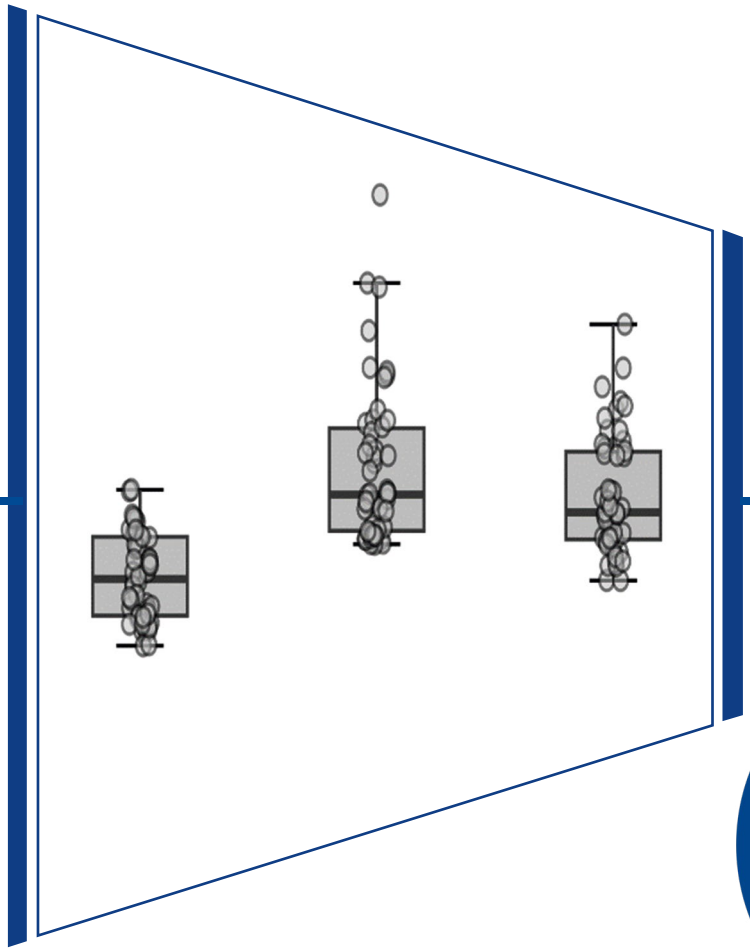
MEDICAL UNIVERSITY OF GDAŃSK

EUROPEAN JOURNAL OF TRANSLATIONAL AND CLINICAL MEDICINE



JOURNAL CITATION REPORT
Clarivate
IMPACT FACTOR
2025
0.4

e-ISSN 2657-3156 2026 Vol. 9 No. 1 ejtcm.gumed.edu.pl

Photo on the cover of the issue
by Lateef et al.
(page 15)

EUROPEAN JOURNAL OF TRANSLATIONAL AND CLINICAL MEDICINE

Editorial Office

Medical University of Gdańsk
European Journal
of Translational Medicine
Dębinki 1 Street,
80-210 Gdańsk, Poland

Phone: +48 58 349 15 37

E-mail: ejtcm@gumed.edu.pl
ejtcm.gumed.edu.pl

Publisher

Medical University of Gdańsk
M. Skłodowskiej-Curie 3 A
80-210 Gdańsk, Poland

Gdańsk 2026
e-ISSN 2657-3156

Online edition is the
original version of the journal.

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):1-93

CONTENTS

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):5-10

INVITED EDITORIAL

Everyone can speak and communicate, but healing with words is an art

Aleksandra Dittmer

Praxis für Psychotherapie, Kiel, Germany

Abstract

According to recent data, nearly 20% of surveyed young people in the United States have used artificial intelligence (AI) in the form of chatbots for personal advice or even psychotherapy. The capabilities of AI are quite tempting: fast, anonymous, affordable and stigma-free access to therapy. In my opinion, it is worth examining what therapy actually is and how it "works." Is it really just a game of questions and answers, questions and instant solutions? Is the opportunity to chat and seek advice 24/7 an effective solution to mental health problems?

Keywords: psychotherapy · artificial intellingence · AI · mental health

Citation

Dittmer A., Everyone can speak and communicate, but healing with words is an art. Eur J Transl Clin Med. 2026;9(1): 5-10.

DOI: 10.31373/ejtcm/224874

Some time ago, I was waiting in a seemingly endless virtual queue for the technical support that manages the medical records system my office is connected to. The first thing I heard from the service technician was the assurance that "99% of the time, the problem is sitting in front of the computer." That system was supposed to be helpful and convenient, but despite the training, I didn't derive any benefits from that technological improvement. In other words, user = loser! I recalled this situation while reading the results of a recent study, which showed that nearly 20% of surveyed young people in the United States have used artificial intelligence (AI) in the form of chatbots for personal advice or even psychotherapy [1]. "Opportunity or risk?" the question is often repeated.

Opportunities

Currently the capabilities of AI are quite tempting: fast, anonymous, affordable and stigma-free access to therapy. But questions arise about the risks posed by a digital

Corresponding author:
Aleksandra Dittmer, Praxis für Psychotherapie, Kiel, Germany
email: praxis.dittmer@gmail.com
Available online: ejtcm.gumed.edu.pl

algorithm in the role of a therapist, and the risks posed by the users themselves, their use of the app and their expectations. In my opinion, it is worth examining what therapy actually is and how it "works." Is it really just a game of questions and answers, questions and instant solutions? Is the ability to speak and hold a conversation enough to heal a person? Does being treated simply mean being able to ask a question, express a goal or express a wish? Is the opportunity to chat and seek advice 24/7 an effective solution to mental health problems?

According to psychotherapy researcher Klaus Grawe, 5 key factors contribute to the success of psychotherapy: the therapeutic relationship, problem activation, active assistance in coping with problems, activation and development of one's own skills, and assistance in clarifying one's own motivation (including actions, needs and wishes) [2].

The need for empathy

A fundamental factor is the creation of a therapeutic relationship, an empathetic and judgment-free space to help "detoxify the problem" (i.e. reveal its universality) and to suggest the first steps towards possible solutions. Research confirms that the patient-therapist relationship (also referred to as the therapeutic alliance) is a key therapeutic factor, regardless of the psychotherapy type [3-4].

From the cradle, we seek contact with living beings. We know that this interaction (consisting of observing facial expressions, vocal melodies, body movements, and individual behaviors) influences the development of mirror neurons, discovered in 1992 by a team of Italian neurophysiologists led by Giacomo Rizzolatti [5]. Since then, numerous neuroimaging studies have confirmed that mirror neurons play a role in the development of empathy and understanding others, which is the basis of all interpersonal skills. The complex activation of multiple brain areas (motor, sensory, and emotional) reveals a common pattern for all of us, leading researchers to conclude that interpersonal understanding may be based on the so-called embodied simulation [6].

The quality of this simulation, however, is highly dependent on the patient's biography and the interpersonal relationships that shape it. Similiar to muscles, mirror neurons gain strength when exercised and deteriorate when neglected. Improving mirroring skills is part of the professional training of psychotherapists, and in the first years of their practice, it is the subject of self-discovery and reflection (e.g. conversation recordings) as part of the supervision by an experienced instructor [7].

Seeing the complexity of this system, one can better understand the task a chatbot is trying to tackle when the user places it in the role of a psychotherapist. Simulating empathy, however, seems to be one of the biggest traps that AI continues to fall into. In the past year the press has been reporting about the extremely tragic consequences of seeking support from chatbots, which not only agreed with users that their current situation was hopeless (and the only solution was suicide), but even offered assistance in composing the suicide note [8-9]. On the other hand, chatbots are quite good at handling the problems of moderately stressed users, as well as at other factors of successful psychotherapy: determining motivation and suggesting new strategies for coping with the problem [10].

Between the lines

The goal of a therapeutic relationship is first and foremost the ability to read between the lines, clarifying what is left unsaid, omitted or even concealed. Sometimes that is more important in therapy. Often, this is the unconscious filter of the person seeking advice. The psychotherapist's active guidance of the conversation aims to gather as much information as possible and to complete the missing pieces of the puzzle. This skill aims to accurately formulate the key problem, as well as to actualize and manage it, which is directly related to the outcome of therapy and is the last of the 5 factors identified by Grawe.

There are numerous causes of missing information: from the patient's ignorance, disregard for their own thoughts and behaviors, lack of awareness of their illness, hidden agendas, through simple shyness (or shame), to blatant lies and manipulation. Facial expressions, voice modulation, pauses, intonation, and psychomotor movements are sources of information and serve to confirm what is said and to decode what is unsaid. A careful conversation is the foundation of the diagnostic process, culminating in a diagnosis, a treatment plan, and an assessment

of prognosis. When we select a voice in the chatbot app, all of the above information is lost (as is currently the case, even in the latest versions, which are designed to process this information) on the way to the virtual interlocutor, preventing responses that may be among the first interventions in the therapeutic process, aimed at clarifying the problem and diagnosis, as well as identifying the patient's real (and unrealistic) expectations regarding the possibilities of psychotherapy. Currently, AI still has difficulty sensing the intent behind our words and weaving threads of a conversation together [11-12].

As the old saying goes, "if you never say *no*, you can't fulfill every *yes*." Empathy and understanding are just one aspect of a therapeutic relationship. One of the challenges in building a therapeutic relationship is maintaining the patient's faith in the therapist's competence and professionalism while simultaneously breaking free from idealizing the psychotherapist and fantasizing about his or her omnipotence. Within the therapeutic relationship, behaviors and responses that disappoint the patient or expose the therapist's imperfections can lead to the patient's frustration or even anger. This requires the psychotherapist to have courage and to demonstrate an attitude that can be an important regulator of the patient's unfavorable thoughts or behaviors. They also signal authenticity in the therapeutic relationship, establish boundaries, and can strengthen the relationship, enabling work on resolving the patient's current problems. They can also lead to a breakdown of the therapeutic cooperation, and in extreme situations in voluntary admission of the patient to the psychiatric ward.

Full availability

Chatbots on the other hand, are programmed to provide services to the client's satisfaction. Try a simple experiment: start an argument with a chatbot or try to force it to remain silent. Research shows that confrontations with a therapist that don't break the therapeutic collaboration, in fact strengthen the relationship and have a positive impact on achieving therapeutic goals [13]. Silence is an active, multifunctional tool in both directive and non-directive psychotherapy and can serve various functions, e.g. it can mark an important moment in the process of self-expression or in deepening one's own emotions. It can also express surprise or sympathy. A psychotherapist can become "speechless"… and this is not necessarily a bad thing! However, the chatbot's silence is most often the result of a disrupted internet connection or a server malfunction.

As mentioned above, developing and stimulating individual skills and competencies is one of the key factors contributing to the success of psychotherapy. Long-term strengthening of self-regulation (except in situations of extreme mental crisis, e.g. severe suicidal ideation) means supporting the patient in maintaining their scheduled appointments within outpatient therapy. The strongest support is in the form of inpatient treatment, where the patient is supervised 24/7, whereas day clinics offer the possibility of daytime supervision and support. This requires the greatest degree of independence from the patient, which through (via successful self-regulation) is the primary goal of therapy. Paradoxically, a 24/7 availability of the therapist would actually be counterproductive. For example, for a patient with hypochondria, somatization disorder or obsessive-compulsive disorder, one of the most difficult challenges is maintaining a waiting period both in contacts with a therapist and also refraining from consultations with the so-called "Doctor Google".

"Trust"?

Thanks to widespread information campaigns, blogs, and articles, e.g. about healthy nutrition, we are more cautious and more aware of our own responsibility when standing in front of a supermarket shelf. We know that the raspberry-flavored juice on the store shelf is not the same as the homemade juice made from fresh raspberries according to Grandma's recipe, but an artificially colored, flavored, and thickened drink sweetened with high-fructose corn syrup. Therefore, it seems worth drawing the attention of those seeking help from AI to the above-mentioned shortcomings of the mental health "services" by chatbots.

Additionally, we don't know what user projections AI is subject to or what human traits and intentions users subconsciously attribute to AI, despite the fact that it doesn't possess them. Is AI brave enough to admit its ignorance? Will the developers allow AI to disappoint or anger its users? Another very problematic phenomenon is the so-called "AI hallucination," a situation in which the AI can't find an answer to a user's question, so it generates false information. In other words, AI can fabricate information without even realizing it's doing so! Hmm, this is almost human behavior, but are we, the users even aware of it?

All of these elements present AI developers with real challenges, but for now, it is us, the users, who need to understand that psychotherapy is more than just the sound of a human voice or a written dialogue generated by a pattern or algorithm. Transparent and reliable psychoeducation is needed, because for now, users must face these challenges on their own. In other words, at this moment chatbot therapy is *de facto* self-therapy and self-help at your own risk!

AI has access to a wealth of information, the most abundant of which is available online and has made its way into the mainstream. This information isn't always accurate or the latest and its sources depend on many factors, e.g.

paid access or copyrights. The quality of information also depends on the chatbot itself (free versus paid versions). In my opinion, the greatest challenge for users today is the ability to select the information the chatbot should receive and to formulate questions and tasks (so-called prompts) to obtain the most relevant information or to initiate a helpful dialogue. Anyone who has used a chatbot has learned that its responses are only as accurate as the questions directed at it. A lack of self-awareness (so essential in the context of psychotherapeutic advice) can significantly limit the effectiveness of these dialogues. A subjective focus on purely emotional/mental problems can increase the risk of missing the somatic etiology of disorders [14].

Prescription for an app

I am confident that eventually we will find a permanent use for AI in psychotherapy. Mobile apps that introduce users to the practice of meditation, support them during anxiety attacks and offer stress reduction assistance are already available for Android and iOS devices. They are intended to help cope with a storm of thoughts, substance cravings or the urge to self-harm. Memory training apps help patients with dementia or stroke to practice speech and memory. In some countries (such as Germany since 2019), selected apps require a prescription. This guarantees partial or full coverage of the purchase and subscription costs. Many of these apps are based on, or directly adapt, proven therapeutic programs from individual or group therapy, focusing on changing previously identified pathological behaviors. However, for the purposes of adapting them to a mobile app format, these programs are often significantly reduced and generalized. Initial analyses, prepared at the request of the German Association of Statutory Health Insurance Funds (*Spitzenverband Bund der Krankenkassen*) point out the disappointing quality of research on the effectiveness of these apps. This was met with considerable disappointment, primarily due to the high costs and the hopes for rapid patient assistance while simultaneously relieving the burden on the public healthcare system [15]. Over the last 4 years, patients in Germany have used somatic and mental health apps 861 000 times, worth €234 million. Mental health apps account for 44% of the catalog and generated 30% of activations [16]. However, the transfer of advice/therapeutic interventions from apps to patients does not seem convincing to the analysts, thus emphasizing the role of regulatory agencies, objective data analysis and independent oversight of the quality of these digital healthcare solutions.

Chatbot for conscious user

While most apps still lag behind in terms of expectations and capabilities, I believe they have great potential. Apps typically focus on Axis I diagnoses (DSM classification), a defined task and are instructional in nature. Chatbots, on the other hand, have a more challenging task. Anyone who has worked with patients with personality disorders or comorbidities understands the complexity of such treatment. What many apps haven't been able to include is working on the (biographical) roots of many dysfunctional behaviors or even working on these behaviors in the context of current events and relationships. Due to the high diversity, this work requires a much more individualized approach, greater flexibility, and more directive behavior on the part of the therapist. Currently, chatbots cannot cope with this, and their effectiveness depends too much on the user's high self-awareness, which, in many therapeutic processes, is a goal in itself. And therein lies the problem. Today, the risk that a patient seeking support from a chatbot will wander in the fog of their own unconsciousness is still high and the promise of quick help can lead to isolation, alienation, and the confirmation of dysfunctional beliefs about one's own inadequacy. However, with high self-awareness and a functional focus on the user's conversation, AI can now enrich advice with information that a psychotherapist typically lacks, e.g. work organization psychology, coaching, exercise therapy.

Final thoughts

AI offers an opportunity for faster, easier, and more affordable access to psychotherapeutic advice for (at this moment) a narrow group of patients with a narrow set of problems. The involvement of academic and training centers is essential for effectively monitoring AI development, and technology companies should be aware of the limitations of their products. The long-awaited and much-needed legal protection of the title of "Psychotherapist" in some countries (e.g. Poland) should also send a strong signal in this direction. Furthermore, we must consider whether the promotion of AI advice in the context of psychotherapy should be driven by materialistic values, e.g. fast, cheap, easy and anonymous access. Technological progress and the associated possibilities are one thing, but what tasks in the therapeutic process do we want to delegate to AI? What is the benefit, the developmental value in this? In the era of digital loneliness, with its emphasis on efficiency and effectiveness, is talking to a machine the right path to a healthy and fully satisfied life among other people? To what extent does the "human element" emphasize the relational and interactive nature of therapy, and act as a reflection and support in the sense of feedback? To what extent does the presence of a living,

breathing *Gegenüber** determine that during versation there is a spiritual and emotional encounter, a contact thanks to which the conversation becomes a source of transformation and the spoken words actually heal?

During the first conversation, I always ask all my patients if they drink alcohol, take drugs, self-harm or use food to regulate their mood. While writing this editorial, I decided to add one more question: "Do you seek personal advice from a chatbot?" Because it is true: a drowning person does clutch at straws…

as a "mirror" or "screen", while according to Jung it is an active participant in the transformation process.

Conflict of interest

None to report.

Funding

None.

References

1. McBain RK, Cantor JH, Breslau J, Diliberti M, Zhang LA, Zhang F, et al. AI Chatbot Use and Disclosure for Mental Health Among US Adolescents and Young Adults. JAMA Pediatr [Internet]. 2026 Jun 1; Available from: https://jamanetwork.com/journals/jamapediatrics/fullarticle/2849307
2. Grawe K, Donati R, Bernauer F. Psychotherapie im Wandel: Von der Konfession zur Profession [Internet]. Göttingen: Hogrefe; 1994. Available from: https://doc1.bibliothek.li/aab/FLMA134816.pdf
3. Baier AL, Kline AC, Feeny NC. Therapeutic alliance as a mediator of change: A systematic review and evaluation of research. Clin Psychol Rev [Internet]. 2020;82:101921. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0272735820301094
4. Finsrud I, Nissen-Lie HA, Vrabel K, Høstmælingen A, Wampold BE, Ulvenes PG. It's the therapist and the treatment: The structure of common therapeutic relationship factors. Psychother Res [Internet]. 2022;32(2):139–50. Available from: https://www.tandfonline.com/doi/full/10.1080/10503307.2021.1916640
5. di Pellegrino G, Fadiga L, Fogassi L, Gallese V, Rizzolatti G. Understanding motor events: a neurophysiological study. Exp Brain Res [Internet]. 1992;91(1):176–80. Available from: http://link.springer.com/10.1007/BF00230027
6. Schmidt SNL, Hass J, Kirsch P, Mier D. The human mirror neuron system – A common neural basis for social cognition? Psychophysiology [Internet]. 2021;58(5). Available from: https://onlinelibrary.wiley.com/doi/10.1111/psyp.13781
7. Knol ASL, Huiskes M, Koole T, Meganck R, Loeys T, Desmet M. Reformulating and Mirroring in Psychotherapy: A Conversation Analytic Perspective. Front Psychol [Internet]. 2020;11. Available from: https://www.frontiersin.org/article/10.3389/fpsyg.2020.00318/full
8. Reiley L. What My Daughter Told ChatGPT Before She Took Her Life. New York Times [Internet]. 2025; Available from: https://www.nytimes.com/2025/08/18/opinion/chat-gpt-mental-health-suicide.html
9. Duffy C. Parents of 16-year-old sue Open AI, claiming ChatGPT advised on his suicide [Internet]. CNN Business. 2025 [cited 2026 Jun 16]. Available from: https://edition.cnn.com/2025/08/26/tech/openai-chatgpt-teen-suicide-lawsuit
10. Hatch SG, Goodman ZT, Vowels L, Hatch HD, Brown AL, Guttman S, et al. Correction: When ELIZA meets therapists: A Turing test for the heart and mind. PLOS Ment Heal [Internet]. 2025;2(8):e0000426. Available from: https://dx.plos.org/10.1371/journal.pmen.0000426
11. Jordan D. AI Can Write Anything, But It Still Doesn't Understand Why Humans Care. Los Angeles Trib [Internet]. 2026;(June 16). Available from: https://thelosangelestribune.com/2026/02/18/ai-can-write-anything-but-it-still-doesnt-understand-why-humans-care/
12. Vutukuri K. Artificial Intelligence Works With Patterns, Not Meaning – and That Changes Everything [Internet]. Medium. 2026 [cited 2026 Jan 6]. Available from: https://medium.com/core-ai/artificial-intelligence-works-with-patterns-not-meaning-and-that-changes-everything-e736da3250c9
13. Eubanks CF, Muran JC, Safran JD. Alliance rupture repair: A meta-analysis. Psychotherapy [Internet]. 2018;55(4):508–19. Available from: https://doi.apa.org/doi/10.1037/pst0000185

14. Grabb D. The impact of prompt engineering in large language model performance: a psychiatric example. J Med Artif Intell [Internet]. 2023;(6):1-5 Available from: https://jmai.amegroups.org/article/view/8190
15. Studie: Zulassungsstudien für DiGA oft mangelhaft [Internet]. Ärzte Zeitung. 2025 [cited 2026 Jun 15]. Available from: https://www.aerztezeitung.de/Wirtschaft/Studie-Zulassungsstudien-fuer-DiGA-oft-mangelhaft-457891.html
16. GKV ärgert sich über steigende Kosten und wenig Nutzen bei DiGA [Internet]. Ärzte Zeitung. 2025 [cited 2026 Jun 5]. Available from: https://www.aerztezeitung.de/Politik/GKV-aergert-sich-ueber-steigende-Kosten-und-wenig-Nutzen-bei-DiGA-457649.html

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):11-19

RESEARCH ARTICLE

Measurement of agouti-related peptide and myeloperoxidase levels and assessment of their potential role in the metabolic pathways of obese individuals with type 2 diabetes

Nuha Dheyaa Lateef, Fayhaa Muqdad Khaleel

Department of Chemistry, College of Science for Women, University of Baghdad, Al-Jadriya, Baghdad, Iraq

Abstract

Background: Agouti-related peptide (AgRP) and myeloperoxidase (MPO) are key markers associated with metabolic and inflammatory dysfunctions linked to type 2 diabetes (T2DM) and obesity through appetite-regulating mechanisms and immune activity. **Material and methods:** Total of 150 participants (30-55 years old) were separated into 3 groups: control group, the obesity group, and the T2DM with obesity group. Fasting blood samples were collected. Biochemical tests (e.g. fasting glycemia and lipid profile), were determined using colorimetric methods, while insulin, AgRP, and MPO were measured using ELISA. Statistical analyses (e.g. one-way ANOVA, multiple linear regression, ROC analysis) were conducted. **Results:** AgRP was significantly higher in the obesity and T2DM with obesity groups. Conversely, MPO levels significantly decreased in these groups ($p < 0.0001$) compared to the control group. The ROC curve showed good discriminatory performance between patient and control groups. **Conclusions:** Increased AgRP levels might reflect alterations of appetite and energy balance, while decreased MPO levels may indicate a complex inflammatory response that could involve enzyme retention in certain tissues. This study confirmed the importance of AgRP and MPO as potential supplementary indicators of metabolic disorders. There is a need for more studies to elucidate the mechanisms underlying the decrease in MPO in these patients.

Keywords: agouti-related peptide · myeloperoxidase · type 2 diabetes mellitus · obesity · insulin resistance

Citation

Lateef ND, Khalee l FM. Measurement of agouti-related peptide and myeloperoxidase levels and assessment of their potential role in the metabolic pathways of obese individuals with Type 2 diabetes. Eur J Transl Clin Med. 2026;9(1):11-19.

DOI: 10.31373/ejtcm/222598

Corresponding author:
Nuha Dheyaa Lateef, Department of Chemistry, College of Science for Women, University of Baghdad, Al-Jadriya, Baghdad, Iraq
e-mail: noha.abd2405m@csw.uobaghdad.edu.iq
Available online: ejtcm.gumed.edu.pl

Introduction

Obesity was originally characterized by excessive adipose tissue accumulation. The understanding of obesity has evolved into a complex illness with significant effects on physiological and metabolic processes, and it is no longer considered strictly a result of lifestyle decisions [1-2]. In type 2 diabetes mellitus (T2DM), the body becomes insulin resistant (IR), which forces the pancreatic beta cells to secrete higher amounts of insulin to produce a glucose-lowering effect. Over time, the pancr eatic beta cells might become unable to produce enough insulin to compensate for IR, which leads to relative insulin deficiency and elevated blood glucose levels [3]. In obese individuals, IR is more common due to shared pathophysiological pathways between obesity and T2DM. Furthermore, changes in adipose tissue biology that link obesity to IR and impaired beta-cell function lead to increased incidence of T2DM, in line with increasing abdominal fat distribution and body mass index (BMI) [4-5]. Despite its common use as a criterion for classifying obesity, the BMI does not accurately reflect the distribution of body fat or the metabolic dysfunction associated with obesity. That is why the European Association for the Study of Obesity (EASO) has indicated that functional impairment associated with fat should be the basis for defining obesity [6].

Agouti-related peptide (AgRP) is a type of neuropeptide produced by the AgRP/NPY neurons (neuropeptide Y) in the ventral part of the arcuate nucleus of the hypothalamus [7-8]. In humans its transcription is composed of 132 amino acids [9]. AgRP acts as an internal competitor to the MC3R and MC4R of melanocortin receptors and is essential for the control of energy balance [10]. If AgRP/NPY neurons' activity increases, then food consumption will increase and less energy will be expended, leading to weight gain. These neurons are a component of a complex neural network that reacts to several energy-related cues, including hormones that signal hunger (ghrelin) or fullness (insulin and leptin). AgRP/NPY neurons are activated during periods of low energy (e.g. fasting), which increases hunger and encourages eating-seeking conduct [11-12]. AgRP neuronal activity modulates feeding and glucose homeostasis, with disrupted insulin signaling contributing to insulin resistance and the etiology of T2DM [13-14].

Myeloperoxidase (MPO, EC 1.11.2.2), is a type of heme peroxidase, generally found in the neutrophils and, with less content, in monocytes. MPO has extensive bactericidal activity by catalyzing the Cl reaction with H2O2 to produce the potent oxidant hypochlorous acid (HOCl). But excessive MPO-derived oxidant generation has harmful effects, particularly in conditions where inflammation is either acute or chronic [15]. Over the past years, there has been a significant shift away from the prevalent and oversimplified understanding of MPO as a cytotoxic agent. It is now clear that MPO has functions that extend beyond its antibacterial properties and contribute to the host sustaining tissue damage. MPO has been shown to directly influence the function of inflammatory cells in addition to neutrophils, endothelium, dendritic cells, macrophages, and epithelial cells, as well as to influence immunity [16-17]. Obesity associated with diabetes increases oxidative stress, which leads to increased MPO activity and exacerbates inflammation, lipid abnormalities, vascular damage, and insulin resistance compared to obese non-diabetic individuals [18]. The relationship between AgRP and MPO and T2DM linked to insulin resistance and obesity remains unclear despite a growing number of studies on the subject. The purpose of this study was to investigate the role of AgRP and MPO in metabolic disorders and oxidative stress in people who are obese with T2DM and without.

Materials and methods

Study design

This research was conducted from January to March 2026 at the Al-Yarmouk Teaching Hospital (Baghdad, Iraq) in collaboration with the College of Science for Women at the University of Baghdad. A total of 150 adults, aged 30-55 years, were enrolled and equally divided into 3 groups according to their clinical status. Group 1 (control group) consisted of 50 individuals with no history of DM, obesity, or other chronic diseases (BMI ≤ 25 kg/m²). Group 2 (obesity only) included 50 people diagnosed with obesity only but without medical history of DM or chronic disease (BMI ≥ 30 kg/m²). Group 3 (DM with obesity) included 50 individuals diagnosed with both obesity and T2DM (BMI ≥ 30 kg/m²). The sample size was determined based on previous studies that investigated the biochemistry that links obesity and T2DM. Exclusion criteria included pregnancy, cardiovascular diseases, thyroid diseases, renal disease, autoimmune disorders, malignancies, insulin injections and use of medications that are known to impair neutrophil activity. The participants provided informed consent and met the diagnostic criteria for obesity and diabetes as applicable. This study was approved by the local Scientific Research Ethics Committee (Approval No. CSW-REC 1018).

Anthropometric measurements

All participants completed a questionnaire and recorded a set of characteristics such as gender, age, and medical history. Weight, height, and waist circumference were also measured in the normal standing position. BMI was computed by dividing weight in kilograms by squared height in meters

(kg/m²). The waist-to-hip ratio (WHR) was computed by dividing the waist circumference in cm by the hip circumference in cm. The waist-to-height ratio (WHtR) was calculated dividing the waist in cm by the height in cm.

Laboratory measurements

Venous blood fasting samples were collected after 8-12 hours of fasting. Biochemical analyses included fasting blood glucose and lipid profile (LDL, HDL, triglycerides, and total cholesterol). Fasting serum insulin, AgRP) and MPO were measured using a human ELISA kit (ELK Biotechnology, Sugar Land, TX, USA) according to the instructions from the manufacturer. The assay sensitivity for AgRP is 3.1 pg/mL, and the detection range is 7.82-500 pg/mL. For MPO, the assay sensitivity was 0.65 ng/mL, and the detection range is 2.34-150 ng/mL; the absorbance was measured using a HumaReader HS microplate reader (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). Insulin resistance was computed using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) model, which is [Insulin (μU/mL) × Glucose (mg/dL)]/405 [19].

Statistical analysis

The Social Sciences Statistical Package (v. 26, SPSS Inc., Chicago, USA) and JASP (v. 0.19.1, JASP Team) were used for the statistical analysis [20]. The data is presented as mean ± SD. Analyses included one-way ANOVA to compare groups, Pearson's correlation analysis, multiple linear regression (including VIF for multicollinearity) and ROC curve analysis. A p-value ≤ 0.05 was deemed significant statistically.

Results

The duration of diabetes in the DM with obesity group ranged from < 3 years to 10 years. The mean ± SD values for BMI, WHR, and WHtR in the obesity group and the DM with obesity group were significantly higher compared with the control group (Table 1). Both fasting glycemia and HOMA-IR showed a higher value in the DM with obesity group relative to the control group. All lipid profile indices were elevated in the obesity and DM with obesity groups, except for HDL-C which decreased in both these groups compared to the control group. VLDL-C levels were noticeably higher in the DM with obesity group compared with the other groups.

