R, Sherman AE, Chen LM, *et al.* Small Cell Carcinoma of the Cervix: Treatment and Survival Outcomes of 188 Patients. Am J Obstet Gynecol 2010;203:347.e1-6.

[33] Chen CC, Wang L, Lin JC, Jan JS. The Prognostic Factors for Locally Advanced Cervical Cancer Patients Treated by Intensity-modulated Radiation Therapy with Concurrent Chemotherapy. J Formos Med Assoc 2015;114:231-7.

[34] Yamashita H, Nakagawa K, Tago M, Shiraishi K, Nakamura N, Ohtomo K. Treatment Results and Prognostic Analysis of Radical Radiotherapy for Locally Advanced Cancer of the Uterine Cervix. Br J Radiol 2005;78:821-6.

[35] Ishikawa M, Kasamatsu T, Tsuda H, Fukunaga M, Sakamoto A, Kaku T, *et al.* A Multi-center Retrospective Study of Neuroendocrine Tumors of the Uterine Cervix: Prognosis According to the New 2018 Staging System, Comparing Outcomes for Different Chemotherapeutic Regimens and Histopathological Subtypes. Gynecol Oncol 2019;155:444-51.

[36] Wang W, Zhou Y, Wang D, Hu K, Zhang F. Prophylactic Extended-field Irradiation in Patients with Cervical Cancer: A Literature Review. Front Oncol 2020;10:579410.

[37] Zivanovic O, Leitao MM Jr., Park KJ, Zhao H, Diaz JP, Konner J, *et al.* Small Cell Neuroendocrine Carcinoma of the Cervix: Analysis of Outcome, Recurrence Pattern and the Impact of Platinum-based Combination Chemotherapy. Gynecol Oncol 2009;112:590-3.

[38] Wang KL, Chang TC, Jung SM, Chen CH, Cheng YM, Wu HH, *et al.* Primary Treatment and Prognostic Factors of Small Cell Neuroendocrine Carcinoma of the Uterine Cervix: A Taiwanese Gynecologic Oncology Group Study. Eur J Cancer 2012;48:1484-94.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

Journal of Clinical and Translational Research 2023; 9(4): 282-289

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

Histopathological findings in celiac disease patients enrolled for duodenal biopsy in Najran, Saudi Arabia: a 5-year retrospective study

Saad Misfer Al-Qahtani[1]*, Mohammed Majeed Alwaily[2], Ali Abdullah Businnah[2], Hussain Ali Al Zamanan[2], Mahdi Turki Alfataih[2], Dhafer Mohammed Al Sagoor[2], Awad Mohammed Al-Qahtani[3]

[1]Department of Pathology, College of Medicine, The University Hospital, Najran University, Najran, Saudi Arabia, [2]College of Medicine, The University Hospital, Najran University, Najran, Saudi Arabia, [3]Department of Family Medicine, College of Medicine, The University Hospital, Najran University, Najran, Saudi Arabia

ARTICLE INFO

Article history:
Received: November 07, 2022
Revised: June 29, 2023
Accepted: July 21, 2023
Published online: August 22, 2023

Keywords:
Celiac disease
Biopsy
Endoscopy
Histopathology
Duodenal

**Corresponding authors:*
Saad Misfer Al-Qahtani
Mahdi Turki Alfataih, Dhafer Mohammed Al Sagoor, Awad Mohammed Al-Qahtani, Najran University College of Medicine, Najran, Najran Saudi Arabia.
Email: smaalqahtany@nu.edu.sa

Abstract

Background: Celiac disease is an autoimmune condition characterized by serological and histopathological manifestations associated with gluten ingestion.
Aim: This report investigates histopathological findings in all celiac disease patients of both genders enrolled for duodenal biopsy from June 2015 to May 2020 in four centers in Najran, Saudi Arabia.
Methods: This retrospective study assessed data retrieved from archived histopathology records. The data were analyzed using Prism GraphPad 6. Categorical variables were examined using descriptive statistics, including frequency and percentages. A Chi-square test was used to assess the association between gender and age, clinical presentation, and histopathological changes. $P < 0.05$ was considered as significant.
Results: The study included 150 celiac disease patients, of whom 104 were female (69.3%), with most aged between 31 and 40 years (33.3%). Regarding clinical presentation, the majority of patients (62%) presented with gastrointestinal symptoms. Almost half of the duodenal biopsies (71 cases, 47.3%) showed shortened villi caused by partial atrophy, which is consistent with Grade B1 according to Corazza and Villanaci criteria, and Type 3A and 3B lesions according to the Marsh–Oberhuber classification. The second most frequent histopathological finding was an increased abundance of intraepithelial lymphocytes in the absence of villous atrophy, which was found in 56 biopsies (37.3%) and categorized as Grade A.
Conclusions: Females are affected by celiac disease more than males in Najran and the majority presented as having typical celiac disease with gastrointestinal symptoms. Most of the diagnosed cases of celiac disease ranged between Grades A and B1, with less involvement of the severe degree Grades B2 and 3C, according to Corazza and Villanaci's criteria and the Marsh–Oberhuber classification. Despite the absence of any association between gender, age, or clinical presentation, there were significant associations between gender and histopathological findings, grading, and classification of celiac disease lesions. Finally, the presence of asymptomatic patients (12.7%) indicates the importance of celiac disease screening.
Relevance of Patients: This study might be considered a reference for pathologists assessing the duodenal biopsies for patients screened for celiac disease in Najran.

1. Introduction

Celiac disease is an autoimmune condition characterized by serological and histopathological manifestations associated with the ingestion of gluten, an alcohol-soluble group of proteins present in different cereals such as wheat, barley, and oats [1]. During the last few decades, there have been significant developments in understanding the diagnosis, pathogenesis, and clinical presentation of this condition [2]. Celiac disease associates

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00189

with gastrointestinal and/or extraintestinal symptoms; typical celiac disease is characterized by varying degrees of severity of gastrointestinal symptoms, while atypical presentation of the disease is more frequent, and is characterized by an absence of gastrointestinal symptoms [3,4].

Gastrointestinal findings in typical celiac disease include persistent diarrhea, abdominal pain, distension, vomiting, and weight loss. The extraintestinal findings are variable and might be non-specific, including chronic fatigue, skin inflammatory disorders, joint pain, anemia, migraines, psychiatric disorders, epilepsy, osteoporosis, infertility, frequent fetal loss, short stature, failure to thrive, dental abnormalities, multiple vitamin deficiencies, and autoimmune disorders [5]. In addition, a latent form of celiac disease is characterized by the presence of predisposing genetic factors such as the presence of human leukocyte antigen (HLA)-DQ2 and/or HLA-DQ8, normal intestinal mucosa, and the usual positive profile of celiac serology [6].

Early diagnosis and treatment of celiac disease are essential mainly in the pediatric age group. This is because certain complications of celiac disease may be irreversible, such as growth retardation, abnormal teething, and osteoporosis. Several studies in the literature suggest prolonged breastfeeding and a delayed gradual introduction of gluten in the 1st year of life to reduce the risk of celiac disease development [7]. The diagnosis of celiac disease is based on the presence of a predisposing genetic factor, positive histopathological biopsy, and the presence of serological antibodies that are released on gluten ingestion [2]. The most available and effective treatment for celiac disease patients is a lifelong gluten-free diet [8]. This generally leads to improvements in patients within weeks, and normal mucosal histology is regained after several years [9]. However, Vitamin B deficiency may affect patients because of long gluten-free diets, and patients are advised to take gluten-free multivitamins [10]. In this context, despite a lack of reports which link the consumption of milk and dairy products to the progression of celiac disease [11], celiac disease patients are advised to avoid such staples because of abnormal intestinal absorption [12].

The current report aims to investigate histopathological findings retrospectively in celiac disease patients enrolled for duodenal biopsy in Najran, since this is considered one of the diagnostic criteria, along with serology and genetic testing.

2. Materials and Methods

A retrospective study was conducted after receiving approval from the Local Ethical Committee at the College of Medicine, Najran University. As mentioned previously, this study aims to investigate histopathological findings in celiac disease patients enrolled for duodenal biopsy. The study included all patients who attended the Departments of Pathology/Histopathology at the King Khalid Hospital, Najran General Hospital, Maternity and Child Hospital, and Najran University Hospital from June 2015 to May 2020, and data were retrieved from the records of confirmed cases. The inclusion criteria comprised all cases that were reported by histopathology, and graded and/or classified according to the Villanaci and Ceppa [13]; Corazza and Villanaci [14]; and/or Marsh–Oberhuber criteria [15]. These criteria were suggested to simplify histopathological reporting and consequently to facilitate communication between pathologists and clinicians. Celiac disease lesions were divided into two categories according to the Corazza and Villanaci criteria, as follows: (1) Grade A non-atrophic lesions, characterized by an increased number of intraepithelial lymphocytes with intact villi and (2) Grade B trophic lesions with further subcategorization into B1, in which villi were still identifiable, and B2, in which villi were totally atrophic [14]. Grade A lesions correspond to Type 1 and Type 2 lesions based on the Marsh–Oberhuber classification and are usually identified by immunohistochemical staining for cluster of differentiation (CD) 3, which is specific for T lymphocytes [2]. Grade B1 lesions correspond to Class 3A and 3B lesions according to the Marsh–Oberhuber classification, while Grade B2 lesions of Corazza and Villanaci correspond to Marsh–Oberhuber class 3C [2].

Crombie's items, the appraisal tool for cross-sectional studies, and the Agency for Health-care Research and Quality methodology checklist for cross-sectional/prevalence studies (Table S1), were used to assess selection bias [16].

Data were analyzed using Prism GraphPad 6 for Windows, version 6.07 (CA, USA). Categorical variables were analyzed using descriptive statistics, including frequency and percentages. A Chi-square test was used to assess the association between gender, age, and clinical presentation. $P < 0.05$ was considered as significant results.

3. Results

This study included 150 celiac disease patients who were diagnosed from June 2015 to May 2020, and no cases have been excluded from the study. As shown in Figure 1 regarding case distribution during this period, most cases were diagnosed in 2019 – 2020 (61, 40.7%). In 2018, there were 51 cases (34%), while there were 18 cases (12%) in 2016 – 2017. The least number of diagnosed cases was in 2015 – 2016 (8 cases, 5.3%), followed by 2017 – 2018 (12 cases, 8%).

As detailed in Table 1, this study included 46 males (30.7%) and 104 females (69.3%), and most of the patients were between 31 and 40 years old (33.3%, 17 males, 33 females). Patients aged between 20 and 30 years comprised 32% (14 males and 34 females) of the diagnosed cases, and those aged between 41 and 50 years accounted for 17.3% (9 males and 17 females) of cases. There were only 15 patients (10%) under 20 years old (three males and 12 females) and 11 patients over 50 years old (6% aged 51 – 60 years, and 1.3% aged more than 60 years). There was no significant association between gender and the different age groups ($P = 0.82$).

Regarding the clinical presentation of the patients, the majority (62%, 28 males, 65 females) presented with gastrointestinal symptoms, including abdominal pain, diarrhea, and abdominal distention. In addition, 20% (10 males and 20 females) of patients presented with anemia, 12.7% (six males and 13 females) were asymptomatic, and 5.3% presented with a history of failure to

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00189

thrive (two males and six females). There was no significant association between gender and the different clinical presentations (P = 0.97).

Table 2 illustrates the histopathological findings of duodenal biopsies, grades of celiac disease according to Corazza and Villanaci criteria, and histopathological classification according to Marsh–Oberhuber criteria. Almost half of duodenal biopsies (71 cases, 47.3%) showed shortened villi caused by partial atrophy, which is consistent with Grade B1 according to Corazza and Villanaci criteria, and Type 3A/3B lesions according to Marsh–Oberhuber classification. Interestingly, most of these patients were females (74.6%), while only 18 were males.

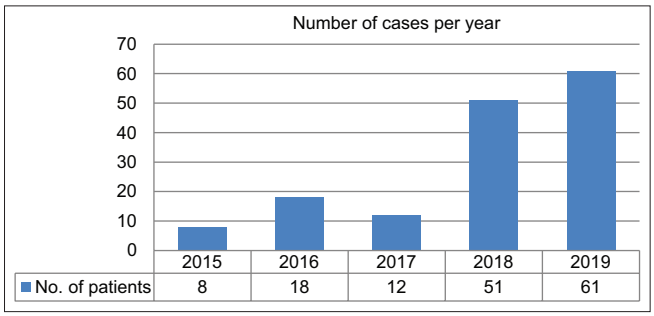


Figure 1. Case distribution of patients diagnosed with celiac disease per year (n = 150).

The second most notable histopathological finding was an increased frequency of intraepithelial lymphocytes without villous atrophy, which was found in 56 biopsies (37.3%) and classified as Grade A according to Corazza and Villanaci criteria, and Type 1/ Type 2 lesions according to the Marsh–Oberhuber classification. In 23 patients (15.3%), there was severe subtotal villous atrophy, assigned as Grade B2 according to Corazza and Villanaci criteria and Type 3C lesions according to the Marsh–Oberhuber classification. This group comprised 13 males (56.5%), compared to only 10 females. Finally, there was a significant association (P = 0.01) between gender and histopathological observations, grading, and classification of celiac disease lesions.

4. Discussion

This 5-year retrospective study aimed to investigate histopathological features in duodenal biopsies from celiac disease patients enrolled in different hospitals in Najran, Saudi Arabia. This work is a continuation of other previously published studies that assessed the histopathological and cytological patterns of different diseases in the region [17,18] and included 150 cases that

Table 1. Gender, age, and presentation of celiac disease patients (n=150)

Parameter	No.	%			
Gender					
Male	46	30.7			
Female	104	69.3			
			Male	**Female**	***P* value (Chi-square, degrees of freedom)**
Age					
<20	15	10	3	12	0.82 (2.22, 5)
20–30	48	32	14	34	
31–40	50	33.3	17	33	
41–50	26	17.3	9	17	
51–60	9	6	3	6	
More than 60	2	1.3	0	2	
Presenting symptoms					
Asymptomatic	19	12.7	6	13	0.97 (0.242, 3)
Gastrointestinal symptoms: abdominal pain, diarrhea, and abdominal distention	93	62	28	65	
Failure to thrive	8	5.3	2	6	
Anemia	30	20	10	20	

Table 2. Histopathological findings, grading, and classification of celiac disease lesions

Histopathological findings	Grade of celiac disease according to Corazza and Villanaci criteria	Histopathological classification according to Marsh–Oberhuber criteria	Number of cases	%	Male	Female	*P* value (Chi-square, degrees of freedom)
Increased intraepithelial lymphocytes without villous atrophy	Grade A/Type 1	Type 1 lesion Type 2 lesion	56	37.3	15	41	*P=0.01 (8.57, 2)
Villi present but shortened as a result of partial atrophy	Grade B1/Type 2	Type 3A lesion Type 3B lesion	71	47.3	18	53	
Subtotal and complete villous atrophy	Grade B2/Type 3	Type 3C lesion	23	15.3	13	10	

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00189

were diagnosed with celiac disease from June 2015 to May 2020. The histopathological grading and classification have clinical importance for clinical follow-up and indicate whether such grades reduce in severity, are maintained, or deteriorate [19]. In this report, according to the criteria of Villanaci and Ceppa [13]; Corazza and Villanaci [14]; and Marsh–Oberhuber [15], Grade A lesions were found in 56 patients (37.3%) as the second most common histopathological pattern after Grade B1 lesions, which were found in 71 patients (47.3%; Table 2). Moreover, only 15.3% of the cases were classified as Grade B2 and Class 3C. This indicates that most of the diagnosed celiac disease cases in Najran range between Grades A and B1, with less involvement of severe Grade B2 and Class 3C. However, despite the absence of any observed associations between gender and age or clinical presentation, contrary to what has been published before [20], there were significant associations between gender and the histopathological findings, grading, and classification of celiac disease lesions. Male patients were diagnosed to have mainly Grades B1 (18 males, 39.1%) and B2 (13 males, 28.3%), while female patients were reported to have mostly Grades A (41 females, 39.4%) and B1 (53 females, 51%) lesions.