The AgRP levels in the obesity group and the DM with obesity group were significantly elevated (mean value ± SD: 227.91 ± 66.46 pg/mL and 204.02 ± 51.15 pg/mL, respectively) compared to the control group. In contrast, MPO was decreased in the patient groups compared with the control group (mean value ± SD: 118.76 ± 16.55 ng/mL).

The correlation analysis showed a positive correlation between WHtR and AgRP ($r = 0.438$, $p < 0.001$), while MPO showed a negative correlation with WHtR ($r = -0.628$, $p < 0.001$) (Table 2). Additionally, AgRP and MPO showed a moderate negative correlation with each other ($r = -0.345$, $p < 0.001$).

Multiple linear regression analysis revealed that AgRP was positively associated with BMI ($\beta = 0.514$, $p < 0.001$) and shows no significant correlation with age or fasting glycemia, while MPO was negatively correlated with both BMI ($\beta = -0.511$, $p < 0.001$) and fasting glycemia ($\beta = -0.311$, $p < 0.001$) (Table 3).

The box plot analysis showed that AgRP levels were higher in both the obesity and DM with obesity groups. In contrast, MPO levels were lower in these groups compared to the control group, as shown in Figure 1.

Table 4 shows the results of the ROC analysis between the control and obesity groups. The area under the AUC of the ROC curve for AgRP and MPO proved to be indicating good discriminatory performance, having values of 0.915 and 0.975, respectively, as shown in Figure 2.

Table 5 presents the ROC analysis, which showed a good discriminatory performance for AgRP and MPO between the control and the DM with obesity groups, with AUC values of 0.857 and 0.976, respectively, as shown in Figure 3.

Discussion

BMI is used to assess obesity, but it doesn't account for fat distribution differences [21]. Recent frameworks by the Lancet Commission and EASO have highlighted the limitations of BMI in diagnosing obesity, however it remains the routinely used criterion for obesity classification in Iraq according to the WHO classification [6, 22-23]. Therefore, BMI was used as the primary criterion for obesity classification among the participants in the present study [23]. As excess weight advances, many individuals develop a characteristic pattern of fat accumulation in the abdominal region, leading to a larger waist circumference and a higher WHR and WHtR. A high WHR is a predictive risk factor that plays a role in several diseases, e.g. T2DM and heart disease [24]. In addition, WHtR may provide additional information for the assessment and classification of obesity [25]. Obesity particularly central obesity, is a metabolic disorder that impairs insulin responsiveness and causes persistent low-grade inflammation. Elevated levels of inflammatory cytokines trigger multiple intracellular signaling pathways, promoting lipid accumulation within adipocytes and, thereby, playing an important part in the progress of IR and T2DM [26-27].

Table 1. Mean ± SD of anthropometric data, biochemical parameters, and main metabolic parameters among three groups

Parameters		Control group (n = 50)	Obesity group (n = 50)	DM with obesity group (n = 50)	P-value
	Anthropometric data				
Age (year)		42.32 ± 7.58	42.36 ± 8.05	44.58 ± 7.06	0.234
BMI (kg/m²)		23.25 ± 1.49	37.45 ± 4.69	37.27 ± 5.30	< 0.0001**
WHR		0.84 ± 0.06	1.06 ± 0.03	1.15 ± 0.03	< 0.0001**
WHtR		0.48 ± 0.036	0.66 ± 0.065	0.65 ± 0.063	< 0.0001**
	Glycemic profile				
Fasting glycemia (mg/dL)		92.54 ± 13.18	111.40 ± 8.63	248.54 ± 33.14	< 0.0001**
Insulin (µIU/mL)		9.18 ± 1.26	14.82 ± 4.34	13.84 ± 2.15	< 0.0001**
HOMA-IR		2.04 ± 0.40	4.06 ± 1.35	8.45 ± 1.68	< 0.0001**
	Lipid pofile				
Cholesterol (mg/dL)		168.26 ± 5.46	203.33 ± 30.35	206.05 ± 22.97	< 0.0001**
Triglyceride (mg/dL)		175.38 ± 18.52	178.64 ± 12.54	244.55 ± 24.05	< 0.0001**
	HDL-C (mg/dL)	59.40 ± 6.81	31.98 ± 7.21	42.82 ± 8.31	< 0.0001**
	LDL-C (mg/dL)	74.50 ± 10.17	135.66 ± 33.10	114.35 ± 26.37	< 0.0001**
	VLDL-C (mg/dL)	35.03 ± 3.71	35.68 ± 2.51	48.87 ± 4.81	< 0.0001**
	Biochemical markers				
	AgRP (pg/mL)	135.60 ± 36.50	227.91 ± 66.46	204.02 ± 51.15	< 0.0001**
	MPO (ng/mL)	118.76 ± 16.55	61.13 ± 19.84	49.45 ± 25.98	< 0.0001**

** ANOVA test significant difference between means at level $p < 0.01$

Table 2. Correlation analysis between WHtR with AgRP and MPO levels

Variables	WHtR	AgRP	MPO
WHtR			
AgRP	r = 0.438 p < 0.001		
MPO	r = -0.628 p < 0.001	r = -0.345 p < 0.001	

At $p < 0.001$, it is considered statistically significant.

Table 3. Multiple linear regression analysis of AgRP and MPO levels

Dependent	predictors	Unstandardized(B)	Standardized(β)	t-value	p-value	VIF
AgRP	**BMI**	4.283	0.514	6.317	< 0.001	1.291
	Age	-0.894	-0.104	-1.435	0.153	1.020
	FBS	-0.031	-0.035	-0.426	0.671	1.307
MPO	**BMI**	-2.402	-0.511	-7.850	< 0.001	1.291
	Age	-0.280	-0.057	-0.994	0.322	1.020
	FBS	-0.158	-0.311	-4.748	< 0.001	1.307

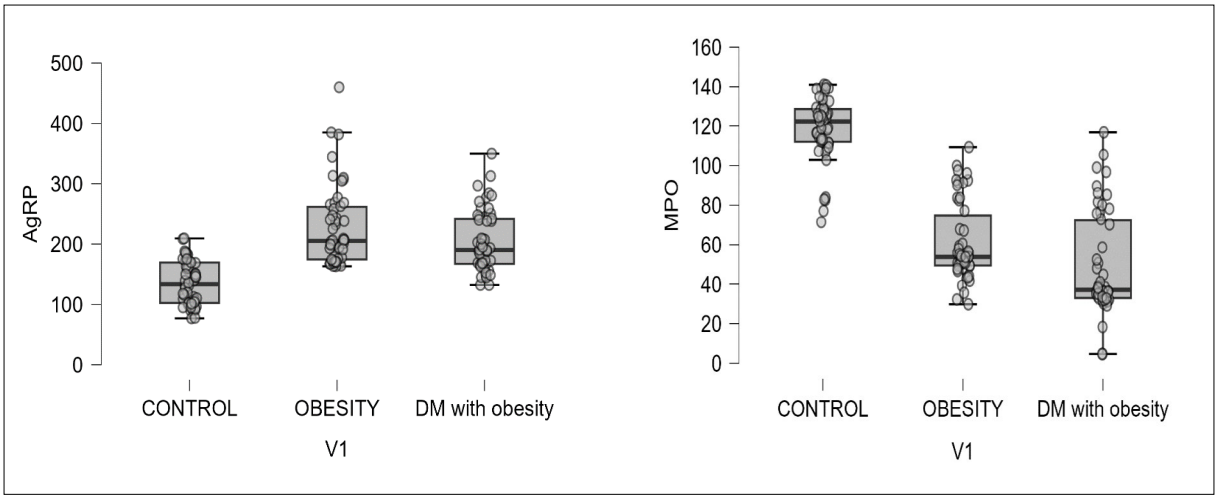


Figure 1. Box plot showing the distribution of serum AgRP and MPO levels among the 3 study groups. The horizontal line represents the median, the box represents the interquartile range, and the circles represent the individual observations.

Table 4. AUC value of the AgRP and MPO between control and obesity groups

Parameters	AUC	Std. error[a]	Asymptotic Sig.[b]	Asymptotic 95% CI		Cut-off value
				Lower bound	**Upper bound**	
AgRP (pg/mL)	**0.915**	0.03	0.000	0.857	0.973	≤ 160.3 pg/mL
MPO (ng/mL)	**0.975**	0.016	0.000	0.943	1.000	> 99.8 ng/mL

Table 5. AUC value of the AgRP and MPO between control and DM with obesity groups

Parameters	AUC	Std. error[a]	Asymptotic Sig.[b]	Asymptotic 95% CI		Cut-off value
				Lower bound	**Upper bound**	
AgRP (pg/mL)	**0.857**	0.038	0.000	0.782	0.932	≤ 140 pg/mL
MPO (ng/mL)	**0.976**	0.014	0.000	0.949	1.000	> 99 ng/m

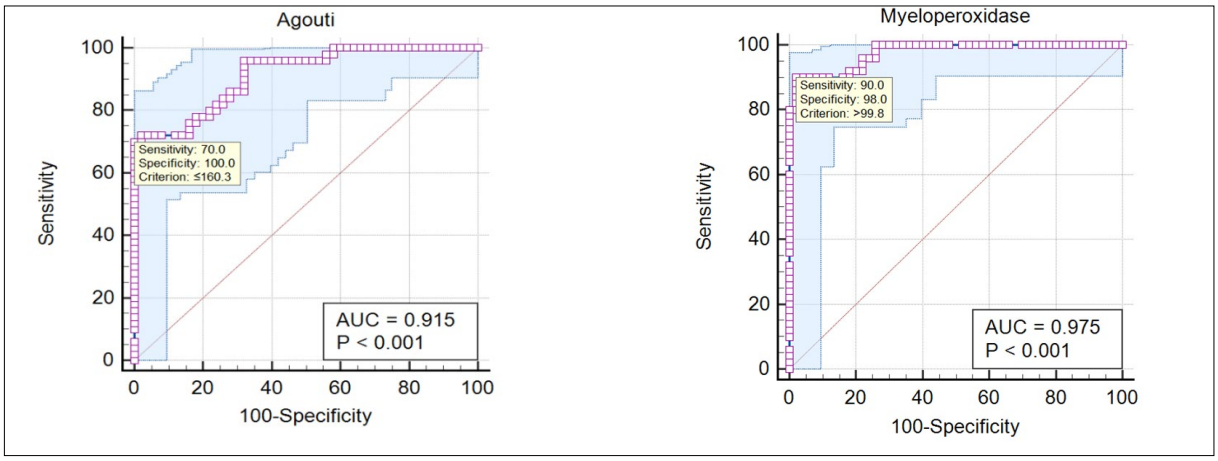


Figure 2. ROC curve analysis of AgRP and MPO between control and obesity groups

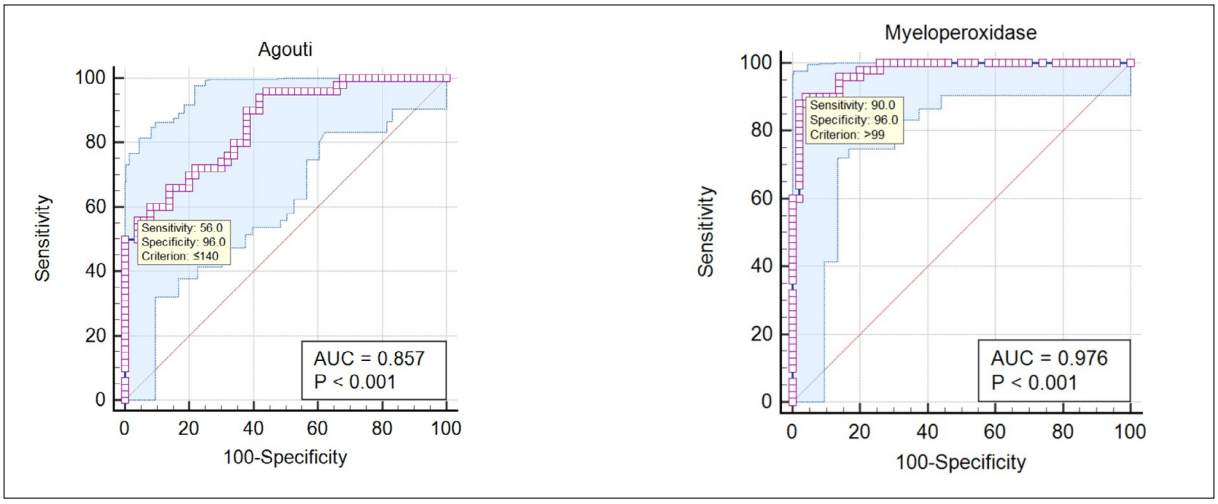


Figure 3. ROC curve analysis of AgRP and MPO between control and DM with obesity groups

Our regression model verified the independent impact of obesity on circulating AgRP, implying that these changes are mostly caused by obesity, independent of age or blood sugar levels. Similarly to Su et al., AgRP is an efficient peptide that enhances appetite; the hormone ghrelin activates its effect on appetite; therefore, increased food intake is associated with increased AgRP expression [28]. AgRP neurons release neurotransmitters, such as NPY, to increase hunger, while the pro-opiomelanocortin (POMC) fibers release α-MSH to reduce hunger and increase satiety. The competitive interaction between these neurotransmitters influences the degree of satiety felt after a meal [29]. During fasting in the arcuate nucleus, decreased leptin plays a role in increasing food consumption and reducing energy spending through POMC inhibition and AgRP/NPY cell activation [30]. Overeating and poor glucose regulation are promoted by obesity's central oxidative stress in the hypothalamus, where increased reactive oxygen species interfere with the function of energy-regulating neural circuits, including AgRP cells [31]. A study conducted by Zhang et al. indicated that AgRP is intimately linked to oxidative stress and metabolic degradation. Increased oxidative load inside AgRP cells themselves, resulting from disturbed mineral balance and metabolism, exacerbates obesity, insulin resistance, and impaired glucose balance [32]. The neural circuits responsible for regulating appetite and energy may be disrupted as a result of hypothalamic dysfunction caused by oxidative imbalance and metabolic stress. Furthermore, circulating AgRP levels may not accurately reflect central hypothalamic activity, therefore the peripheral measurements should be interpreted with caution.

Although inconsistent with several previous studies, the low levels of MPO in our study among participants with T2DM and obesity, may be explained by multiple mechanisms. Labato et al. indicated that neutrophil secretion may be impaired or affected by chronic metabolic dysregulation in obese individuals with T2DM, leading to a disruption in the granule release process and consequently reducing the release of MPO into the bloodstream [33]. Similarly, evidence suggests that

circulating MPO levels in chronic inflammation are subject to a complex regulatory mechanism. This complexity indicates that MPO levels may not always follow a linear pattern because their release into the circulation may not strictly correspond to their total protein content or enzymatic activity, or their functions may be actively suppressed [34]. In addition, MPO is considered a bridge link between oxidative stress, inflammation and systemic diseases. Studies have reported changes in oxidative stress in patients with T2DM, which may contribute to metabolic dysfunction or disorder [35-36]. Due to MPO's high reactivity with various substrates such as lipids and proteins, the enzyme may be consumed in multiple pathways in metabolic states characterized by high oxidative burden, such as obesity and T2DM [15, 37]. Therefore, decreased circulation of MPO should be interpreted cautiously and does not imply the absence of inflammatory activity. Furthermore, intracellular enzyme activity and tissue retention in chronic metabolic conditions require more studies.

The ROC analysis in our study for both AgRP and MPO showed a good AUC value. This may indicate that AgRP and MPO are sensitive to early metabolic and oxidative alterations, which could support their potential use as early biomarkers of metabolic dysregulation. However, further validation studies are still needed to define their precise role.

Study limitations

Despite the importance of its findings, this study has many limitations. Firstly, serum MPO measurements do not necessarily represent its real functional activity within tissues. Additionally, the cross-sectional design of the study may not demonstrate a causal relationship between AgRP and MPO. We measured only peripheral AgRP, which may not fully reflect central neuroendocrine signaling pathways. Another limitation is the exclusion of the BMI 25-30 kg/m² group. In individuals with a BMI between 25 and 30 kg/m², there may be increased cardiometabolic risk and metabolic dysfunction associated with obesity [38]. Our sample size and the fact that the participants were recruited within a single geographical area may restrict the generalizability of our results. In addition, lifestyle factors, dietary habits, physical activity, smoking status, ethnic differences, and the use of oral antidiabetic medications were also not fully evaluated among our study participants, which may have affected some anthropometric indicators and circulating biomarker levels. Furthermore, the lack of HbA1c measurements may restrict the thorough evaluation of long-term glucose control.

Conclusions

AgRP and MPO are associated with metabolic disorders observed in both T2DM and obesity. An increase in AgRP levels may reflect a potential disruption in the neural control of appetite and energy balance, which may contribute to overeating and impaired glucose regulation. In contrast, the observed decrease in MPO may suggest a complex inflammatory response possibly related to enzyme consumption in tissues as a result of chronic oxidative stress. Further studies are needed to test this hypothesis. Although there is no direct link between AgRP and MPO, these biomarkers may be indirectly connected through pathways associated with insulin resistance and the onset of T2DM due to the metabolic dysfunction in obesity. This supports their potential role as complementary markers of metabolic disorders rather than as stand-alone diagnostic tools. Long-term and longitudinal research is required to confirm these biomarkers' clinical relevance and usefulness.

Acknowledgements

We extend our sincere thanks to everyone who contributed to and supported this study and helped in its successful completion, including the doctors and laboratory staff at the Yarmouk Teaching Hospital.

Conflicts of interest

The authors confirm that no a conflicts of interest were present.

Data availability

The datasets generated or analyzed in this study is not publicly available due to ethical and participant privacy considerations, but it is available upon reasonable request.

References

1. Bond ST, Calkin AC, Drew BG. Adipose-Derived Extracellular Vesicles: Systemic Messengers and Metabolic Regulators in Health and Disease. Front Physiol [Internet]. 2022;13. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2022.837001/full

2. Sharma G, Woods CD, Rajkarnikar R, Hathaway HJ, Prossnitz ER. LBODP005 Gper Modulates Multiple Functions In Adipose Tissue To Promote An Anti-obese Phenotype. J Endocr Soc [Internet]. 2022;6(Supplement_1):A3–A3. Available from: https://academic.oup.com/jes/article/6/Supplement_1/A3/6787312
3. Parcha V, Heindl B, Kalra R, Li P, Gower B, Arora G, et al. Insulin Resistance and Cardiometabolic Risk Profile Among Non-diabetic American Young Adults: Insights From NHANES. J Clin Endocrinol Metab [Internet]. 2022;107(1):e25–37. Available from: https://academic.oup.com/jcem/article/107/1/e25/6362635
4. Klein S, Gastaldelli A, Yki-Järvinen H, Scherer PE. Why does obesity cause diabetes? Cell Metab [Internet]. 2022;34(1):11–20. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1550413121006318
5. Kadium TE, Alrubaie A, Ghanim SAM. The Link between Serum Omentin Level and Insulin Resistance Biomarkers, Lipid Profile, and Atherogenic Indices in Iraqi Obese Patients. Baghdad Sci J [Internet]. 2023;20(1):0074. Available from: https://bsj.uobaghdad.edu.iq/home/vol20/iss1/5
6. Busetto L, Dicker D, Frühbeck G, Halford JCG, Sbraccia P, Yumuk V, et al. A new framework for the diagnosis, staging and management of obesity in adults. Nat Med [Internet]. 2024;30(9):2395–9. Available from: https://www.nature.com/articles/s41591-024-03095-3
7. Vohra MS, Benchoula K, Serpell CJ, Hwa WE. AgRP/NPY and POMC neurons in the arcuate nucleus and their potential role in treatment of obesity. Eur J Pharmacol [Internet]. 2022;915:174611. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0014299921007676
8. Katsu Y, Iguchi T, Takei Y, Ando H, Tsutsui K. Agouti-related protein. In: Takei Y, Ando H, Tsutsui K, editors. Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research. Academic Press; 2015. p. 70–1.
9. Han J, Liang X, Guo Y, Wu X, Li Z, Hong T. Agouti-related protein as the glucose signaling sensor in the central melanocortin circuits in regulating fish food intake. Front Endocrinol (Lausanne) [Internet]. 2022;13. Available from: https://www.frontiersin.org/articles/10.3389/fendo.2022.1010472/full
10. Takeuchi S. Agouti-related protein. In: Handbook of Hormones [Internet]. Elsevier; 2021. p. 115–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128206492000309
11. Atasoy D, Betley JN, Su HH, Sternson SM. Deconstruction of a neural circuit for hunger. Nature [Internet]. 2012;488(7410):172–7. Available from: https://www.nature.com/articles/nature11270
12. Chen Y, Lin Y-C, Zimmerman CA, Essner RA, Knight ZA. Hunger neurons drive feeding through a sustained, positive reinforcement signal. Elife [Internet]. 2016;5. Available from: https://elifesciences.org/articles/18640
13. Dodd GT, Kim SJ, Méquinion M, Xirouchaki CE, Brüning JC, Andrews ZB, et al. Insulin signaling in AgRP neurons regulates meal size to limit glucose excursions and insulin resistance. Sci Adv [Internet]. 2021;7(9). Available from: https://www.science.org/doi/10.1126/sciadv.abf4100
14. Abed BA, Farhan LO, Dawood AS. Relationship between serum Nesfatin-1, Adiponectin, Resistin Concentration, and Obesity with Type 2 Diabetes Mellitus. Baghdad Sci J [Internet]. 2023; Available from: https://bsj.uobaghdad.edu.iq/home/vol21/iss1/22
15. Lin W, Chen H, Chen X, Guo C. The Roles of Neutrophil-Derived Myeloperoxidase (MPO) in Diseases: The New Progress. Antioxidants [Internet]. 2024;13(1):132. Available from: https://www.mdpi.com/2076-3921/13/1/132
16. Silvestre-Roig C, Hidalgo A, Soehnlein O. Neutrophil heterogeneity: implications for homeostasis and pathogenesis. Blood [Internet]. 2016;127(18):2173–81. Available from: https://ashpublications.org/blood/article/127/18/2173/35073/Neutrophil-heterogeneity-implications-for
17. Filep JG, Ariel A. Neutrophil heterogeneity and fate in inflamed tissues: implications for the resolution of inflammation. Am J Physiol Physiol [Internet]. 2020;319(3):C510–32. Available from: https://journals.physiology.org/doi/10.1152/ajpcell.00181.2020
18. Qaddoumi MG, Alanbaei M, Hammad MM, Al Khairi I, Cherian P, Channanath A, et al. Investigating the Role of Myeloperoxidase and Angiopoietin-like Protein 6 in Obesity and Diabetes. Sci Rep [Internet]. 2020;10(1):6170. Available from: https://www.nature.com/articles/s41598-020-63149-7
19. Lin S-Y, Li W-C, Yang T-A, Chen Y-C, Yu W, Huang H-Y, et al. Optimal Threshold of Homeostasis Model Assessment of Insulin Resistance to Identify Metabolic Syndrome in a Chinese Population Aged 45 Years or Younger. Front Endocrinol (Lausanne) [Internet]. 2022;12. Available from: https://www.frontiersin.org/articles/10.3389/fendo.2021.746747/full
20. JASP Team (2026). JASP (Version 0.97.1) [Computer software].
21. Destra E, Anggraeni N, Firmansyah Y, Santoso AH. Waist to hip ratio in Cardiovascular Disease Risk : A Review of the Literature. MAHESA Malahayati Heal Student J [Internet]. 2023;3(6):1770–81. Available from: https://ejurnalmalahayati.ac.id/index.php/MAHESA/article/view/10595

22. Correction to Lancet Diabetes Endocrinol 2025; 13: 221–62. Lancet Diabetes Endocrinol [Internet]. 2025;13(3):e6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2213858725000063
23. World Health Organization [Internet]. Obesity and overweight. 2025 [cited 2026 May 26]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
24. Aghaei M, Joukar F, Hasanipour S, Ranjbar ZA, Naghipour M, Mansour-Ghanaei F. The association between waist-to-hip ratio (WHR) with diabetes in the PERSIAN Guilan cohort study population. BMC Endocr Disord [Internet]. 2024;24(1):113. Available from: https://bmcendocrdisord.biomedcentral.com/articles/10.1186/s12902-024-01641-1
25. Chan V, Cao L, Wong MMH, Lo K, Tam W. Diagnostic Accuracy of Waist-to-Height Ratio, Waist Circumference, and Body Mass Index in Identifying Metabolic Syndrome and Its Components in Older Adults: A Systematic Review and Meta-Analysis. Curr Dev Nutr [Internet]. 2024;8(1):102061. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2475299123266457
26. Taha BE, Majeed MJ. Estimation of Insulin Resistance in Obese Adults in Baghdad. J Fac Med Baghdad [Internet]. 2024;65(4):335–40. Available from: https://iqjmc.uobaghdad.edu.iq/index.php/19JFacMedBaghdad36/article/view/2118
27. Ameen EM, Mohammed HY. Correlation between Tumor Necrosis Factor–Alfa and Anti-tyrosine Phosphatase with Obesity and Diabetes Type 2. Iraqi J Sci [Internet]. 2022;3322–31. Available from: https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/4351
28. Su M, Yan M, Gong Y. Ghrelin fiber projections from the hypothalamic arcuate nucleus into the dorsal vagal complex and the regulation of glycolipid metabolism. Neuropeptides [Internet]. 2019;78:101972. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0143417919300289
29. Zhang SX, Kim A, Madara JC, Zhu PK, Christenson LF, Lutas A, et al. Stochastic neuropeptide signals compete to calibrate the rate of satiation. Nature [Internet]. 2025;637(8044):137–44. Available from: https://www.nature.com/articles/s41586-024-08164-8
30. Park H-K, Ahima RS. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. Metabolism [Internet]. 2015;64(1):24–34. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0026049514002418
31. Minakhina S, Kim SY, Wondisford FE. Regulation of hypothalamic reactive oxygen species and feeding behavior by phosphorylation of the beta 2 thyroid hormone receptor isoform. Sci Rep [Internet]. 2024;14(1):7200. Available from: https://www.nature.com/articles/s41598-024-57364-9
32. Zhang Y, Chen L, Xuan Y, Zhang L, Tian W, Zhu Y, et al. Iron overload in hypothalamic AgRP neurons contributes to obesity and related metabolic disorders. Cell Rep [Internet]. 2024;43(3):113900. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2211124724002286
33. Lobato TB, Gennari-Felipe M, Pauferro JRB, Correa IS, Santos BF, Dias BB, et al. Leukocyte metabolism in obese type 2 diabetic individuals associated with COVID-19 severity. Front Microbiol [Internet]. 2022;13. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1037469/full
34. Rizo-Téllez SA, Sekheri M, Filep JG. Myeloperoxidase: Regulation of Neutrophil Function and Target for Therapy. Antioxidants [Internet]. 2022;11(11):2302. Available from: https://www.mdpi.com/2076-3921/11/11/2302
35. Ndrepepa G. Myeloperoxidase – A bridge linking inflammation and oxidative stress with cardiovascular disease. Clin Chim Acta [Internet]. 2019;493:36–51. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0009898119300841
36. Al-Musawi HS, Al-Lami M, Al-Saadi AH. Assessment of Glycemic Control, Renal Function, and Oxidative Stress Parameters in Type 2 Diabetes MellitusPatients. Iraqi J Sci [Internet]. 2021;4628–38. Available from: https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/3613
37. Mhaibes SH, Ali SH. Biomarkers of Oxidative Stress in Diabetic Microvascular Complications Review Article. Iraqi J Pharm Sci [Internet]. 2024;33(3):1–16. Available from: https://bijps.uobaghdad.edu.iq/index.php/bijps/article/view/2743
38. Rubino F, Cummings DE, Eckel RH, Cohen R V, Wilding JPH, Brown WA, et al. Definition and diagnostic criteria of clinical obesity. Lancet Diabetes Endocrinol [Internet]. 2025;13(3):221–62. Available from: https://doi.org/10.1016/S2213-8587(24)00316-4

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):20-26

RESEARCH ARTICLE

Istel HR 2000 in clinical practice: diagnostic accuracy versus standard ECG using aVR lead data

Agata Firkowska[1], Julia Stepnowska[1], Karolina Śron[1], Mikołaj Młyński[2], Anna Bavnbek[3], Karol Kroczak[4], Montse Gonzalez[4], Paweł Spychalski[5]

[1] 2nd Department of Cardiology and Electrotherapy, University Clinical Centre, Gdańsk, Poland
[2] Department of Cardiology and Electrotherapy, Medical University of Gdańsk, Poland
[3] Regionshospitalet Horsens, Ortopeadisk sengeafsnit, Horsens, Denmark
[4] Complejo Hospitalario Universitario Insular-Materno Infantil, Las Palmas de Gran Canaria, Spain
[5] Emergency Department, Municipal Hospital, Gdynia, Poland

Abstract

Background: Mobile ECG devices are increasingly used in telecardiology, but the diagnostic utility of the Istel HR 2000 ECG recorder has not been fully established beyond AF detection. This study evaluated the accuracy and comparability of Istel recordings versus standard limb 6-lead ECG, with particular emphasis on the QRS vector in aVR lead. **Material and methods:** We compared standard ECGs of 59 adults with their Istel ECGs (recorded in up to 3 positions). The aVR vector was calculated by adding R and S/Q wave amplitudes. The correlations between aVR vectors were evaluated. Istel recordings with an aVR vector ≤ -1 mm in all positions were included for further comparative analysis of ECGs parameters. **Results:** The mean difference in aVR vector between standard and Istel ECGs was similar across all device positions. Based on the aVR criteria, 37 patients (62.7%) were eligible for further analysis. Istel recordings more frequently showed right axis deviation and T-wave abnormalities than standard ECG. Device position influenced ECG interpretation, with position 3 showing the highest concordance. **Conclusions:** Istel HR 2000 provides reproducible ECG recordings but cannot replace the standard limb ECG. Device position and altered lead geometry affect ECG interpretation. The aVR vector may help assess recording validity, although further studies are required.

Keywords: electrocardiogram · telemedicine · aVR lead · stel

Corresponding author:
Agata Firkowska, Department of Cardiology and Electrotherapy, University Clinical Centre, Gdańsk, Poland
e-mail: agata.firkowska@gmail.com
Available online: ejtcm.gumed.edu.pl

Citation

Firkowska A, Stepnowska J, Śron K, Młyński M, Bavnbek A, Kroczak K, Gonzalez M, Spychalski P. Istel HR 2000 in clinical practice: diagnostic accuracy versus standard ECG using aVR lead data. Eur J Transl Clin Med. 2026;9(1):20-26.