The prevalence of celiac disease in Western countries ranges from 1% to 2%. In Saudi Arabia, while there is no clear data regarding the prevalence of celiac disease, studies from different cities and regions have estimated a prevalence range of 1%–3% [21-23]. A meta-analysis conducted in 2018 concluded that the prevalence of histopathology-proven celiac disease cases is about 1.4%, and that seroprevalence is around 2.7% [24]. One of the largest studies in Saudi Arabia was performed in 2013, and included 1167 healthy adolescents for screening in three different regions [25]; this investigation revealed a celiac disease seroprevalence rate of 2.2%. A notable mass screening study to determine the prevalence of celiac disease in Riyadh reported a high prevalence of the disease (1.5%) among Saudi children, which is at least double that in Europe and North America [26]. In this report, the prevalence was not studied in detail and was not one of the objectives. However, during the collection of gastrointestinal cases assessed by histopathology in our 5-year study period, the number of celiac disease cases was noted to be 150 out of 9406 (1.6%), which is consistent with previous reports in other regions of the country.

Regarding celiac disease case distribution through the study period (Figure 1), the highest frequency was in 2019, with 61 cases diagnosed with celiac disease (40.7%). The second highest frequency was in 2018, with 51 cases (34%), followed by 2016 (18 cases, 12%) and 2017 (12 cases, 8%), and the lowest in 2015 (8 cases, 5.3%). Regarding gender differences, males and females are comparable in terms of prevalence and presentation [27]. However, the number of affected females has been reported to be higher than that of affected males [28,29], consistent with the observations of this study (Table 1). Females comprised more than two-thirds of the cases (104 cases, 69.3%). Conversely, gender is not of clinical significance in follow-up, and males and females have comparable disease courses after adhering to a gluten-free diet [20].

Celiac disease patients present with various signs and symptoms, such as abdominal distention, diarrhea, abdominal pain, weight loss, anemia, and bone disease. Despite the increased global prevalence of celiac disease, a significant number of celiac disease patients are still undiagnosed [30]. A variety of reasons for this have been discussed in the literature, including the patchy appearance of mucosal pathology in celiac disease, insufficient or non-representative biopsy for histopathological assessment, variability in histopathology reporting, and the presence of asymptomatic patients [31-34]. The last observation is consistent with the results of this study (Table 1), where the asymptomatic patient group consisted of 19 cases (12.7%). Although typical celiac disease presenting with gastrointestinal symptoms was less common than that presenting with extra-intestinal symptoms [2-4], the former group comprised 93 cases (62%) in this report, and the most affected age group was 31 – 40 years (33.3%), followed by 21 – 30 years (32%). In this report, the atypical extraintestinal celiac disease presentation group [35] included 30 cases of anemia (20%) and 8 cases of failure to thrive (5.3%).

Finally, the pathogenesis of celiac disease has been linked to various microbial species, including *Helicobacter pylori*. In this study, only 2% of the patients had any history of *H. pylori* infection (data not shown). This was contrary to several studies that found a high prevalence of *H. pylori* infection in celiac disease patients [36]. However, some studies have reported no relationship or correlation between the presence of *H. pylori* and pathogenesis of celiac disease [37,38], which may support the observation of this report in this regard.

In conclusion, females are affected by celiac disease more than males and most of the patients are aged between 31 and 40 years. Regarding the clinical presentation of the patients, the majority (62%) presented with typical celiac disease with gastrointestinal symptoms, including abdominal pain, diarrhea, and abdominal distention. Most of the diagnosed cases of celiac disease in Najran range between Grades A and B1, with less involvement of the severe degree Grade B2 and Class 3C, according to Corazza and Villanaci criteria and the Marsh–Oberhuber classification, respectively. Despite the absence of any association between gender and age or clinical presentations, there was a significant association between gender and the histopathological findings, grading, and classification of celiac disease lesions. Finally, the presence of asymptomatic patients (12.7%) may indicate the importance of celiac disease screening.

Acknowledgments

The authors gratefully acknowledge their colleagues from the different hospitals who facilitated their work during data collection.

Funding

None.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00189

Ethics Approval and Consent to Participate

The study was conducted after receiving approval from the Local Ethical Committee at the College of Medicine, Najran University. Consents have been obtained from the human subjects prior to this study.

Consent to Publication

Not applicable.

References

[1] Fasano A, Catassi C. Clinical Practice. Celiac Disease. N Engl J Med 2012;367:2419-26.

[2] Caio G, Volta U, Sapone A, Leffler DA, De Giorgio R, Catassi C, *et al.* Celiac Disease: A Comprehensive Current Review. BMC Med 2019;17:142.

[3] Rodrigo L, Beteta-Gorriti V, Alvarez N, Gómez de Castro C, de Dios A, Palacios L, *et al.* Cutaneous and Mucosal Manifestations Associated with Celiac Disease. Nutrients 2018;10:800.

[4] Livshits OE, Shauol R, Reifen R, Matthias T, Lerner A. Can Celiac Disease Present Along with Childhood Obesity. Int J Celiac Dis 2017;5:19-23.

[5] Ehsani-Ardakani MJ, Rostami Nejad M, Villanacci V, Volta U, Manenti S, Caio G, *et al.* Gastrointestinal and Non-gastrointestinal Presentation in Patients with Celiac Disease. Arch Iran Med 2013;16:78-82.

[6] Sciurti M, Fornaroli F, Gaiani F, Bonaguri C, Leandro G, Di Mario F, *et al.* Genetic Susceptibilty and Celiac Disease: What Role do HLA Haplotypes Play? Acta Biomed 2018;89:17-21.

[7] Szajewska H, Shamir R, Mearin L, Ribes-Koninckx C, Catassi C, Domellöf M, *et al.* Gluten Introduction and the Risk of Coeliac Disease: A Position Paper by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr 2016;62:507-13.

[8] Makovicky P, Makovicky P, Caja F, Rimarova K, Samasca G, Vannucci L. Celiac Disease and Gluten-free Diet: Past, Present, and Future. Gastroenterol Hepatol Bed Bench 2020;13:1-7.

[9] Newnham ED, Shepherd SJ, Strauss BJ, Hosking P, Gibson PR. Adherence to the Gluten-free Diet Can Achieve the Therapeutic Goals in Almost All Patients with Coeliac Disease: A 5-year Longitudinal Study From Diagnosis. J Gastroenterol Hepatol 2016;31:342-9.

[10] Rondanelli M, Faliva MA, Gasparri C, Peroni G, Naso M, Picciotto G, *et al.* Micronutrients Dietary Supplementation Advices for Celiac Patients on Long-term Gluten-free Diet with Good Compliance: A Review. Medicina (Kaunas) 2019;55:337.

[11] Zingone F, Iovino P, Bucci C, Ciacci C. Coeliac Disease: No Difference in Milk and Dairy Products Consumption in Comparison with Controls. BMJ Nutr Prev Health 2019;2:39-42.

[12] Rubin JE, Crowe SE. Celiac Disease. Ann Intern Med 2020;172:C1-16.

[13] Villanacci V, Ceppa P, Tavani E, Vindigni C, Volta U, Gruppo Italiano Patologi Apparato Digerente (GIPAD), *et al.* Coeliac Disease: The Histology Report. Dig Liver Dis 2011;43:S385-95.

[14] Corazza GR, Villanacci V. Coeliac Disease. J Clin Pathol 2005;58:573-4.

[15] Oberhuber G, Granditsch G, Vogelsang H. The Histopathology of Coeliac Disease: Time for a Standardized Report Scheme for Pathologists. Eur J Gastroenterol Hepatol 1999;11:1185-94.

[16] Ma LL, Wang YY, Yang ZH, Huang D, Weng H, Zeng XT. Methodological Quality (Risk of Bias) Assessment Tools for Primary and Secondary Medical Studies: What are they and which is Better? Mil Med Res 2020;7:7.

[17] Alqahtani SM. Cytological Patterns of Thyroid Lesions in Najran, Saudi Arabia: A 5-year Retrospective Study. Saudi Med J 2022;43:735-42.

[18] Al-Qahtani SM, Uz Zafar MN, Assiri AM, Bashanfer G, Alwaily MM, Alshaiban MH, *et al.* Pattern of Malignant Tumors in Najran, Saudi Arabia: A 5-Year Retrospective Study. Int J Biomed 2021;11:498-504.

[19] Elli L, Zini E, Tomba C, Bardella MT, Bosari S, Conte D, *et al.* Histological Evaluation of Duodenal Biopsies from Coeliac Patients: The Need for Different Grading Criteria During Follow-Up. BMC Gastroenterol 2015;15:133.

[20] Galli G, Amici G, Conti L, Lahner E, Annibale B, Carabotti M. Sex-Gender Differences in Adult Coeliac Disease at Diagnosis and Gluten-free-diet Follow-Up. Nutrients 2022;14:3192.

[21] Al-Hussaini A, Alharthi H, Osman A, Eltayeb-Elsheikh N, Chentoufi A. Genetic Susceptibility for Celiac Disease is Highly Prevalent in the Saudi Population. Saudi J Gastroenterol 2018;24:268-273.

[22] Al Hatlani MM. Prevalence of Celiac Disease among Symptom-free Children from the Eastern Province of Saudi Arabia. Saudi J Gastroenterol 2015;21:367-71.

[23] Saeed A, Assiri A, Assiri H, Ullah A, Rashid M. Celiac Disease in Saudi Children. Evaluation of Clinical Features and Diagnosis. Saudi Med J 2017;38:895-9.

[24] Safi MA. Prevalence of Celiac Disease in Saudi Arabia: Meta-analysis. Glob Vaccines Immunol. 2018;3:1-6.

[25] Aljebreen AM, Almadi MA, Alhammad A, Al Faleh FZ. Seroprevalence of Celiac Disease among Healthy Adolescents in Saudi Arabia. World J Gastroenterol 2013;19:2374-8.

[26] Al-Hussaini A, Troncone R, Khormi M, AlTuraiki M, Alkhamis W, Alrajhi M, *et al.* Mass Screening for Celiac Disease Among School-aged Children: Toward Exploring Celiac Iceberg in Saudi Arabia. J Pediatr Gastroenterol

Nutr 2017;65:646-51.

[27] Jansson-Knodell CL, King KS, Larson JJ, Van Dyke CT, Murray JA, Rubio-Tapia A. Gender-based Differences in a Population-based Cohort with Celiac Disease: More Alike than Unalike. Dig Dis Sci 2018;63:184-92.

[28] Volta U, Caio G, Stanghellini V, De Giorgio R. The Changing Clinical Profile of Celiac Disease: A 15-Year Experience (1998-2012) in an Italian Referral Center. BMC Gastroenterol 2014;14:194.

[29] Choung RS, Ditah IC, Nadeau AM, Rubio-Tapia A, Marietta EV, Brantner TL, *et al.* Trends and Racial/Ethnic Disparities in Gluten-Sensitive Problems in the United States: Findings From the National Health and Nutrition Examination Surveys from 1988 to 2012. Am J Gastroenterol 2015;110:455-61.

[30] Lionetti E, Gatti S, Pulvirenti A, Catassi C. Celiac Disease from a Global Perspective. Best Pract Res Clin Gastroenterol 2015;29:365-79.

[31] Brown I, Bettington M, Rosty C. The Role of Histopathology in the Diagnosis and Management of Coeliac Disease and Other Malabsorptive Conditions. Histopathology 2021;78:88-105.

[32] Dai Y, Zhang Q, Olofson AM, Jhala N, Liu X. Celiac Disease: Updates on Pathology and Differential Diagnosis. Adv Anat Pathol 2019;26:292-312.

[33] Bao F, Bhagat G. Histopathology of Celiac Disease. Gastrointest Endosc Clin N Am 2012;22:679-94.

[34] Montén C, Bjelkenkrantz K, Gudjonsdottir AH, Browaldh L, Arnell H, Naluai ÅT, *et al.* Validity of Histology for the Diagnosis of Paediatric Coeliac Disease: A Swedish Multicentre Study. Scand J Gastroenterol 2016;51:427-33.

[35] Durazzo M, Ferro A, Brascugli I, Mattivi S, Fagoonee S, Pellicano R. Extra-intestinal Manifestations of Celiac Disease: What Should We Know in 2022? J Clin Med 2022;11:258.

[36] Størdal K, Kahrs C, Tapia G, Agardh D, Kurppa K, Stene LC. Review Article: Exposure to Microbes and Risk of Coeliac Disease. Aliment Pharmacol Ther 2021;53:43-62.

[37] Amlashi FI, Norouzi Z, Sohrabi A, Shirzad-Aski H, Norouzi A, Ashkbari A, *et al.* A Systematic Review and Meta-analysis for Association of Helicobacter Pylori Colonization and Celiac Disease. PLoS One 2021;16:e0241156.

[38] Basyigit S, Unsal O, Uzman M, Sapmaz F, Dogan OC, Kefeli A, *et al.* Relationship between Helicobacter Pylori Infection and Celiac Disease: A Cross-sectional Study and a Brief Review of the Literature. Prz Gastroenterol 2017;12:49-54.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00189

Journal of Clinical and Translational Research 2023; 9(4): 282-289

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

Histopathological findings in celiac disease patients enrolled for duodenal biopsy in Najran, Saudi Arabia: a 5-year retrospective study

Supplementary File

Table S1. Checklist for bias assessment

Major components	Response options		
The Appraisal tool for Cross-Sectional Studies (AXIS tool; last introduced on December 8, 2016)			
Introduction			
1. Were the aims/objectives of the study clear?	Yes	No	Do not know/comment
Methods			
2. Was the study design appropriate for the stated aim (s)?	Yes	No	Do not know/comment
3. Was the sample size justified?	Yes	No	Do not know/comment
4. Was the target/reference population clearly defined? (Is it clear who the research was about?)	Yes	No	Do not know/comment
5. Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?	Yes	No	Do not know/comment
6. Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation?	Yes	No	Do not know/comment Not applicable
7. Were measures undertaken to address and categorise non-responders?	Yes	No	Do not know/comment Not applicable
8. Were the risk factor and outcome variables measured appropriate to the aims of the study?	Yes	No	Do not know/comment Not applicable
9. Were the risk factor and outcome variables measured correctly using instruments/measurements that had been trialed, piloted or published previously?	Yes	No	Do not know/comment Not applicable
10. Is it clear what was used to determined statistical significance and/or precision estimates? (e.g., *P* values, CIs)	Yes	No	Do not know/comment
11. Were the methods (including statistical methods) sufficiently described to enable them to be repeated?	Yes	No	Do not know/comment
Results			
12. Were the basic data adequately described?	Yes	No	Do not know/comment
13. Does the response rate raise concerns about non-response bias? Not applicable	Yes	No	Do not know/comment
14. If appropriate, was information about non-responders described? Not applicable	Yes	No	Do not know/comment
15. Were the results internally consistent?	Yes	No	Do not know/comment
16. Were the results for the analyses described in the methods, presented?	Yes	No	Do not know/comment

(*Contd...*)

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00189

Table S1. (*Continued*)

Major components	Response options		
The Agency for Healthcare Research and Quality (AHRQ) Methodology Checklist for Cross-Sectional/Prevalence Study Website: http://www.ncbi.nlm.nih.gov/books/NBK35156/			
1. Define the source of information (survey, record review)	Yes	No	Unclear
2. List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications	Yes	No	Unclear
3. Indicate time period used for identifying patients	Yes	No	Unclear
4. Indicate whether or not subjects were consecutive if not population-based	Yes	No	Unclear
5. Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants	Yes	No	Unclear
6. Describe any assessments undertaken for quality assurance purposes (e.g., test/retest of primary outcome measurements) Not applicable	Yes	No	Unclear
7. Explain any patient exclusions from analysis	Yes	No	Unclear
8. Describe how confounding was assessed and/or controlled Not applicable	Yes	No	Unclear
9. If applicable, explain how missing data were handled in the analysis Not applicable	Yes	No	Unclear
10. Summarize patient response rates and completeness of data collection Not applicable	Yes	No	Unclear
11. Clarify what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained Not applicable	Yes	No	Unclear
Crombie's items			
1. Appropriateness of design to meet the aims	Yes (1 point)	Unclear (0.5 point)	No (0 point)
2. Adequate description of the data	Yes (1 point)	Unclear (0.5 point)	No (0 point)
3. Report the response rates: not applicable	Yes (1 point)	Unclear (0.5 point)	No (0 point)
4. Adequate representativeness of the sample to total	Yes (1 point)	Unclear (0.5 point)	No (0 point)
5. Clearly stated aims and likelihood of reliable and valid measurements	Yes (1 point)	Unclear (0.5 point)	No (0 point)
6. Assessment of statistical significance	Yes (1 point)	Unclear (0.5 point)	No (0 point)
7. Adequate description of statistical methods	Yes (1 point)	Unclear (0.5 point)	No (0 point)

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00189

Journal of Clinical and Translational Research 2023; 9(4): 290-296

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

Does the endoscopic keyhole technique have advantages over the microscopic keyhole technique for treating cervical radiculopathy?

Shutong Xu[1], Junlong Zhong[1], Zhenhai Zhou[1], Hao Lv[1], Jiachao Xiong[1], Shengbiao Ma[1], Zhimin Pan[1], Yong Zhang[2], Kai Cao[1]*

[1]The Orthopedic Hospital, The First Affiliated Hospital of Nanchang University, Nanchang, 330006, China, [2]Department of Pain, The First Affiliated Hospital of Nanchang University, Nanchang, 330006, China

ARTICLE INFO

Article history:
Received: February 6, 2023
Revised: March 27, 2023
Accepted: June 10, 2023
Published online: July 28, 2023

Keywords:
Cervical radiculopathy
Endoscopy
Keyhole discectomy
Microscopy
Learning curve
Microscopic keyhole technique
Endoscopic keyhole technique

**Corresponding authors:*
Kai Cao,
The Orthopedic Hospital, The First Affiliated Hospital of Nanchang University, Nanchang, 330006, China.
E-mail: kaichaw@126.com
Yong Zhang,
Department of Pain Clinic, The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi Province, 330209, China.
E-mail: zy830226@163.com

Abstract

Background: Both endoscopic keyhole and microscopic keyhole techniques are considered minimally invasive approaches. However, it is still unclear which is superior in treating cervical radiculopathy.
Aim: This study aimed to compare the clinical outcomes of the two methods for cervical radiculopathy.
Methods: Seventy-one patients with cervical radiculopathy caused by single-level disc herniation were retrospectively reviewed. These patients were treated with the endoscopic keyhole technique (EKT) (34 cases, classified as EKT group) or the microscopic keyhole technique (37 cases, classified as MKT group). Magnetic resonance imaging (MRI), neck disability index (NDI), and visual analog scores (VAS) were employed to assess clinical outcomes. All patients were followed up for at least 24 months.
Results: The average operative time (71.0 ± 15.2 min vs. 63.7 ± 18.9 min, $P = 0.131$), blood loss (56.1 ± 18.2 ml vs. 64.4 ± 13.5 ml, $P = 0.068$), and hospital stay (24.9 ± 5.6 h vs. 28.3 ± 7.1 h, $P = 0.061$) between the EKT and MKT groups were not significantly different. Postoperative MRI demonstrated that effective neural decompression was obtained in all cases after surgery. The NDI in both groups was significantly decreased from pre- to postoperatively (EKT group: 32.8 ± 9.4 vs. 9.2 ± 3.6, $P < 0.001$; MKT group: 36.2 ± 11.3 vs. 10.5 ± 4.1, $P < 0.001$), VAS (EKT group: 5.6 ± 2.3 vs. 1. 5 ± 1.0, $P < 0.001$; MKT group: 6.2 ± 2.1 vs. 1.9 ± 0.8, $P < 0.001$). Nine patients in the EKT group underwent revision surgery due to recurrent disc herniation compared with 2 patients in the MKT group ($P = 0.034$). The interval time from primary surgery to revisional surgery was shorter in the EKT group than in the MKT group (21 ± 5.8 weeks vs. 29 ± 7.2 weeks, $P < 0.001$). There were 2 patients with temporary nerve root irritation and 1 patient with cerebrospinal fluid leak that occurred in the EKT group versus 1 patient who suffered nerve root irritation in the MKT group ($P = 0.547$).
Conclusions: Both EKT keyhole and microscopic keyhole techniques are effective in treating cervical radiculopathy. However, compared with the microscopic keyhole technique, the EKT brings about a higher revision surgery rate with a shorter interval time from index surgery to revision surgery.
Relevance for Patients: These findings suggest that the microscopic keyhole technique seems to be a better way of treating cervical radiculopathy.

1. Introduction

Cervical radiculopathy is defined as a clinical syndrome of sensorimotor deficits due to compression on the cervical nerve root [1]. Facet joint spondylosis and herniation of the intervertebral disc are the most common causes of nerve root compression [2]. Patients present with pain, tingling, numbness, or even weakness in the upper extremity [2].

Surgical management for cervical radiculopathy mainly includes anterior cervical discectomy and fusion (ACDF), cervical foraminotomy via an anterior or posterior approach,

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00023

and cervical arthroplasty with decompression [3-6]. ACDF has been widely performed and is considered the standard surgical treatment for cervical degenerative disc disease [7]. This procedure, however, usually results in the loss of motion at the operated level and accelerates adjacent segmental degeneration [8,9]. In addition to graft-site complications, dysphagia, esophageal perforation, and pseudoarthrosis may also occur in ACDF. Posterior cervical foraminotomy is an appropriate alternative since it is a motion-preserving and minimizing adjacent segmental degeneration technique. The posterior approach is especially feasible for patients whose soft disc herniation originates from the posterolateral location, lying lateral to the cord and compressing the nerve root. It is also appropriate for osteophytes originating from the facet joint, and arm symptoms are more severe than neck symptoms [10,11].

The importance of reducing damage, particularly to muscles that maintain segmental stability, has been widely recognized [12]. The concept that less invasive decompression could yield better results has given rise to the development of minimally invasive techniques, such as microscope-assisted keyhole discectomy and the recently developed percutaneous endoscopic keyhole discectomy. Both of them are considered minimally invasive approaches. However, no literature has reported which one is superior in treating cervical radiculopathy. This study aimed to compare the clinical outcomes of endoscopic keyhole and microscopic keyhole discectomy in treating cervical radiculopathy.

2. Materials and Methods

2.1. Patients

From September 2018 to November 2022, 71 consecutive patients aged 29–75 years with single-level cervical radiculopathy were reviewed in four hospitals. A retrospective study was performed in patients treated with endoscopic keyhole discectomy (n = 34) and microscopic keyhole discectomy (n = 37). The inclusion criteria for this study were (1) unilateral posterolateral soft disc herniation demonstrated by magnetic resonance imaging (MRI), (2) unilateral radicular symptoms with or without neck pain consistent with MRI findings, and (3) failure of conservative treatment for at least 6 weeks. The exclusion criteria were as follows: previous cervical surgical history, myelopathic symptoms, segmental instability, cervical kyphosis, massive, sequestered disc prolapse, cervical axial pain, and discitis. This study was designed in conformity with the Declaration of Helsinki, and informed consent was obtained from eligible patients. The demographic data of the patients are shown in Table 1.

Table 1. Patient demographics

Parameter	EKT (n=34)	MKT (n=37)	P-value
Age (year)	56.5±12.8	61.7±14.2	0.172
Gender (M/F)	15/19	20/17	0.549
Follow-up time (month)	31.8±6.3	29.5±5.1	0.154
Operative time (min)	71.0±15.2	63.7±18.9	0.131
blood loss (ml)	56.1±18.2	64.4±13.5	0.068
hospital stay (h)	24.9±5.6	28.3±7.1	0.061
Operative level			
C3/4	3	1	
C4/5	8	9	
C5/6	13	17	
C6/7	10	8	
C7/T1	0	2	

EKT: Endoscopic keyhole technique; M: Male; F: Female

2.2. Surgical procedures

In the microscopic keyhole group, the patient's head was fixed by the Mayfield frame in the Concorde position after general anesthesia. The incision level was determined by fluorography. First, a longitudinal initial incision approximately 10 mm lateral to the midline was made on the pathologic side. Under fluoroscopic guidance, a K-wire was advanced from the incision and was docked at the inferomedial portion of the lateral mass of the surgical level. The incision was elongated to 20 mm, followed by muscular blunt dissection with tubular dilators (Figure 1A). An 18- or 20-mm tubular retractor was placed around the dilator and fixed on the laminofacet junction with a table-mounted flexible arm (Figure 1B). Next, the dilator was removed, and the surgical field was amplified and focused under the microscope. Bipolar cautery and pituitary rongeurs were used to conduct hemostasis and clear the remaining soft tissue off the lateral mass and lamina (Figure 1C). Then, a high-speed burr was utilized to resect the medial one-third of the inferior articular process of the cephalad vertebra until the superior articular process of the caudal vertebrae could be visualized (Figure 1D). After that, a small upangled curette was used to gently detach the ligamentum flavum from the undersurface of the inferior edge of the lamina, and a Kerrison rongeur was used to resect the medial one-third of the exposed superior articular process of the caudad vertebra. Finally, the herniated disc fragment was exposed and removed by a pituitary rongeur after slightly retracting away the dura and nerve root (Figure 1E). The target nerve root could be completely decompressed and checked under microscopic visualization (Figure 1F). A typical case treated by microscopic keyhole discectomy is presented on MRI (Figure 2).

Compared with the microscopic keyhole technique, the procedures of the endoscopic keyhole technique (EKT) were different as follows: the patient laid in the same position as mentioned above after general anesthesia. First, under fluoroscopic guidance, a K-wire was advanced from a 7 mm incision and docked at the inferomedial portion of the lateral mass of the surgical level. Tubular dilators were used to bluntly dissect muscles, and then the dilator was removed after a working channel was established. Second, a 5.9 mm endoscope was inserted through the working channel to obtain the vision of the margin of the superior lamina, inferior lamina, and medial facet joint after clearing off the attached soft tissue. Third, a keyhole foraminotomy was performed at the lamina-facet junction by using a 3 mm diamond burr and a bone punch. Then, the lateral edge of the dura and the nerve root was identified, and discectomy was performed using micropituitary forceps (Figure 3). A typical case treated by endoscopic keyhole discectomy is presented in Figure 4.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00023

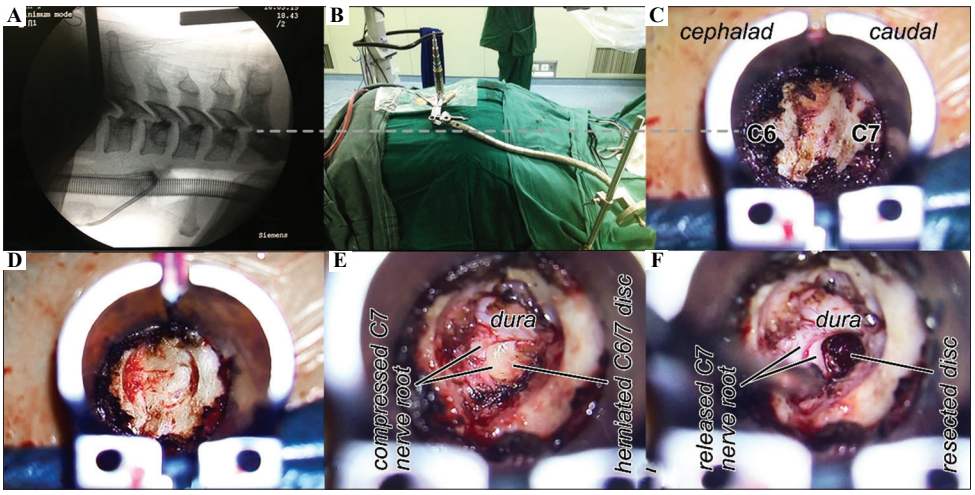


Figure 1. The procedure of the microscopic keyhole technique. (A) Lateral fluoroscopic image confirmed the interest level and demonstrated the starting dilator advancement over the target level. (B) The final 20-mm tubular retractor was placed over the dilators and fixed into place over the laminofacet junction with a table-mounted flexible retractor arm. (C) The surgical field was amplified and focused under the microscope, and the inferior articular process of C6 and the superior articular process of C7 were visualized. (D) After a high-speed burr was utilized to resect the medial third of the inferior articular process of C6 and the superior articular process C7, the yellow ligament was presented. (E) After detachment of the ligamentum flavum from the undersurface of the inferior edge of the lamina and resection of the medial third of the exposed facet joint of C6-C7, the herniated disc and compressed C7 nerve root were exposed. (F) The herniated disc fragment was removed by a pituitary rongeur, and then the C7 nerve root was completely decompressed.

2.3. Clinical evaluations and management

Preoperative and postoperative MRI was compared to evaluate neural decompression. The neck disability index (NDI) and visual analog scale (VAS) scores were recorded to assess intragroup and intergroup neurological functions. Operative time, blood loss, and hospital stay were documented. Surgery-related complications such as neurological deficits and leakage of cerebrospinal fluid (CSF) were recorded to evaluate surgical safety. All patients were followed up for at least 24 months.

Antimicrobials were intravenously administered half an hour before surgery in all patients just once. Analgetic acid was routinely administered for all patients for 72 h postoperatively. General activity was suggested on the 2nd day after surgery. A cervical collar was suggested for use for 2 weeks.

2.4. Statistical analysis

All statistical analyses were performed by IBM SPSS Statistics ver. 19.0 (IBM, Armonk, NY, USA). Preoperative and postoperative data were compared by paired t-tests. Independent samples t-tests were used to compare corresponding data between EKT and MKT groups. The revision surgery rate and complication rate were compared by the Chi-square test. Data are presented as mean ± standard deviation. A $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Operative outcomes

Seventy-one consecutive patients were retrospectively reviewed in this study. All patients' incisions were primarily healed. The average operative time was 71.0 ± 15.2 min in the EKT group and 63.7 ± 18.9 min in the MKT group ($P = 0.131$). The estimated blood loss was 56.1 ± 18.2 ml in the EKT group versus 64.4 ± 13.5 ml in the MKT group ($P = 0.068$). Additionally, the hospital stay (24.9 ± 5.6 h vs. 28.3 ± 7.1 h for EKT vs. MKT group, respectively, $P = 0.061$) was not significantly different.