DOI: 10.31373/ejtcm/218602

Introduction

Nowadays, the use of telemonitoring in medicine is increasingly widespread, particularly in cardiology. Electrocardiographic telemonitoring (TM-ECG) using external recording devices is a fundamental tool of cardiological telediagnostics. This method is based on the remote acquisition and transmission of electrocardiographic (ECG) recordings. TM-ECG enables the detection, documentation, and evaluation of abnormalities in cardiac electrical activity during patients' everyday activities, thereby increasing the likelihood of establishing an accurate diagnosis. Although it is not the most efficient, the most commonly used device for the initial screening of arrhythmias is the Holter ECG monitor [1]. Its most important limitation is the fact that it does not provide real-time ECG, thus delaying the diagnosis [2]. Recent advances in long-term ambulatory ECG recording can be a solution. We can distinguish devices that are handheld (e.g. KardiaMobile, KM) or attached to the chest, wearable (e.g. Holter recorders, smartwatches or the Cardiovest system) as well as implantable loop recorders and mobile recorders (based on ECG or photoplethysmography) [3-5].

To emphasize the importance and usefulness of the topic, the authors of an European Heart Rhythm Association document indicated that both photoplethysmography and ECG-based mobile health devices can be used as screening tools for atrial fibrillation (AF) and also can be used to diagnose arrhythmias [6]. The example of a mobile electrocardiogram (ECG) recorder is the Istel 2000, produced in Poland (IS). Its purpose is to assess the heart rate and to detect AF (Figure 1).

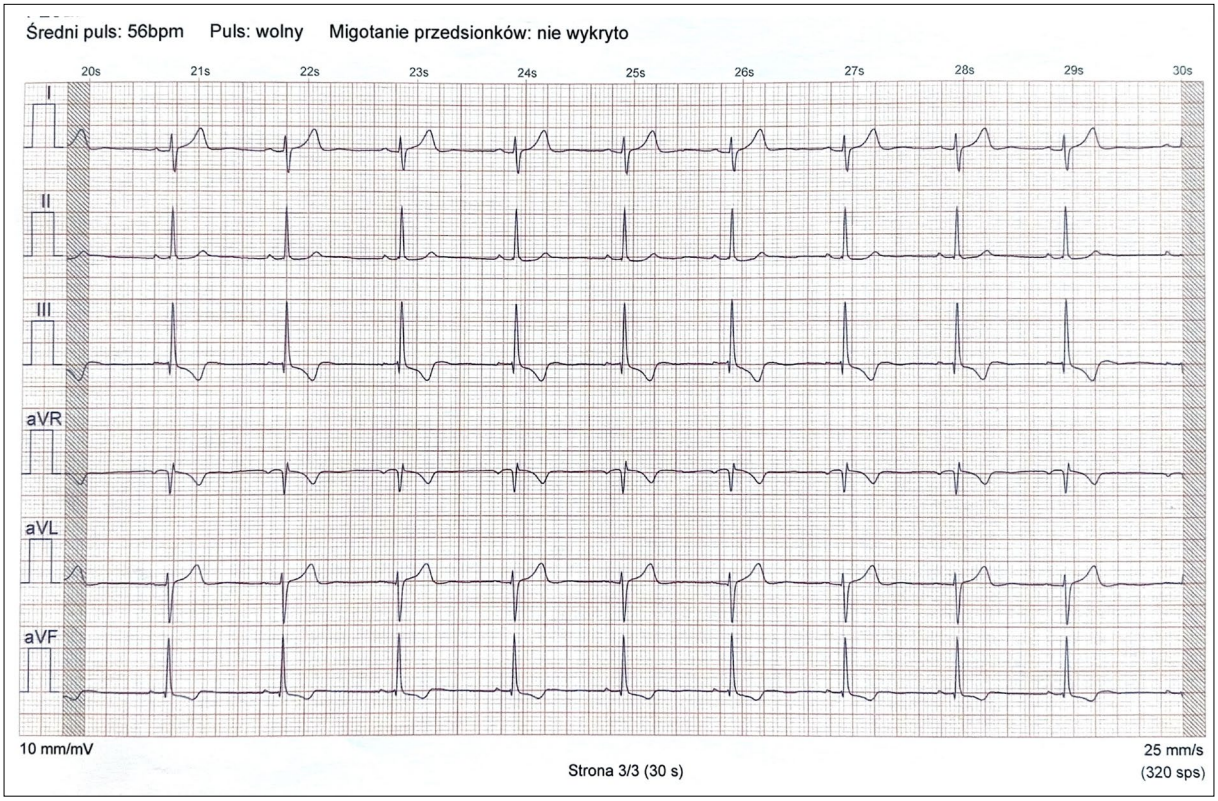


Figure 1. Example of an ECG tracing recorded by the Istel HR 2000 device
Średni puls – average pulse; *puls: wolny* – pulse: slow; *Migotanie przedsionków: nie wykryto* – atrial fibrillation: not detected; *strona* – page

It contains 4 integrated electrodes, which provide a wireless 6-lead (I, II, III, aVL, aVR, aVF) ECG recording. The IS device is easy to use (ECG is obtained by placing the IS in the middle of a bare chest and holding it for 30 seconds) and can be performed by patients outside medical facilities. The ECG recordings can be uploaded from the IS to a dedicated smartphone app (Android or iOS).

The aim of this study was to estimate the diagnostic accuracy of the IS ECG recordings based on the device's placement on the chest and aVR lead deflection. By comparing the IS ECG recordings to standard 6-lead limb lead ECGs (SE), we intended to observe whether IS allows a reliable assessment of ECG parameters, (beyond the presence of AF and heart rate changes) and can be used to support therapeutic decisions. As a point of reference we choose the aVR lead deflection.

Materials and methods

The study population included a total of 59 adult patients (16 women and 43 men) and their median age was 38 (21--75) years. The median age for women was 40.5 (21-65) years and for men was 36 (21-75) years. All the participants were patients of a cardiology outpatient clinic and were in the diagnostic process of heart palpitations. At the time of the study, the participants were not diagnosed with any specific cardiovascular diseases. Between 20th March 2021 and 2nd October 2022 during their scheduled appointments at the clinic, we used an Aspel AsCARD Grey v.07.205 ECG device (Aspel SA, Zabierzów, Poland) to obtain SE from each participant. During the same visit, we recorded an ECG using the Istel HR 2000 (Diagnosis S.A., Białystok, Poland) from each participant.

On the 30 mm long superior part of the IS device there was a designated measuring area. In that area we distinguished 3 sectors serving as the indicators of the IS position on the patient's chest (Figure 2). The initial positioning of the device was achieved by placing the palmar surface of the patient's right hand on the chest and setting the patient's thumb in the jugular notch of the sternum (position 1) (Figure 3). The IS was then located in the middle of the chest by putting its superior margin parallel to the edge of the 5th finger. To obtain further positions we shifted the device 10 mm superiorly (position 2) and then another 10 mm superiorly (position 3). In each of the 3 positions the IS device was aligned with the horizontal nipple line (Figure 4).

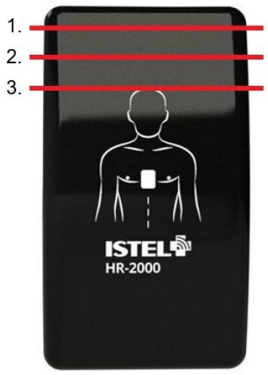


Figure 2. Istel HR 2000 placement indicators

All ECGs were obtained at rest under standardized conditions. Patients were placed in supine position on the examination bed. During IS assessment, we focused primarily on the evaluation of the aVR lead QRS complex deflection as a method for selecting patients with ECG-recordings that were most comparable to SE. In each IS recording we calculated the net vector of the QRS complex by adding the amplitudes of the R wave with S and/or Q wave. IS ECGs with aVR vector ≤ -0.1mV in each device position were included in further analysis of ECG parameters. The ECGs which did not meet the criteria were out of analysis in order to eliminate the possibility of human mistake and achieve more reliable results. All IS ECGs were assessed in terms of the changes of aVR vector depending on measurement position. The IS ECGs meeting the above criteria were analyzed in accordance with the Polish ECG guidelines and focused on the presence of sinus rhythm (SR), electrical heart axis, changes in ST-segments and T waves [7].

Results

Standard ECG (SE)

A total of 59 SE were obtained and all were good quality (clear isoelectric line, no artifacts, well-defined waves). Sinus rhythm was present in all of the SEs obtained from the participants. Intermediate axis was seen in 54 out of 59 participants (91.53%), 3 had left axis deviation (5.08%) and 2 had right axis deviation (3.39%). In 37.29% of the ECGs there were T wave alterations such as: negative T wave in III lead (n = 18); negative T wave in aVL lead (n = 2) and negative-positive T wave in III lead (n = 2). None of the patients had significant ST segment changes. Among the 59 participants with SEs, 98.31% of them (n = 58) had a negative aVR vector (ranging from -0.15 mV to -1.05 mV). The patient with a positive aVR vector (+0,3 mV) had left axis deviation. No arrhythmias were found in the SE tracings.

Istel ECG (IS)

Overall, we recorded a total of 135 IS tracings in 59 patients. There were 18 participants with IS recordings made in 3 positions. 40 participants had IS recorded in 2 positions (21 in positions 1 & 3, 19 in positions 1 & 2) and 1 participant in only 1 position. There were 38 patients with negative aVR vectors in each IS position. Among them there was 1 person with an aVR vector > -0,1mV in at least one position. Based on the aVR lead vector and the criteria mentioned in the

"Material and methods" section, among all 59 participants 37 people were included in further analysis (62.71%). In these 37 patients we assessed their IS recordings (n = 87) and compared them to SE. 22 people were not included into further analysis. There was no arrhythmia found in any IS ECGs.

AVR vector

Taking the IS recordings into assessment, in 15 participants we observed a smaller (more negative) aVR vector in position 1 than in position 2. The difference in deflection ranged from 0.5 mm to 3 mm. 22 participants had an aVR vector either greater or equal in position 1 than in position 2 (9 had greater, 13 equal). 39 participants had IS recorded in positions number 1 & 3. In 11 participants we noted an aVR vector smaller in position 1 rather than in position 3. 28 participants had an aVR vector greater or equal in position 1 than in position 3 (14 equal, 14 greater). There were 18 measurements made between aVR vector on IS ECG in position 2 and in position 3. 27.78% (n = 5) of these patients had aVR deflection smaller in position 2. The remaining 13 measurements had aVR vector equal in both positions in 61.11% (11 out of 18) cases and greater in position 2 in 11.11% (2 out of 18).

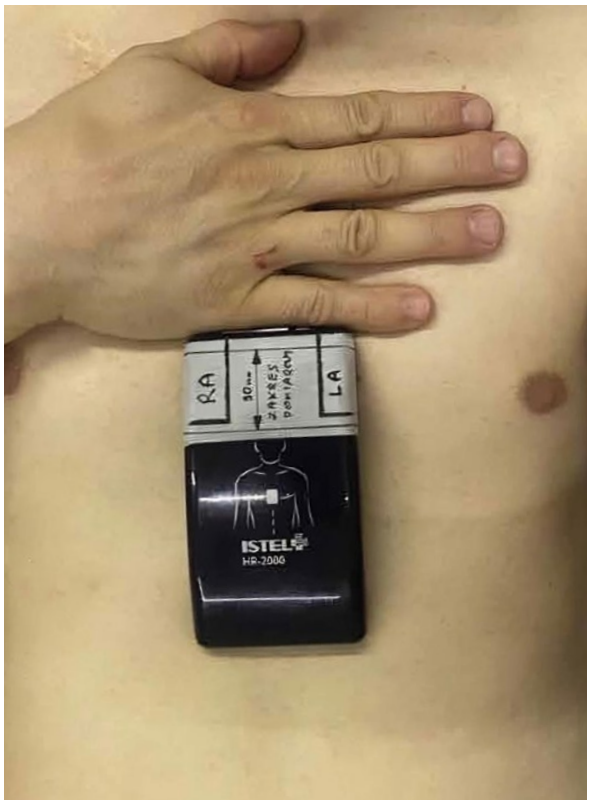


Figure 3. Initial position of the device

Correlations between the aVR vectors in different IS positions are shown in Figure 5. The black line depicts the situation where vectors in both positions are equal. For example: in the graph showing vectors in position 1 and 2, points under the line show results where the aVR vector in position 1 is greater than in position 2, and points over the line depict results where the aVR vector is smaller in position 1 than in position 2. It was also observed that the mean difference between the aVR vector in the IS ECGs compared to the aVR vector on the SE was similar across IS positions 1, 2 and 3: 0.351 mV, 0.326 mV, and 0.344 mV respectively.

Heart axis

Taking position number 1 into assessment, there were 37 measurements. Nearly half of the group (48.65%, n = 18) had right axis deviation. The intermediate axis was noted as well at 48.65% (n = 18). Left axis deviation was observed in only 1 patient. In the IS position number 2, there were 25 recordings taken into account. The intermediate heart axis was present in 40% (n = 10) of records. In 60% (n = 15) of participants there was noted right axis deviation. 25 IS recorded in the 3rd IS position were taken into account. There were 64% (n = 16) of people with intermediate heart axis and 36% (n = 9) with right axis deviation.

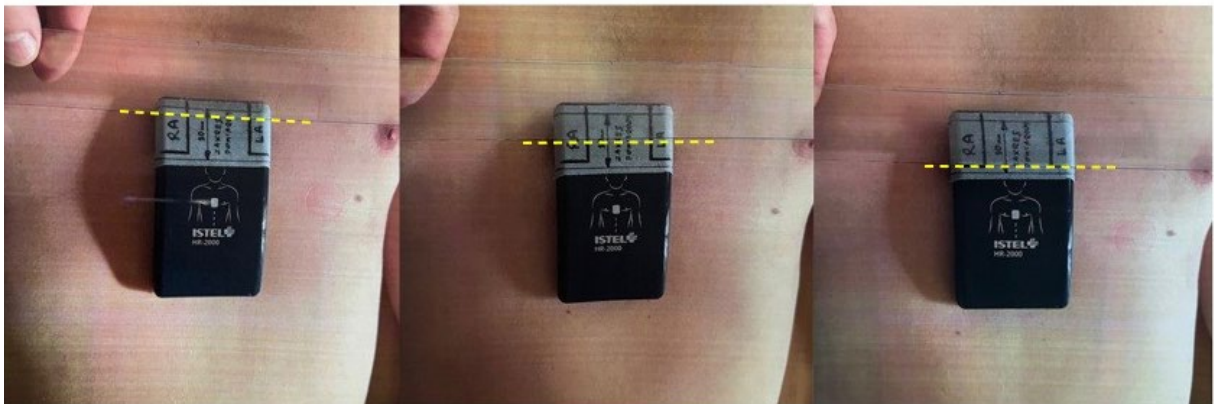


Figure 4. Istel HR 2000 in 3 positions on patient's chest; from right: position 1, position 2, position 3

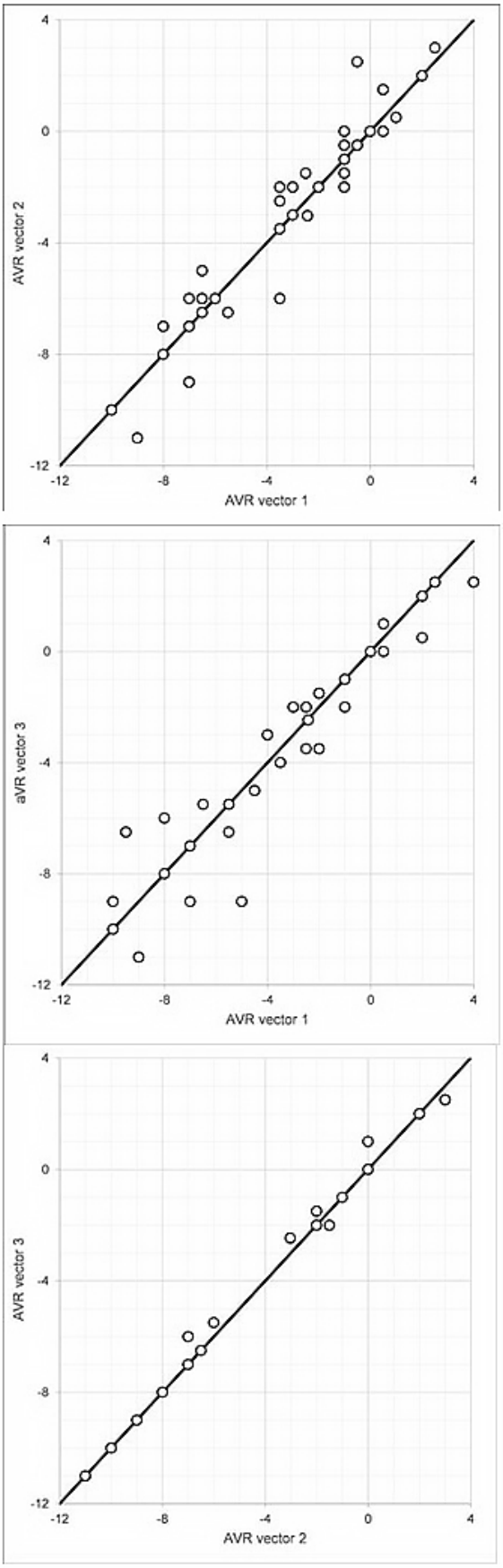


Figure 5. Comparison of aVR vectors in different Istel positions

Heart rhythm

In position 1 SR was not found in 10.81% (n = 4) of recorded IS. This results in 89.19% (n = 33) of patients with detected SR. In position 2 the criteria for SR were fulfilled in IS tracings of 80% (n = 20) of patients. In 88% (n = 22) of patients SR was detectable in position 3.

ST-T changes

In all IS positions, there were no significant ST segment changes observed.

In position 1, T wave changes were observed in 89.19% (n = 33). There were such changes as negative T wave in lead III (n = 15), negative T wave in lead aVF and III (n = 15) and other nonspecific changes in 3 patients.

In 2nd position, T wave changes were observed in 96% (n = 24) of participants. There was noted negative T wave in lead III and aVF (n = 14), negative T in lead III (n = 8), negative-positive T wave in lead III and aVF (n = 1) and negative-positive T wave in aVF lead with negative T in III (n = 1).

Finally, in 3rd position, T wave abnormalities were found in 100% patients. Negative T wave in lead III was observed in 32% (n = 8); negative T wave in leads III and aVF leads in 48% (n = 12); other changes were observed in 5 records.

The data are summarized in Table 1.

Discussion

There are a few articles particularly taking up the topic of clinical use of the IS. Usually, the publications take into account the problem of AF and describe a variety of diagnostic devices and solutions. Bonini et al. focused on the types of mobile health solutions [8]. The authors described the role of photoplethysmography-based devices and ECG-based devices (KM, smartwatches) in detecting paroxysmal arrhythmias, although the IS device was not mentioned [8]. Książczyk et al. covered the topic of smartphone and smartwatch apps in arrhythmia detection [9]. Although they briefly described the IS device and its “device-dependent” app, no further analysis of IS ECG was conducted [9]. Krzowski et al. compared SE, IS and the KM device [10]. They observed that normal heart axis was less common in ECG tracings obtained from IS (18%) than from KM (61%) or SE (69%) [10]. Our data confirmed that IS recordings included an intermediate heart axis less often than limb ECG.

In our analysis, the aVR lead proved to be a useful parameter. Physiologically, the QRS complexes in the aVR are predominantly negative, as the mean cardiac electrical vector is oriented away from the right arm electrode. Positive or atypical deflections in aVR may indicate an underlying pathology,

Table 1. Comparison of ECG parameters among 6-lead limb ECGs and chosen Istel HR 2000 recordings in 3 positions

Analised variables	Limb ECG	IS 1	IS 2	IS 3
No. of records	59	37	25	25
Sinus rhythm	100%	89.19%	80.00%	88.00%
Intermediate axis	91.53%	48.65%	40.00%	64.00%
Right axis	3.39%	48.65%	60.00%	36.00%
Left axis	5.08%	2.70%	0.00%	0.00%
T wave changes	37.29%	89.19%	96.00%	100%

e.g. right atrial enlargement, right ventricular hypertrophy or extensive myocardial ischemia [11-12]. Nearly all of our participants had a negative aVR vector on SE. After applying our aVR-based criteria (see "Material and methods") to IS recordings, approximately two-thirds of participants (n = 37) were eligible for further analysis of IS ECG parameters. This highlights aVR lead's potential role as a screening tool for the validity of single-device ECG recordings.

Compared to ES, right axis deviation was observed more frequently in IS recordings, particularly in positions 1 and 2. These findings likely reflect the altered lead geometry inherent to the IS device rather than true pathological axis deviation. In SE the electrodes are located on the limbs and create the Einthoven triangle with the heart in the middle of it. IS lacks this classic orientation, as the IS device electrodes are much closer to each other and the heart (source of the electrical signal) is not located at the geometric center of the measurement system. The lead axes and angles are altered and the distribution of the heart's electrical potentials changes. Position 3 yielded a higher proportion of intermediate axis recordings, suggesting that this placement may more closely approximate SE lead placement and therefore provide more physiological results.

Sinus rhythm identification was generally possible in IS recordings, although in a small subset (n = 12) of these recordings sinus rhythm could not be confidently identified (most notably in position 2). This limitation is likely related to the reduced P-wave amplitude or suboptimal lead alignment, indicating that device position may limit the reliability of rhythm analysis in single- or limited-lead EKG devices such as IS.

Repolarization abnormalities, particularly T-wave changes, were markedly more frequent in IS recordings than in ES. Negative T waves in leads III and aVF were noted across all device positions, with the majority in position 3. Importantly, these T-wave changes were not accompanied by ST-segment deviations, suggesting that they are most likely attributable to differences in lead orientation and vector projection. This finding highlights the need for caution when interpreting T-wave morphology in non-standard ECG lead placement.

Conclusions

This study evaluated electrocardiographic parameters obtained from standard limb ECG and IS recordings performed in different device positions, with particular emphasis on aVR vector changes. Our findings demonstrate that while standard ECG results were largely normal across the study population, IS recordings showed some differences that must be considered in clinical interpretation. At this moment, it cannot be said that IS recorder can be a substitute for standard ECG. Future studies should focus on optimizing the device position, correlating findings in IS recordings with 6-lead ECGs in diverse patient populations and defining position-specific reference values to improve diagnostic accuracy.

Funding

None.

Conflicts of interest

None to report.

References

1. Shen Q, Li J, Cui C, Wang X, Gao H, Liu C, et al. A wearable real-time telemonitoring electrocardiogram device compared with traditional Holter monitoring. J Biomed Res [Internet]. 2021;35(3):238. Available from: http://www.jbr-pub.org.cn/article/doi/10.7555/JBR.34.20200074?viewType=HTML
2. Kędzierski K, Radziejewska J, Sławuta A, Wawrzyńska M, Arkowski J. Telemedicine in Cardiology: Modern Technologies to Improve Cardiovascular Patients' Outcomes — A Narrative Review. Medicina (B Aires) [Internet]. 2022;58(2):210. Available from: https://www.mdpi.com/1648-9144/58/2/210
3. Suchecka J, Homenda W, Kukulski S, Młyński M, Kozłowski D. A novel method for the diagnosis and monitoring of cardiac arrhythmias in patients with chronic lymphocytic leukemia treated with ibrutinib. Polish Arch Intern Med [Internet]. 2025;135(7–8). Available from: https://www.mp.pl/paim/issue/article/17060
4. Dahiya ES, Kalra AM, Lowe A, Anand G. Wearable Technology for Monitoring Electrocardiograms (ECGs) in Adults: A Scoping Review. Sensors [Internet]. 2024 ;24(4):1318. Available from: https://www.mdpi.com/1424-8220/24/4/1318
5. Suchecka J, Świątczak M, Młyński M, Daniłowicz-Szymanowicz L, Kozłowski D. Electrocardiogram recording vest: A useful tool in explaining recurrent syncope. Cardiol J [Internet]. 2024;31(1):168–70. Available from: https://journals.viamedica.pl/cardiology_journal/article/view/87231
6. Svennberg E, Tjong F, Goette A, Akoum N, Di Biase L, Bordachar P, et al. How to use digital devices to detect and manage arrhythmias: an EHRA practical guide. Europace [Internet]. 2022;24(6):979–1005. Available from: https://academic.oup.com/europace/article/24/6/979/6561927
7. Baranowski R, Wojciechowski D, Kozłowski D, Kukla P, Kurpesa M, Lelakowski J, et al. Compendium for performing and describing the resting electrocardiogram. Diagnostic criteria describe rhythm, electrical axis of the heart, QRS voltage, automaticity and conduction disorders. Experts' group statement of the Working Group on Noninvasive Ele. Polish Hear J (Kardiologia Pol [Internet]. 2016;74(5):493–500. Available from: https://doi.org/10.5603/KP.2016.0070
8. Bonini N, Vitolo M, Imberti JF, Proietti M, Romiti GF, Boriani G, et al. Mobile health technology in atrial fibrillation. Expert Rev Med Devices [Internet]. 2022;19(4):327–40. Available from: https://www.tandfonline.com/doi/full/10.1080/17434440.2022.2070005
9. Książczyk M, Dębska-Kozłowska A, Warchoł I, Lubiński A. Enhancing Healthcare Access–Smartphone Apps in Arrhythmia Screening: Viewpoint. JMIR mHealth uHealth [Internet]. 2021;9(8):e23425. Available from: https://mhealth.jmir.org/2021/8/e23425
10. Krzowski B, Skoczylas K, Osak G, Żurawska N, Peller M, Kołtowski Ł, et al. Kardia Mobile and ISTEL HR applicability in clinical practice: a comparison of Kardia Mobile, ISTEL HR, and standard 12-lead electrocardiogram records in 98 consecutive patients of a tertiary cardiovascular care centre. Eur Hear J - Digit Heal [Internet]. 2021;2(3):467–76. Available from: https://academic.oup.com/ehjdh/article/2/3/467/6274659
11. Riera ARP, Ferreira C, Ferreira Filho C, Dubner S, Barbosa Barros R, Femenía F, et al. Clinical Value of Lead aVR. Ann Noninvasive Electrocardiol [Internet]. 2011;16(3):295–302. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1542-474X.2011.00435.x
12. Kozłowski D. Method in the Chaos – a step-by-step approach to ECG Interpretation. Eur J Transl Clin Med [Internet]. 2018;1(1):76–92. Available from: https://ejtcm.gumed.edu.pl/articles/8

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):27-35

RESEARCH ARTICLE

Nearly half of the patients are anemic: seasonal and demographic patterns in rural Madagascar

Daniel Kasprowicz, Franco Cyrille Rajaomalala

Clinique Médicale Beyzym, Manerinerina, Ambatoboeny District, Madagascar

Abstract

Background: Anemia is a major public health concern in Sub-Saharan Africa, but data from rural Madagascar are scarce. This study assessed monthly anemia prevalence and demographic and seasonal patterns in northern Madagascar. **Material and methods:** We conducted a retrospective cross-sectional analysis of hemoglobin levels of patients routinely tested at a secondary-level rural referral clinic in Manerinerina (Madagascar) between January and December 2024. Hemoglobin was measured using automated methods and anemia in adults was defined as < 12.0 g/dL. Monthly prevalence and demographic associations were examined using chi-square tests and odds ratios (ORs). **Results:** Among the 1714 patients, 45.4% (n = 778) were anemic. Children < 15 years of age had the highest prevalence (boys 79.3%, girls 74.6%) and more than six-fold higher odds of anemia than adults aged 15-49 (OR 6.24, 95% CI 4.80-8.11). Women were more often anemic than men (48.9% vs. 40.6%; OR 1.40, 95% CI 1.15-1.70). Monthly prevalence peaked during the rainy season: December (60.2%) to March (24.0%). **Conclusions:** Anemia is highly prevalent in this rural Malagasy population, particularly among children and women of reproductive age. Pronounced seasonal variation suggests that cyclical food insecurity substantially increases vulnerability to anemia. Strengthening routine screening and access to qualified healthcare providers should be prioritized, particularly during the rainy season.

Keywords: anemia · Madagascar · malnutrition · food insecurity · hemoglobin

Citation

Kasprowicz D, Rajaomalala FC. Nearly half of the patients are anemic: seasonal and demographic patterns in rural Madagascar. Eur J Transl Clin Med. 2026;9(1):27-35.

DOI: 10.31373/ejtcm/217895

Corresponding author:
Daniel Kasprowicz, Clinique Médicale Beyzym, Manerinerina, Ambatoboeny District, Madagascar
e-mail: daniel.kasprowicz@icloud.com
Available online: ejtcm.gumed.edu.pl

Introduction

Anemia is a reduction in red blood cell number or hemoglobin concentration that impairs oxygen transport [1]. According to the World Health Organization (WHO) 2024 guidelines, diagnostic cut-off values vary by age, sex, physiological status and altitude; at sea level, anemia is defined as hemoglobin < 11.0 g/dL in children < 5 years of age and pregnant women, and < 12.0 g/dL in non-pregnant women [2]. This condition remains a major public-health problem in many low- and middle-income countries, particularly in sub-Saharan Africa where nutritional deficiencies, infections and parasitic diseases are highly prevalent [3].

Due to its substantial impact on child development, maternal health and economic productivity, anemia reduction has been designated a global health priority. The WHO aims to decrease anemia in women of reproductive age by 50% by 2030 [4]. Meanwhile, the United Nations incorporated anemia control into its Sustainable Development Goal 2 (target 2.2), which calls for eliminating all forms of malnutrition by 2030 [5]. The African region continues to report the highest anemia prevalence worldwide – 43.0% in 2000, 36.0% in 2012 and 36.5% in 2023 [3]. Despite the fact that the recent WHO estimates indicate that anemia affects over one-third of women aged 15-49 years in Madagascar, national and region-specific data (particularly from the rural northern areas) from that country remain limited [6]. Such region-specific estimates are required to identify periods of increased vulnerability, support resource allocation and contextualize local epidemiological patterns within national trends. Therefore, the aim of this study was to assess monthly anemia prevalence over a full calendar year among patients attending a rural referral clinic in northern Madagascar and to contextualize these findings within national estimates.

Material and methods

This retrospective cross-sectional study analyzed routine clinical and laboratory data collected over a 12-month period (January–December 2024) at Clinique Médicale Beyzym (CMB), a secondary-level referral facility located in Manerinerina (Ambatoboeny District of northwestern Madagascar 16°17′02″ S, 47°17′05″ E). This clinic serves a predominantly rural population within the Boeny Region, characterized by low population density, widespread poverty, agrarian livelihood, and limited access to formal healthcare and diagnostic services.

All hemoglobin measurements recorded as part of routine clinical care during the study period were eligible for inclusion. No additional exclusion criteria were applied. Data were extracted from monthly medical activity reports and laboratory registers.

Blood sampling and hemoglobin assessment

Venous blood was collected into EDTA tubes using a vacuum-assisted blood collection system by trained nursing personnel in accordance with internal Standard Operating Procedures (SOP). Hemoglobin levels were measured using a Mindray M-30 hematology (Mindray Bio-Medical Electronics Co., Shenzhen, China). All laboratory procedures were performed by qualified diagnostic staff, and the final validation of results was conducted by a supervising clinical biologist responsible for quality assurance. Manual May-Grünwald blood smears were prepared as part of routine internal quality control procedures.

Definition and classification of anemia

Although the WHO 2024 guidelines state that hemoglobin thresholds for anemia vary by age, sex, and physiological status, a simplified operational threshold (< 12.0 g/dL) was applied in routine clinical practice at the CMB for all adult patients, regardless of sex [2]. For consistency with local diagnostic practice and to ensure a coherent analytical framework, in this study we adopted the same uniform cut-off.

Statistical analysis

Monthly anemia prevalence was calculated as the proportion of individuals with hemoglobin concentrations < 12.0 g/dL. Differences across months were assessed using the chi-square test (χ^2), and effect size was estimated using Cramér's V. Data entry and organization were performed using Apple Numbers version 6.1 (Apple Inc., Cupertino, California, USA). All statistical analyses were conducted using Python version 3.11 (Python Software Fundation, Wilmington, Delaware, USA) with the SciPy library (scipy.stats).

Ethical considerations

The study protocol was authorized by the Ambatoboeny District Public Health Office and approved by the Hospital Supervisory Committee of the CMB. Only anonymized, routinely collected clinical data were used. No names, addresses, dates of birth, or other identifying information were extracted from the patient records. Data were handled exclusively in aggregated form, in accordance with local ethical guidelines and international standards for the secondary use of clinical information.

Results

A total of 1714 patients were included in this analysis. Hemoglobin concentrations < 12.0 g/dL were documented in 45.4% individuals (n = 778), whereas 54.6% (n = 936) had hemoglobin concentrations ≥ 12.0 g/dL. Among the anemic patients, 57.2% (n = 445) had hemoglobin concentrations between 10.0 and 11.9 g/dL, 28.3% (n = 220) had 8.0 to 9.9 g/dL and 14.5% (n = 113) had hemoglobin < 8.0 g/dL (Table 1).

Anemia was more frequently observed among women than men. Among the 988 women, 48.9% (n = 483) were anemic, compared with 40.6% of the 726 men (n = 295) (χ^2 = 11.50, df = 1; p = 0.0007). Women had higher odds of anemia than men (OR 1.40, 95% CI 1.15-1.70), although the effect size was small (Cramér's V = 0.08). Sex-specific hemoglobin distributions are shown in Table 2.

Marked differences were also observed within specific age-sex strata. Among children 5-14 years of age, anemia affected 79.3% of boys and 74.6% of girls. In adults 15-49 years of age, anemia was identified in 20.3% of men and 43.8% of women, demonstrating a substantially higher burden among women of reproductive age. In adults ≥ 50 years, anemia prevalence reached 40.4% among men and 34.8% among women. Overall, age group was strongly associated with anemia (χ^2 = 218.70, degrees of freedom = 2; p < 0.0001), with a large effect size (Cramér's V = 0.36). Children had over six-fold higher odds of anemia than adults aged 15-49 years (odds ratio (OR) 6.24, 95% confidence interval (CI) 4.80-8.11). Fully stratified distributions of hemoglobin concentrations, including anemia prevalence with 95% CI, are presented in Table 3.

Anemia prevalence varied during the year, ranging from 24.0% in March to 60.2% in December. Higher proportions were observed in January (58.5%), February (57.3%), May (57.4%), November (58.0%), and December (60.2%), while lower proportions were recorded in March (24.0%) and July (34.2%). Month of the year was significantly associated with anemia (χ^2 = 99.02, df = 11; p < 0.0001), with a moderate effect size (Cramér's V = 0.24). The monthly distribution of hemoglobin categories, expressed as proportions of all patients, is shown in Figure 1.

Overall, anemia affected nearly half of the studied population, with statistically significant differences across sex and age groups and marked variation across months of the year. The highest burden was observed among children < 15 years of age, followed by older adults (≥ 50 years of age) and women of reproductive age (15-49 years of age). Several monthly anemia proportions exceeded 55% and coincided numerically with periods of increased rainfall, suggesting a potential link between seasonal environmental conditions and anemia burden in this rural population.

Discussion

This study demonstrated a substantial burden of anemia in the rural population of northwestern Madagas-

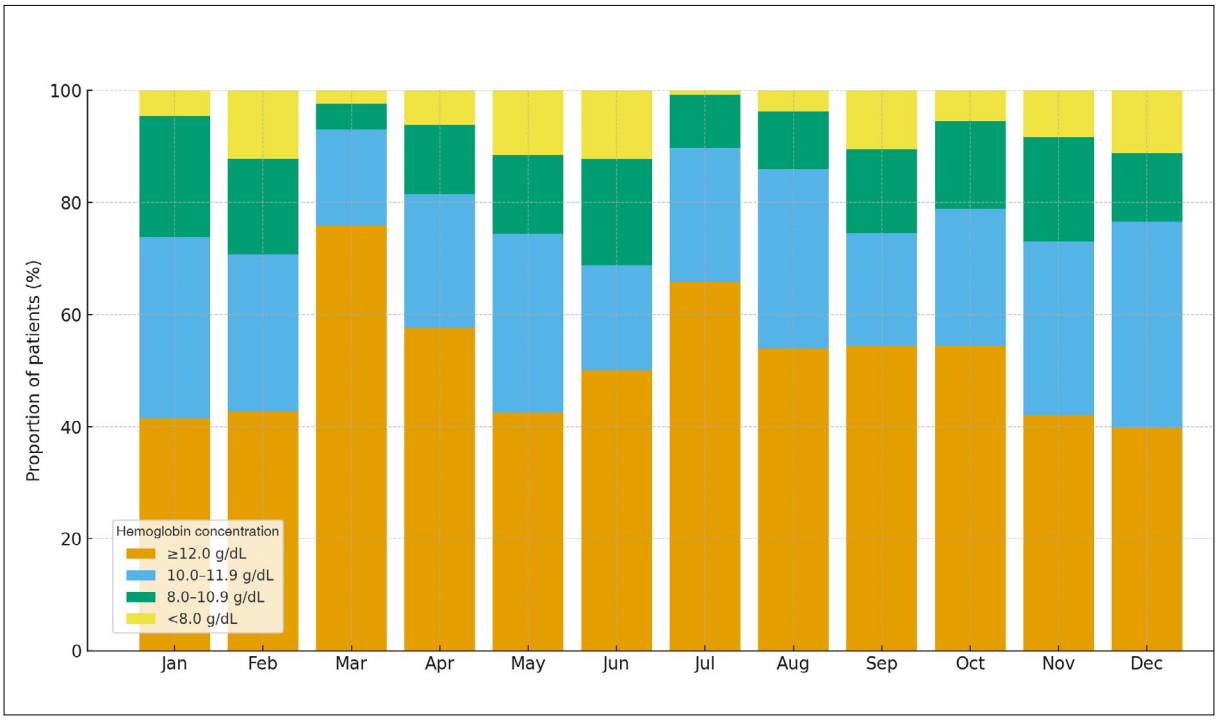


Figure 1. Monthly distribution of hemoglobin concentration expressed as proportions of all patients

Table 1. Distribution of hemoglobin concentrations among anemic individuals

Hemoglobin concentration (g/dL)*	n (%)**	% of study group (n = 1714)
< 8.0	113 (14.5%)	6.6%
8.0-10.9	220 (28.3%)	12.8%
10.0-11.9	445 (57.2%)	26.0%
Total anemia cases	778 (100%)	45.4%

* Anemia was defined according to the WHO hemoglobin guidelines [2].
** Percentages were calculated within the subgroup of individuals with anemia (n = 778).

Table 2. Hemoglobin distribution by sex

Sex	Anemia n (%)*	Hb ≥ 12.0 g/dL n (%)	Total
Men	295 (40.6%)	431 (59.4%)	726
Women	483 (48.9%)	505 (51.1%)	988
Total	778	936	1714

* Anemia was defined as hemoglobin (Hb) < 12 g/dL. Percentages were calculated for each sex (row).

Table 3. Age- and sex-specific distribution of hemoglobin concentrations with anemia prevalence

	Hemoglobin concentration (g/dl)					
Group	< 8.0 n (%)	8.0-10.0 n (%)	10.0-11.9 n (%)	≥ 12.0 n (%)	Total n*	Anemia n (%) [95% CI]**
5-14 years old						
boys	30 (14.1)	47 (22.1)	92 (43.2)	44 (20.7)	213	169 (79.3) [73.4; 84.2]
girls	18 (9.1)	51 (25.9)	78 (39.6)	50 (25.4)	197	147 (74.6) [68.1; 80.2]
15-49 years old						
men	14 (3.5)	20 (5.0)	48 (11.9)	322 (79.7)	404	82 (20.3) [16.7; 24.5]
women	40 (5.9)	84 (12.4)	172 (25.4)	380 (56.2)	676	296 (43.8) [40.1; 47.6]
≥ 50 years old						
men	8 (7.3)	8 (7.3)	28 (25.7)	65 (59.6)	109	44 (40.4) [31.6; 49.8]
women	3 (2.6)	10 (8.7)	27 (23.5)	75 (65.2)	115	40 (34.8) [26.7; 43.9]
Total	113 (6.6)	220 (12.8)	445 (26.0)	936 (54.6)	1714	778 (45.4) [—]

* Percentages within hemoglobin categories are calculated within each age- and sex-specific subgroup (row-based percentages).
** Anemia prevalence is reported as the proportion of individuals with hemoglobin values below the WHO–defined thresholds within each subgroup; 95% confidence intervals were calculated using the binomial method.

car, with nearly half of all patients presenting with hemoglobin concentrations below the WHO-defined thresholds. The prevalence of anemia was particularly high among children < 15 years of age (almost 4 out of 5 were affected), indicating the most vulnerable demographic subgroup. Marked sex-related differences were also evident: women showed a significantly higher prevalence of anemia than men, and this disparity was most pronounced among women of reproductive age. In older adults, anemia remained common in both sexes, affecting more than one-third of individuals. Moreover, although anemia was observed throughout the entire year, its prevalence peaked in December, coinciding with the onset of the rainy season – a period typically associated with reduced food security and limited access to affordable nutritional resources in many rural areas of Madagascar. These findings underscore the heterogeneous distribution of anemia across demographic strata and highlight the relevance of age-sex-specific and seasonal patterns when assessing the local epidemiology of anemia in resource-limited settings.

When placed within the broader sub-Saharan African context, the anemia prevalence identified in our study stands notably above the expectations. Across sub-Saharan Africa, anemia remains one of the most persistent public health challenges, with children and women of reproductive age carrying the greatest burden despite global improvement efforts [7]. Regional analyses consistently show that anemia prevalence among children often exceeds 60%, is substantially higher in rural areas and these patterns are attributed to chronic undernutrition, high rates of infectious diseases, and limited healthcare access [8]. Among women, long-term data reveal only slow and uneven declines in anemia prevalence, with many African countries exhibiting stagnation linked to persistent poverty, food insecurity, and constrained availability of micronutrient supplementation [9]. These structural determinants are further compounded by environmental and seasonal pressures, including fluctuating food supply, rainfall-linked disease transmission patterns, and cyclical nutritional shortages all of which can produce sharp local variations in anemia prevalence not captured by the national or continental averages [10]. In this context, the markedly higher rates detected in our study, together with the clear seasonal peak, suggest that localized socioeconomic and ecological stressors may amplify anemia risk far beyond the trends typically reported for sub-Saharan Africa.

The disproportionately high prevalence of anemia we observed among children aligns with previous findings from Madagascar, where pediatric populations consistently demonstrate substantial hematologic vulnerability. Studies conducted across different regions of the country report anemia in 58-100% of examined children, often with a considerable share of moderate or severe cases [11-14]. For instance, a recent hematological assessment in northern Madagascar found that all participating children were anemic, with nearly 80% classified as moderately anemic and approximately 20% severely anemic [11]. Similar trends were reported in preschool and early school-age populations, where more than half of children had hemoglobin concentrations < 12 g/dL [12], and nearly 60% of children were anemic in larger community-based cohorts [13]. Investigations focusing on younger children (particularly those under 10 years of age) also documented high rates of anemia associated with chronic undernutrition, recurrent infections, and limited dietary diversity, further underscoring the systemic nature of this problem [14]. Importantly, most Malagasy pediatric studies, consistent with our findings, report minimal sex differences and limited associations between anemia and narrow age brackets within childhood, although some analyses show that hemoglobin concentrations tend to be lowest among children < 5 years of age [12]. This relationship between malnutrition and hematologic impairment is consistent with broader analyses of feeding challenges and metabolic vulnerabilities among malnourished children in low-resource settings, including Madagascar [15]. Researchers have demonstrated that although parasitic infections (including schistosomiasis and intestinal helminthiasis) are common, they have an inconsistent relationship with hemoglobin concentrations, thus suggesting that pediatric anemia in Madagascar is driven by multifactorial determinants rather than single etiologies [12, 16]. Collectively, these data confirm that anemia is a pervasive and entrenched health burden among Malagasy children, and its exceptionally high prevalence in our results is fully consistent with national pediatric trends.

The high prevalence of anemia among women of reproductive age observed in our data closely reflects the substantial burden documented across sub-Saharan Africa. Recent WHO estimates for Madagascar show that anemia in women aged 15-49 years has remained persistently high over the past decade, increasing from 32.1% in 2010 to 33.3% in 2015 and 35.3% in 2020, reaching 37.2% in 2023 – one of the highest levels reported globally, with wide uncertainty intervals indicative of pronounced regional disparities [6]. Our 2024 data exceed these national estimates, suggesting that women in remote rural districts may experience considerably greater vulnerability than reflected in national averages. This aligns with evidence from large African meta-analyses, which consistently demonstrate that anemia affects between one-third and one-half of women of reproductive age across the continent, with several analyses estimating pooled prevalences ranging from 38% to over 50% depending on geographic region and socioeconomic context [17-18]. Key determinants identified across African studies include chronic undernutrition, iron deficiency, malaria, intestinal parasitic infections, short interpregnancy intervals, adolescent pregnancies, and limited access to antenatal supplementation programs [19].

More recent analyses further highlight the substantial contribution of food insecurity, poor dietary diversity, and cyclical shortages of staple foods – factors that disproportionately affect rural populations [20]. Environmental stressors, including seasonal peaks in infectious diseases and fluctuations in agricultural productivity, have also been shown to exacerbate anemia risk in African women [21]. Taken together, these continental trends suggest that the disproportionately high prevalence recorded in our study represents an intensified expression of broader African patterns, magnified by the intersection of poverty, environmental instability, and limited health system reach in rural Madagascar.

The marked seasonal variation observed in our dataset, with anemia peaking between November and March, corresponds to the well-documented "lean season" in many rural regions of sub-Saharan Africa. This period, which follows the depletion of stored crops and precedes the next harvest, is characterized by reduced food availability, sharp increases in staple food prices, and widespread dietary insufficiency [22]. In the context of Madagascar, this seasonal window often represents the most acute phase of food insecurity, during which families experience prolonged caloric deficits and markedly limited access to iron-rich or protein-dense foods [23-24]. Such nutritional constraints are strongly reflected in our findings, which show the highest proportions of anemic individuals precisely during months when agricultural production is minimal and household food reserves are exhausted.

The rainy season further compounds these vulnerabilities: although rainfall in the Boeny region is relatively modest compared with other tropical zones, these intermittent wet conditions create favorable breeding sites for the Anopheles mosquitoes, contributing to seasonal surges of malaria [25]. Malaria-associated hemolysis and inflammation, superimposed on chronic undernutrition, may further exacerbate anemia risk during this period [26]. Taken together, these overlapping environmental and socioeconomic pressures suggest that seasonal instability in food security (rather than isolated infectious or nutritional factors) acts as a dominant driver of the pronounced fluctuations in anemia prevalence observed in this rural Malagasy population.

From a public health perspective, the high burden of anemia observed in this population underscores the potential relevance of integrated intervention strategies. Evidence from low-resource settings indicates that effective anemia control requires multifaceted approaches, including iron supplementation, food fortification, maternal and child nutrition programs, and infection control measures, rather than single-component interventions alone [27-29]. Importantly, the effectiveness and safety of these strategies are highly context-dependent. In malaria-endemic and infection-prone regions, indiscriminate iron supplementation may be associated with limited benefit or potential risks, thus emphasizing the need for targeted, screening-based approaches [28, 30]. In such settings, combining nutritional support with infection control, deworming, and strengthened primary healthcare services may offer more sustainable population-level benefits [27, 29]. While our study was not designed to assess the etiology of anemia or intervention efficacy, these findings highlight the need for context-adapted public health strategies and further research to inform evidence-based anemia control programs in rural Madagascar and comparable settings.

Overall, the interplay of nutritional deficits, infection dynamics, and seasonal instability appears to shape the anemia burden observed in this population, pointing to a multifactorial problem that warrants further investigation.

Limitations

Our study has several important limitations. First, we analysed routinely collected clinical data from a single rural referral clinic. As such, our findings reflect anemia patterns among individuals seeking care rather than providing population-level prevalence for the Ambatoboeny District. However, clinic-based datasets remain highly informative for detecting local trends, seasonal variations, high-risk demographic groups, and they offer essential insight in settings where community-level epidemiological data are scarce.

Second, although hemoglobin measurements were performed using standardized procedures and subjected to internal quality control, we relied on a simplified clinical threshold for anemia classification that does not fully align with age- and sex-specific WHO cut-offs. This operational choice may have led to some misclassification of anemia status, particularly among men and younger adolescents, and could have influenced comparisons with external datasets. Furthermore, the analysis was based on single time-point hemoglobin assessments, without complementary biomarkers such as the serum concentrations of ferritin, C-reactive protein, vitamin B12, folate or transferrin saturation. Consequently, we were unable to distinguish iron-deficiency anemia from anemia of inflammation, hemoglobinopathies, or other etiologies.

Third, because we did not aim to determine the etiology of anemia, routinely collected registers did not include information about nutritional biomarkers and co-morbidities (e.g. malaria, helminthic infections, protozoal diseases). While these factors are known to contribute to anemia in Madagascar (as shown in previous studies from the region), our analysis was designed to describe overall anemia prevalence and its demographic/seasonal patterns rather than to attribute causality. Although the absence of etiological variables limits the interpretation of mechanisms, it does not affect our primary objective of quantifying anemia distribution in this population. Despite these limitations, our study provides much-needed, context-specific evidence from a specific re-

gion of Madagascar, where health data are limited. The observed age-, sex- and season-related patterns of anemia prevalence offer a valuable basis for future community-based or longitudinal investigations.

Conclusions

This study confirms that anemia is a major and persistent health problem in rural Madagascar, with a particularly high burden among children < 15 years of age and women of reproductive age. The observed seasonal variation in hemoglobin levels suggests that periods of increased vulnerability, potentially related to food insecurity and the burden of infectious and parasitic diseases, are associated with lower hemoglobin concentrations. These findings underscore the need to strengthen routine hemoglobin screening in primary care, particularly during periods when population-level vulnerability is expected to peak.

Given that anemia prevalence in this rural setting exceeds national and regional estimates, targeted public health interventions are urgently needed. Priorities should include improving access to qualified healthcare providers (e.g. who are capable of evaluating anemia, identifying its likely contributors, and initiating appropriate management), as well as expanding local monitoring systems to better capture anemia trends in remote communities. Further research is essential to clarify the multifactorial drivers of anemia in these settings and to inform the development of context-appropriate strategies for prevention, early detection, and clinical decision-making.

Acknowledgements

The authors express their gratitude to all the clinical and laboratory staff of Clinique Médicale Beyzym in Manerinerina for their daily commitment to patient care and their invaluable assistance in maintaining high-quality medical records. In particular, we wish to thank Razanatsimialintsoa Fidelia for her meticulous work in organizing and validating monthly laboratory reports, Ravolanjafy Razely for her support in retrieving and interpreting ward activity registers, and Dr Mazava Sabbat Dieudonné (head physician) for his continuous clinical guidance and supervision of diagnostic decision-making.

We are also grateful to the District Public Health Office of Ambatoboeny staff for their administrative support and for authorizing the use of routine clinical data for this analysis. Finally, we sincerely thank all the patients and their families who sought care at Clinique Médicale Beyzym; without their trust in this clinic, our study would not have been possible.

Funding

The authors did not receive any specific external funding for this study.

Conflicts of interest

None to report.

References

1. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity [Internet]. Geneva: World Health Organization; 2011 [cited 2026 Feb 18]. Available from: https://iris.who.int/server/api/core/bitstreams/b82789de-df63-41ba-8058-25f9f5da0c40/content
2. Guideline on haemoglobin cutoffs to define anaemia in individuals and populations [Internet]. Geneva: World Health Organization; 2024 [cited 2026 Feb 18]. Available from: https://www.who.int/publications/i/item/9789240088542
3. WHO global anaemia estimates. Key findings 2025 [Internet]. Geneva: World Health Organization; 2023 [cited 2026 Feb 18]. Available from: https://iris.who.int/server/api/core/bitstreams/ffbc07c6-420c-4711-8589-2a7b6f3a3da2/content
4. Global nutrition targets 2025: anaemia policy brief [Internet]. Geneva: World Health Organization; 2014 [cited 2026 Feb 18]. Available from: https://www.who.int/publications/i/item/WHO-NMH-NHD-14.4
5. Sustainable Development Goal 2.2: Malnutrition. Anaemia among women aged 15 to 49 Years [Internet]. Nearest East and North Africa. Regional Overview of Food Security and Nutrition. Statistics and Trends. Rome; 2023 [cited 2026 Feb 16]. Available from: https://openknowledge.fao.org/server/api/core/bitstreams/d18451b6-1c80-40be-b30b-dea116c532d7/content/sofi-statistics-rne-2023/aneamia-among-women.html
6. Anaemia in women of reproductive age (aged 15-49), prevalence (%), by pregnancy status [Internet]. World Health Organization. 2023 [cited 2026 Feb 16]. Available from: https://www.who.int/data/gho/data/indicators/indicator-details/GHO/prevalence-of-anaemia-in-women-of-reproductive-age-(-)

7. Makubi A, Lwakatare J, Ogah OS, Rydén L, Lund LH, Makani J. Anaemia and iron deficiency in heart failure: epidemiological gaps, diagnostic challenges and therapeutic barriers in sub-Saharan Africa. Cardiovasc J Afr [Internet]. 2017 Oct 27;28(5): 331–7. Available from: https://cvja.co.za/onlinejournal/vol28/vol28_issue5/files/assets/basic-html/page-57.html#
8. Makani J, Roberts DJ. Hematology in Africa. Hematol Oncol Clin North Am [Internet]. 2016;30(2):457–75. Available from: https://www.sciencedirect.com/science/article/pii/S0889858815002087
9. Zhu Y, He C, Gasparrini A, Vicedo-Cabrera AM, Liu C, Bachwenkizi J, et al. Global warming may significantly increase childhood anemia burden in sub-Saharan Africa. One Earth [Internet]. 2023 Oct 20;6(10):1388–99. Available from: https://doi.org/10.1016/j.oneear.2023.09.003
10. Abaynew Y, Ali A, Taye G, Shenkut M. Prevalence and types of anemia among people with tuberculosis in Africa: a systematic review and meta-analysis. Sci Rep [Internet]. 2023;13(1):5385. Available from: https://doi.org/10.1038/s41598-023-32609-1
11. Alfano MV, Gozalbo M, Tapia-Veloz G, Guirao V, Soriano JM, Trelis M. Nutrimetry and Evaluation of Intestinal Parasites and Anaemia in Malnourished Schoolchildren from Toliara (Madagascar). Children [Internet]. 2025 Feb 13;12(2):225. Available from: https://www.mdpi.com/2227-9067/12/2/225
12. Richert W, Kołodziej D, Zarudzka D, Kasprowicz D, Świetlik D, Korzeniewski K. Intestinal Parasites and Hematological Parameters in Children Living in Ambatoboeny District, Madagascar. Pathogens [Internet]. 2024 Oct 25;13(11):930. Available from: https://www.mdpi.com/2076-0817/13/11/930
13. Tapia-Veloz G, Gozalbo M, Guirao V, Dinari H, Fuentes MV, Trelis M. Integrated Evaluation of Undernutrition, Anaemia, and Intestinal Parasitic Infections in School-Aged Children: A Cross-Sectional Study in Three Regions of Southern Madagascar. Children [Internet]. 2025 Jul 28;12(8):990. Available from: https://www.mdpi.com/2227-9067/12/8/990
14. Wilczyńska W, Kasprowicz D, Świetlik D, Korzeniewski K. The Prevalence of Schistosoma haematobium and Its Impact on the Hematological Profile of Children Living in Northern Madagascar. Pathogens [Internet]. 2025 Feb 9;14(2):172. Available from: https://www.mdpi.com/2076-0817/14/2/172
15. Kasprowicz D, Rajaomalala FC. The problem of paediatric patients in developing countries: do we actually know how to feed the malnourished children? Eur J Transl Clin Med [Internet]. 2020 Jan 9;2(2):7–18. Available from: https://ejtcm.gumed.edu.pl/articles/39
16. Abdulkareem BO, Adam AO, Ahmed AO, Mariam AA, Samuel UU. Malaria-induced anaemia and serum micronutrients in asymptomatic Plasmodium falciparum infected patients. J Parasit Dis [Internet]. 2017;41(4):1093–7. Available from: https://doi.org/10.1007/s12639-017-0940-4
17. Liyew AM, Tesema GA, Alamneh TS, Worku MG, Teshale AB, Alem AZ, et al. Prevalence and determinants of anemia among pregnant women in East Africa; A multi-level analysis of recent Demographic and Health Surveys. PLoS One [Internet]. 2021;16(4):e0250560. Available from: https://doi.org/10.1371/journal.pone.0250560
18. Teshale AB, Tesema GA, Worku MG, Yeshaw Y, Tessema ZT. Anemia and its associated factors among women of reproductive age in eastern Africa: A multilevel mixed-effects generalized linear model. PLoS One [Internet]. 2020;15(9):e0238957. Available from: https://doi.org/10.1371/journal.pone.0238957
19. Tirore LL, Areba AS, Habte A, Desalegn M, Kebede AS. Prevalence and associated factors of severity levels of anemia among women of reproductive age in sub-Saharan Africa: a multilevel ordinal logistic regression analysis. Front Public Heal [Internet]. 2024;Volume 11. Available from: https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2023.1349174
20. Seifu BL, Negussie YM, Asnake AA, Fente BM, Asmare ZA, Melkam M, et al. Modifiable risk factors for anemia among women of childbearing age in sub-saharan Africa (2015–2023). BMC Public Health [Internet]. 2025;25(1):267. Available from: https://doi.org/10.1186/s12889-025-21427-x
21. Correa-Agudelo E, Kim H-Y, Musuka GN, Mukandavire Z, Miller FD, Tanser F, et al. The epidemiological landscape of anemia in women of reproductive age in sub-Saharan Africa. Sci Rep [Internet]. 2021;11(1):11955. Available from: https://doi.org/10.1038/s41598-021-91198-z
22. Mohajan H. Food Insecurity and Malnutrition of Africa : A Combined Attempt Can Reduce Them. J Econ Dev Environ People [Internet]. 2022;11(1):24–34. Available from: https://mpra.ub.uni-muenchen.de/112609/
23. Herrera JP, Rabezara JY, Ravelomanantsoa NAF, Metz M, France C, Owens A, et al. Food insecurity related to agricultural practices and household characteristics in rural communities of northeast Madagascar. Food Secur [Internet]. 2021 Dec 24;13(6):1393–405. Available from: https://link.springer.com/10.1007/s12571-021-01179-3
24. Raholiarimanana F, Rakotomanana H, Ishida A. Does Raising Livestock Improve Household Food Security and Child Dietary Diversity in a Rural Region of Madagascar? Children [Internet]. 2023 Apr 23;10(5):765. Available from: https://www.mdpi.com/2227-9067/10/5/765

25. Roberts SA, Brabin L, Tinto H, Gies S, Diallo S, Brabin B. Seasonal patterns of malaria, genital infection, nutritional and iron status in non-pregnant and pregnant adolescents in Burkina Faso: a secondary analysis of trial data. BMC Public Health [Internet]. 2021 Dec 27;21(1):1764. Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-021-11819-0
26. Egbi G, Tohuoenou MM, Glover-Amengor M, Adom T. The impact of seasonal variation on anemia and nutritional status with associated factors in 6–12 years Ghanaian school age children in peri-urban communities. Hum Nutr Metab [Internet]. 2021 Dec;26:200135. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2666149721000177
27. Itzkovich M, Neuberger A, Harris I, Yahav D, Paul M, Gilboa M. Oral iron supplements for children in malaria-endemic areas. Cochrane Database Syst Rev [Internet]. 2026 Jan 9;2026(1). Available from: http://doi.wiley.com/10.1002/14651858.CD006589.pub5
28. Crider KS, Williams JL, Qi YP, Gutman J, Yeung LF, Mai CT, et al. Folic acid supplementation and malaria susceptibility and severity among people taking antifolate antimalarial drugs in endemic areas. Cochrane Database Syst Rev [Internet]. 2022 Feb 1;2022(2). Available from: http://doi.wiley.com/10.1002/14651858.CD014217
29. Okebe JU, Yahav D, Shbita R, Paul M. Oral iron supplements for children in malaria-endemic areas. In: Paul M, editor. Cochrane Database of Systematic Reviews [Internet]. Chichester, UK: John Wiley & Sons, Ltd; 2011. Available from: https://doi.wiley.com/10.1002/14651858.CD006589.pub3
30. Moya-Alvarez V, Ouédraogo S, Accrombessi M, Cot M. High folate levels are not associated with increased malaria risk but with reduced anaemia rates in the context of high-dosed folate supplements and intermittent preventive treatment against malaria in pregnancy with sulphadoxine–pyrimethamine in Benin. Trop Med Int Heal [Internet]. 2018 Jun 21;23(6):582–8. Available from: https://onlinelibrary.wiley.com/doi/10.1111/tmi.13064

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):36-45

RESEARCH ARTICLE

Novel cardiovascular risk factors in young women with classical risk profiles

Abdalhaleem Ibdah[1], Mahmoud Alkhawaldeh[2], Rashid Ibdah[3], Atef Hulliel[2], Sukaina Rawashdeh[3], Ashaar Al-Akhras[2], Kenda Abedal-Kareem[2], Lina Wahbeh[4], Sarah Khader[2], Mohammed Baker[2], Sadeen Eid[2], Jalal Al Mansi[2], Rajae Khraisat[5,2], Mohamad Hitham S. Alsahan[2], Yamameh Al-Rhayyel[6], Mohmmad M. Alawajneh[2], Suhaib Abu Dayah[2], Saad Mahmoud[2], Ayman Hammoudeh[7]

[1] Faculty of Medicine, Department of Special Surgery, The Hashemite University, Zarqa, Jordan
[2] Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan
[3] Department of Internal Medicine, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan
[4] Faculty of Medicine, University of Jordan, Amman, Jordan
[5] Department of Family Medicine, King Abdullah University Hospital, Ar Ramtha, Jordan
[6] Faculty of Medicine, Yarmouk University, Irbid, Jordan
[7] Department of Clinical Research, Istishari Hospital, Amman, Jordan

Abstract

Introduction: Urbanization plays a growing role in the development of atherosclerotic cardiovascular disease (ASCVD) across the Middle East, particularly among younger adults. Yet, we still know very little about how the health profiles of young women differ between urban and rural settings. In this study, we aim to bridge that gap by examining the demographic characteristics and the prevalence of both classical and novel ASCVD risk factors in young Middle Eastern women. **Methods:** We analyzed the demographic, social, and clinical profiles of women aged 18-50 years, including 19 classical and novel ASCVD risk factors. We included participants living in either urban or rural areas, recruited from 11 centers across Jordan and 1 in the Gaza Strip between August 2020 and October 2023. **Results:** Of the 627 women studied (mean age 44.2 ± 5.1 years), 74% lived in urban areas and 26% in rural areas. ASCVD was present in 32.0% of urban and 37.3% of rural participants. Rural women had higher rates of physical inactivity, obesity, and elevated BMI, while smoking was more common among urban women. Among novel risk factors, premature menopause and depression were more frequent in urban women, whereas hypertensive disorders of pregnancy were more common in rural women. **Conclusion:** Urban young women demonstrated a worse clinical profile, with higher prevalence of several key ASCVD risk factors.