3.2. NDI and VAS assessments

The NDI in the EKT group was significantly decreased from 32.8 ± 9.4 preoperatively to 9.2 ± 3.6 ($P < 0.001$) 2 years postoperatively. The NDI in the MKT group decreased from 36.2 ± 11.3 preoperatively to 10.5 ± 4.1 2 years postoperatively ($P < 0.001$). VAS in the EKT group decreased from preoperative 5.6 ± 2.3 to postoperative 2 years 1.5 ± 1.0 ($P < 0.001$), while in the MKT group, VAS decreased from 6.2 ± 2.1 to 1.9 ± 0.8 after surgery 2 years ($P < 0.001$). The improvement in NDI in the EKT group and that in the MKT group were not significantly different (23.4 ± 5.7 vs. 25.3 ± 7.6, $P = 0.313$). The same was true for the improvements in VAS between the two groups (4.1 ± 1.2 vs. 4.3 ± 1.4, $P = 0.583$). Comparing with the EKT group, the VAS and NDI were similarly ameliorated in the MKT group at 3 months and 2 years postoperatively (Table 2).

3.3. Surgery-related complications and revision surgery

MRI demonstrated that effective neural decompression was observed in all cases after primary surgery. Nine patients in the EKT group underwent revision surgery because of recurrent disc herniation versus 2 patients in the MKT group ($P = 0.034$). The interval time from primary surgery to revision surgery was shorter in the EKT group than in the MKT group (Table 3). There were 2 patients with temporary nerve root irritation and 1 patient with

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00023

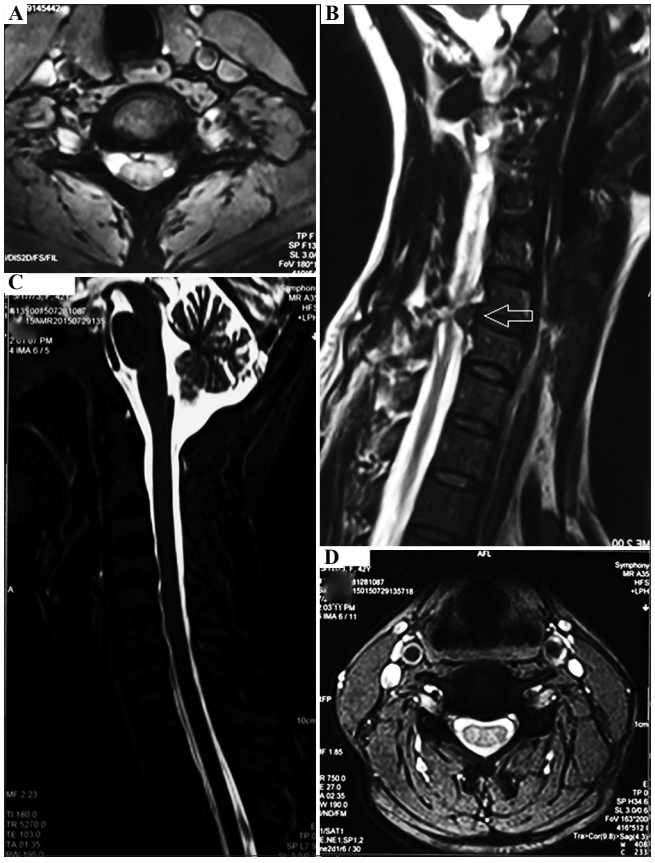


Figure 2. Preoperative and postoperative magnetic resonance imaging (MRI) preoperative cervical MRI showed the herniated fragment located lateral to the cord and compressing the nerve root of C7 (axial view in (A) and sagittal view in (B), white arrow). Postoperative cervical MRI demonstrated that the herniated fragment was completely resected by microscopic keyhole discectomy, and the nerve root of C7 was decompressed (sagittal view in (C) and axial view in (D)).

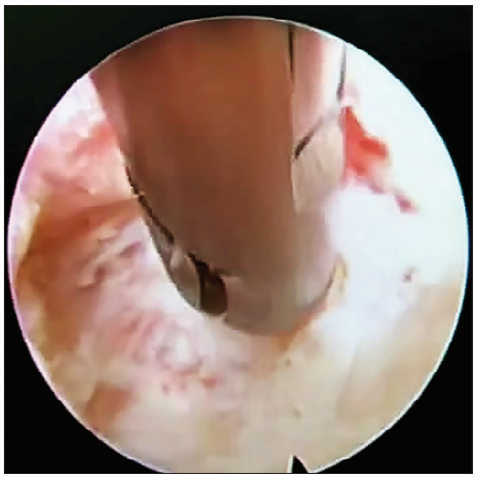


Figure 3. Endoscopic keyhole discectomy was performed by using micropituitary forceps.

CSF leakage due to a dural tear that occurred in the EKT group versus 1 patient who suffered nerve root temporary irritation in the MKT group. There was no significant difference in surgery-related complications between the EKT and the microscopic keyhole technique ($P = 0.547$).

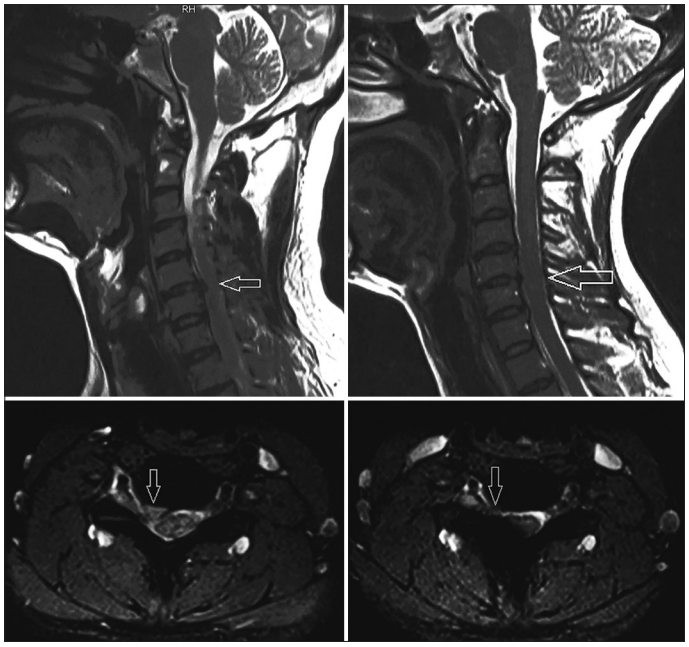


Figure 4. Preoperative and postoperative magnetic resonance imaging (MRI) in the endoscopic keyhole group. Preoperative cervical MRI showed the herniated fragment located lateral to the cord and compressing the nerve root of C6 (A and C, white arrow). Postoperative cervical MRI demonstrated that the herniated fragment was completely resected by endoscopic keyhole discectomy, and the nerve root of C6 was decompressed (B and D, white arrow).

4. Discussion

The posterior approach has distinct advantages in patients with posterolateral disc herniation [13,14], including direct decompression of the involved nerve root without much disruption of the disc and preservation of spinal segmental mobility [15]. In addition, it avoids the risk of injuring the front vital structures of the cervical spine. However, conventional posterior cervical approaches have some drawbacks, such as C5 palsy, kyphosis, and neck pain associated with extensor muscle detachment and atrophy [16,17]. Minimally invasive cervical spinal surgeries were developed to overcome the aforementioned shortcomings. Of those, the keyhole technique is an effective method for treating posterolateral cervical disc herniation which results in cervical radiculopathy. In this study, we compared the clinical outcomes of endoscopic keyhole and microscopic keyhole discectomy in treating cervical radiculopathy and found that both endoscopic keyhole and microscopic keyhole techniques were effective in treating cervical radiculopathy, but the latter had advantages in reducing the revision surgery rate and complications.

Adamson reported that endoscopic posterior lamino-foraminotomy was an effective alternative for treating unilateral cervical radiculopathy secondary to lateral or foraminal disc herniations or spondylosis [18]. In a cadaveric and clinical

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00023

Table 2. NDI and VAS in the EKT group and MKT group

Variable	EKT (*n*=34)	MKT (*n*=37)	*P*-value
VAS			
Preoperative	5.6±2.3	6.2±2.1	0.331
Postoperative 3 months	2.4±1.2	2.7±1.0	0.332
Postoperative 2 years	1.5±1.0	1.9±0.8	0.118
P-value	<0.001	<0.001	
Δ Pre- and post operative	4.1±1.2	4.3±1.4	0.583
NDI			
Preoperative	32.8±9.4	36.2±11.3	0.244
Postoperative 3 month	19.2±6.0	16.8±5.4	0.136
Postoperative 2 years	9.2±3.6	10.5±4.1	0.230
P-value	<0.001	<0.001	
*Δ Pre- and post operative	23.4±5.7	25.3±7.6	0.313

*Δpre- and post operative indicates the difference between preoperative VAS/NDI and VAS/NDI at 2 years postoperatively. NDI: Neck disability index; VAS: Visual analog scores; EKT: Endoscopic keyhole technique

Table 3. Surgery-related complications and revision surgery

Item	EKT (*n*=34)	MKT group (*n*=37)	*P*-value
Complications			
Nerve root irritation	2	1	0.547
Cerebrospinal fluid	1	0	
Revision surgery	9	2	0.034
*Interval time (week)	21.0±6	29.0±7	<0.001

*Interval time means the interval time from primary surgery to revisional surgery. EKT: Endoscopic keyhole technique

combined study, it was demonstrated that a viable, minimally invasive technique could provide exceptional visualization and an improvement in postoperative recovery time [19]. In this study, the NDI and VAS were also significantly decreased after endoscopic keyhole surgery, which confirmed the effectiveness of this minimally invasive surgery (MIS) method. Theoretically, endoscopic keyhole surgery is less invasive than microscopic keyhole surgery. However, we found that both keyhole techniques had similar MIS characteristics regarding operative time, estimated blood loss, and hospital stay. In the MKT group, a slightly longer incision and involved dissection might not affect the abovementioned aspects. Xu *et al.* considered that the tubular retractor system used in the MKT group was fixed by a free arm, so the traction force on posterior extensors was evenly dispersed, and excessive muscular traction could be avoided [20]. Hence, there was no severe postoperative muscle atrophy that occurred in the MKT group. The limited surgery time might be another explanation for the similar invasiveness between the two groups.

Although the incisions of both keyhole techniques were small, intraoperative neural decompression could be performed effectively. The current study showed that NDI and VAS in both groups were significantly decreased after surgery ($P < 0.001$ in both groups), which revealed valid neural decompression resulting from both MKT keyhole and EKTs. The improvements in VAS and NDI between the two groups were significant. Considering that we treated cervical radiculopathy rather than myelopathy in the current study, we did not employ the JOA score to assess clinical outcomes.

Interestingly, in this study, the occurrence rate of revision surgery because of recurrent disc herniation in the EKT group was significantly higher than that in the MKT group ($P = 0.034$). Although the endoscopic technique can provide a minimally invasive approach, it only provides two-dimensional visualization, and surgical vision is often blurred by bleeding or obscured by tissue fragments during operation. The microscopic keyhole technique could provide a three-dimensional and amplified visualization of the surgical field, in coordination with coaxial illumination, and the tubular retractor system also provided more space for performance, which allowed the surgeon to resect the herniated disc more thoroughly and minimized neurological injury. Furthermore, the interval time from primary surgery to revision surgery was longer in the MKT group than in the EKT group ($P < 0.001$). This might reveal that the residual fragments of the disc could reherniate in an earlier stage in the EKT group and that the effectiveness of the microscopic keyhole technique in treating cervical radiculopathy was more durable. This is also the case because of the steep learning curve of the EKT, which has been one of its disadvantages. Furthermore, unskilled operation in the early stage of the steep learning curve is also the reason for the higher recurrence rate in the EKT group. Concerning surgery-related complications, there was more but no significant difference in the EKT group versus the MKT group ($P = 0.547$). Therefore, both techniques could be considered safe methods in the treatment of cervical radiculopathy.

To master the endoscopic technique in clinical practice, surgeons need to know the anatomic landmarks under endoscopy and acquire a way to minimize bone resection. Bony resection of endoscopic keyhole laminoforaminotomy was limited as follows: 1. superior limit, inferior border of the superior facet; 2. inferior limit, superior border of the inferior facet; 3. lateral limit, the junction of the lamina and facet; and 4. medial limit, lateral aspect of the dural sac. To avoid confusion, we considered all superior and inferior anatomic structures of a superior vertebra as superior and all superior and inferior structures of an inferior vertebra as inferior. Hence, instead of using anatomic nomenclature, we identified the facets and laminae based on their relative surgical perspectives. Although the amount of bony resection depends on the patient's anatomy and surgeon's experience, facet resection is usually not more than 25% of the facet joint and very rarely 50% to avoid segmental disability.

After the nerve root has been exposed, it is vital to discern whether the dorsal sensory and ventral motor roots are combined in a single dural sleeve or if the ventral motor root has a separate, thinner, dusky dural mater. This identification is critical to avoid confusing a tethered ventral motor root surrounded by perineural adhesions with the disc herniation itself. Typically, a compressed nerve root is surrounded by an engorged epidural venous plexus that must be coagulated, where feasible, with bipolar forceps. Electrocoagulation should be precise, especially when it is used in the spinal canal, and the electrode should be turned down to reduce damage to the nerve. Surgeons who are just getting

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00023

involved in this field can start with endoscopic lumbar discectomy in a transforaminal approach, which is easier for beginners.

There were some limitations in this study. First, the sample size in this study was limited, which may increase bias. Second, the proficiency of surgery influences the clinical outcomes. Third, this study was a retrospective cohort study. A randomized controlled trial (RCT) would be better to illuminate the clinical outcome difference between the two different posterior MIS techniques. However, to our knowledge, this is the first comparative study between these different keyhole surgeries despite the abovementioned limitations. In future, a multicenter RCT study with a larger number of cases will be required to clarify the effectiveness and safety of both techniques.

With similar surgical complication rates, both endoscopic keyhole and microscopic keyhole techniques are effective in treating cervical radiculopathy resulting from posterolateral disc herniation. However, compared with the microscopic keyhole technique, the EKT brings about a higher revision surgery rate with a shorter interval time from index surgery to revisional surgery.

Acknowledgments

We appreciate the editors and reviewers for the helpful comments on this manuscript.

Funding

This study was supported by Double-thousand Plan Program of Jiangxi Province (JXSQ2023201023) and Clinical Cultivation Project of the First Affiliated Hospital of Nanchang University (YFYLCYJPY202202).

Conflicts of Interest

The authors have declared no conflicts of interest.

Ethics Approval and Consent to Participate

This study has been approved by The First Affiliated Hospital of Nanchang University.

Consent for Publication

All patients gave their oral and written consent.

References

[1] Carette S, Fehlings MG. Clinical Practice. Cervical Radiculopathy. N Engl J Med 2005;353:392-9.