Keywords: risk factors · ASCVD · atherosclerotic cardiovascular disease · urbanization · rural

Corresponding author:
Atef Hulliel, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan
e-mail: afhulliel181@med.just.edu.jo
Available online: ejtcm.gumed.edu.pl

Citation

Ibdah A, Alkhawaldeh M, Ibdah R, Hulliel A, Rawashdeh S, Al-Akhras A, Abedal-Kareem K, Wahbeh L, Khader S, Baker M, Eid S, Al Mansi J, Khraisat R, S. Alsahan MH, Yamameh Al-Rhayyel Y, M. Alawajneh M, Abu Dayah S, Mahmoud S, Hammoudeh A. Novel cardiovascular risk factors in young women with classical risk profiles. Eur J Transl Clin Med. 2026;9(1):36-45.

DOI: 10.31373/ejtcm/217511

Introduction

Atherosclerotic cardiovascular disease (ASCVD) has become a major global epidemic, accounting for nearly one-third of all deaths worldwide [1]. In Jordan, ASCVD was responsible for 45% of all deaths in 2019, according to the Ministry of Health [2]. Across the Middle East and North Africa (MENA), the number of ASCVD cases increased by 140.9% between 1990 and 2019 [3]. Although women experience ASCVD at rates similar to men, evidence shows they often suffer higher mortality and worse outcomes after acute cardiovascular (CV) events [4]. The long-held belief that women are protected from ASCVD is false because the data shows that almost half of all women will develop heart disease or experience a stroke during their lifetime [5-6]. Notably, acute myocardial infarction in the Middle East occurs at a younger age compared with other regions [3, 7]. Classical (traditional) ASCVD risk factors (e.g. diabetes mellitus (DM), hypertension (HTN), smoking, dyslipidemia, obesity, family history of premature ASCVD, physical inactivity) vary even within the same country. One in six women with type 2 diabetes (T2DM) in the Middle East and Africa experience early ASCVD, with most at high or very high risk for an acute CV event [8]. HTN is also highly prevalent, and about 10% of untreated individuals develop ASCVD [9-10]. Smoking further increases risk, with women who smoke facing a 25% higher CV risk than men of the same age. Hypercholesterolemia contributes substantially as well, accounting for 47% of the population-adjusted CV risk in women [11]. Family history of premature ASCVD remains another key risk factor, affecting around 2.3% of the population and associated with recurrent events [12]. Emerging evidence has highlighted several female-specific and reproductive age-related novel risk factors (including gestational diabetes mellitus (GDM), hypertensive disorders of pregnancy (HDP), preterm delivery, postpartum weight gain, polycystic ovary syndrome, and premature menopause) that contribute to ASCVD development in young women [13]. Our aim was to bridge this gap by investigating the variations in clinical and socioeconomic characteristics encompassing classical and novel CV risk factors within a cohort of Middle Eastern women.

Material and methods

This study is based on data from the Novel and Classical Atherosclerotic Cardiovascular Risk Factors in Middle Eastern Young Women (ANCORS-YW) Study. The detailed methodology has been described earlier [14]. In summary, this was a case-control, cross-sectional multicenter study conducted between August 2021 and October 2023, and it follows the STROBE guidelines for reporting observational research. Participants were recruited from 12 centers, including 11 across Jordan and one in the Gaza Strip. The sample size was calculated using a significance level of 0.05, a power of 80%, and an estimated frequency of 10% for each variable. Based on these assumptions, and to detect a clinically meaningful difference between the study groups (odds ratio = 2%), the required total sample size was 627 participants (209 cases and 418 controls).

Cases included married women aged 18-50 years who had ASCVD and at least 1 previous pregnancy. Each case was matched by age (± 5 years) to 2 healthy controls. We collected detailed information on ASCVD diagnosis, anthropometric measurements, demographics (age, education, residency, health insurance, physical activity), blood test results, chronic medication history, and any history of heart failure or sleep apnea. Both classical and novel (emerging) CV risk factors were recorded for all cases and their matched controls. “Urban communities” were defined as communities with high population density, determined by the government as a “city” [15]. On the other hand, “rural society” is any community of people outside the urban settlements.

Definition of ASCVD

ASCVD was defined as the presence of at least one major atherosclerotic condition. These included acute coronary syndrome (STEMI, NSTEMI, or unstable angina), significant epicardial coronary artery disease identified on CCTA (≥ 50% stenosis in the left main artery or ≥ 70% stenosis in any other major coronary vessel), ischemic or hemorrhagic stroke, transient ischemic attack (TIA), extracranial carotid artery disease (ECCAD), or peripheral arterial disease (PAD).

STEMI was defined as ischemic chest pain accompanied by ST elevation (≥ 1.5 mm in leads V2-V3 or ≥ 1 mm in two or more contiguous leads) along with elevated cardiac biomarkers. NSTEMI was characterized by ischemic symptoms with ST depression, T-wave inversion, or even a normal ECG, but with elevated cardiac enzymes. Unstable angina was defined as ischemic chest pain with ECG evidence of ischemia but without biomarker elevation. ECCAD and PAD diagnoses were based on clinical symptoms supported by Doppler studies, CT imaging, or angiographic confirmation of atherosclerosis. Stroke and TIA diagnoses were confirmed by a neurologist and verified with the appropriate neuroimaging.

Classical risk factors

Classical ASCVD risk factors in this study included HTN, smoking, obesity, metabolic syndrome, dyslipidemia, low HDL-C, and T2DM. HTN was defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg on at least 2 occasions or the use of antihypertensive medication. T2DM was diagnosed based on classic hyperglycemic symptoms with random glucose ≥ 200 mg/dl, fasting glucose ≥ 126 mg/dl, HbA1c ≥ 6.5%, or documented history of DM or use of DM medications. Dyslipidemia was defined as LDL-C >70 mg/dl in patients with ASCVD or T2DM, and > 116 mg/dl in others. Low HDL-C was defined as < 50 mg/dl. Obesity was defined as BMI ≥ 30 kg/m^2. Metabolic syndrome was defined by the presence of 4 components: HTN, obesity, low HDL-C, and hypertriglyceridemia (> 200 mg/dl) at the same time.

Novel risk factors

We also assessed several novel ASCVD risk factors among urban and rural women, including preterm delivery, HDP, GDM, premature menopause, polycystic ovary syndrome (PCOS), and depression. Preterm delivery was defined as birth before 37 weeks' gestation. HDP included gestational HTN (onset after 20 weeks of pregnancy), chronic HTN, preeclampsia (BP > 140 mmHg with end-organ damage), and eclampsia (seizures following preeclampsia). GDM was defined as glucose intolerance first recognized during pregnancy. PCOS was diagnosed using the Rotterdam criteria (≥ 2 criteria) or prior gynecologic diagnosis [16]. Premature menopause was defined by a prior diagnosis or by oligomenorrhea before age 40 lasting > 4 months with elevated gonadotropins on two tests 4-6 weeks apart. Depression was defined by a documented psychiatric diagnosis or prescribed antidepressant therapy.

Statistical analysis

Descriptive statistics are presented as means with standard deviation (SD) in case of continuous variables, or as frequencies and percentages in case of categorical variables. To compare continuous variables, means were compared using an independent t-test, while categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate. Statistical significance was determined when $p < 0.05$. Statistical analysis was computed using the R Language and Environment for Statistical Computing (version 4.1.2, R Core Team, Vienna, Austria).

Ethical considerations

This observational study was carried out according to the Declaration of Helsinki [17], which was registered in the ClinicalTrials.gov database (NCT04975503). This study obtained ethical approval from an institutional review board at each participating institute in the 3 target regions (approval number IH-IRB-IRC-7-29-2021). In addition, written informed consent was obtained from each patient.

Results

A total of 627 women between 18 and 50 years of age were included in the study, with a mean age of 44.2 ± 5.1 years. Most participants lived in urban areas (73.8%), while 164 of them (26.2%) were residents of rural regions. Baseline demographics, ASCVD prevalence, and CV risk factors by residence type are summarized in Table 1.

Age distribution was similar between urban and rural participants, and there was no significant difference in the type of ASCVD diagnosed in the two groups (32.0% vs. 37.2%, $p = 0.5$). Among the classical risk factors, smoking was more common among urban residents compared to rural residents (33.5% vs. 17.1%, $p < 0.001$). In contrast, rural residents demonstrated higher rates of physical inactivity and obesity and had a higher mean BMI ($p < 0.05$). Serum LDL concentrations were available for 252 participants (40.2%). Specifically, hypercholesterolemia was identified in 132 of 160 urban women (82.5%) and 67 of 92 rural women (72.8%), with no statistically significant difference ($p = 0.07$). HTN, DM, and family history of ASCVD were similar between groups (Figure 1).

Regarding novel ASCVD risk factors, HDP were more common among rural women. In contrast, depression and premature menopause were significantly more prevalent among urban women. GDM, preterm delivery, persistent postpartum weight gain, PCOS, and prior breast radiation (due to cancer) were comparable between the two groups ($p > 0.05$) (Figure 2). Additionally, women living in urban areas were more likely to have a university-level or higher education, while being less likely to have health insurance coverage.

Table 1. Demographics, risk factors, and social attributes of young women living in urban vs. rural residence

Characteristic	Overall (n = 627)	Urban (n = 463)	Rural (n = 164)	p-value
Age (years), mean ± SD	44.2 ± 5.1	44.3 ± 4.8	43.8 ± 5.9	0.3
Age (years)				
≤ 40	127 (20.3%)	87 (18.8%)	40 (24.4%)	0.13
> 40	500 (79.7%)	376 (81.2%)	124 (75.6%)	
ASCVD patients	209 (33.3%)	148 (32.0%)	61 (37.2%)	0.2
ASCVD classification				0.5
ACS	169/209 (80.9%)	120/148 (81.1%)	49/61 (80.3%)	
Non-obstructive CAD	10/209 (4.8%)	8/148 (5.4%)	2/61 (3.3%)	
PAD or stroke	30/209 (14.4%)	20/148 (13.5%)	10/61 (16.4%)	
Classical risk factors				
HTN	232 (37.0%)	163 (35.2%)	69 (42.1%)	0.12
DM	132 (21.1%)	93 (20.1%)	39 (23.8%)	0.3
Smoking	183 (29.2%)	155 (33.5%)	28 (17.1%)	**< 0.001**
Family history	199 (31.7%)	148 (32.0%)	51 (31.1%)	0.8
Hypercholesterolemia (n = 252)	199 (79.0%)	132 (82.5.5%)	67 (72.8%)	0.070
Physical inactivity	470 (74.9%)	332 (71.7%)	138 (84.1%)	**0.002**
Obesity	269 (42.9%)	187 (40.4%)	82 (50.0%)	**0.033**
BMI, mean ± SD (kg/m²)	29.5 ± 5.9	29.2 ± 5.7	30.4 ± 6.4	**0.039**
Novel risk factors				
Hypertensive disease of pregnancy	159 (25.4%)	108 (23.3%)	51 (31.1%)	**0.049**
Gestational DM	77 (12.3%)	51 (11.0%)	26 (15.9%)	0.10
Preterm delivery	125 (19.9%)	98 (21.2%)	27 (16.5%)	0.2
Persistent weight gain after delivery	120 (19.1%)	91 (19.7%)	29 (17.7%)	0.6
PCOS	42 (6.7%)	35 (7.6%)	7 (4.3%)	0.15
Premature menopause	62 (9.9%)	55 (11.9%)	7 (4.3%)	**0.005**
Radiation to the breast	5 (0.8%)	4 (0.9%)	1 (0.6%)	< 0.9
Social determinants of health				
University or higher education	308 (49.1%)	242 (52.3%)	66 (40.2%)	**0.008**
Insurance status	387 (61.8%)	257 (55.6%)	130 (79.3%)	**< 0.001**
Depression	54 (8.6%)	48 (10.4%)	6 (3.7%)	**0.009**

ACS – acute coronary syndrome, ASCVD – atherosclerotic cardiovascular disease, BMI – body mass index, CAD – coronary artery disease, DM – diabetes mellitus, HTN – hypertension, PAD – peripheral artery disease, PCOS – polycystic ovary syndrome, SD – standard deviation

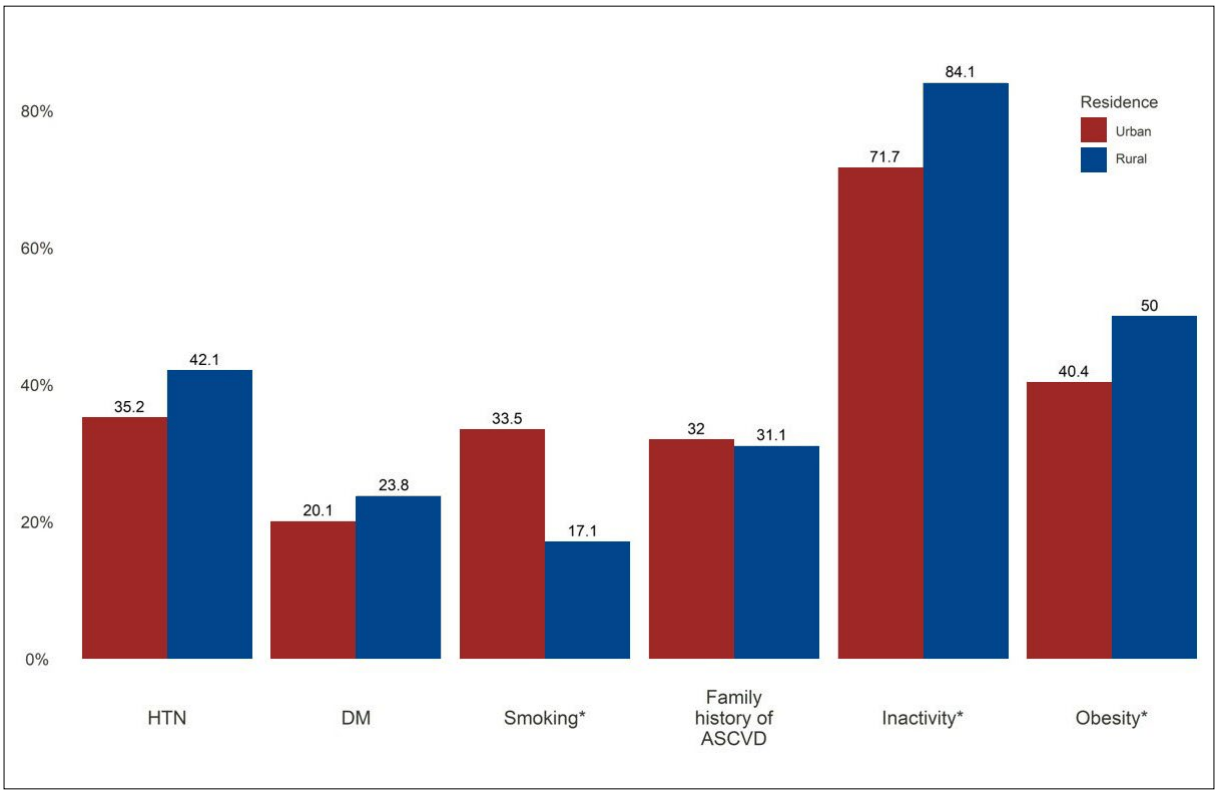


Figure 1. Prevalence of classical ASCVD risk factors among urban and rural young women
ASCVD – atherosclerotic cardiovascular disease; DM – diabetes mellitus; HTN – hypertension; *p < 0.05 between groups

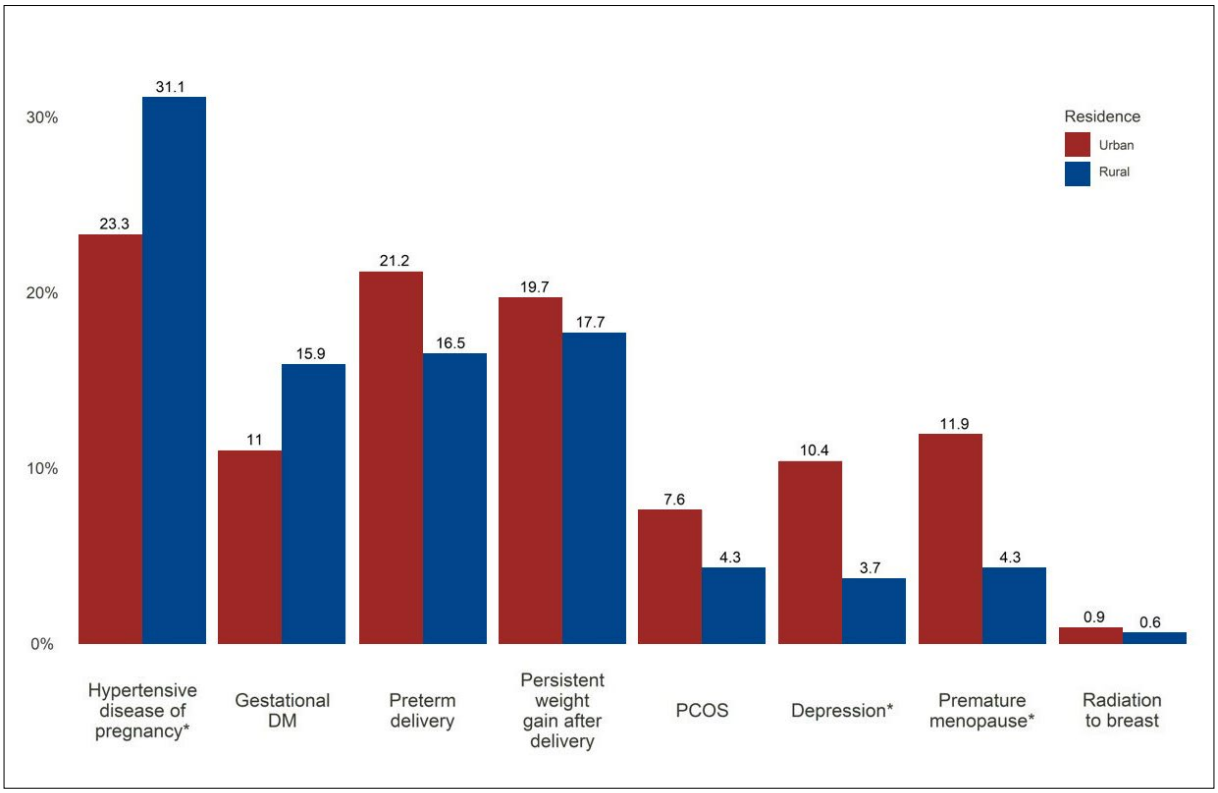


Figure 2. Prevalence of novel ASCVD risk factors among urban and rural young women
DM – diabetes mellitus; PCOS – polycystic ovary syndrome; *p < 0.05 between groups

Discussion

In this study, physical inactivity or sedentary lifestyle was the most prevalent classical risk factor among women with an overall percentage of (74.9%) comprising of 71.7% and 84.1% in urban and rural areas' residents respectively. Our results were consistent with the observations of the 2009 Northern Sweden MONICA cohort [18]. Another study established a positive correlation between sedentary lifestyle and mortality among rural inhabitants in the USA regardless of depressive symptoms [19]. According to the 2019 Jordan National Stepwise Survey (STEPs) for Noncommunicable Disease Risk Factors, 25% of respondents didn't meet the WHO recommendations for physical activity for health, with an average of (24%) women being physically inactive [2]. Another Jordanian study reported lower prevalence of physical activity practice in women than in men [20]. Such findings of high rural than urban prevalence of inactivity could be related to the deficiency of sporting facilities in addition to the more conservative culture of rural areas than those living in cities. Doctors and other medical care givers should encourage women of any age to engage in regular physical activity, as it reduces the risk of mortality and cardiovascular disease (CVD) events [21]. Similar results were reported by Kazemi et al. [22]. It is worth mentioning the impact of a 10-minute substitution of sedentary time with light-intensity physical activity on all-cause and CVD-specific mortality risk reduction among patients with heart failure [23].

In another study, the prevalence of obesity (BMI ≥ 30) among Jordanian women was higher than those reported in Saudi, Iranian and American women, while similar to that reported among Turkish women [24]. Obesity in our results accounts for an overall prevalence of (42.9%), recording a higher percentage among rural dwellers (50.0%) than in urban (40.4%). The overall mean BMI among our study's participants was in the overweight range (29.5). Urban residents had a similar mean BMI (29.2), while rural women's mean BMI fell within the obesity range (30.4). In rural areas, women exhibit lower levels of physical activity, often attributed to a higher percentage of women engaged in homemaking duties, in contrast to their urban counterparts. Our findings align with those of the Swedish MONICA study [18], but contradict those reported in India [25].

An overall 29.2% of women in this study reported smoking, with higher percent 33.5% in urban areas versus 17.1% in rural. Such findings could be attributed to the relatively higher incomes and financial prosperity among people living in urban areas compared to rural dwellers. Opposite results with rural predominance were found in India, such different findings could be correlated to the diversity and the wide spread of tobacco products in India, particularly the cheap smokeless tobacco [25]. Whilst our results and the study from India showed urban-rural distribution difference in tobacco consumption, such difference was not observed in the Swedish MONICA study [18]. Medical intervention regarding smoking cessation should be taken into consideration, particularly because females are more prone than males for coronary heart events [26-27].

Contrary to existing literature, our study did not reveal statistically significant urban-rural disparities in the distribution of HTN, DM, and hypercholesterolemia. Such similarity in the distribution of these 3 risk factors could be influenced by the ongoing process of urbanization within rural communities and the substantial migration rates of rural residents to urban areas. This socio-economic situation may contribute to a convergence in health profiles between urban and rural populations, blurring traditional distinctions.

A 2019 study conducted in Jordan revealed that almost one-third of Jordanian adults had HTN [28]. We noted a similar distribution of HTN among the urban rural groups of our study cohort, which is in contrast to the findings of the Swedish MONICA study, where rural dwellers reported higher burden of increased systolic blood pressure in both men and women [18].

DM is a global health problem which besides end-organ damage also causes and accelerates atherosclerotic CV events [29]. Similar urban-rural distribution was noted by a study in India [25] and the Swedish MONICA [18] which consists with our findings. A Chinese study discussed sex and urban-rural distribution difference of dyslipidemia, revealed higher percentages of dyslipidemia (apart from low HDL-C) in rural residents with females exhibiting higher whole lipid profile readings compared to males in both areas of residence contrasting to our results [30]. Higher education in the Arab world is rapidly evolving, shaped by the diverse cultural and socio-economic contexts of the 22 countries in the MENA [31]. Higher level of education is inversely linked to smoking and high salt intake on the one hand, while positively linked to increase in physical activity on the other hand, as both of these lifestyle habits may provide a clue for such a relationship [32]. In our study, nearly half of the participants (49.1%) had higher education. Women in urban areas r eported a higher prevalence of higher education (52.3%) compared to their rural counterparts (40.2%). These numbers are not so different as the mean age of most participants in both groups is quite high.

As for the novel ASCVD risk factors, HDP (including pre-eclampsia, gestational HTN and eclampsia) are common complications of pregnancy globally and are the top causes of maternal and fetal morbidity and mortality [32]. In 2017, the incidence of preeclampsia in Jordan was approximately 1.5%, which was similar to the other countries in the region [33]. In our study, we found that there is a significant difference in the prevalence of HDP between urban and rural residents, with

a p-value of 0.049. The difference was more obvious among rural participants, where 31.1% were affected compared to 23.4% of their urban counterparts. Similarly, a study conducted in South Africa revealed that most of the women with HTN over the age of 65 were living in rural areas [34]. This variability in prevalence might be explained by the variety of maternal risk factors, including age, parity, obesity, high blood pressure and the region.

Depression is a well-established independent risk factor for CVD in the non-pregnant population. However, the risk of a new CVD diagnosis diagnosed postpartum was elevated among patients with prenatal depression [35]. A Swedish nationwide study demonstrated that the risk of developing CVD varies between women with antepartum depression (APD) and postpartum depression (PPD), with higher risk among women with PPD [36]. We found a significant difference in depression prevalence (p = 0.009) between women living in urban (n = 48, 10.4%) and rural (n = 6, 3.7%) areas. A study conducted on the prevalence of depression and mood disorders among African American women and non-Hispanic white women revealed that rural residence is linked to lower rates of depression: because these women have developed resources and coping mechanisms to deal with stressful circumstances (including supportive social ties and high levels of religious participation) [37].

The American College of Cardiology (ACC) and American Heart Association (AHA) guidelines have demonstrated that premature menopause (at > 40 years of age) might pose as a risk for ASCVD due to the cardiometabolic changes that occur earlier [38]. A 2023 study of a large Korean cohort found that premature menopause was associated with 1.5 times greater risk of MI, 1.25 times risk of ischemic stroke, and 1.2 times the risk of all-cause mortality when compared to women with normal menopause [39]. In our study, 62 women had premature menopause, 55 of them were living in urban areas (11.9%), and 7 were in rural (4.3%), p = 0.005. This difference is consistent with the findings of an Indonesian study, that revealed women living in rural areas lowered their potential for premature menopause by 0.67 times compared to women who live in urban areas, who are 1.46 times more likely to experience premature menopause [40].

Limitations

It is important to acknowledge several limitations that may have influenced the interpretation and generalizability of our findings. The small sample size in this study may have limited the ability to draw definitive conclusions and generalize the findings to broader populations. Furthermore, the limited availability of resource data for comparison with other studies restricted our ability to assess the study.

Our findings study underscore the critical need for sex-specific approaches in CV care. Recent international collaborations have emphasized the necessity of timely management of acute coronary syndrome in women to improve their prognosis which often is worse compared to men [41]. Furthermore, the establishment of specialized heart centers for women has been proposed as a vital modality to enhance long-term CV, metabolic, and reproductive care, offering a comprehensive solution to the regional disparities identified in our cohort [42].

Conclusions

Our cohort demonstrated clear differences in ASCVD risk factor patterns between urban and rural women in Jordan and Gaza. Urban participants showed worse overall clinical profiles, with higher rates of premature menopause, depression, and smoking, whereas hypertensive disorders of pregnancy emerged as the most significant novel risk factor among rural women. Physical inactivity and obesity were also more common in rural areas. Other classical risk factors showed no significant differences. Our findings highlight the need for population-specific strategies addressing both classical and novel ASCVD risk factors.

Funding

The authors relied on self-financing sources to complete the preparation and publication of their research article.

Conflict of interest

All authors assure that there is no conflict of interest with any party and that the research was prepared within the scientific methodology that does not contain sensitive personal data or conflict with the interest of any party.

References

1. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart Disease and Stroke Statistics – 2017 Update: A Report From the American Heart Association. Circulation [Internet]. 2017;135(10). Available from: https://www.ahajournals.org/doi/10.1161/CIR.0000000000000485
2. Jaber S. Jordan National Stepwise Survey (STEPs) for Noncommunicable Diseases Risk Factors 2019 [Internet]. 2019 STEPS Country Report Jordan. Ministry of Health, Jordan; 2020. 1–118 p. Available from: https://www.who.int/publications/m/item/2019-steps-country-report-jordan
3. Alhuneafat L, Ta'ani O Al, Jabri A, Tarawneh T, ElHamdan A, Naser A, et al. Cardiovascular disease burden in the Middle East and North Africa region. Curr Probl Cardiol [Internet]. 2024;49(3):102341. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0146280623007582
4. Dewan P, Rørth R, Jhund PS, Shen L, Raparelli V, Petrie MC, et al. Differential Impact of Heart Failure With Reduced Ejection Fraction on Men and Women. J Am Coll Cardiol [Internet]. 2019;73(1):29–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30621948
5. Parapid B. Cardiovascular risk in women: here, there and everywhere. Eur J Transl Clin Med [Internet]. 2025;8(2):5–8. Available from: https://ejtcm.gumed.edu.pl/articles/208813
6. Vogel B, Acevedo M, Appelman Y, Bairey Merz CN, Chieffo A, Figtree GA, et al. The Lancet women and cardiovascular disease Commission: reducing the global burden by 2030. Lancet [Internet]. 2021;397(10292):2385–438. Available from: https://doi.org/10.1016/S0140-6736(21)00684-X
7. Xu X, Bao H, Strait KM, Edmondson DE, Davidson KW, Beltrame JF, et al. Perceived Stress After Acute Myocardial Infarction: A Comparison Between Young and Middle-Aged Women Versus Men. Psychosom Med [Internet]. 2017;79(1):50–8. Available from: https://journals.lww.com/00006842-201701000-00007
8. Alawadi F, Assaad-Khalil SH, Almahmeed W, Alamuddin N, Alkandari H, Haddad JA, et al. 21-LB: Prevalence of Atherosclerotic Cardiovascular Diseases in Women with Type 2 Diabetes across the Middle East and Africa — Primary Gender Analysis of the PACT-MEA Study. Diabetes [Internet]. 2023;72(Supplement_1). Available from: https://diabetesjournals.org/diabetes/article/72/Supplement_1/21-LB/149270/21-LB-Prevalence-of-Atherosclerotic-Cardiovascular
9. Lamprea-Montealegre JA, Zelnick LR, Hall YN, Bansal N, de Boer IH. Prevalence of Hypertension and Cardiovascular Risk According to Blood Pressure Thresholds Used for Diagnosis. Hypertension [Internet]. 2018;72(3):602–9. Available from: https://www.ahajournals.org/doi/10.1161/HYPERTENSIONAHA.118.11609
10. Wenger NK, Arnold A, Bairey Merz CN, Cooper-DeHoff RM, Ferdinand KC, Fleg JL, et al. Hypertension Across a Woman's Life Cycle. J Am Coll Cardiol [Internet]. 2018;71(16):1797–813. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0735109718333126
11. Yusuf S, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet [Internet]. 2004;364(9438):937–52. Available from: https://doi.org/10.1016/S0140-6736(04)17018-9
12. Wahrenberg A, Kuja-Halkola R, Magnusson PKE, Häbel H, Warnqvist A, Hambraeus K, et al. Cardiovascular Family History Increases the Risk of Disease Recurrence After a First Myocardial Infarction. J Am Heart Assoc [Internet]. 2021;10(23). Available from: https://www.ahajournals.org/doi/10.1161/JAHA.121.022264
13. Theodorou A, Karagiannakis DS, Stefanaki K, Kassi E, Peppa M, Vryonidou A, et al. Female-specific risk factors for cardiovascular disease: an update. Hormones (Athens) [Internet]. 2024;23(4):637–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/38922384
14. Hammoudeh AJ, Jallad M, Khader Y, Badaineh Y, Tabbalat RA, Zammar H, et al. Atherosclerotic Cardiovascular Disease Novel and Traditional Risk Factors in Middle Eastern Young Women. The ANCORS-YW Study. Glob Heart [Internet]. 2024;19(1):59. Available from: http://www.ncbi.nlm.nih.gov/pubmed/39035774
15. Sclar ED, Volavka-Close N. Urban Health: an Overview ED 2021. Elsevier BV; 2020.
16. Hmoud TR, Jawad MM, Alredha RDA. Hormonal profiles and the diagnostic utility of serum dihydrotestosterone in polycystic ovary syndrome: a comparative case-control study. Eur J Transl Clin Med [Internet]. 2025;8(2):17–24. Available from: https://doi.org/10.31373/ejtcm/208403
17. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA [Internet]. 2013;310(20):2191–4. Available from: https://pubmed.ncbi.nlm.nih.gov/24141714/

18. Lindroth M, Lundqvist R, Lilja M, Eliasson M. Cardiovascular risk factors differ between rural and urban Sweden: the 2009 Northern Sweden MONICA cohort. BMC Public Health [Internet]. 2014;14(1):825. Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-14-825
19. Park LG, Dracup K, Whooley MA, McCulloch C, Lai S, Howie-Esquivel J. Sedentary lifestyle associated with mortality in rural patients with heart failure. Eur J Cardiovasc Nurs [Internet]. 2019;18(4):318–24. Available from: https://academic.oup.com/eurjcn/article/18/4/318-324/5925232
20. Barghouti F, Jaghbir M, Aburmaileh N, Jallad D, Qudah Y. Leisure Time Physical Activity in Jordan: Knowledge and Sociodemographic Determinants. Int Med J [Internet]. 2015 1;22:183–287. Available from: https://www.researchgate.net/publication/281740602_Leisure_Time_Physical_Activity_in_Jordan_Knowledge_and_Sociodemographic_Determinants
21. Lear SA, Hu W, Rangarajan S, Gasevic D, Leong D, Iqbal R, et al. The effect of physical activity on mortality and cardiovascular disease in 130 000 people from 17 high-income, middle-income, and low-income countries: the PURE study. Lancet [Internet]. 2017;390(10113):2643–54. Available from: https://doi.org/10.1016/S0140-6736(17)31634-3
22. Kazemi A, Soltani S, Aune D, Hosseini E, Mokhtari Z, Hassanzadeh Z, et al. Leisure-time and occupational physical activity and risk of cardiovascular disease incidence: a systematic-review and dose-response meta-analysis of prospective cohort studies. Int J Behav Nutr Phys Act [Internet]. 2024;21(1):45. Available from: https://ijbnpa.biomedcentral.com/articles/10.1186/s12966-024-01593-8
23. Kim Y, Canada JM, Kenyon J, Billingsley HE, Arena R, Lavie CJ, et al. Effects of Replacing Sedentary Time With Physical Activity on Mortality Among Patients With Heart Failure: National Health and Nutrition Examination Survey Follow-Up Study. Mayo Clin Proc [Internet]. 2022;97(10):1897–903. Available from: https://doi.org/10.1016/j.mayocp.2022.05.009
24. Khader Y, Batieha A, Ajlouni H, El-Khateeb M, Ajlouni K. Obesity in Jordan: prevalence, associated factors, comorbidities, and change in prevalence over ten years. Metab Syndr Relat Disord [Internet]. 2008;6(2):113–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18510436
25. Behera S, Sharma R, Yadav K, Chhabra P, Das M, Goel S. Prevalence and predictors of risk factors for cardiovascular diseases among women aged 15–49 years across urban and rural India: findings from a nationwide survey. BMC Womens Health [Internet]. 2024;24(1):77. Available from: https://bmcwomenshealth.biomedcentral.com/articles/10.1186/s12905-023-02869-0
26. Huxley RR, Woodward M. Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. Lancet [Internet]. 2011;378(9799):1297–305. Available from: https://doi.org/10.1016/S0140-6736(11)60781-2
27. King A. Risk factors: Cigarette smoking increases the risk of coronary heart disease in women more than in men. Nat Rev Cardiol [Internet]. 2011;8(11):612. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21878881
28. Khader Y, Batieha A, Jaddou H, Rawashdeh SI, El-Khateeb M, Hyassat D, et al. Hypertension in Jordan: Prevalence, Awareness, Control, and Its Associated Factors. Int J Hypertens [Internet]. 2019;2019:3210617. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31186953
29. Global report on diabetes [Internet]. 2016. Available from: https://www.who.int/publications/i/item/9789241565257
30. Opoku S, Gan Y, Fu W, Chen D, Addo-Yobo E, Trofimovitch D, et al. Prevalence and risk factors for dyslipidemia among adults in rural and urban China: findings from the China National Stroke Screening and prevention project (CNSSPP). BMC Public Health [Internet]. 2019;19(1):1500. Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-019-7827-5
31. Altbach PG, Reisberg L, Rumbley LE. Trends in Global Higher Education: Tracking an Academic Revolution. A Report Prepared for the UNESCO 2009 World Conference on Higher Education [Internet]. Paris: UNESCO; 2009. Available from: https://unesdoc.unesco.org/ark:/48223/pf0000183168
32. Hu M, Yang T, Yang Y. Causal Associations of Education Level With Cardiovascular Diseases, Cardiovascular Biomarkers, and Socioeconomic Factors. Am J Cardiol [Internet]. 2024;213:76–85. Available from: https://doi.org/10.1016/j.amjcard.2023.06.044
33. Khader YS, Batieha A, Al-Njadat RA, Hijazi SS. Preeclampsia in Jordan: incidence, risk factors, and its associated maternal and neonatal outcomes. J Matern Fetal Neonatal Med [Internet]. 2018;31(6):770–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28274172
34. Mphekgwana PM, Malema N, Monyeki KD, Mothiba TM, Makgahlela M, Kgatla N, et al. Hypertension Prevalence and Determinants among Black South African Adults in Semi-Urban and Rural Areas. Int J Environ Res Public Health [Internet]. 2020;17(20). Available from: http://www.ncbi.nlm.nih.gov/pubmed/33066410

35. Ackerman-Banks CM, Lipkind HS, Palmsten K, Pfeiffer M, Gelsinger C, Ahrens KA. Association of Prenatal Depression With New Cardiovascular Disease Within 24 Months Postpartum. J Am Heart Assoc [Internet]. 2023;12(9):e028133. Available from: https://doi.org/10.1161/JAHA.122.028133
36. Lu D, Valdimarsdóttir UA, Wei D, Chen Y, Andreassen OA, Fang F, et al. Perinatal depression and risk of maternal cardiovascular disease: a Swedish nationwide study. Eur Heart J [Internet]. 2024;45(31):2865–75. Available from: https://academic.oup.com/eurheartj/article/45/31/2865/7693155
37. Weaver A, Himle JA, Taylor RJ, Matusko NN, Abelson JM. Urban vs Rural Residence and the Prevalence of Depression and Mood Disorder Among African American Women and Non-Hispanic White Women. JAMA psychiatry [Internet]. 2015;72(6):576–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25853939
38. Torbati T, Shufelt C, Wei J, Noel Bairey Merz C. Premature menopause and cardiovascular disease: can we blame estrogen? Eur Heart J [Internet]. 2022;43(40):4158–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/36239222
39. Lee GB, Nam GE, Kim W, Han B, Cho KH, Kim SM, et al. Association Between Premature Menopause and Cardiovascular Diseases and All-Cause Mortality in Korean Women. J Am Heart Assoc [Internet]. 2023;12(22):e030117. Available from: http://www.ncbi.nlm.nih.gov/pubmed/37947103
40. Leone T, Brown L, Gemmill A. Secular trends in premature and early menopause in low-income and middle-income countries. BMJ Glob Heal [Internet]. 2023;8(6):e012312. Available from: https://doi.org/10.1136/bmjgh-2023-012312
41. Manzo-Silberman S, Hawranek M, Banerjee S, Kaluzna-Oleksy M, Alasnag M, Paradies V, et al. Call to action for acute myocardial infarction in women: international multi-disciplinary practical roadmap. Eur Hear J open [Internet]. 2024;4(6):oeae087. Available from: http://www.ncbi.nlm.nih.gov/pubmed/39507804
42. Parapid B, Kanjuh V, Kostic V, Polovina S, Dinic M, Loncar Z, et al. Women's health in Serbia - past, present, and future. Srp Arh Celok Lek [Internet]. 2021;149(11–12):745–54. Available from: https://doiserbia.nb.rs/Article.aspx?ID=0370-81792100105P

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):46-58

RESEARCH ARTICLE

Differential roles of the interleukin 10 family (IL-10, IL-19 and IL-22) in the pathogenesis of type 2 diabetes mellitus in Southern Saudi Arabia

Mohammad Muzaffar Mir[1], Syeda Fatima Rizvi[2], Shahzada Khalid Sohail[2], Rashid Mir[3], Javed Iqbal Wani[4], Zia Ul Sabah[4], Fahad Alremthi[5], Muffarah Alharthi[6], Imadeldin Elfaki[7], Hany M. A. Sonpol[8], Mushabab Alghamdi[9], Jaber Alfaifi[10], AbdulElah Al Jarallah AlQahtani[9]

[1] Department of Clinical Biochemistry, College of Medicine, University of Bisha, Saudi Arabia
[2] Department of Pathology, College of Medicine, University of Bisha, Saudi Arabia
[3] Prince Fahd Bin Sultan Research Chair, Department of MLT, Faculty of Applied Medical Sciences, University of Tabuk, Saudi Arabia
[4] Department of Internal Medicine, College of Medicine, King Khalid University, Abha, Saudi Arabia
[5] Diabetes and Endocrine Center, King Abdullah Hospital, Ministry of Health, Bisha, Saudi Arabia
[6] Department of Family and Community Medicine, College of Medicine, University of Bisha, Saudi Arabia
[7] Department of Biochemistry, Faculty of Science, University of Tabuk, Saudi Arabia
[8] Department of Anatomy, College of Medicine, University of Bisha, Saudi Arabia / Anatomy Department, Faculty of Medicine, Mansoura University, Egypt
[9] Department of Internal Medicine, College of Medicine, University of Bisha, Saudi Arabia
[10] Department of Child Health, College of Medicine, University of Bisha, Saudi Arabia

Abstract

Background: Type 2 diabetes mellitus (T2DM) is a growing global health burden strongly associated with obesity and lifestyle changes. Inflammatory cytokines, particularly from the interleukin-10 (IL-10) family, are increasingly implicated in the pathogenesis of T2DM. This study assessed circulating IL-10, IL-19, and IL-22 levels in T2DM patients across obesity categories in southern Saudi Arabia. **Materials and methods:** A total of 320 T2DM patients and 196 healthy controls were enrolled. All participants were classified by body mass index (BMI) as normal weight, overweight or obese (class I, class II or class III). Anthropometric and biochemical parameters were re-

Corresponding author:
Mohammad Muzaffar Mir, Department of Clinical Biochemistry, College of Medicine, University of Bisha, Saudi Arabia
e-mail: mirmuzaffar11@gmail.com
Available online: ejtcm.gumed.edu.pl

corded, and serum IL-10, IL-19, and IL-22 levels were measured using standardized immunoassays. **Results:** IL-10 levels were significantly reduced in normal weight and overweight T2DM patients of both sexes ($p < 0.001$) and remained lower in obesity class I ($p < 0.01$) and class III ($p < 0.05$). IL-19 levels were markedly elevated in obesity class I and class III across sexes ($p < 0.001$). IL-22 concentrations increased progressively with BMI, with significant elevations in obesity class I and class III groups. **Conclusions:** Reduced IL-10 showed a strong association with T2DM, while higher levels in obese females suggest a possible sex-related protective effect. IL-19 was consistently associated with T2DM, whereas IL-22 correlated more closely with obesity severity.

Keywords: T2DM · IL-10 · Saudi Arabia · IL-22 · IL-19

Citation

Mir MM, Rizvi SF, Sohail SK, Mir R, Wani JI, Sabah ZU, Alremthi F, Alharthi M, Elfaki I, Sonpol HMA, Alghamdi M, Alfaifi J, AlQahtani AAJ. Differential roles of the interleukin 10 family (IL-10, IL-19 and IL-22) in the pathogenesis of type 2 diabetes mellitus in Southern Saudi Arabia. Eur J Transl Clin Med. 2026;9(1):46-58.

DOI: 10.31373/ejtcm/220579

Introduction

Type 2 diabetes mellitus (T2DM) is a major global public health challenge, particularly in developing regions, driven by rapid urbanization, sedentary lifestyles, and increased consumption of calorie-dense diets [1-5]. According to the International Diabetes Federation (IDF), the global number of individuals with diabetes is projected to reach approximately 852.5 million (13% of the population) by 2045 [6]. A marked rise in prevalence has also been observed in China and other developing nations [7]. Saudi Arabia is among the top 10 countries with the highest diabetes prevalence in the world among individuals 20-79 years of age, with approximately 5.3 million cases in 2024 and projections of 9.5 million by 2050 [8]. Diabetes is a leading cause of morbidity and mortality, conferring a two- to three-fold increased risk of death [1-2]. Approximately 95% of cases are T2DM, characterized by chronic hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both [8-9]. T2DM prevalence varies across populations, with higher susceptibility reported among Asian, Hispanic, Native American, and Black populations [9-13].

T2DM arises from complex interactions between genetic predisposition, environmental influences, obesity, and socioeconomic factors [14-15]. Genome-wide association studies have identified multiple loci associated with T2DM risk, primarily affecting β-cell function and insulin secretion [14-15]. Acute complications include diabetic ketoacidosis and hyperosmolar hyperglycemic state, whereas chronic complications include nephropathy, retinopathy, neuropathy, and cardiovascular disease [16]. Notably, approximately 90% of individuals with T2DM are overweight or obese, emphasizing obesity as a key modifiable risk factor [17-18].

Emerging evidence highlights the role of chronic low-grade inflammation, adipokine imbalance, gut microbiota alterations, and immune dysregulation in T2DM pathogenesis [3, 14]. Periodontal disease has also been associated with T2DM, although the bidirectional relationship remains incompletely understood [19]. Several cytokines, particularly members of the interleukin (IL)-10 family, have been implicated in metabolic inflammation [14, 20-21]. These cytokines share structural homology and are produced by immune cells as well as adipose tissue, linking immune signaling with metabolic regulation [22-23].

IL-10 is a key anti-inflammatory cytokine that regulates inflammatory mediators including IL-19 and IL-22, and reduced circulating levels have been reported in obesity, metabolic syndrome, and T2DM [21-22]. IL-19, produced mainly by activated B cells and monocytes, can induce IL-10 production and modulate Th2 responses implicated in inflammatory and cardiovascular diseases [24-29]. It also exhibits angiogenic effects, although its role in T2DM remains incompletely defined [29-31]. IL-22, secreted by CD4+ T helper subsets and innate lymphoid cells, demonstrates functional divergence from IL-10 despite structural homology.

This purpose of this study was to investigate the association between selected members of the IL-10 family, namely IL-10, IL-19, and IL-22 and the etiopathogenesis of T2DM

across the degrees of obesity among the patients from the Asir (southern) region of Saudi Arabia. The study further examined correlations between obesity severity and IL levels to elucidate potential links between adiposity, metabolic control, and inflammatory-immune profiles in T2DM.

Materials and methods

Study design

This was a multi-center study of age-matched T2DM patients and healthy control participants, carried out in two cities (Bisha and Abha) of the Asir province in the southern part of Saudi Arabia. The patients and controls were recruited between March 2020 and May 2023.

Study group

In this study included only Saudi residents of the Asir region, particularly in the cities of Bisha and Abha. The study had a total of 348 patients diagnosed T2DM (210 male and 138 female). All patients were currently undergoing treatment with oral hypoglycemic agents and/or insulin. Patients with gestational diabetes or other significant chronic diseases (e.g. hypertension, cardiovascular diseases, asthma, chronic kidney diseases and cancer) were excluded from the study group.

Control group

A total of 196 healthy people (102 men and 94 women) were recruited in the study. All had fasting glucose levels, glycated hemoglobin (HbA1c), kidney and liver function tests within the reference ranges. Chronic diseases (e.g. hypertension, cardiovascular diseases, asthma, chronic kidney diseases and cancer) were also an exclusion criterion.

Ethics

This study was approved by the local RELOC Committee of the College of Medicine at the University of Bisha (Ref. No. UB-RELOC H-06-BH-087/ (0504 of 23), Dated January 6th, 2023 extended on March 4th, 2025), in accordance with the local guidelines that aligned with the fundamental principles of the Helsinki Declaration. Informed written consent was obtained from all participants.

Data collection

Finally, 320 participants with confirmed T2DM (195 males and 125 females) who visited the Diabetic Centre of King Abdullah Hospital (KAH) in Bisha, and at the Asir General Hospital (AGH) in Abha for regular follow-up and met the inclusion criteria were included in the study. T2DM was diagnosed in accordance with the WHO guidelines. The classification of obesity was based on the degree of obesity as reported earlier [3, 40]. The case histories, age, sex, BMI, blood glucose (both fasting and random), glycated hemoglobin, triacylglycerol, high-density lipoprotein-cholesterol and low-density lipoprotein-cholesterol levels were analyzed. Standardized procedures were used to evaluate the anthropometric and biochemical parameters.

Blood specimen collection

All blood samples were collected from the participants at the Diabetic Centre of KAH, and at the AGH. Venous blood samples were collected in standard sample collection tube without the use of any anticoagulants. One aliquot of serum (approximately 0.5 mL) was kept at -20 °C until the IL estimation. A second serum aliquot was used for the analysis of biochemical and other parameters on a routine basis.

Stratification on the basis of BMI

Height (cm) was recorded using a wall-mounted scale, and weight (kg) was determined with electronic weighing scale. The BMI of the patients was calculated using the formula; BMI = weight (kg)/m^2 (m is height in meters). The patient cohort was divided into 5 groups: normal weight (BMI 18.5-24.9), overweight (BMI 25.0-29.9), class I obesity (BMI 30.0-34.9), class II obesity (BMI 35.0-39.9), class III (severe) obesity (BMI ≥ 40.0).

Biochemical analyses

The biochemical parameters were analyzed using fully automatic random access, multi-channel analyzer (Cobas c311, Roche Diagnostics, Basel, Switzerland). For these studies we utilized commercially available test kits from (Randox Laboratories, Crumlin, Northern Ireland, UK), which included calibrators and internal quality control samples provided by the manufacturer. The manufacturers' protocols were followed during the analysis of biochemical parameters.

Estimation of interleukins

Serum concentrations of IL-10, IL-19, and IL-22 were quantified using human ELISA kits from different sources and according to the manufacturers' instructions. IL-10 was measured using the Human IL-10 ELISA Kit (Thermo Fisher Scientific, Carlsbad, USA, Cat. No. BMS215HS; sensitivity 0.03 pg/mL), IL-19 using the Human IL-19 ELISA Kit (Abcam

Limited, Cambridge, UK; Cat. No. ab231922; sensitivity 0.65 pg/mL), and IL-22 using the Human IL-22 ELISA Kit (Thermo Fisher Scientific, Carlsbad, USA; Cat. No. BMS2047; sensitivity 5 pg/mL). Absorbance was read using a Bio-Rad Model 680 XR microplate reader (Bio-Rad Laboratories, Hercules, USA). All samples were analyzed following standardized protocols, and cytokine concentrations were expressed in pg/mL. Intra- and inter-assay coefficients of variation were < 10% for all measurements.

Statistical analyses

The Statistical Package for Social Sciences software (SPSS v. 20, IBM Corp., Armonk, USA) was used for data analysis. Unlike data with a normal distribution, which was expressed as the mean ± standard deviation (Shapiro-Wilk test), data that were not normally distributed were represented as medians (Q1–Q3) and analyzed by Chi-Square test. For normally distributed variables with homogeneous variances, the significance of differences was determined by the one-way analysis of variance with Tukey HSD test. In all other cases the Kruskal-Wallis one-way analysis of variance on ranks and the multiple comparison post hoc test were used. The p-value was considered statistically significant when it was ≤ 0.05.

Results

Baseline characteristics of male participants

The biochemical and anthropometric characteristics of male T2DM patients and controls are summarized in Table 1. Fasting glucose levels were comparable to controls in normal weight, overweight, and class I obese T2DM groups but were significantly higher in the class III obese group ($p < 0.001$). HbA1c levels were significantly elevated in all T2DM groups compared with controls ($p < 0.001$), with no significant differences among patient categories, although relatively higher values were observed in the class I obese group.

Total cholesterol (TC) was significantly increased in normal weight, overweight, and class I obese T2DM groups compared with controls ($p < 0.01$), while class III obese patients exhibited a greater elevation ($p < 0.001$). TC levels did not differ significantly among the first three T2DM groups. HDL levels were not significantly different between patients and controls, although a decreasing trend was observed in T2DM groups.

LDL levels were similar in controls and normal weight T2DM patients but were significantly higher in overweight and class I obese groups ($p < 0.001$). Class III obese patients demonstrated significantly higher LDL levels compared with both controls and overweight T2DM patients ($p < 0.001$). Triglyceride levels were significantly elevated in all T2DM groups compared with controls ($p < 0.01$), with the highest levels observed in class III obese patients ($p < 0.001$), which were also significantly greater than those in the other T2DM groups ($p < 0.01$).

Male T2DM patients (n = 195) aged 28-69 years were categorized according to BMI into normal weight (n = 52; 21.82 ± 2.08 kg/m²), overweight (n = 46; 27.68 ± 2.32 kg/m²), class I obese (n = 48; 35.06 ± 3.22 kg/m²), and class III obese (n = 49; 47.68 ± 5.72 kg/m²). Controls (n = 102) had a mean BMI of 21.74 ± 1.72 kg/m² (Table 1). WHR did not differ significantly between controls and normal weight or overweight T2DM patients but was significantly higher in class I and class III obese groups. Class III obese patients showed the greatest increase in WHR compared with normal weight and overweight groups ($p < 0.001$), while class I obese patients also demonstrated a significant increase ($p < 0.01$).

BMI did not differ significantly between controls and normal weight or overweight T2DM patients. However, BMI was significantly higher in class I obese patients compared with normal weight and overweight groups ($p < 0.001$). Class III obese patients showed the highest BMI values, significantly exceeding those of all other groups, including class I obesity ($p < 0.01$).

Baseline features of the female participants

The biochemical and anthropometric characteristics of female T2DM patients and controls are summarized in Table 2. Fasting plasma glucose levels were significantly higher in normal weight, overweight, and class I obese T2DM patients compared with controls ($p < 0.01$), while class III obese group showed a more pronounced elevation ($p < 0.001$). HbA1c levels were significantly increased in all T2DM groups compared with controls ($p < 0.001$), with no significant differences observed among the BMI categories.

Total cholesterol (TC) levels were significantly elevated in normal weight and overweight T2DM patients compared with controls ($p < 0.01$), whereas class I and class III obese patients exhibited more marked increases ($p < 0.001$). TC levels in class I and class III obese patients were also significantly higher than those in normal weight and overweight T2DM groups ($p < 0.01$). HDL-cholesterol levels showed no significant differences between controls and normal weight or overweight T2DM patients, although a non-significant decrease was observed in class I and class III obese groups.

LDL-cholesterol levels were comparable between controls and normal weight T2DM patients but were significantly increased in class I and class III obese groups ($p < 0.001$).

Table 1. Biochemical and anthropometric parameters of males with T2DM (n = 195) and healthy male controls (n = 102)

	Controls (n = 102)	Normal weight group (n = 52)	Overweight group (n = 46)	Class I obesity group (n = 48)	Class III (severe) obesity Group (n = 49)
FSG (mg/dL)	95 (82-115)	117 (91-133)	121 (94-142)	118 (88-148)	125 (102-155) *
HbA1c (g/dL)	5.1 ± 0.82	7.5 ± 0.99*	8.05 ± 1.1*	8.6 ± 1.42*S	7.8 ± 0.82*
TC (mg/dL)	182 (132-216)	208** (174-240)	220** (146-238)	218** (147-248)	232* (169-272)
HDL-C (mg/dL)	48 (36-66)	44 (39-58)	47 (41-57)	44 (39-56)	45 (34-55)
LDL-C (mg/dL)	96 (85-116)	108 (78-134)	120** (87-142)	1124** (88-150)	138*^{W} (92-165)
TG (mg/dL)	122 (82-138)	134 (92-145)	155 (100-195)	236* (172-262)	244*^{W} (168-290)
Age (years)	45 (29-61)	44 (28-54)	49 (34-60)	48 (35-64)	49 (35-69)
BMI (kg/m²)	21.74 ± 1.72	21.82 ± 2.08	27.68 ± 2.32	35.06 ± 3.22*T	47.68 ± 5.72*^{WX}
WHR	0.85 (0.78-0.94)	0.94 (0.82-0.1.04)	0.95 (0.84-1.08)	1.08**^{T} (0.96-1.12)	1.16*^{S} (1.08-1.22)

Values for various analytes and anthropometric parameters are presented as mean with ranges in parentheses. The values followed by ± sign represent standard deviation.
WHR – waist to hip ratio; BMI – body mass index; FSG – fasting glucose; TC – total cholesterol; HbA1c – glycated hemoglobin; HD – high density lipoprotein; LDL – low density lipoprotein; TG – triglycerides
*p < 0.001 vs. control group; **p < 0.01 vs. control group, S p < 0.001 vs. normal weight and overweight groups,
T p < 0.01 vs. overweight and class I obesity; W p < 0.001 vs. controls and normal weight group; X p < 0.01 vs. class I obesity group

Table 2. Biochemical and anthropometric parameters of female T2DM patients (n = 125) and the female control group (n = 94)

	Controls (n = 94)	Normal weight group (n = 31)	Overweight group (n = 32)	Class I obesity group (n = 33)	Class III (severe) obesity Group (n = 29)
FSG (mg/dL)	94 (80-112)	120** (88-142)	116** (90-140)	118** (99-142)	126* (102-155)
HbA1c (g/dL)	4.8 ± 0.48	7.94 ± 1.10*	8.3 ± 1.28*	8.6 ± 1.52*^{S}	8.5 ± 1.06*
TC (mg/dL)	172 (129-216)	196** (152-242)	208** (164-246)	222*^{T} (178-246)	232*^{T} (172-264)
HDL-C (mg/dL)	55 (44-64)	54 (40-60)	50 (38-58)	45 (34-59)	42 (35-60)
LDL-C (mg/dL)	106 ± 26.88	98 ± 27.65	118** ± 28.46	116** ± 29.72	136* ± 32.04
TG (mg/dL)	102 (90-128)	126** (102-158)	146** (94-192)	158** (104-218)	188*^{WX} (112-254)
Age (years)	47 (28-65)	46 (27-64)	48 (27-65)	45 (27-60)	48 (32-68)
BMI (kg/m²)	21.28 ± 1.58	21.44 ± 1.98	28.96 ± 2.12	34.12 ± 2.98*^{T}	46.72 ± 4.96**^{WX}
WHR	0.84 (0.77-0.95)	0.92 (0.80-0.1.03)	0.94 (0.86-1.08)	1.08**^{T} (0.96-1.14)	1.12*^{S} (0.98-1.24)

Values for various analytes and anthropometric parameters are presented as mean with ranges in parentheses. The values followed by ± sign represent standard deviation.
FSG – fasting plasma glucose; TC – total cholesterol; HbA1c – glycated hemoglobin; HDL – high density lipoprotein; LDL – low density lipoprotein; TG – triglycerides; WHR – waist to hip ratio; BMI – body mass index
*p < 0.001 vs. control group; **p < 0.01 vs. control group, S p < 0.001 vs. normal weight and overweight groups, T p < 0.01 vs. normal weight and overweight groups; W p < 0.001 vs. controls and normal weight group; X p < 0.01 vs. class I obesity group

Class III obese patients demonstrated significantly higher LDL levels compared with normal weight patients ($p < 0.001$). Triglyceride (TG) levels were significantly elevated in normal weight, overweight, and class III obese T2DM patients compared with controls ($p < 0.01$), with the class III obese group showing the highest increase ($p < 0.001$). TG levels in class III obese patients were also significantly higher than those in normal weight and class I obese groups ($p < 0.01$).