[2] Caridi JM, Pumberger M, Hughes AP. Cervical Radiculopathy: A Review. HSS J 2011;7:265-72.

[3] Skovrlj B, Gologorsky Y, Haque R, Fessler RG, Qureshi SA. Complications, Outcomes, and Need for Fusion after Minimally Invasive Posterior Cervical Foraminotomy and Microdiscectomy. Spine J 2014;14:2405-11.

[4] Ghori A, Konopka JF, Makanji H, Cha TD, Bono CM. Long Term Societal Costs of Anterior Discectomy and Fusion (acdf) Versus Cervical Disc Arthroplasty (cda) for Treatment of Cervical Radiculopathy. Int J Spine Surg 2016;10:1.

[5] Gao Y, Liu M, Li T, Huang F, Tang T, Xiang Z. A Meta-Analysis Comparing the Results of Cervical Disc Arthroplasty with Anterior Cervical Discectomy and Fusion (acdf) for the Treatment of Symptomatic Cervical Disc Disease. J Bone Joint Surg Am 2013;95:555-61.

[6] Chung SW, Kim HJ, Lee SH, Lee SY, Kang MS, Shin YH, *et al*. Posterior Cervical Foraminotomy for Cervical Radiculopathy: Should Cervical Alignment be Considered? J Spine Surg 2019;5:541-8.

[7] Song KJ, Choi BY. Current Concepts of Anterior Cervical Discectomy and Fusion: A Review of Literature. Asian Spine J 2014;8:531-9.

[8] Shin JJ. Comparison of Adjacent Segment Degeneration, Cervical Alignment, and Clinical Outcomes After One- and Multilevel Anterior Cervical Discectomy and Fusion. Neurospine 2019;16:589-600.

[9] Hilton DL Jr. Minimally Invasive Tubular Access for Posterior Cervical Foraminotomy with Three-Dimensional Microscopic Visualization and Localization with Anterior/Posterior Imaging. Spine J 2007;7:154-8.

[10] Kiely PD, Quinn JC, Du JY, Lebl DR. Posterior Surgical Treatment of Cervical Spondylotic Myelopathy: Review Article. HSS J 2015;11:36-42.

[11] Saadeh YS, Sabbagh MA, Smith BW, Joseph JR, Buckingham MJ. Technique for Open Posterior Cervical Foraminotomy: 2-Dimensional Operative Video. Oper Neurosurg (Hagerstown) 2020;18:E120.

[12] Ward SR, Kim CW, Eng CM, Gottschalk LJ 4th, Tomiya A, Garfin SR, *et al*. Architectural Analysis and Intraoperative Measurements Demonstrate the Unique Design of the Multifidus Muscle for Lumbar Spine Stability. J Bone Joint Surg Am 2009;91:176-85.

[13] Aldrich F. Posterolateral Microdisectomy for Cervical Monoradiculopathy Caused by Posterolateral Soft Cervical Disc Sequestration. J Neurosurg 1990;72:370-7.

[14] Kunert P, Prokopienko M, Marchel A. Posterior Microlaminoforaminotomy for Cervical Disc Herniation. Neurol Neurochir Pol 2010;44:375-84.

[15] Yolas C, Ozdemir NG, Okay HO, Kanat A, Senol M, Atci IB, *et al*. Cervical disc hernia operations through posterior laminoforaminotomy. J Craniovertebr Junction Spine 2016;7:91-5.

[16] Oh JK, Hong JT, Kang DH, Kim SW, Kim SW, Kim YJ, *et al*. Epidemiology of c5 Palsy After Cervical Spine Surgery: A 21-center study. Neurospine 2019;16:558-62.

[17] Harel R, Stylianou P, Knoller N. Cervical Spine Surgery: Approach-Related Complications. World Neurosurg 2016;94:1-5.

[18] Adamson TE. Microendoscopic Posterior Cervical Laminoforaminotomy for Unilateral Radiculopathy: Results of a New Technique in 100 Cases. J Neurosurg

2001;95:51-7.

[19] Burke TG, Caputy A. Microendoscopic Posterior Cervical Foraminotomy: A Cadaveric Model and Clinical Application for Cervical Radiculopathy. J Neurosurg 2000;93:126-9.

[20] Xu J, Yu BF, Liu CH, Zheng W, Xiao YH, Lin Y. Microscopic Keyhole Technique for Surgical Removal of Thoracic Spinal Meningiomas. World Neurosurg 2019;124:e373-9.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00023

Journal of Clinical and Translational Research 2023; 9(4): 297-307

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

Racial and gender-based disparities and trends in common psychiatric conditions for patients with inflammatory bowel disease in the United States: an 11-year national cross-sectional study

Hassam Ali[1], Faisal Inayat[2]*, Talia F. Malik[3], Pratik Patel[4], Sobaan Taj[5], Arslan Afzal[1], Gul Nawaz[2], Rizwan Ishtiaq[6], Ali Jaan[7], Lucia Angela Smith-Martinez[8], Karina Fatakhova[4], Ramona Rajapakse[4]

[1]Department of Internal Medicine, East Carolina University Brody School of Medicine, Greenville, NC, USA, [2]Department of Internal Medicine, Allama Iqbal Medical College, Lahore, Punjab, Pakistan, [3]Department of Internal Medicine, Chicago Medical School at Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA, [4]Department of Gastroenterology, Mather Hospital and Hofstra University Zucker School of Medicine, Port Jefferson, NY, USA, [5]Department of Internal Medicine, Jersey Shore University Medical Center, Neptune, NJ, USA, [6]Department of Internal Medicine, Saint Francis Hospital and Medical Center, Hartford, CT, USA, [7]Department of Internal Medicine, Rochester General Hospital, Rochester, NY, USA, [8]Department of Psychiatry, East Carolina University Brody School of Medicine, Greenville, NC, USA

ARTICLE INFO

Article history:
Received: December 23, 2022
Revised: June 09, 2023
Accepted: July 11, 2023
Published online: August 22, 2023

Keywords:
Inflammatory bowel disease
Psychiatric disorders
Race
Gender
Disparities
Population-based trends

**Corresponding authors:*
Faisal Inayat
Allama Iqbal Medical College, Allama Shabbir Ahmad Usmani Road, Faisal Town, Lahore, 54550, Punjab, Pakistan.
Cell: +92 321 774 3758
Fax: +92 42 9923 1443
Email: faisalinayat@hotmail.com

Abstract

Background and Aim: Inflammatory bowel disease (IBD) is a chronic, debilitating disease that has been extensively studied. However, the clinical evidence remains limited regarding the racial and gender-based disparities in psychiatric illnesses in IBD patients. We aim to evaluate trends and socio-demographic disparities in psychiatric disorders in patients with IBD.

Methods: The United States National Inpatient Sample (NIS) database was retrospectively investigated from 2009 to 2019 to report trends and disparities in common psychiatric comorbidities in hospitalized patients with IBD.

Results: For the study period (2009–2019), the prevalence of generalized anxiety disorder (GAD) in IBD patients increased from 0.36% to 1.78%, depression increased from 9% to 13%, attention-deficit hyperactivity disorder increased from 0.49% to 2%, and post-traumatic stress disorder (PTSD) increased from 0.39% to 1.23% ($P < 0.001$). The prevalence of somatization (0.004%), schizophrenia (0.43%), schizoaffective disorder (0.18%), and bipolar disorder (2.28%) showed no significant trend ($P > 0.05$). Compared to males, females had a higher association with GAD, with an adjusted odds ratio (aOR) of 1.74 (95% confidence interval [CI]: 1.54–1.97, $P < 0.001$), depression (aOR = 1.85 [95% CI: 1.79–1.92] $P < 0.001$), bipolar disorder (aOR = 1.39 [95% CI: 1.29–1.51] $P < 0.001$), PTSD (aOR = 1.38 [95% CI: 1.21–1.57] $P < 0.001$), and chronic fatigue (aOR = 2.91 [95% CI: 1.71–4.95] $P < 0.001$). Blacks, Hispanics, and Asian/Native Americans had a lower association with psychiatric illnesses compared to Whites ($P < 0.001$).

Conclusions: This population-based study shows a rising prevalence of common psychiatric disorders in hospitalized patients with IBD, particularly in females. These mental illnesses were more commonly associated with Whites than Blacks, Hispanics, and Asian/Native Americans.

Relevance for Patients: Our findings highlight the need for effective screening and treatment protocols for psychiatric disorders in patients with IBD. It can potentially improve the quality of life and medication adherence and reduce the use of valuable healthcare resources. Prompt recognition of these mental illnesses followed by early treatment initiation can be of paramount clinical importance for sustained IBD remission.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic, progressive condition affecting an estimated 4.90 million individuals worldwide [1]. Conventionally, it is considered a

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00231

disease of the Western world; contemporary research highlights an increasing incidence in the Middle East, Asia, and South America [2]. Despite advances in IBD therapeutics, disparities in care persist across different races and ethnicities. The previous research has documented disparities in treatment initiation and utilization of advanced therapies among different ethnic groups [3,4]. Furthermore, it is recognized that IBD exhibits gender-specific alterations, highlighting the need for targeted care strategies for female patients [5]. Liu *et al.* further emphasized the importance of understanding and addressing these disparities to promote health equity among IBD populations [6]. Mental illnesses, affecting nearly one in five adults in the United States, represent a considerable public health concern [7]. Therefore, it is critical to understand the influence of racial and gender-based disparities on the clinical care of IBD patients, particularly concerning psychiatric comorbidities.

The incidence of psychiatric illnesses has increased among IBD patients compared to the general population [8-10]. It can significantly impact disease progression and healthcare utilization [11]. Comorbid depression and anxiety are linked to poorer clinical outcomes in IBD patients, including higher odds of emergency room visits and hospitalizations [12]. IBD has been independently associated with an increased risk of deliberate self-harm and other mental health illnesses [13]. Specific attention must be paid to gender-based disparities in the IBD population, as some studies suggest an association between female gender, active IBD, and the onset of depression [14]. Therefore, early detection and treatment of psychiatric disorders in the IBD population may become vital components of clinical management. It may have crucial clinical importance regarding disease progression, treatment compliance, and quality of life.

Despite the recognized importance of these topics, there is a lack of large-scale, data-driven studies investigating racial and gender-based disparities and trends in psychiatric comorbidities among IBD patients. To the best of our knowledge, this article represents the first National Inpatient Sample (NIS)-based retrospective study, providing a comprehensive evaluation of these disparities in common psychiatric conditions among IBD patients over a decade. Our findings hold significant clinical implications, offering a foundation of data-driven evidence that highlights racial and gender-based disparities in psychiatric comorbidities among IBD patients. This work is anticipated to heighten community awareness, support the establishment of effective psychiatric screening protocols, and promote timely referrals to mental health professionals. These measures will contribute to improve clinical care and health outcomes for patients with IBD.

2. Materials and Methods

2.1. Design and data source

The NIS is designed by the Agency for Healthcare Research and Quality [15]. It is the largest inpatient database in the United States [15]. The design of this particular database is to approximate a 20% stratified sample of hospitals along with sampling weights to calculate national estimates [15]. Additional information on the design of NIS and sampling methods is available at https://www.hcup-us.ahrq.gov. The data in NIS are provided using the International Classification of Diseases (ICD) 9 (before September 2015) and 10 (after October 2015) coding systems. The present retrospective study utilized the NIS database to identify patients with a primary diagnosis of IBD from January 2009 to December 2019. All patients below the age of 18 were excluded. The codes utilized for each variable in this study are outlined in Table S1.

2.2. Outcome measures

Primary outcomes included the prevalence of common psychiatric conditions that included generalized anxiety disorder (GAD), depression, somatization, bipolar disorder, attention-deficit hyperactivity disorder (ADHD), schizophrenia, schizoaffective disorder, post-traumatic stress disorder (PTSD), and chronic fatigue in IBD patients. Trend analysis for respective outcomes was also reported to ascertain any time-based shifts. Secondary outcomes were associations between gender, race, and psychiatric disorders among hospitalized IBD patients.

2.3. Statistical analysis

This study utilized Statistical Software for Data Science (STATA) (StataCorp LLC, College Station, TX, USA), version 16.0. The analysis had 0.05 as the threshold for statistical significance, and all *P*-values were 2-sided. Bivariate analysis was conducted using a Chi-square test for categorical variables and an independent-samples t-test for continuous variables. Categorical variables were presented as frequency (N) and percentage (%), and continuous variables were reported as mean with standard deviation (SD), as appropriate. For outcomes such as the length of stay and mean inpatient charges, a hierarchical multivariate linear regression analysis was conducted to adjust patient- or hospital-level factors. Multivariate logistic regression was conducted to assess the relationship between gender, race, and psychiatric conditions among hospitalized patients with IBD. The outcomes were reported as adjusted odds ratios (aOR) with 95% confidence intervals (CI) and *P*-values. The adjusted Wald test was utilized to compare slopes of time-based linear regression outcomes and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) to generate figures.

2.4. Ethical consideration

The NIS database contains de-identified information for the protection of the privacy of patients, physicians, and hospitals. Therefore, it did not require institutional review board approval. Patient consent was also waived, as each hospitalization was stripped of any patient identifiers.

3. Results

3.1. Demographic characteristics of study sample

The total IBD hospitalizations decreased from 75,813 (200/100,000 total NIS hospitalizations) in 2009 to 70,210 (198/100,000 total NIS hospitalizations) in 2019, without

any statistical significance ($P = 0.30$) (Figure 1). During the study period, there was a higher frequency of females compared to males (54% vs. 46%) in hospitalized patients with IBD ($P < 0.001$). Most patients belonged to the age group 18–33 years (35%), followed by 34–49 years (27%) and 50–64 years (21%) ($P < 0.001$). There was a higher frequency of IBD hospitalizations among Whites (77%), followed by Blacks (14%), and Hispanics (8%) ($P = 0.052$). A vast majority of hospitalized patients with IBD had a Charlson Comorbidity Index (CCI) score of 0 (70%) ($P < 0.001$). Urban teaching hospitals had the highest frequency of IBD hospitalizations (62%), followed by urban non-teaching (29%) and rural (8%) hospitals ($P < 0.001$). Private insurance remained the primary payer for 50% of hospitalized patients with IBD, followed by Medicare (26%) and Medicaid (17%) ($P < 0.001$). Inpatient mortality significantly decreased from 0.51% in 2009 to 0.32% in 2019 ($P = 0.016$). The outcomes such as length of stay, mean inpatient charges, and additional demographic characteristics over the study period are described in Table 1.

3.2. Prevalence and trends of common psychiatric conditions in the IBD population

The prevalence of GAD in hospitalized patients with IBD was 0.83% for the study period, with increasing trends from 0.36% in 2009 (2.91/1000 IBD patients) to 1.78% in 2019 (17.8/1000 IBD patients) ($P < 0.001$). The prevalence of depression was 11.81%, with increasing trends from 9% in 2009 (85.7/1000 IBD patients) to 13% in 2019 (133.5/1000 IBD patients) ($P < 0.001$). The prevalence of ADHD was 1.04%, with increasing trends from 0.49% in 2009 (4.9/1000 IBD patients) to 2% in 2019 (15.5/1000 IBD patients) ($P < 0.001$). The prevalence of PTSD was 0.74%, with increasing trends from 0.39% in 2009 (3.9/1000 IBD patients) to 1.23% in 2019 (12.3/1000 IBD patients) ($P < 0.001$). The prevalence of somatization (0.004%), schizophrenia (0.43%), schizoaffective disorder (0.18%), and bipolar disorder (2.28%) showed no significant trend ($P > 0.05$) (Table 2).