Female T2DM patients ranged in age from 27-68 years, while controls ranged from 28-65 years. Based on BMI, T2DM patients were categorized into 4 groups: normal weight (n = 31; BMI 21.44 ± 1.98 kg/m²), overweight (n = 32; BMI 28.96 ± 2.12 kg/m²), class I obese (n = 33; BMI 34.12 ± 2.98 kg/m²), and class III obese (n = 29; BMI 46.72 ± 4.96 kg/m²). Controls had a mean BMI of 21.28 ± 1.58 kg/m² (Table 2).

WHR was significantly higher in class I obese ($p < 0.01$) and class III obese ($p < 0.001$) T2DM patients compared with controls. Additionally, class III obese patients exhibited significantly higher WHR compared with normal weight and overweight groups ($p < 0.001$). BMI did not differ significantly between controls and normal weight or overweight T2DM patients. However, BMI was significantly elevated in class I and class III obese groups compared with controls ($p < 0.001$). Class III obese patients demonstrated significantly higher BMI than normal weight and overweight groups ($p < 0.001$), as well as higher BMI compared with class I obese patients ($p < 0.01$). Class I obese patients also showed significantly higher BMI than normal weight and overweight groups ($p < 0.01$).

Interleukin levels in male participants

Table 3 presents a summary of the blood concentrations of IL 10, 19 and 22 and the results are graphically depicted in Figure 1.

Interleukin-10 in male participants

The average blood IL-10 concentration in the control group of healthy males (n = 102) was 18.78 ± 2.21 pg/mL. T2DM patients in normal weight and overweight groups exhibited significant reductions ($p < 0.001$) in IL-10 levels, measuring 7.89 ± 1.45 and 7.32 ± 1.36 pg/mL, respectively, as compared to the control group. T2DM patients in in class I and III obesity groups had statistically significant reductions in IL-10 levels (11.78 ± 1.87 and 13.56 ± 1.92 pg/mL, respectively) when compared to the control group. The p values for these comparisons were < 0.01 and < 0.05, respectively. The IL-10 levels of class I and III obesity groups were significantly higher than those in normal weight and overweight groups ($p < 0.05$).

Interleukin-19

The IL-19 level (mean ± SD) in the male controls (n = 102) was 72 ± 21.45 pg/mL. Male T2DM patients in in normal weight and overweight groups had a statistically significant increase ($p < 0.01$) in IL-19 concentrations (232 ± 21.45 and 273 ± 29.66 pg/mL) respectively, as compared to controls. T2DM patients in class I and III obesity groups displayed very significant increases in IL-19 values (310 ± 32.24 and 356 ± 43.64 pg/mL respectively) in comparison with controls with p values of < 0.001 and < 0.001. Patients in class I and III obesity groups also had significantly greater IL-19 levels in comparison with those in normal weight and overweight groups ($p < 0.05$).

Interleukin-22 in male participants

The average serum IL-22 concentration in the male controls (n = 102) was 46 ± 12.48 pg/mL. The T2DM patients with normal weight exhibited a slight, albeit statistically insignificant elevation in IL-22 levels (52 ± 17.45 versus 46 ± 12.48 pg/mL). Overweight patients also had significant elevation in IL-22 levels in comparison to both the control group and normal weight group ($p < 0.05$). The levels of IL-22 in class I obesity group exhibited a statistically significant rise when contrasted with controls, $p < 0.01$. Class I obesity T2DM patients had a notable increase in IL-22 levels in comparison to overweight T2DM patients, with $p < 0.04$. While comparing to both the controls and normal weight group, IL-22 levels were considerably greater in patients with class III obesity ($p < 0.001$). Figure 1 presents the cluster charts depicting the levels of serum IL 10 family members in both male control and patient groups.

Interleukin-10 in female groups

Table 4 provides a summary of the serum levels of IL 10, 19 and 22 in T2DM female patients (n = 125) and 94 individuals serving as control subjects. The average serum IL-10 concentration in the control group of healthy females was 19.12 ± 2.28 pg/mL, a value comparable to that observed in the control group of males. Normal weight female T2DM patients exhibited a statistically significant reduction ($p < 0.001$) in IL-10 concentrations as compared to the control subjects. The female patients in overweight group exhibited a noteworthy reduction in IL-10 levels (11.68 ± 1.55 pg/mL) in contrast to controls, with $p < 0.01$. T2DM patients with class I and III obesity showed a slight, albeit statistically insignificant reduction in IL-10 levels in comparison with the controls. The results for female subjects are summarized in table 4 and are graphically illustrated in Figure 2.

Table 3: Serum levels of IL 10, 19 and 22 in male T2DM patients (n = 195) and normal controls (n = 102)

	IL-10 (pg/mL)	IL-19 (pg/mL)	IL-22 (pg/mL)
Controls (n = 102)	18.78 ± 2.21	72 ± 21.45	46 ± 12.48
Normal weight group (n = 52)	7.89 ± 1.45*	232 ± 21.45**	52 ± 17.45
Overweight group (n = 46)	7.32 ± 1.36*	273 ± 29.66**	98 ± 26.12***^{M}
Class I obesity group (n = 48)	11.78 ± 1.87**S	310 ± 32.24*^{S}	147 ± 34.65**^{L}
Class III (severe) obesity group (n = 49)	13.56 ± 1.92***^{T}	356 ± 43.64*^{T}	178 ± 38.28*^{P}

Values for various analytes are presented as mean with ranges in parentheses ± standard deviation.
*p < 0.001 vs. control group; ** p < 0.01 vs. control group; *** p < 0.05 vs. control group; Lp < 0.04 vs. overweight group; Sp < 0.05 compared to normal weight and overweight T2DM patients; Tp < 0.05 compared to normal weight and overweight T2DM patients; M p < 0.05 compared to controls and normal weight group; N p < 0.01 compared to controls and normal weight group; Pp < 0.001 compared to controls and normal weight group

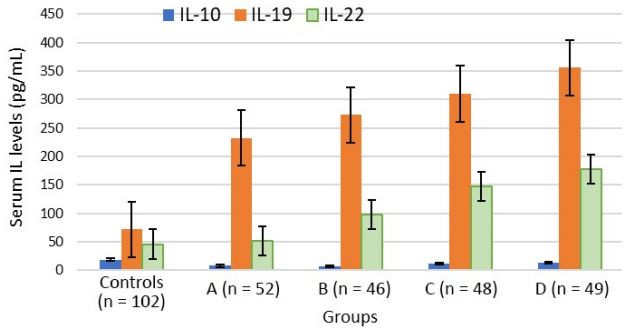


Figure 1. Cluster charts of IL 10, 19 and 22 levels in male controls and T2DM patients. A represents T2DM patients with normal weight; B represents overweight group; C represents class I obeseity group and D represents class III obesity group respectively.

Interleukin-19 in female T2DM patients

The average blood IL-19 concentration in the female control group (n = 94) was 67 ± 17.42 pg/mL. Female T2DM normal weight and overweight patients exhibited statistically significant increases in IL-19 concentrations (168 ± 26.78 and 212 ± 28.22 pg/mL, respectively) in comparison to controls with p < 0.01. Simultaneously, it was observed that overweight T2DM patients exhibited significantly higher IL-19 levels in comparison to normal weight patients (p < 0.01). Patients with class I and III obesity exhibited a very significant elevation in IL-19 levels (276 ± 35.14 and 288 ± 36.98 pg/mL, respectively) when contrasted with controls (p < 0.0001). In addition, IL-19 levels were significantly higher in class I and III obesity groups compared to the normal weight group (p < 0.001).

Interleukin-22 in female T2DM patients

The average blood IL-22 concentration in the control group of healthy females (n = 94) was 44 ± 12.02 pg/mL as are presented in Table 4. The patients with normal weight exhibited a small and statistically insignificant rise in IL-22 levels (48 ± 14.08 versus 44 ± 12.02 pg/mL). Overweight patients with T2DM had a statistically significant (p < 0.01) elevation in IL-22 levels in comparison to both the controls and patients with normal weight. IL-22 values in patient group with class I obesity exhibited a statistically significant rise in comparison to the controls, as well as normal weight and overweight patients, with p values < 0.01, < 0.01, and < 0.04, respectively. The IL-22 levels in patients with class III obesity were significantly higher than those in the controls (p < 0.001), and also, those of normal weight group A (p < 0.001) and overweight group p < 0.04). The cluster charts of serum IL concentrations in 74 female patients and 41 controls are displayed in Figure 2.

Sex-based comparison of IL levels

Comparative analysis of serum IL levels between male and female T2DM patients revealed distinct sex-related patterns across BMI categories. IL-10 levels were significantly reduced in normal-weight and overweight patients of both sexes compared with controls, however, suppression was more pronounced in males (7.32-7.89 pg/mL) than females (9.23-11.68 pg/mL). With increasing obesity severity, IL-10 levels showed partial restoration, approaching control values more closely in females than males. IL-19 concentrations were markedly elevated across all BMI categories in both groups, with a substantially greater increase observed in males (232-356 pg/mL)

Table 4. Serum levels of IL 10, 19 and 22 in female T2DM patients (n = 125) and normal controls (n = 94)

	IL-10 (pg/mL)	IL-19 (pg/ mL)	IL-22 (pg/ mL)
Controls (n = 94)	19.12 ± 2.28	67 ± 17.42	44 ± 12.02
Normal weight group (n = 31)	9.23 ± 1.52*	168 ± 26.78*	48 ± 14.08
Overweight group (n = 32)	11.68 ± 1.55**	212 ± 28.22*X	112 ± 29.56**S
Class I obesity group (n= 33)	17.46 ± 2.10	276 ± 35.14***Y	165 ± 33.24**SM
Class III (severe) obesity group (n = 29)	18.02 ± 2.14	288 ± 36.98***Y	198 ± 37.82***TN

Values for various analytes are presented as mean with ranges in parentheses ± standard deviation.
*p < 0.001 vs. control group; ** p < 0.01 vs. control group; *** p < 0.0001 vs. control group; X p < 0.01 compared to normal weight patient group; Y p < 0.001 compared to normal weight patient group; S p < 0.01 compared to controls and normal weight patient group; T p < 0.001 compared to normal weight patient group and controls; M p < 0.04 compared to overweight patients; N p < 0.01 compared to overweight patient group

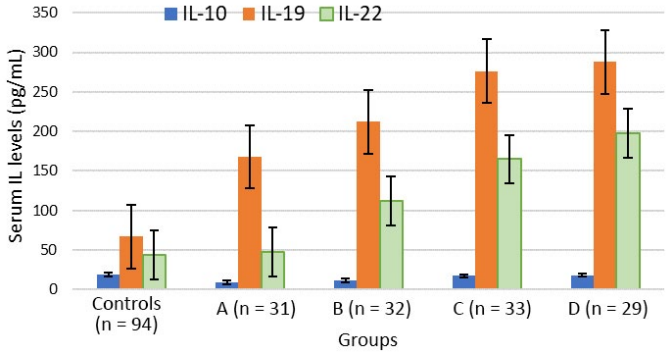


Figure 2. Cluster charts of IL 10, 19 and 22 levels in female controls and T2DM patients. A represents normal weight group; B represents overweight group; C represents class I obesity group and D represents class III obesity group of T2DM patients.

compared to females (168-288 pg/mL). IL-22 levels demonstrated a progressive rise with increasing adiposity in both sexes. Although both sexes exhibited significant elevations in overweight and obese categories, males showed higher levels in most BMI strata, while severely obese females demonstrated comparable peak values. Overall, male T2DM patients exhibited a stronger pro-inflammatory IL profile, whereas females demonstrated partial preservation of anti-inflammatory IL-10 with increasing adiposity.

IL levels in all T2DM patients (male & female) vs. controls

A comparison of the serum levels of ILs 10, 19 and 22 in T2DM patients with controls has been summarized in Table 5 and the results are graphically presented in Figure 3. The average serum IL-10 concentration in T2DM patients was 12.52 ± 1.84 pg/mL as compared to 18.95 ± 2.25 pg/mL for those of controls showing a statistically significant decrease in IL-10 levels with $p < 0.01$. The average serum IL-19 concentration in T2DM patients was 264.44 ± 34.12 pg/mL in comparison to 69.50 ± 20.34 pg/mL for those of controls, showing a statistically significant increase in IL-19 levels with $p < 0.0001$. The serum IL-22 level in T2DM patients was 124.68 ± 30.24 pg/mL as compared to 45.0 ± 12.42 pg/mL for those of controls, depicting a very significant increase in IL-19 levels with $p < 0.001$.

Discussion

To the best of our knowledge, this is the first study to explore the interplay between obesity-related anthropometric measures and IL-10 family cytokines in the context of T2DM in the population of the Asir region of Saudi Arabia.

IL-10 levels were comparable between male (n = 102) and female (n = 94) controls, in agreement with previous studies [21, 23, 32-33]. In contrast, normal-weight T2DM patients demonstrated a significant reduction in IL-10, suggesting a diminished anti-inflammatory milieu that may contribute to disease onset [21, 23, 33]. Among overweight individuals, male patients showed a marked decline in IL-10 ($p < 0.001$), while females exhibited a more modest reduction. The anti-inflammatory role of IL-10 is well-established [21-22, 24], and it is often viewed as protective in metabolic disorders, although excessively elevated levels may have paradoxical effects [22]. Notably, IL-10 levels were significantly reduced in class I and III obese males, whereas females in the same categories showed comparatively higher levels ($p < 0.01$). This divergence reinforces the role of obesity as an independent

Table 5. Comparison of serum levels of ILs 10, 19, and 22 of all T2DM patients (males and females combined n = 320) with normal controls (= 196)

	IL-10 (pg/mL)	IL-19 (pg/ mL)	IL-22 (pg/ mL)
Controls (n =196)	18.95 ± 2.25	69.50 ± 20.34	45.0 ± 12.42
T2DM patients (n = 320)	12.52 ± 1.84*	264.44 ± 34.12***	124.68 ± 30.24**
Overweight group (n = 32)	11.68 ± 1.55**	212 ± 28.22*^{x}	112 ± 29.56**^{s}

* Values for various analytes are presented as mean with ranges in parentheses ± standard deviation.
p < 0.01 for combined patients vs. control group; ** p < 0.001 for combined patients vs. control group; *** p < 0.0001 for combined patients vs. control group

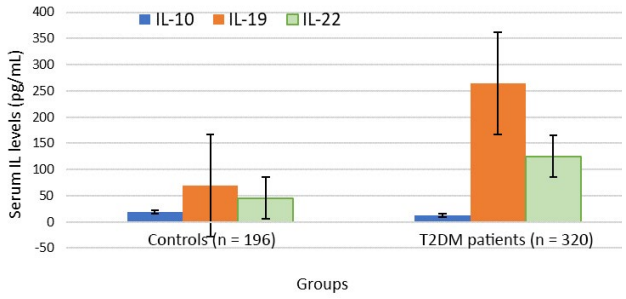


Figure 3. Cluster charts of IL 10, 19 and 22 levels in combined controls(n = 196) and T2DM patients (n = 320)

risk factor and points toward a possible attenuation of anti-inflammatory signaling in males. Similar patterns have been described previously [34].

Sex-based differences in cytokine responses are well documented [35-37]. The relatively higher IL-10 levels observed in obese females may be influenced by sex hormones, particularly given the role of adipose tissue in hormone metabolism [34, 37-40]. Elevated IL-10 levels in post-menopausal women further support this association [34, 40]. These observations warrant deeper exploration, and comparisons between pre- and post-menopausal states are being addressed in our ongoing work.

The mechanisms underlying elevated IL-10 in obese female T2DM patients remain incompletely understood but may involve transcriptional regulation [34]. It is plausible that this reflects a compensatory anti-inflammatory response, however this speculation requires further validation.

IL-19, a structurally distinct member of the IL-10 family with an α-helical configuration [26], has increasingly been implicated in metabolic disorders [29, 40]. In our study, IL-19 levels were significantly altered across all T2DM groups, irrespective of sex, consistently with prior reports [29, 40]. Patients with class I and III obesity exhibited marked elevations compared to both controls and normal-weight T2DM individuals ($p < 0.001$), suggesting a role not only in T2DM but also in the gradation of obesity [29, 40]. IL-19 has been associated with anti-inflammatory, antiatherogenic, and vasculoprotective effects, indicating potential clinical relevance [26,41-44]. However, these observations need confirmation in larger, well-characterized cohorts [41, 44]. IL-22 demonstrated a progressive increase across T2DM groups, with statistically significant elevations primarily in overweight and obese individuals, aligning with earlier findings [45-46]. These results support the view that IL-22 alone may not serve as a definitive biomarker for T2DM, and its levels appear largely independent of sex hormones [45-46]. IL-22 signals through a receptor complex comprising IL-22R1 and IL-10R2, the latter also shared by IL-10, IL-26, IL-28, and IL-29 [45]. When analyzed collectively, T2DM patients showed significant alterations in all three interleukins compared to controls, consistently with existing literature [22, 27, 47-48].

Previous studies have reported higher IL-22 levels and increased frequencies of IL-22-producing CD4+ T cells in insulin-resistant and obese individuals, although many of these studies are limited by small sample sizes and lack of replication [46, 49-50]. Experimental models suggest that IL-22 may mitigate oxidative and endoplasmic reticulum stress, and exogenous IL-22 has shown beneficial metabolic effects in animal studies [46, 51]. Unfortunately, human data remain limited. There is a clear need for carefully designed translational studies to better define the role of IL-22 in T2DM and to assess its potential as a therapeutic target.

Limitations

All the T2DM patients enrolled for this study belonged to one ethnic group which limits the generalizability of our

results. Additional investigations using larger sample sizes in multiple ethnic groups are necessary to explore the IL-10 family's role in the pathogenesis of T2DM and its potential therapeutic value. Another limitation of this study is the fact that our control group did not include a sub-group of obese participants without T2DM.

Conclusions

IL-10 levels were significantly reduced in both male and female normal-weight T2DM patients compared to controls. With increasing adiposity, males consistently showed lower IL-10 levels than females, while females in class I and III obesity groups exhibited relatively higher levels within comparable metabolic categories. IL-19 levels were significantly elevated across all T2DM groups, irrespective of sex, with a more pronounced increase in obese and severely obese patients compared to controls and normal-weight individuals, indicating a potential role in both T2DM and obesity.

IL-22 levels showed a progressive rise across T2DM groups, although significant changes were limited to overweight and obese categories, suggesting a stronger association with obesity rather than with T2DM *per se*.

Conflict of interest

None

Acknowledgments

The authors are thankful to the Deanship of Graduate Studies and Scientific Research at the University of Bisha for supporting this work through the Fast-Track Research Support Program.

Data availability statement

Data used in this study is available upon reasonable request.

References

1. Huang X, Wu Y, Ni Y, Xu H, He Y. Global, regional, and national burden of type 2 diabetes mellitus caused by high BMI from 1990 to 2021, and forecasts to 2045: analysis from the global burden of disease study 2021. Front Public Heal [Internet]. 2025;13:151579. Available from: https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2025.1515797
2. Ba T, Ren Q, Gong S, Li M, Lian H, Cai X, et al. Prevalence and Clinical Characteristics of NEUROD1-MODY in Chinese Early-Onset Type 2 Diabetes Mellitus and a Literature Review. J Diabetes [Internet]. 2025;17(4):e70080. Available from: https://doi.org/10.1111/1753-0407.70080
3. Mir MM, Mir R, Alghamdi MAA, Wani JI, Sabah ZU, Jeelani M, et al. Differential Association of Selected Adipocytokines, Adiponectin, Leptin, Resistin, Visfatin and Chemerin, with the Pathogenesis and Progression of Type 2 Diabetes Mellitus (T2DM) in the Asir Region of Saudi Arabia: A Case Control Study. J Pers Med [Internet]. 2022;12(5):735. Available from: https://www.mdpi.com/2075-4426/12/5/735
4. Mir M, Mir R, Alghamdi M, Wani J, Elfaki I, Sabah Z, et al. Potential impact of GCK , MIR-196A-2 and MIR-423 gene abnormalities on the development and progression of type 2 diabetes mellitus in Asir and Tabuk regions of Saudi Arabia. Mol Med Rep [Internet]. 2022;25(5):162. Available from: http://www.spandidos-publications.com/10.3892/mmr.2022.12675
5. Qadir MI, Ahmed Z. lep Expression and Its Role in Obesity and Type-2 Diabetes. Crit Rev Eukaryot Gene Expr [Internet]. 2017;27(1):47–51. Available from: http://www.dl.begellhouse.com/journals/6dbf508d3b17c437,7963fdcd2773abac,7c1a039e7f33b3cb.html
6. Magliano D, Boyko E. The global picture of diabetes. In: Diabetes Atlas [Internet]. 11th ed. Brussels: International Diabetes Federation; 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK618744/
7. Zhou X, Guan H, Zheng L, Li Z, Guo X, Yang H, et al. Prevalence and awareness of diabetes mellitus among a rural population in China: results from Liaoning Province. Diabet Med [Internet]. 2015;32(3):332–42. Available from: https://onlinelibrary.wiley.com/doi/10.1111/dme.12599
8. Moin ASM, Butler AE. Alterations in Beta Cell Identity in Type 1 and Type 2 Diabetes. Curr Diab Rep [Internet]. 2019;19(9):83. Available from: http://link.springer.com/10.1007/s11892-019-1194-6
9. Yin J, Yeung R, Luk A, Tutino G, Zhang Y, Kong A, et al. Gender, diabetes education, and psychosocial factors are associated with persistent poor glycemic control in patients with type 2 diabetes in the in the Joint Asia Diabetes Evaluation (JADE) program. J Diabetes [Internet]. 2016;8(1):109–19. Available from: https://onlinelibrary.wiley.com/doi/10.1111/1753-0407.12262

10. Liu M, Lv X, Li Y, Li J, He Y. Prevalence and Control Status of Diabetes and Related Risk Factors Among 4196 Chinese Male Older Elderly Aged ≥80 Years. Int J Gerontol [Internet]. 2018;12(2):122–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1873959816302083
11. Wang H, Yao J, Yin X, Guo X, Yin J, Qu H, et al. Organisational and individual characteristics associated with glycaemic control among patients with type 2 diabetes: cross-sectional study in China. BMJ Open [Internet]. 2020;10(4):e036331. Available from: https://bmjopen.bmj.com/lookup/doi/10.1136/bmjopen-2019-036331
12. Irazola V, Rubinstein A, Bazzano L, Calandrelli M, Chung-Shiuan C, Elorriaga N, et al. Prevalence, awareness, treatment and control of diabetes and impaired fasting glucose in the Southern Cone of Latin America. Alway SE, editor. PLoS One [Internet]. 2017;12(9):e0183953. Available from: https://dx.plos.org/10.1371/journal.pone.0183953
13. Hu D, Fu P, Xie J, Chen C-S, Yu D, Whelton PK, et al. Increasing prevalence and low awareness, treatment and control of diabetes mellitus among Chinese adults: The InterASIA study. Diabetes Res Clin Pract [Internet]. 2008;81(2):250–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168822708001897
14. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci [Internet]. 2020;21(17):6275. Available from: https://www.mdpi.com/1422-0067/21/17/6275
15. Abdul-Ghani MA, Jayyousi A, DeFronzo RA, Asaad N, Al-Suwaidi J. Insulin Resistance the Link between T2DM and CVD: Basic Mechanisms and Clinical Implications. Curr Vasc Pharmacol [Internet]. 2019;17(2):153–63. Available from: http://www.eurekaselect.com/156313/article
16. Forbes JM, Cooper ME. Mechanisms of Diabetic Complications. Physiol Rev [Internet]. 2013;93(1):137–88. Available from: https://www.physiology.org/doi/10.1152/physrev.00045.2011
17. Niu G, Li J, Wang H, Ren Y, Bai J. Associations of A-FABP with Anthropometric and Metabolic Indices and Inflammatory Cytokines in Obese Patients with Newly Diagnosed Type 2 Diabetes. Biomed Res Int [Internet]. 2016;2016:1–6. Available from: https://www.hindawi.com/journals/bmri/2016/9s382092/
18. Urbanavičius V, Abalikšta T, Brimas G, Abraitienė A, Gogelienė L, Strupas K. Comparison of changes in blood glucose, insulin resistance indices, and adipokine levels in diabetic and nondiabetic subjects with morbid obesity after laparoscopic adjustable gastric banding. Medicina (B Aires) [Internet]. 2013;49(1):2. Available from: https://medicina.lsmuni.lt/med/1301/1301-02e.pdf.
19. Păunică I, Giurgiu M, Dumitriu AS, Păunică S, Pantea Stoian AM, Martu M-A, et al. The Bidirectional Relationship between Periodontal Disease and Diabetes Mellitus—A Review. Diagnostics [Internet]. 2023;13(4):681. Available from: https://www.mdpi.com/2075-4418/13/4/681
20. Brocker C, Thompson D, Matsumoto A, Nebert DW, Vasiliou V. Evolutionary divergence and functions of the human interleukin (IL) gene family. Hum Genomics [Internet]. 2010;5(1):30. Available from: https://doi.org/10.1186/1479-7364-5-1-30
21. Iyer SS, Cheng G. Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease. Crit Rev Immunol [Internet]. 2012;32(1):23–63. Available from: http://www.dl.begellhouse.com/journals/2ff21abf44b19838,76944bab5d-debfc1,2a93e3a12bdb71e5.html
22. van Exel E, Gussekloo J, de Craen AJM, Frölich M, Bootsma-van der Wiel A, Westendorp RGJ. Low Production Capacity of Interleukin-10 Associates With the Metabolic Syndrome and Type 2 Diabetes. Diabetes [Internet]. 2002;51(4):1088–92. Available from: https://diabetesjournals.org/diabetes/article/51/4/1088/34607/Low-Production-Capacity-of-Interleukin-10
23. Snijder MB, Heine RJ, Seidell JC, Bouter LM, Stehouwer CDA, Nijpels G, et al. Associations of Adiponectin Levels With Incident Impaired Glucose Metabolism and Type 2 Diabetes in Older Men and Women. Diabetes Care [Internet]. 2006;29(11):2498–503. Available from: https://diabetesjournals.org/care/article/29/11/2498/24584/Associations-of-Adiponectin-Levels-With--Incident
24. Hofmann SR, Rösen-Wolff A, Tsokos GC, Hedrich CM. Biological properties and regulation of IL-10 related cytokines and their contribution to autoimmune disease and tissue injury. Clin Immunol [Internet]. 2012;143(2):116–27. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1521661612000642
25. Jordan WJ, Eskdale J, Boniotto M, Lennon GP, Peat J, Campbell JDM, et al. Human IL-19 regulates immunity through auto-induction of IL-19 and production of IL-10. Eur J Immunol [Internet]. 2005;35(5):1576–82. Available from: https://onlinelibrary.wiley.com/doi/10.1002/eji.200425317
26. Ray M, Autieri M V. Regulation of pro- and anti-atherogenic cytokines. Cytokine [Internet]. 2019;122:154175. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1043466617302892
27. Jain S, Gabunia K, Kelemen SE, Panetti TS, Autieri M V. The Anti-Inflammatory Cytokine Interleukin 19 Is Expressed By and Angiogenic for Human Endothelial Cells. Arterioscler Thromb Vasc Biol [Internet]. 2011;31(1):167–75. Available from: https://www.ahajournals.org/doi/10.1161/ATVBAHA.110.214916

28. England RN, Autieri M V. Anti-Inflammatory Effects of Interleukin-19 in Vascular Disease. Int J Inflam [Internet]. 2012;2012:1–10. Available from: http://www.hindawi.com/journals/iji/2012/253583/
29. Li L, ZHeng-Qing Y, Juan-Yu H, Jian-Yong X, Fan L, Guang-Chun Z, et al. Association between interleukin-19 and angiopoietin-2 with vascular complications in type 2 diabetes. J Diabetes Investig [Internet]. 2016;7(6):895–900. Available from: https://onlinelibrary.wiley.com/doi/10.1111/jdi.12519
30. Li L, Yu Zh-Q, Qian L, Xu J-Y, Liu F, Zhao G-C, et al. Interleukin-19 and angiopoietin-2 can enhance angiogenesis of diabetic complications. J Diabetes Complications [Internet]. 2016;30(2):386–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1056872715004390
31. Chen J, Caspi RR, Chong WP. IL-20 receptor cytokines in autoimmune diseases. J Leukoc Biol [Internet]. 2018;104(5):953–9. Available from: https://academic.oup.com/jleukbio/article/104/5/953/6935471
32. Yaghini N, Mahmoodi M, Asadikaram GR, Hassanshahi GH, Khoramdelazad H, Arababadi MK. Serum levels of interleukin 10 (IL-10) in patients with type 2 diabetes. Iran Red Crescent Med J [Internet]. 2011;13(10):752. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3371882/
33. Giulietti A, van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile. Diabetes Res Clin Pract [Internet]. 2007;77(1):47–57. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168822706004736
34. Subramanian N, Tavira B, Hofwimmer K, Gutsmann B, Massier L, Abildgaard J, et al. Sex-specific regulation of IL-10 production in human adipose tissue in obesity. Front Endocrinol (Lausanne) [Internet]. 2022;13. Available from: https://www.frontiersin.org/articles/10.3389/fendo.2022.996954/full
35. Cartier A, Côté M, Lemieux I, Pérusse L, Tremblay A, Bouchard C, et al. Sex differences in inflammatory markers: what is the contribution of visceral adiposity? Am J Clin Nutr [Internet]. 2009;89(5):1307–14. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002916523237880
36. Camporez JP, Lyu K, Goldberg EL, Zhang D, Cline GW, Jurczak MJ, et al. Anti-inflammatory effects of oestrogen mediate the sexual dimorphic response to lipid-induced insulin resistance. J Physiol [Internet]. 2019;597(15):3885–903. Available from: https://physoc.onlinelibrary.wiley.com/doi/10.1113/JP277270
37. Shepherd R, Cheung AS, Pang K, Saffery R, Novakovic B. Sexual Dimorphism in Innate Immunity: The Role of Sex Hormones and Epigenetics. Front Immunol [Internet]. 2021;11. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2020.604000/full
38. Verthelyi D, Klinman DM. Sex hormone levels correlate with the activity of cytokine-secreting cells in vivo. Immunology [Internet]. 2000;100(3):384–90. Available from: https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2567.2000.00047.x
39. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The Role of Estrogens in Control of Energy Balance and Glucose Homeostasis. Endocr Rev [Internet]. 2013;34(3):309–38. Available from: https://academic.oup.com/edrv/article/34/3/309/2354631
40. Jgarkava M, Pantsulaia I, Rukhadze R, Karanadze N, Chikovani T. Association of il-10 and resistin in apparently healthy elderly population. Georg Med News [Internet]. 2021;318:119–24. Available from: https://www.researchgate.net/profile/Tinatin-Chikovani/publication/355250290_ASSOCIATION_OF_IL-10_AND_RESISTIN_IN_APPARENTLY_HEALTHY_ELDERLY_POPULATION/links/61950c7507be5f31b78ec9cc/ASSOCIATION-OF-IL-10-AND-RESISTIN-IN-APPARENTLY-HEALTHY-ELDERLY-POPULATI
41. Cuneo AA, Herrick D, Autieri M V. Il-19 reduces VSMC activation by regulation of mRNA regulatory factor HuR and reduction of mRNA stability. J Mol Cell Cardiol [Internet]. 2010;49(4):647–54. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022282810001793
42. Ellison S, Gabunia K, Kelemen SE, England RN, Scalia R, Richards JM, et al. Attenuation of Experimental Atherosclerosis by Interleukin-19. Arterioscler Thromb Vasc Biol [Internet]. 2013;33(10):2316–24. Available from: https://www.ahajournals.org/doi/10.1161/ATVBAHA.113.301521
43. England RN, Preston KJ, Scalia R, Autieri M V. Interleukin-19 decreases leukocyte-endothelial cell interactions by reduction in endothelial cell adhesion molecule mRNA stability. Am J Physiol Physiol [Internet]. 2013;305(3):C255–65. Available from: https://journals.physiology.org/doi/10.1152/ajpcell.00069.2013
44. Ouyang W, O'Garra A. IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation. Immunity [Internet]. 2019;50(4):871–91. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1074761319301372
45. Sabat R, Wolk K. Deciphering the role of interleukin-22 in metabolic alterations. Cell Biosci [Internet]. 2015;5(1):68. Available from: http://www.cellandbioscience.com/content/5/1/68