3.3. Gender-based disparities of common psychiatric conditions in IBD population

Among hospitalized patients with IBD, females had a higher association with GAD (aOR = 1.74 [95% CI: 1.54–1.97] $P < 0.001$), depression (aOR = 1.85 [95% CI: 1.79–1.92] $P < 0.001$), bipolar disorder (aOR = 1.39 [95% CI: 1.29–1.51] $P < 0.001$), PTSD (aOR = 1.38 [95% CI: 1.21–1.57] $P < 0.001$), and chronic fatigue (aOR = 2.91 [95% CI: 1.71–4.95] $P < 0.001$), compared to males. There was a lower association with ADHD, schizophrenia, and schizoaffective disorders for females when compared to males with IBD (Table 3).

3.4. Race-based disparities of common psychiatric conditions in IBD population

Blacks, Hispanics, and Asian/Native Americans had a lower association with GAD, depression, bipolar disorder, PTSD, and ADHD compared to Whites in hospitalized patients with IBD ($P < 0.001$). Blacks and Hispanics had a higher association with schizophrenia than Whites ($P < 0.001$). Blacks also had a higher association with schizoaffective disorder (aOR = 1.66 [95% CI: 1.22–2.25] $P = 0.001$) compared to Whites with IBD. There was no significant difference in the association among Hispanics and Asian/Native Americans when compared to Whites for schizoaffective disorder. Blacks had a lower association with chronic fatigue compared to Whites (aOR = 0.43 [95% CI: 0.18–1.001] $P = 0.05$). For chronic fatigue, Hispanics showed no significant difference, whereas Asian/Native Americans could not be compared due to the smaller sample size (Table 4).

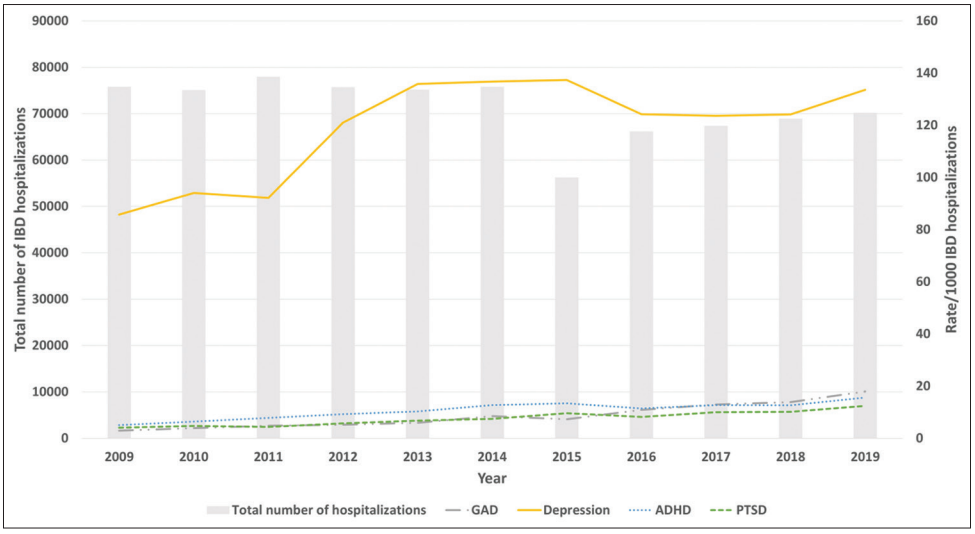


Figure 1. Rate of common psychiatric conditions in primary inflammatory bowel disease (IBD) hospitalizations in the National Inpatient Sample from 2009 to 2019. Bars show the total IBD hospitalizations per year. The line shows the rate per 1000 IBD hospitalizations for the study period for psychiatric conditions with significant trends in the present study ($P < 0.05$).
GAD: Generalized anxiety disorder; ADHD: Attention-deficit hyperactivity disorder; PTSD: Post-traumatic stress disorder.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00231

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00231

Table 1. Sociodemographic trends of inflammatory bowel disease hospitalizations in the National Inpatient Sample from 2009 to 2019

Variables	Years											*P*-values
	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	
Total hospitalizations	75813	75092	77943	75720	75169	75815	56219	66164	67364	68939	70210	0.30
Mean age (years)	44.9±0.69	44.6±0.77	45.0±0.36	44.9±0.12	44.7±0.69	44.5±0.78	44.8±0.71	45.5±0.62	45.7±0.29	46.2±0.17	46.6±0.86	<0.001
Adjusted mean length of stay (days)	5.53±0.1	5.51±0.1	5.29±0.08	5.18±0.06	5.24±0.05	5.19±0.07	5.01±0.07	5.40±0.06	5.18±0.06	5.16±0.06	5.17±0.06	<0.001
Adjusted mean inpatient cost ($)	33090±1331	34998±1469	36352±1477	36784±960	38810±788	40546±868	40639±904	47318±1126	47989±1155	51201±1303	55138±1600	<0.001
Age groups (years)												<0.001
18–33	26124 (34%)	26741 (36%)	27616 (35%)	26620 (35 %)	26849 (35%)	27440 (36%)	19920 (35%)	22589 (34%)	22594 (34%)	22615 (33%)	22335 (32%)	
34–49	21731 (29%)	21111 (28%)	20747 (27%)	20405 (27%)	20389 (27%)	20510 (27%)	15055 (27%)	17360 (26%)	18065 (27%)	17864 (26%)	18310 (26%)	
50–64	15497 (20%)	15206 (20%)	16584 (21%)	15995 (21%)	15604 (21%)	15685 (21%)	11964 (21%)	14339 (22%)	14830 (22%)	15260 (22%)	15350 (22%)	
65–79	8776 (12%)	8454 (11%)	9106 (12%)	9325 (12%)	9099 (12%)	8750 (12%)	6915 (12%)	8909 (13%)	8789 (13%)	10019 (15%)	10995 (16%)	
≥80	3683 (5%)	3577 (5%)	3888 (5%)	3375 (4%)	3225 (4%)	3430 (5%)	2365 (4%)	2965 (4%)	3085 (5%)	3180 (5%)	3220 (5%)	
Gender												<0.001
Male	33377 (44%)	33263 (44%)	34227 (44%)	33915 (45%)	34249 (46%)	34935 (46%)	25709 (46%)	32429 (49%)	32754 (49%)	32665 (47%)	33660 (48%)	
Female	42246 (56%)	41700 (56%)	43574 (56%)	41805 (55%)	40909 (54%)	40855 (54%)	30485 (54%)	33664 (51%)	34610 (51%)	36269 (53%)	36340 (52%)	
Race												0.052
White	50281 (80%)	51659 (77%)	53655 (76%)	55305 (77%)	54099 (76%)	55025 (77%)	40244 (75%)	48849 (77%)	49864 (77%)	51439 (77%)	53255 (77%)	
Black	7259 (12%)	9638 (14%)	10268 (15%)	10170 (14%)	9775 (14%)	10000 (14%)	8104 (15%)	8234 (13%)	8615 (13%)	8725 (13%)	9035 (13%)	
Hispanic	4409 (7%)	5053 (8 %)	5496 (8 %)	5385 (8 %)	5960 (8 %)	5785 (8 %)	4270 (8 %)	5050 (8 %)	5420 (8 %)	5880 (9%)	5395 (8 %)	
Asian/Native American	797 (1%)	919 (1%)	943 (1%)	900 (1%)	955 (1%)	1020 (2%)	900 (2%)	930 (1%)	1010 (2%)	1145 (2%)	1070 (2%)	
CCI												0.001
CCI=0	55726 (74%)	54253 (72%)	55454 (71%)	54060 (71%)	53129 (71%)	54370 (72%)	39979 (71%)	45564 (69%)	45819 (68%)	45309 (66%)	45640 (65%)	
CCI=1	12791 (17%)	13108 (17%)	13668 (18%)	13370 (18%)	13399 (18%)	13140 (17%)	9735 (17%)	11749 (18%)	12215 (18%)	13175 (19%)	13290 (19%)	
CCI=2	4066 (5%)	4217 (6%)	4626 (6%)	4410 (6%)	4590 (6%)	4370 (6%)	3435 (6%)	4570 (7%)	4475 (7%)	5260 (8%)	5325 (8%)	
CCI≥3	3229 (4%)	3512 (5%)	4194 (5%)	3880 (5%)	4050 (5%)	3935 (5%)	3070 (5%)	4279 (6%)	4855 (7%)	5195 (8%)	5955 (8%)	
Hospital location and teaching status												<0.001
Rural	7855 (10%)	8215 (11%)	7437 (10%)	7610 (10%)	7324 (10%)	6240 (8%)	4520 (8%)	4274 (6%)	4369 (6%)	4109 (6%)	4335 (6%)	
Urban nonteaching	30154 (40%)	29896 (40%)	29840 (39%)	27169 (36%)	26345 (35%)	18775 (25%)	14254 (25%)	14724 (22%)	13379 (20%)	12275 (18%)	10749 (15%)	
Urban teaching	36819 (49%)	36146 (49%)	39860 (53%)	40940 (54%)	41499 (55%)	50800 (67%)	37444 (67%)	47165 (71%)	49614 (74%)	52554 (76%)	55125 (79%)	
Primary payer												<0.001
Medicare	17782 (25%)	18097 (26%)	19503 (26%)	19185 (27%)	19044 (27%)	18660 (26%)	14310 (26%)	16384 (26%)	16589 (26%)	17679 (27%)	18565 (27%)	
Medicaid	9608 (13%)	11278 (16%)	11376 (15%)	11195 (16%)	11195 (16%)	14239 (20%)	10835 (20%)	11819 (18%)	11744 (18%)	12235 (18%)	11925 (18%)	
Private	37915 (53%)	34791 (49%)	36892 (50%)	34739 (48%)	34334 (48%)	34895 (48%)	25744 (48%)	32454 (51%)	32890 (51%)	32754 (49%)	33270 (49%)	
Other	6427 (9%)	6645 (9%)	6107 (8%)	6525 (9%)	6644 (9%)	4875 (7%)	3150 (6%)	3270 (5%)	3685 (6%)	3590 (5%)	3815 (6%)	
Died	384 (0.51%)	357 (0.47%)	385 (0.49%)	275 (0.36%)	265 (0.35%)	220 (0.29%)	180 (0.32%)	225 (0.34%)	255 (0.37%)	235 (0.34%)	230 (0.32%)	0.016

CCI: Charlson Comorbidity Index

Table 2. Trends of psychiatric comorbidities in patients hospitalized with a primary diagnosis of inflammatory bowel disease in the National Inpatient Sample database from 2009 to 2019

Variables	Years										*P*-values
	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	
GAD	221 (0.36%)	292 (0.38%)	374 (0.47%)	390 (0.51%)	445 (0.59%)	640 (0.84%)	410 (0.72%)	720 (1.1%)	870 (1.29%)	955 (1.38%)	1255 (1.78%)	<0.001
Depression	6500 (9%)	7060 (9%)	7182 (9%)	9160 (12%)	10210 (14%)	10365 (14%)	7720 (14%)	8219 (12%)	8325 (12%)	8555 (12%)	9375 (13%)	<0.001
Somatization	0 (0%)	10 (<1%)	4 (<1%)	5 (<1%)	0 (0%)	5 (<1%)	0 (0%)	0 (0%)	5 (<1%)	0 (0%)	5 (<1%)	0.31
Bipolar disorder	1748 (2%)	1860 (2%)	1826 (2%)	1895 (2%)	1690 (2%)	1865 (2%)	1365 (2%)	1325 (2%)	1415 (2%)	1350 (2%)	1590 (2%)	0.37
ADHD	377 (0.49%)	479 (1%)	606 (1%)	700 (1%)	775 (1%)	960 (1%)	750 (1%)	755 (1%)	855 (1%)	870 (1%)	1090 (2%)	<0.001
Schizophrenia	331 (0.43%)	283 (<1%)	278 (<1%)	300 (<1%)	300 (<1%)	270 (<1%)	265 (<1%)	290 (<1%)	365 (1%)	365 (1%)	340 (0.48%)	0.90
Schizoaffective disorder	123 (<1%)	117 (<1%)	82 (<1%)	165 (<1%)	125 (<1%)	115 (<1%)	85 (<1%)	120 (<1%)	210 (<1%)	165 (<1%)	145 (<1%)	0.56
PTSD	302 (0.39%)	350 (0.46%)	336 (0.43%)	430 (0%)	505 (1%)	560 (1%)	540 (1%)	540 (1%)	675 (1%)	700 (1%)	870 (1.23%)	<0.001
Chronic fatigue	30 (<1%)	22 (<1%)	37 (<1%)	29 (<1%)	50 (<1%)	50 (<1%)	20 (<1%)	35 (<1%)	55 (<1%)	55 (<1%)	60 (<1%)	0.16

GAD: Generalized anxiety disorder; ADHD: Attention-deficit hyperactivity disorder; PTSD: Post-traumatic stress disorder.

Table 3. Gender-based disparities with common psychiatric conditions in inflammatory bowel disease hospitalizations (females compared against males)

Variables	Adjusted odds ratio with 95% confidence interval	*P*-values
GAD	1.74 (1.54–1.97)	<0.001
Depression	1.85 (1.79–1.92)	<0.001
Somatization	4.8 (0.57–3.98)	0.14
Bipolar disorder	1.39 (1.29–1.51)	<0.001
ADHD	0.77 (0.69–0.86)	<0.001
Schizophrenia	0.43 (0.36–0.51)	<0.001
Schizoaffective disorder	0.67 (0.52–0.86)	0.002
PTSD	1.38 (1.21–1.57)	<0.001
Chronic fatigue	2.91 (1.71–4.95)	<0.001

GAD: Generalized anxiety disorder; ADHD: Attention-deficit hyperactivity disorder; PTSD: Post-traumatic stress disorder

4. Discussion

This study found a decrease in hospitalizations with IBD as a primary diagnosis. While there has been an overall increase in IBD cases, newer and more effective treatments have possibly resulted in a drop in hospital admissions. Our findings show that several psychiatric disorders are becoming more common in hospitalized patients with IBD. Whites were more commonly associated with GAD, depression, bipolar disorder, PTSD, and ADHD compared to Blacks, Hispanics, and Asian/Native Americans. Furthermore, females had a higher association with GAD, depression, bipolar disorder, PTSD, and chronic fatigue than male IBD patients.

IBD patients may have a significantly higher prevalence of serious psychological distress (7.4% vs. 3.4%) compared to those without IBD [16]. A retrospective cohort study from Canada revealed an increased incidence and prevalence of psychiatric disorders in IBD patients than in the general population, including anxiety, depression, and bipolar disorder [9]. In our study, depression was the most common psychiatric disorder among IBD patients. Mardini *et al.* demonstrated that in patients with Crohn's disease, the presence of depressive symptoms was associated with increased disease activity over an 18-month follow-up [17]. The presence of depression has also been shown to increase the risk of relapse in IBD and can lead to reduced quality of life and low medication adherence [18-20].

IBD patients with anxiety have been shown to have an increased risk of surgery, poorer treatment compliance, and decreased quality of life [18,21,22]. A study in Switzerland showed an increased recurrence of IBD in patients with depression and anxiety [23]. A Korean study revealed that IBD patients in remission with concomitant functional gastrointestinal and mood disorders demonstrated a lower health-related quality of life [24]. The young adult patients face multiple financial, academic, and personal challenges, which may lead to an increased risk of developing psychiatric disorders [25]. Our study also showed an increased rate of IBD hospitalizations in young adults. It may potentially be attributed to the worsening of IBD disease activity due to the aforementioned factors in this age group.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00231

Table 4. Racial disparities with common psychiatric conditions in inflammatory bowel disease hospitalizations (compared against White race)

Variables	Adjusted odds ratio with 95% confidence interval	*P*-values
GAD		
White	-	
Black	0.42 (0.34–0.53)	<0.001
Hispanic	0.53 (0.40–0.69)	<0.001
Asian/Native American	0.15 (0.04–0.47)	0.001
Depression		
White	-	
Black	0.62 (0.59–0.66)	<0.001
Hispanic	0.60 (0.56–0.65)	<0.001
Asian/Native American	0.39 (0.32–0.48)	<0.001
Bipolar disorder		
White	-	
Black	0.77 (0.68–0.86)	<0.001
Hispanic	0.71 (0.61–0.83)	<0.001
Asian/Native American	0.30 (0.18–0.51)	<0.001
ADHD		
White	-	
Black	0.22 (0.17–0.28)	<0.001
Hispanic	0.33 (0.25–0.44)	<0.001
Asian/Native American	0.22 (0.10–0.47)	<0.001
Schizophrenia		
White	-	
Black	3.84 (3.2–4.60)	<0.001
Hispanic	1.52 (1.14–2.02)	0.004
Asian/Native American	0.56 (0.21–1.51)	0.25
Schizoaffective disorder		
White	-	
Black	1.66 (1.22–2.25)	0.001
Hispanic	0.88 (0.54–1.44)	0.62
Asian/Native American	0.79 (0.25–2.45)	0.69
PTSD		
White		
Black	0.57 (0.46–0.72)	<0.001
Hispanic	0.62 (0.47–0.81)	0.001
Asian/Native American	0.46 (0.23–0.93)	0.032
Chronic fatigue		
White	-	
Black	0.43 (0.18–1.001)	0.05
Hispanic	0.39 (0.12–1.26)	0.11
Asian/Native American	-	-

GAD: Generalized anxiety disorder; ADHD: Attention-deficit hyperactivity disorder; PTSD: Post-traumatic stress disorder.

In our study, females had a higher frequency of IBD hospitalizations than males. Furthermore, female IBD patients were more commonly associated with diagnoses of GAD, depression, bipolar disorder, PTSD, and chronic fatigue. An increased genetic susceptibility to psychiatric disorders, hormonal fluctuations, alterations in microbiome composition, and various environmental factors can possibly contribute to these trends [26]. In addition, it is also critical to take into account the fact that women are more likely than men to face physical, sexual, and emotional abuse [27]. These factors could also be driving the observed gender differences in mental health disorders among IBD patients in our analysis. Moreover, it is known that women with IBD show increased odds of poor maternal and fetal outcomes, and a pre-pregnancy IBD diagnosis may increase the risk for mood and anxiety disorders [28,29].

Smoking is a known risk factor for the development of Crohn's disease in both males and females [30]. A Swiss study found that young women had a higher smoking rate of 51.7% than young men [31]. Similarly, a Dutch study revealed that there were more female current smokers among patients with IBD than males [32]. The higher smoking rate in young females may contribute to the increased IBD-related hospitalizations observed in our study. The present study showed an increased prevalence of anxiety in female IBD patients, which mirrors the gender differences in anxiety among the general population [33]. Genetic predisposition due to known susceptibility gene variants is one of the causes of the increased prevalence of IBD in the female population. Females are more commonly associated with familial than sporadic IBD (61% vs. 54%) [34]. Females are also more likely to develop ulcerative colitis if they have a single nucleotide polymorphism in the promoter region of interleukin-10 [35]. A number of immune-mediated diseases result from the loss of the X-chromosome in peripheral T and B lymphocytes, including primary biliary cholangitis, autoimmune thyroid disease, Raynaud's syndrome, and systemic sclerosis [36,37]. A similar mechanism may contribute to the prevalence of IBD in females. However, the exact mechanism remains unclear. Compared to males, IBD in females is more often associated with a negative body image and reduced sexual activity [38,39]. This may further contribute to the development of psychiatric disorders. Other factors that result in gender disparities among IBD patients include occupation, lifestyle, and dietary habits. However, a clear causal relationship remains to be established.

Racial disparities among IBD patients have previously been studied. However, less is known about their impact on IBD patients with common psychiatric conditions. IBD is typically more prevalent among populations in Western and Northern Europe [40]. However, its incidence among non-White populations has also increased in recent years [41]. In our study, Whites had the highest rate of IBD hospitalizations (77%), followed by Blacks (14%) and Hispanics (8%). In a large survey-based study, Wang *et al.* revealed similar findings in the United States [42]. Moreover, in a recent retrospective study based on 132,894 IBD hospitalizations with substance use disorder, White was the most common race (N = 98,147; 79%) [43]. Our data showed that GAD, depression, bipolar disorder, PTSD, and ADHD were more prevalent in White IBD patients than in Black, Hispanic, and Asian/Native American IBD patients. In contrast, a retrospective cohort study conducted at a large urban outpatient center revealed

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00231

that Black patients had an increased prevalence of depression with low screening rates [44]. Several studies have demonstrated racial disparities in outcomes in patients hospitalized with IBD, with Blacks having higher readmission rates, a longer length of stay, and increased morbidity and mortality compared to White patients [45,46]. This may be due to several sociocultural factors and barriers to healthcare utilization. One study revealed that Whites were more likely to be seen by gastroenterologists annually than Black IBD patients [47]. These disparities have been attributed to the underutilization of specialist care as a result of difficulties in obtaining specialist referrals and financial concerns among Black patients [47]. Similar reasons may contribute to decreasing healthcare utilization for psychiatric disorders in non-White patients, leading to poor prognoses and worse outcomes. Contrarily, schizophrenia and schizoaffective disorder were less likely to be seen in Whites than in Blacks and Hispanics. This trend may be attributed to the disproportionate number of schizophrenia diagnoses among African Americans and Latino Americans [48].

The prognosis of IBD can be negatively impacted by psychiatric disorders, often due to medication non-adherence, resulting in worsened clinical outcomes, functional disability, and reduced quality of life [18-21,49,50]. The previous investigations have examined the impact of psychiatric disorders on the IBD patient population [11,49,50]. However, the clinical evidence on race and gender disparities in IBD patients remains sparse. Our study represents one of the largest analyses exploring racial and gender-based disparities in psychiatric comorbidities in IBD patients, identifying females and Whites as particularly susceptible. It is essential to consider how social and structural norms may influence the diagnosis of IBD and psychiatric illnesses. For instance, it is known that women are more likely to access health services, particularly mental health support. This observation could possibly be overrepresented in our findings. Similarly, the barriers faced by racial and ethnic minorities in accessing healthcare could lead to delayed or missed diagnoses. As the prevalence of psychiatric comorbidities increases in chronic medical conditions, it is important for clinicians and policymakers to consider the racial and gender-based disparities in these psychiatric illnesses [51,52]. Our results highlight the need for further research to develop effective screening and treatment guidelines for IBD patients with psychiatric disorders. This could improve the prognosis, enhance the quality of life, and reduce healthcare costs. Beyond medical treatment, addressing psychosocial concerns in IBD is critical. Mussell *et al.* described the efficacy of outpatient psychological group therapy in the short- and long-term reduction of psychological distress in IBD patients, but they simultaneously emphasized gender-specific interventions [53]. Further studies examining the correlation between IBD and psychiatric comorbidities across different genders and races are warranted. It would be helpful to shed light on the interplay of psychiatric disorders, IBD, and potential genetic, environmental, and social factors affecting outcomes.

It is critical to consider implementing culturally sensitive mental health screening and treatment protocols. It can help to cater to the specific needs of diverse patient populations to address the observed disparities in psychiatric comorbidities among IBD patients. This strategy may assist in lowering barriers to receiving care for mental health and enhancing the general standard of care for IBD patients with psychiatric comorbidities. The complex and potentially bidirectional relationship between IBD and psychiatric disorders should be the subject of further investigation, considering elements such as cultural background, gender, and social determinants of health [54]. A deeper understanding of these relationships will facilitate the development of focused interventions to address these disparities. It will improve the overall clinical care and outcomes for IBD patients with comorbid psychiatric conditions. In light of the trends observed over a decade-long span in our study, we speculate that increased disease awareness, advancements in diagnostic methodologies, societal attitudes toward mental health, and potential changes in lifestyle and environmental factors may have influenced the prevalence and recognition of IBD and associated psychiatric illnesses. This further emphasizes the importance of ongoing research to better understand and address these evolving trends.

5. Limitations

We acknowledge several limitations to our study, primarily grounded in the inherent restrictions of using the NIS. While it presents a large database for generalized interpretation, hospital data in NIS do not evaluate non-hospitalized individuals. Therefore, this dataset could lead to an underrepresentation of psychiatric disorders within the broader IBD population. The database also lacks supplementary data on treatments such as antidepressants and antipsychotic usage. It could provide a more holistic view of the prevalence of psychiatric disorders in IBD patients. Another limitation is the reliance on ICD codes as the primary indicator of psychiatric disorders. Despite being systematic in their disease classification, these codes may not fully capture the complexity of mental health diagnoses [55]. The exclusive reliance on ICD codes could also lead to the misclassification or underrepresentation of certain mental health conditions. The study does not account for changes in mental health reporting patterns due to increasing societal awareness. The increased awareness may lead to improved identification and reporting of mental health disorders, causing an apparent rise in prevalence. Simultaneously, heightened awareness might also encourage individuals to report milder distress as mental health issues, potentially overestimating the prevalence rates. The study does highlight ethnic disparities in the prevalence of psychiatric disorders, observing higher rates among White IBD patients compared to other ethnic groups. However, these findings should be interpreted cautiously due to potential confounding factors. Sociocultural influences, healthcare access disparities, and systemic biases could potentially contribute to underdiagnosis or underreporting in minority populations.

6. Conclusions

This study revealed that females had a higher frequency of IBD hospitalizations compared to males. Most patients were in the younger age group of 18–33 years. The most frequent

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00231

psychiatric diagnosis among hospitalized IBD patients was depression, which was followed by bipolar disorder, ADHD, GAD, PTSD, schizophrenia, schizoaffective disorder, chronic fatigue, and somatization. Depression, GAD, bipolar disorder, PTSD, and chronic fatigue were more commonly associated with females compared to males. Whites had the highest rate of IBD hospitalizations, followed by Blacks and Hispanics. GAD, depression, bipolar disorder, PTSD, and ADHD were more commonly associated with Whites compared to Blacks, Hispanics, and Asian/Native Americans. However, schizophrenia and schizoaffective disorder were less likely to be seen in Whites than in Blacks and Hispanics. Our findings highlight the importance of recognizing these racial and gender-based disparities and trends among IBD patients. Effective screening and treatment protocols for psychiatric comorbidities in IBD patients may aid in their early recognition and management. It will also increase IBD treatment compliance, which will help in achieving sustained remission. The quality of life for IBD patients will be improved and valuable healthcare resources will be saved.

Acknowledgments

We would like to thank the NIS for providing a free public database. Thanks to the editors and reviewers for their insightful critique of the manuscript.

Funding

The authors report that no external funding was used for this work.

Conflicts of Interest

There are no conflicts of interest associated with the publication of this manuscript.

Ethics Approval and Consent to Participate

The data of patients was not acquired from any specific institution but rather open-access United States National Inpatient Sample (NIS) database. The NIS contains de-identified information, protecting the privacy of patients, physicians, and hospitals. Therefore, ethics approval and consent to participate were deemed exempt for this study.

Consent for Publication

Participants were not required to give informed consent for publication of this study since the analysis of baseline characteristics used anonymized clinical data.

References

[1] Wang R, Li Z, Liu S, Zhang D. Global, Regional and National Burden of Inflammatory Bowel Disease in 204 Countries and Territories from 1990 to 2019: A Systematic Analysis Based on the Global Burden of Disease Study 2019. BMJ Open 2023;13:e065186.

[2] Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, *et al*. Worldwide Incidence and Prevalence of Inflammatory Bowel Disease in the 21st Century: A Systematic Review of Population-Based Studies. Lancet 2017;390:2769-78.

[3] Flasar M, Mulani PM, Yang M, Chao J, Lu M, Cross R. Racial Differences in Use of Biologics for Crohn's Disease in a Medicaid Population. Gut 2011;60:A138.

[4] Tandon P, Chhibba T, Natt N, Brar GS, Malhi G, Nguyen GC. Significant Racial and Ethnic Disparities Exist in Health Care Utilization in Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. Inflamm Bowel Dis 2023;izad045. https://doi.org/10.1093/ibd/izad045

[5] Lungaro L, Costanzini A, Manza F, Barbalinardo M, Gentili D, Guarino M, *et al*. Impact of Female Gender in Inflammatory Bowel Diseases: A Narrative Review. J Pers Med 2023;13:165.

[6] Liu JJ, Abraham BP, Adamson P, Barnes EL, Brister KA, Damas OM, *et al*. The Current State of Care for Black and Hispanic Inflammatory Bowel Disease Patients. Inflamm Bowel Dis 2023;29:297-307.

[7] Mental Illness. National Institute of Mental Health (NIMH); 2023. Available from: https://www.nimh.nih.gov/health/statistics/mental-illness [Last accessed on 2022 Dec 23].

[8] Tarar ZI, Zafar MU, Farooq U, Ghous G, Aslam A, Inayat F, *et al*. Burden of Depression and Anxiety Among Patients with Inflammatory Bowel Disease: Results of a Nationwide Analysis. Int J Colorectal Dis 2022;37:313-21.

[9] Barberio B, Zamani M, Black CJ, Savarino EV, Ford AC. Prevalence of Symptoms of Anxiety and Depression in Patients with Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis. Lancet Gastroenterol Hepatol 2021;6:359-70.

[10] Hu S, Chen Y, Chen Y, Wang C. Depression and Anxiety Disorders in Patients With Inflammatory Bowel Disease. Front Psychiatry 2021;12:714057.

[11] Bernstein CN, Hitchon CA, Walld R, Bolton JM, Lix LM, El-Gabalawy R, *et al*. The Impact of Psychiatric Comorbidity on Health Care Utilization in Inflammatory Bowel Disease: A Population-Based Study. Inflamm Bowel Dis 2021;27:1462-74.

[12] Kim S, Lee S, Han K, Koh SJ, Im JP, Kim JS, *et al*. Depression and Anxiety are Associated with Poor Outcomes in Patients with Inflammatory Bowel Disease: A Nationwide Population-Based Cohort Study in South Korea. Gen Hosp Psychiatry 2023;81:68-75.

[13] Umar N, King D, Chandan JS, Bhala N, Nirantharakumar K, Adderley N, *et al*. The Association between Inflammatory Bowel Disease and Mental ill Health: A Retrospective Cohort Study Using Data from UK Primary Care. Aliment Pharmacol Ther 2022;56:814-22.

[14] Panara AJ, Yarur AJ, Rieders B, Proksell S, Deshpande AR, Abreu MT, *et al*. The Incidence and Risk Factors for Developing Depression after Being Diagnosed with

Inflammatory Bowel Disease: A Cohort Study. Aliment Pharmacol Ther 2014;39:802-10.

[15] Nationwide Inpatient Sample (NIS). Rockville, MD: Agency for Healthcare Research and Quality. Available from: http://www.ahrq.gov/data/hcup/hcupnis.htm [Last accessed on 2022 Dec 23].

[16] Xu F, Dahlhamer JM, Zammitti EP, Wheaton AG, Croft JB. Health-Risk Behaviors and Chronic Conditions among Adults with Inflammatory Bowel Disease-United States, 2015 and 2016. MMWR Morb Mortal Wkly Rep 2018;67:190-5.

[17] Mardini HE, Kip KE, Wilson JW. Crohn's Disease: A Two-Year Prospective Study of the Association between Psychological Distress and Disease Activity. Dig Dis Sci 2004;49:492-7.

[18] Regueiro M, Greer JB, Szigethy E. Etiology and Treatment of Pain and Psychosocial Issues in Patients with Inflammatory Bowel Diseases. Gastroenterology 2017;152:430-9.e4.

[19] Mittermaier C, Dejaco C, Waldhoer T, Oefferlbauer-Ernst A, Miehsler W, Beier M, *et al*. Impact of Depressive Mood on Relapse in Patients with Inflammatory Bowel Disease: A Prospective 18-Month Follow-up Study. Psychosom Med 2004;66:79-84.

[20] Persoons P, Vermeire S, Demyttenaere K, Fischler B, Vandenberghe J, Van Oudenhove L, *et al*. The Impact of Major Depressive Disorder on the Short-and Long-Term Outcome of Crohn's Disease Treatment with Infliximab. Aliment Pharmacol Ther 2005;22:101-10.

[21] Graff LA, Walker JR, Bernstein CN. Depression and Anxiety in Inflammatory Bowel Disease: A Review of Comorbidity and Management. Inflamm Bowel Dis 2009;15:1105-18.

[22] Gray WN, Denson LA, Baldassano RN, Hommel KA. Treatment Adherence in Adolescents with Inflammatory Bowel Disease: The Collective Impact of Barriers to Adherence and Anxiety/Depressive Symptoms. J Pediatr Psychol 2012;37:282-91.

[23] Mikocka-Walus A, Pittet V, Rossel JB, von Känel R, Swiss IB Cohort Study Group. Symptoms of Depression and Anxiety are Independently Associated with Clinical Recurrence of Inflammatory Bowel Disease. Clin Gastroenterol Hepatol 2016;14:829-35.e1.

[24] Kim ES, Cho KB, Park KS, Jang BI, Kim KO, Jeon SW, *et al*. Predictive Factors of Impaired Quality of Life in Korean Patients with Inactive Inflammatory Bowel Disease: Association with Functional Gastrointestinal Disorders and Mood Disorders. J Clin Gastroenterol 2013;47:e38-44.

[25] Filatova S, Marttila R, Koivumaa-Honkanen H, Nordström T, Veijola J, Mäki P, *et al*. A Comparison of the Cumulative Incidence and Early Risk Factors for Psychotic Disorder in Young Adults in the Northern Finland Birth Cohorts 1966 and 1986. Epidemiol Psychiatr Sci 2017;26:314-24.

[26] Fracas E, Costantino A, Vecchi M, Buoli M. Depressive and Anxiety Disorders in Patients with Inflammatory Bowel Diseases: Are there any Gender Differences? Int J Environ Res Public Health 2023;20:6255.

[27] Sardinha L, Maheu-Giroux M, Stöckl H, Meyer SR, García-Moreno C. Global, Regional, and National Prevalence Estimates of Physical or Sexual, or Both, Intimate Partner Violence Against Women in 2018. Lancet 2022;399:803-13.

[28] Tarar ZI, Farooq U, Zafar MU, Saleem S, Nawaz A, Kamal F, *et al*. A National Study of Pregnancy-Related Maternal and Fetal Outcomes in Women with Inflammatory Bowel Disease. Int J Colorectal Dis 2022;37:1535-43.

[29] Vigod SN, Kurdyak P, Brown HK, Nguyen GC, Targownik LE, Seow CH, *et al*. Inflammatory Bowel Disease and New-Onset Psychiatric Disorders in Pregnancy and Post-Partum: A Population-Based Cohort Study. Gut 2019;68:1597-605.

[30] Lakatos PL, Vegh Z, Lovasz BD, David G, Pandur T, Erdelyi Z, *et al*. Is Current Smoking Still an Important Environmental Factor in Inflammatory Bowel Diseases? Results from a Population-Based Incident Cohort. Inflamm Bowel Dis 2013;19:1010-7.

[31] Biedermann L, Fournier N, Misselwitz B, Frei P, Zeitz J, Manser CN, *et al*. High Rates of Smoking Especially in Female Crohn's Disease Patients and Low Use of Supportive Measures to Achieve Smoking Cessation--Data from the Swiss IBD Cohort Study. J Crohns Colitis 2015;9:819-29.

[32] Severs M, Spekhorst LM, Mangen MJ, Dijkstra G, Löwenberg M, Hoentjen F, *et al*. Sex-Related Differences in Patients with Inflammatory Bowel Disease: Results of 2 Prospective Cohort Studies. Inflamm Bowel Dis 2018;24:1298-306.

[33] McLean CP, Asnaani A, Litz BT, Hofmann SG. Gender Differences in Anxiety Disorders: Prevalence, Course of Illness, Comorbidity and Burden of Illness. J Psychiatr Res 2011;45:1027-35.

[34] Zelinkova Z, Stokkers PC, van der Linde K, Kuipers EJ, Peppelenbosch MP, van der Woude CP. Maternal Imprinting and Female Predominance in Familial Crohn's Disease. J Crohns Colitis 2012;6:771-6.

[35] Tedde A, Putignano AL, Bagnoli S, Congregati C, Milla M, Sorbi S, *et al*. Interleukin-10 Promoter Polymorphisms Influence Susceptibility to Ulcerative Colitis in a Gender-Specific Manner. Scand J Gastroenterol 2008;43:712-8.

[36] Svyryd Y, Hernández-Molina G, Vargas F, Sánchez-Guerrero J, Segovia DA, Mutchinick OM. X Chromosome Monosomy in Primary and Overlapping Autoimmune Diseases. Autoimmun Rev 2012;11:301-4.

[37] Invernizzi P, Miozzo M, Selmi C, Persani L, Battezzati PM,

Zuin M, *et al*. X Chromosome Monosomy: A Common Mechanism for Autoimmune Diseases. J Immunol 2005;175:575-8.

[38] Trindade IA, Ferreira C, Pinto-Gouveia J. The Effects of Body Image Impairment on the Quality of Life of Non-Operated Portuguese Female IBD Patients. Qual Life Res 2017;26:429-36.

[39] Jedel S, Hood MM, Keshavarzian A. Getting Personal: A Review of Sexual Functioning, Body Image, and Their Impact on Quality of Life in Patients with Inflammatory Bowel Disease. Inflamm Bowel Dis 2015;21:923-38.

[40] Lakatos PL. Recent Trends in the Epidemiology of Inflammatory Bowel Diseases: Up or Down? World J Gastroenterol 2006;12:6102-8.

[41] Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, *et al*. Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases with Time, Based on Systematic Review. Gastroenterology 2012;142:46-54.e42.

[42] Wang YR, Loftus EV Jr., Cangemi JR, Picco MF. Racial/Ethnic and Regional Differences in the Prevalence of Inflammatory Bowel Disease in the United States. Digestion 2013;88:20-5.

[43] Fatakhova K, Patel P, Inayat F, Dhillon R, Ali H, Taj S, *et al*. Trends in Hospital Admissions and Mortality Among Inflammatory Bowel Disease Patients with Substance Use Disorder: A 10-Year United States Nationwide Analysis. Proc (Bayl Univ Med Cent) 2023;36:427-33.

[44] Jordan A, Mills K, Sobukonla T, Bredy S, Kelly A, Flood M. Depression Rates Among African American Inflammatory Bowel Disease Patients at a Large Safety Net Hospital. Colorectal Dis 2022;24:1550-5.

[45] Gunnells DJ Jr., Morris MS, DeRussy A, Gullick AA, Malik TA, Cannon JA, *et al*. Racial Disparities in Readmissions for Patients with Inflammatory Bowel Disease (IBD) After Colorectal Surgery. J Gastrointest Surg 2016;20:985-93.

[46] Montgomery SR Jr., Butler PD, Wirtalla CJ, Collier KT, Hoffman RL, Aarons CB, *et al*. Racial Disparities in Surgical Outcomes of Patients with Inflammatory Bowel Disease. Am J Surg 2018;215:1046-50.

[47] Nguyen GC, LaVeist TA, Harris ML, Wang MH, Datta LW, Brant SR. Racial Disparities in Utilization of Specialist Care and Medications in Inflammatory Bowel Disease. Am J Gastroenterol 2010;105:2202-8.

[48] Schwartz RC, Blankenship DM. Racial Disparities in Psychotic Disorder Diagnosis: A Review of Empirical Literature. World J Psychiatry 2014;4:133-40.

[49] Sajadinejad MS, Asgari K, Molavi H, Kalantari M, Adibi P. Psychological Issues in Inflammatory Bowel Disease: An Overview. Gastroenterol Res Pract 2012;2012:106502.

[50] Marafini I, Longo L, Lavasani DM, Rossi R, Salvatori S, Pianigiani F, *et al*. High Frequency of Undiagnosed Psychiatric Disorders in Inflammatory Bowel Diseases. J Clin Med 2020;9:1387.

[51] Kallio M, Tornivuori A, Miettinen P, Kolho KL, Culnane E, Sawyer S, *et al*. Disease Control and Psychiatric Comorbidity Among Adolescents with Chronic Medical Conditions: A Single-Centre Retrospective Study. BMJ Paediatr Open 2023;7:e001605.

[52] Patel P, Ali H, Inayat F, Pamarthy R, Giammarino A, Ilyas F, *et al*. Racial and Gender-Based Disparities and Trends in Common Psychiatric Conditions in Liver Cirrhosis Hospitalizations: A Ten-Year United States Study. World J Hepatol 2023;15:289-302.

[53] Mussell M, Böcker U, Nagel N, Olbrich R, Singer MV. Reducing Psychological Distress in Patients with Inflammatory Bowel Disease by Cognitive-Behavioural Treatment: Exploratory Study of Effectiveness. Scand J Gastroenterol 2003;38:755-62.

[54] Bisgaard TH, Allin KH, Elmahdi R, Jess T. The Bidirectional Risk of Inflammatory Bowel Disease and Anxiety or Depression: A Systematic Review and Meta-Analysis. Gen Hosp Psychiatry 2023;83:109-16.

[55] American Psychiatric Association, DSM-5 Task Force. Diagnostic and Statistical Manual of Mental Disorders: DSM-5™. 5th ed. Washington, DC: American Psychiatric Publishing, Inc.; 2013. Available from: https://dsm.psychiatryonline.org/doi/book/10.1176/appi.books.9780890425596 [Last accessed on 2022 Dec 23].

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00231

Journal of Clinical and Translational Research 2023; 9(4): 297-307

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

Racial and gender-based disparities and trends in common psychiatric conditions for patients with inflammatory bowel disease in the United States: an 11-year national cross-sectional study

Supplementary File

Table S1. ICD 9 and ICD 10 codes for the present study

Category	ICD-9-CM Codes	ICD-10-CM Codes
Inflammatory bowel disease	5560, 5561, 5562, 5563, 5565, 5566, 5568, 5569, 5550, 5551, 5552, and 5559	K5100, K51011, K51012, K51013, K51014, K51015, K51016, K51017, K51018, K51019, K5120, K51211, K51212, K51213, K51214, K51218, K51219, K5130, K51311, K51312, K51313, K51314, K51318, K51319, K5140, K51411, K51412, K51512, K51513, K51514, K51518, K51519, K5180, K51811, K51812, K51813, K51814, K51818, K51819, K5190, K51911, K51912, K51913, K51914, K51918, K51919, K50011, K50012, K50013, K50014, K50018, K50019, k5010, K50111, K50112, K50113, K50114, K50118, K50119, k5080, K50811, K50812, K50813, K50814, K50818, K50819, k5090, K50911, K50912, K50913, K50914, K50918, K50919, and k5000
Generalized anxiety disorder	30002	F411
Depression	29621, 29622, F29623, 29624, 2980, 29625, 29626, 29682, 29620, and 311	F320, F321, F322, F323, F324, F325, F3289, and F329
Somatization	30081	F450
Bipolar disorder	29640, 29641, 29642, 29643, 29644, 29650, 29651, 29652, 29653, 29654, 29660, 29661, 29662, 29663, 29664, 2967, 29645, 29646, 29655, 29656, 29665, 29666, 29689, 29640, and 29680	F310, F3110, F3111, F3112, F3113, F312, F3130, F3131, F3132, F314, F315, F3160, F3161, F3162, F3163, F3164, F3170, F3171, F3172, F3173, F3174, F3175, F3176, F3177, F3178, F3181, F3189, and F319
Attention-deficit hyperactivity disorder	31400, 31401, and 3142	F900, F901, F902, F908, and F909
Schizophrenia	29530, 29510, 29520, 29590, 29560, 29540, and 29590	F200, F201, F202, F203, F205, F2081, F2089, and F209
Schizoaffective disorder	29570	F250, F251, F258, and F259
Post-traumatic stress disorder	30981	F4310, F4311, and F4312
Anorexia	3071	F5000, F5001, and F5002
Binge eating disorder	30759	F5081
Chronic fatigue	78071	R5382

ICD: International Classification of Disease

DOI: http://dx.doi.org/10.18053/jctres.09.202304.22-00231

Journal of Clinical and Translational Research

Journal of Clinical and Translational Research (JCTR) welcomes submissions from various research topics that are centered on solving clinically-driven issues to ultimately benefit patients.

You will benefit from the following key features of JCTR as our author:

- Open access
- Author-friendly guidelines: 'your paper, your way'
- Reputable editorial board
- No word count or reference restrictions
- Double-blind review process to minimize bias
- Rapid production and publication
- Broad scope, interdisciplinary research exchange platform

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

Internal medicine (all branches)	Gastroenterology and hepatology
Vascular medicine and phlebology	Surgery and transplantation
Oncology	Hematology
Cardiology	Nephrology
Intensive care medicine	Dermatology
Ophthalmology	Endocrinology and metabolism
Neurology and neurosciences	Anesthesiology
Anatomy, physiology, and embryology	Radiology and nuclear medicine
Pathology	Clinical chemistry
Clinical physics	Genetics and epigenetics
Epidemiology	Global health
Medical devices	Nutrition
Pharmacology	Immunology
Microbiology	Virology
Parasitology	Biomedical engineering

Biomedical spectroscopy and spectrometry

Thanks for considering the Journal of Clinical and Translational Research.

Editorial team JCTR

Journal of Clinical and Translational Research is an independent open access journal published by ACCSCIENCE PUBLISHING

Operating office: APPLIVE BV, the Netherlands
Contact: info@jctres.com • Tel: +65 62215600
www.jctres.com

ISSN 2382-6533

ACCSCIENCE PUBLISHING
8 Burn Road, #15-03 Trivex, Singapore 369977.
Tel: +65 62215600