46. Herder C, Kannenberg JM, Carstensen-Kirberg M, Huth C, Meisinger C, Koenig W, et al. Serum levels of interleukin-22, cardiometabolic risk factors and incident type 2 diabetes: KORA F4/FF4 study. Cardiovasc Diabetol [Internet]. 2017;16(1):17. Available from: http://cardiab.biomedcentral.com/articles/10.1186/s12933-017-0498-6
47. Keir ME, Yi T, Lu TT, Ghilardi N. The role of IL-22 in intestinal health and disease. J Exp Med [Internet]. 2020;217(3). Available from: https://rupress.org/jem/article/217/3/e20192195/133771/The-role-of-IL-22-in-intestinal-health-and
48. Sabat R, Ouyang W, Wolk K. Therapeutic opportunities of the IL-22–IL-22R1 system. Nat Rev Drug Discov [Internet]. 2014;13(1):21–38. Available from: https://www.nature.com/articles/nrd4176
49. Fabbrini E, Cella M, Mccartney SA, Fuchs A, Abumrad NA, Pietka TA, et al. Association Between Specific Adipose Tissue CD4+ T-Cell Populations and Insulin Resistance in Obese Individuals. Gastroenterology [Internet]. 2013;145(2):366-374.e3. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0016508513005118
50. Dalmas E, Venteclef N, Caer C, Poitou C, Cremer I, Aron-Wisnewsky J, et al. T Cell–Derived IL-22 Amplifies IL-1β–Driven Inflammation in Human Adipose Tissue: Relevance to Obesity and Type 2 Diabetes. Diabetes [Internet]. 2014;63(6):1966–77. Available from: https://diabetesjournals.org/diabetes/article/63/6/1966/34320/T-Cell-Derived-IL-22-Amplifies-IL-1-Driven
51. Abdalla MMI. Advancing diabetes management: Exploring pancreatic beta-cell restoration's potential and challenges. World J Gastroenterol [Internet]. 2024;30(40):4339–53. Available from: https://www.wjgnet.com/1007-9327/full/v30/i40/4339.htm

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):59-65

RESEARCH ARTICLE

Endoscopic spine surgery: methodology and outcomes based on patient feedback

Mateusz Krakowiak[1], Damian Krystian Palus[1], Vishnu Prakash Mundayadan[2], Barbara Maria Myszkowska[1], Cezary Grochowski[3], Bartłomiej Kulesza[3], Ewelina Nadolska[4], Paweł Sokal[1]

[1] Department of Neurosurgery and Neurology, Jan Biziel University Hospital No. 2, Bydgoszcz, Poland
[2] Nicolaus Copernicus University, Collegium Medicum, Bydgoszcz, Poland
[3] The John Paul II Catholic University of Lublin Neurosurgery Clinic, Lublin, Poland
[4] Faculty of Medical Sciences, Kujawy and Pomorze University in Bydgoszcz, Bydgoszcz, Poland

Abstract

Background: Endoscopic lumbar decompression (ELD) is emerging as a minimally invasive alternative to traditional microdiscectomy, potentially offering faster recovery and reduced tissue damage. Our aim was to analyze clinical outcomes following the introduction of single-port ELD at our institution. **Material and methods:** A retrospective analysis of 28 consecutive patients undergoing single-port ELD (2019-2023) was conducted. Most surgeries involved the L5-S1 level (77.8%) and utilized a translaminar approach (85.7%). Primary outcomes included the Oswestry Disability Index (ODI) and Numerical Rating Scale (NRS) pain scores. **Results:** Mean ODI improved significantly from 65.1% pre-operative to 15.1% post-operative ($p < 0.0001$). Mean NRS scores improved from 8.33 to 2.36 ($p < 0.0001$). A minimal clinically important difference (MCID) was achieved in 92.9% of patients. Intraoperative conversions to open surgery occurred in 10.7% of cases. Post-operative complications were observed in 10.7% of patients (2 instances of paresis and 1 recurrence), with one case requiring reoperation. **Conclusions:** Single-port ELD yields substantial improvements in both functional outcomes and pain relief. However, moderate complication and conversion rates reflect the technical learning curve. Extended follow-up is required to assess long-term recurrence and durability.

Keywords: spine · spine surgery · neurosurgery · endoscopy

Citation

Krakowiak M, Palus DK, Mundayadan VP, Myszkowska BM, Grochowski C, Kulesza B, Nadolska E, Sokal P. Endoscopic spine surgery: methodology and outcomes based on patient feedback. Eur J Transl Clin Med. 2026;9(1):59-65.

DOI: 10.31373/ejtcm/221061

Corresponding author:
Vishnu Prakash Mundayadan, Nicolaus Copernicus University, Collegium Medicum, Bydgoszcz, Poland
e-mail: vishnuprakas.ncu@gmail.com
Available online: ejtcm.gumed.edu.pl

Introduction

Endoscopic lumbar decompression (ELD) has evolved considerably since Kambin and Gellman first described percutaneous lateral discectomy of the lumbar spine in 1983 [1]. Over the subsequent four decades, advances in optics, instrumentation, and surgical technique have transformed ELD from an experimental concept into a viable minimally invasive alternative to conventional open discectomy (OD) and microdiscectomy (MD) for the treatment of lumbar disc herniation and spinal stenosis [2-3]. Despite this progress, traditional techniques such as MD remain the predominant surgical method worldwide, as they are long-established and familiar to the majority of surgeons [4].

A growing body of evidence supports the benefits of ELD over conventional approaches. Comparative studies have demonstrated that ELD is associated with smaller incisions (often 10-16 mm), reduced paraspinal muscle disruption, less intraoperative blood loss, shorter operating times, and decreased post-operative pain compared to traditional microscopic discectomy [5-6]. These advantages lead to faster recovery, shorter hospital stays, and an earlier return to daily activities [7]. However, adoption of ELD remains limited due to the prolonged learning curve and the need for specialized instruments [3, 8]. For example, rigid endoscopy-assisted procedures require neurosurgeons to develop specialized skills for handling single-port endoscopes and adapting to two-dimensional video-guided navigation in confined surgical corridors (Figure 1) [9].

Despite these challenges, the continued development of less invasive surgical techniques has driven interest in ELD as the preferred alternative to MD and OD [10]. In this retrospective study, we aimed to assess the surgical outcomes of ELD and patient-reported feedback following the adoption of this technique at our institution.

Materials and methods

Patient selection and demographics

We retrospectively analyzed 28 consecutive patients with complete pre-operative and post-operative outcome data who underwent single-port ELD between January 2019 and December 2023. These 28 patients were selected from a total of 40 endoscopic lumbar procedures performed at our institution during this period by 3 neurosurgeons in the early phases of acquiring endoscopic surgical expertise (< 100 cumulative endoscopic cases at study initiation).

The 28-patient cohort consisted of 14 males (50.0%) and 14 females (50.0%), with a mean age of 46.1 ± 8.8 years (range, 26-58 years). The following levels of the spine were operated: L5-S1 (n = 21, 77.8%), L4-L5 (n = 5, 18.5%), and L3-L4 (n = 1, 3.7%). One patient's operative level was not recorded in the database and was excluded from level-specific analysis (n = 27 for level analysis).

Inclusion criteria

The patient inclusion criteria were: age (≥ 18 years at time of surgery), primary lumbar disc herniation confirmed on magnetic resonance imaging (MRI), lumbar spinal stenosis (with or without associated disc herniation) confirmed on MRI, radiculopathy or neurogenic claudication refractory to conservative management (≥ 6 weeks of non-operative treatment, including physical therapy and/or pharmacologic management), ODI score > 40% at baseline, sufficient cognitive and language capacity to independently complete standardized outcome questionnaires during the pre-operative and post-operative assessments.

Exclusion criteria

The exclusion criteria were: recurrent disc herniation at the same operative level, spinal instability (spondylolisthesis > Grade 1, spondylolysis with translation, or scoliosis > 20°), prior surgical intervention at the same spinal level as the one considered for endoscopic treatment, cauda equina syndrome presenting as an emergency requiring urgent decompression, severe systemic comorbidities (uncontrolled cardiac arrhythmia, severe pulmonary dysfunction, severe hepatic disease, uncontrolled diabetes), and active coagulopathy (or anticoagulation therapy that could not be safely discontinued perioperatively).

Anesthesia and positioning

Patients were positioned prone on a radiolucent operating table under general endotracheal anesthesia. Neuromonitoring (including transcranial motor evoked potentials (tcMEP) and somatosensory evoked potentials (SSEP)) was recorded from bilateral lower extremities to assess nerve root and spinal cord function throughout the procedure. Vital and peri-operative parameters (including continuous electrocardiography, pulse oximetry, end-tidal capnography and core temperature) were monitored in accordance with the American Society of Anesthesiologists (ASA) standards.

Surgical technique and operative parameters

All procedures utilized a single-port rigid endoscopic approach. A rigid 4-mm single-port endoscope with integrated

visualization and bimanual working channels (Karl Storz SE & Co. KG, Tuttlingen, Germany) was connected to a high-definition video system and an appropriate illumination source. Surgeon preference, anatomical considerations, and the site of pathology determined the selection of surgical approach (transforaminal vs. translaminar). Four patients (14.3%) were operated via transforaminal approach, while the translaminar approach was chosen in 24 patients (85.7%)

Translaminar approach (n = 24)

Following skin sterilization, a small midline skin incision (approximately 10-16 mm) was made over the lumbodorsal (thoracolumbar) fascia at the affected spinal level under fluoroscopic guidance. The fascia was incised, and sequential dilation of the interlaminar space was performed using dilators. The endoscope was advanced into the interlaminar space, providing direct visualization of the ligamentum flavum, dura mater, nerve root, and any herniated disc material or osteophytes. Endoscopic decompression was achieved through the removal of ligamentous and osseous structures using endoscopic graspers, curettes, and a 3000 RPM endoscopic burr under direct visualization. Hemostasis was maintained using bipolar radiofrequency coagulation. Adequate decompression was confirmed by direct visualization of the nerve root and traversing dura without compression.

Transforaminal approach (n = 4)

Following fluoroscopic localization of the target foramen, a small lateral skin incision was made. Sequential dilators (6 mm and 8 mm) were used to dilate the intervertebral foramen through the paravertebral musculature under fluoroscopic guidance in a progressive manner. The endoscope was advanced through the dilators, permitting visualization of the foraminal anatomy, exiting nerve root, and any herniated disc material within the foramen or subarticular space. Endoscopic decompression involved the removal of disc material and osteophytes compressing the nerve root, followed by hemostasis with bipolar coagulation.

Intraoperative conversion

If adequate decompression could not be achieved via the endoscopic approach (due to technical factors or anatomical constraints), conversion to open microscopic or open surgical approach was planned and documented. Three patients (10.7%) underwent intraoperative conversion: 2 to open microscopic decompression and 1 to wide surgical decompression.

Follow-up assessment

Post-operative clinical assessments were conducted at standardized intervals: at post-operative week 2 (wound assessment), week 6 (clinical evaluation), week 12 (clinical and functional assessment), and at final follow-up (mean 4.5 months, range 3-6 months). Imaging included post-operative MRI at a mean of 6 weeks to assess adequacy of neural decompression, exclude recurrent or persistent herniation, and rule out complications.

Outcome measurement

Primary outcome measures

We used the Oswestry Disability Index (ODI) version 2.0, a 10-item condition-specific disability questionnaire that quantifies functional limitations attributable to low back pain [11]. Scores range from 0 to 100%, with higher scores indicating greater functional disability. Standard disability categories are defined as follows: 0-20% (minimal disability), 21--40% (moderate disability), 41-60% (severe disability), 61-80% (very severe disability), and 81-100% (bedbound/extreme functional impairment).

To standardize the patients' assessment of lower back and radicular pain intensity, we used the 11-point Numerical Rating Scale (NRS), ranging from 0 (no pain) to 10 (worst possible pain) [12]. The minimal clinically important difference (MCID) for NRS in lower extremity pain is generally considered to be ≥ 2.0 points [13].

The ODI and NRS questionnaires were administered in a structured format using a standardized script to minimize interviewer variability. Patients were asked to respond to each item independently without guidance or suggestion.

Secondary outcome measures

Secondary outcomes included: return to work status (percentage of patients returning to baseline occupation or equivalent duties), resumption of activities of daily living without significant limitation, and patient satisfaction with surgical outcome, (assessed using a 5-point Likert scale: very dissatisfied, dissatisfied, neutral, satisfied, very satisfied) [14]. Structured interviews were administered by trained research staff at baseline (pre-operatively) and at final follow-up (mean 4.5 months post-operatively).

Results

Primary outcome: disability (Oswestry Disability Index)

Table 1 presents the ODI scores before and after ELD. The mean change in the ODI score from baseline to post-operative assessment was 50.0 percentage points (95% CI: 44.3--55.7). A paired t-test demonstrated that this change was statistically significant: $t(27) = 27.3$, $p < 0.0001$, representing a large effect size (Cohen's $d = 5.14$). Table 2 presents the distribution of patients across ODI disability categories before and after surgery (see Figures 2.1 and 2.2). This represents a clinically and statistically significant shift toward lower disability categories, with 82.1% of patients achieving minimal disability status post-operatively compared to 0% pre-operatively.

Primary outcome: pain (Numerical Rating Scale)

The mean reduction in pain was 5.97 points on the NRS scale (95% CI: 5.09-6.85). A paired t-test demonstrated statistical significance: $t(27) = 19.2$, $p < 0.0001$, representing a large effect size (Cohen's $d = 3.64$). In the current cohort, 26 of 28 patients (92.9%) met the MCID threshold of ≥ 2.0 points, indicating substantial clinically meaningful improvement in pain (Table 3 and Figure 3).

Secondary outcomes: functional recovery and satisfaction

Of the 28 patients, 24 (85.7%) reported return to their baseline occupation or equivalent employment duties by final follow-up assessment. Twenty-six of 28 patients (92.9%) reported the successful resumption of activities of daily

Table 1. Summary of Oswestry Disability Index (ODI) scores before and after endoscopic lumbar decompression (n = 28)

Parameter	Pre-operative	Post-operative
Range	41-82%	2-48%
Mean ± SD	65.1% ± 11.2%	15.1% ± 14.6%
95% CI	60.8-69.4%	9.8-20.4%
Median	68%	10%

Table 2. Distribution of patients by ODI disability category before and after endoscopic lumbar decompression (n = 28)

Disability category	Pre-operative n (%)	Post-operative n (%)
Minimal (0-20%)	0 (0%)	23 (82.1%)
Moderate (21-40%)	5 (17.9%)	5 (17.9%)
Severe (41-60%)	7 (25.0%)	0 (0%)
Very severe (61-80%)	12 (42.9%)	0 (0%)
Bedbound (81-100%)	4 (14.3%)	0 (0%)
Total	28 (100%)	28 (100%)

Table 3. Summary of Numerical Rating Scale (NRS) pain scores before and after endoscopic lumbar decompression (n = 28)

Parameter	Pre-operative	Post-operative
Range	6-10	0-6
Mean ± SD	8.33 ± 1.69	2.36 ± 2.44
95% CI	7.85-8.81	1.39-3.33
Median	8.0	2.0

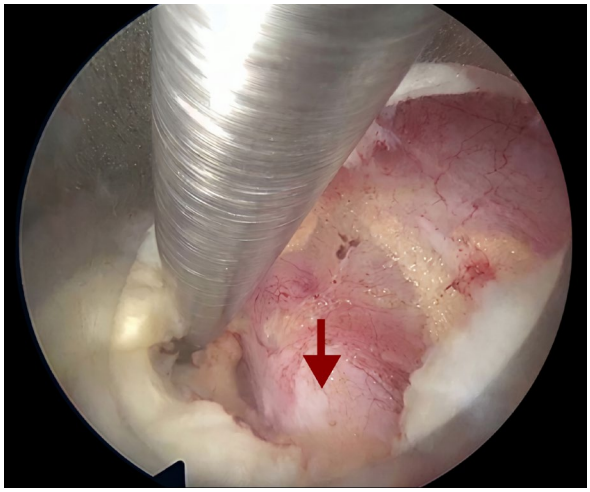


Figure 1. A compressed nerve root (red arrow) in the endoscopic view

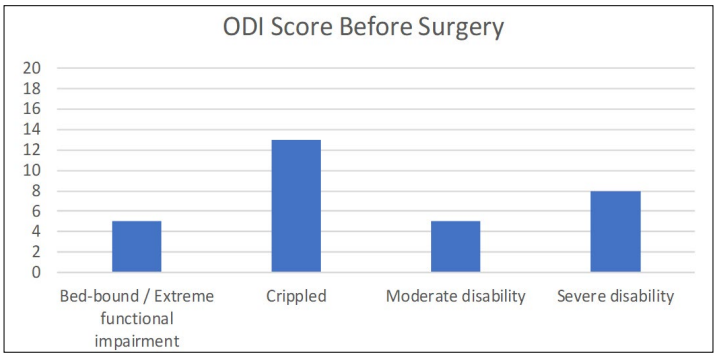


Figure 2.1. Preoperative Oswestry Disability Index (ODI) scores recorded before ELD (n = 28)

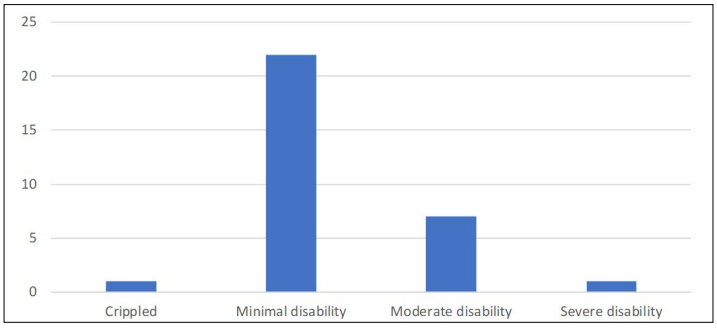


Figure 2.2. ODI Score after ELD

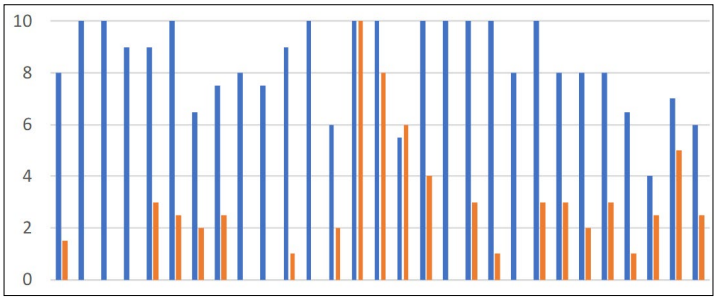


Figure 3. Chart comparing patient self-assessment of pain on the numerical rating scale (NRS) before and after (ELD) (n = 28)
blue bars – NRS assessment before ELD, orange bars – NRS assessment after ELD

living (including household activities, self-care, and personal hygiene) without significant functional limitations at final follow-up.

Patient satisfaction, assessed via a Likert scale, was highly favorable: 26 of 28 patients (92.9%) reported being satisfied or very satisfied with the procedure, 2 of 28 patients (7.1%) reported neutral satisfaction, and zero patients (0%) reported dissatisfaction.

Discussion

In this retrospective study we observed significant improvements in both functional disability and pain outcomes of 28 patients, who underwent single-port ELD during the early adoption phase of this procedure at our institution. Mean ODI scores decreased by 50.0 percentage points (from 65.1% to 15.1%, $p < 0.0001$), with 82.1% of patients transitioning from severe or very severe disability categories to minimal disability post-operatively. Similarly, mean NRS pain scores decreased by 5.97 points (from 8.33 to 2.36, $p < 0.0001$), with 92.9% of patients achieving the MCID threshold. These results demonstrate that even when performed by surgeons in the early stages of their learning curve, the ELD procedure can produce clinically meaningful improvements in patient-reported outcomes.

Our findings are consistent with those reported by Leyendecker et al., who demonstrated significant early improvements in both ODI and NRS scores after full endoscopic spine surgery in a larger cohort [7]. The magnitude of improvement observed in our study (Cohen's $d = 5.14$ for ODI and 3.64 for NRS) indicates large effect sizes, suggesting that the clinical benefits of ELD are substantial and not merely statistically significant. The high rate of return to work (85.7%) and patient satisfaction (92.9%) further supports the clinical effectiveness of this approach.

However, equally notable are the 10.7% intraoperative conversion rate and the 10.7% complication rate (2 instances of post-operative paresis and 1 recurrence) in our series. Both rates are higher than those reported in large-volume centers with experienced endoscopic surgeons and likely reflect the early learning curve of our surgical team [5-6]. Sun et al. have previously documented that complication rates decrease as surgeons progress along the

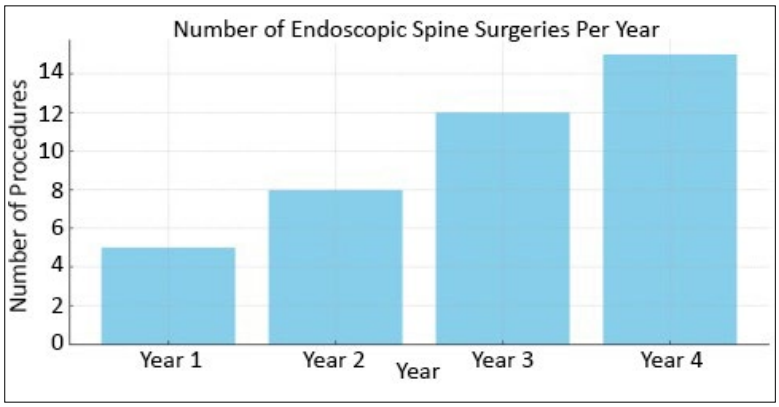


Figure 4. The increasing number of endoscopic spine surgeries performed by our team
This upward trend reflects the learning curve associated with the adoption of the technique, as well as the growing experience and confidence of the surgical team in performing these procedures.

learning curve for percutaneous endoscopic lumbar discectomy [8]. Similarly, Kang et al. found that outcomes of endoscopic surgery improve significantly with increasing surgeon experience [4]. Our surgeons reported subjective improvement in their endoscopic skills during the study period, however, we lacked specific analytical data, such as changes in operating time, to objectively quantify this progression.

The long-term recurrence rate of disc-related pathology following endoscopic procedures remains an important area of ongoing investigation. Several studies have reported recurrence rates ranging from 3% to 12% [15-17], but extended follow-up data beyond 5 years remain limited [18].

Study limitations

Our study has several limitations that should be acknowledged when interpreting the results. The small sample size (n = 28) limits the generalizability of our findings to larger patient populations. Although the data shows promising improvements in both pain and disability, larger cohorts are needed to confirm these findings with more robust statistical power.

We tracked outcomes during a short post-operative period (mean 4.5 months), and therefore we did not collect long-term follow-up data (e.g. recurrence) of disc-related issues or lasting functional improvements. We assessed cases from a single center, focusing on the experiences of a particular surgical team. Therefore, our findings may not apply to other settings with different levels of ELD expertise or resources.

Patient-reported outcome measures (e.g. the ODI and NRS) may demonstrate only subjective improvements. We were unable to assess objective functional tests or radiological images systematically, which could have enhanced the thoroughness and relevance of the results.

The early stage of adopting ELD at our center involved a significant learning curve (Figure 4). Operator experience and skill levels during this initial period could have influenced the surgical results (e.g. differences in surgery duration, complication rates) and patient outcomes. We lacked specific data to quantify the surgeons' actual improvement on this procedure's learning curve (e.g. decrease in operating time over the years), therefore, our findings regarding learning curve progression were based solely on the surgeons' self-reported opinions.

To confirm these preliminary findings and to better understand the full scope and limits of ELD, multicenter studies with larger cohorts, standardized methods, and extended follow-up are needed.

Conclusions

Single-port ELD yielded substantial and statistically significant improvements in both functional outcomes and pain relief in this cohort of 28 patients, as demonstrated by a 50.0 percentage-point mean reduction in ODI scores and a 5.97-point mean reduction in NRS pain scores, with 92.9% of patients achieving the MCID threshold. However, the moderate complication rate (10.7%) and intraoperative conversion rate (10.7%) reflect the challenges associated with the early stages of the technical learning curve. Extended follow-up with larger, multicenter cohorts is required to assess long-term recurrence rates, durability of clinical outcomes, and the trajectory of the learning curve for this procedure.

Funding

None.

Conflicts of interest

None to report.

References

1. Kambin P, Gellman H. Percutaneous Lateral Discectomy of the Lumbar Spine A Preliminary Report. Clin Orthop Relat Res [Internet]. 1983;174:127–32. Available from: https://journals.lww.com/corr/fulltext/1983/04000/percutaneous_lateral_discectomy_of_the_lumbar.17.aspx
2. Mayer HM, Brock M. Percutaneous endoscopic discectomy: surgical technique and preliminary results compared to microsurgical discectomy. J Neurosurg [Internet]. 1993;78(2):216–25. Available from: https://thejns.org/view/journals/j-neurosurg/78/2/article-p216.xml
3. Kim M, Kim H-S, Oh SW, Adsul NM, Singh R, Kashlan ON, et al. Evolution of Spinal Endoscopic Surgery. Neurospine [Internet]. 2019;16(1):6–14. Available from: http://e-neurospine.org/journal/view.php?doi=10.14245/ns.1836322.161
4. Kang K-B, Shin Y-S, Seo E-M. Endoscopic Spinal Surgery (BESS and UESS) Versus Microscopic Surgery in Lumbar Spinal Stenosis: Systematic Review and Meta-Analysis. Glob Spine J [Internet]. 2022;12(8):1943–55. Available from: https://journals.sagepub.com/doi/10.1177/21925682221083271
5. Yang X, Wu Z. Comparison of postoperative outcomes between endoscopic and traditional lumbar spine surgery. Spine J. 2018;18(4):684–690. DOI: 10.1016/j.spinee.2017.08.260
6. Ruetten S, Komp M, Merk H, Godolias G. Full-Endoscopic Interlaminar and Transforaminal Lumbar Discectomy Versus Conventional Microsurgical Technique. Spine (Phila Pa 1976) [Internet]. 2008;33(9):931–9. Available from: http://journals.lww.com/00007632-200804200-00002
7. Leyendecker J, Prasse T, Park C, Payne C, Rückels P, Bieler E, et al. Pain alleviation and functional improvement: ultra-early patient-reported outcome measures after full endoscopic spine surgery. J Neurosurg Spine [Internet]. 2024;40(4):465–74. Available from: https://thejns.org/view/journals/j-neurosurg-spine/40/4/article-p465.xml
8. Sun B, Shi C, Xu Z, Wu H, Zhang Y, Chen Y, et al. Learning Curve for Percutaneous Endoscopic Lumbar Diskectomy in Bi-needle Technique Using Cumulative Summation Test for Learning Curve. World Neurosurg [Internet]. 2019;129:e586–93. Available from: https://www.sciencedirect.com/science/article/pii/S1878875019315049
9. Chung AS, Wang JC. The Rationale for Endoscopic Spinal Surgery. Neurospine [Internet]. 2020;17(Suppl 1):S9–12. Available from: http://e-neurospine.org/journal/view.php?doi=10.14245/ns.2040104.052
10. Kim H-S, Wu PH, Jang I-T. Commentary on "Complications and Management of Endoscopic Spinal Surgery". Neurospine [Internet]. 2023;20(1):78–9. Available from: http://e-neurospine.org/journal/view.php?doi=10.14245/ns.2346308.154
11. Fairbank JCT, Pynsent PB. The Oswestry disability index. Spine (Phila Pa 1976) [Internet]. 2000;25(22):2940–53. Available from: https://journals.lww.com/spinejournal/_layouts/15/oaks.journals/downloadpdf.aspx?an=00007632-200011150-00017
12. Hjermstad MJ, Fayers PM, Haugen DF, Caraceni A, Hanks GW, Loge JH, et al. Studies Comparing Numerical Rating Scales, Verbal Rating Scales, and Visual Analogue Scales for Assessment of Pain Intensity in Adults: A Systematic Literature Review. J Pain Symptom Manage [Internet]. 2011;41(6):1073–93. Available from: https://www.sciencedirect.com/science/article/pii/S0885392411000145
13. Copay AG, Glassman SD, Subach BR, Berven S, Schuler TC, Carreon LY. Minimum clinically important difference in lumbar spine surgery patients: a choice of methods using the Oswestry Disability Index, Medical Outcomes Study questionnaire Short Form 36, and Pain Scales. Spine J [Internet]. 2008;8(6):968–74. Available from: https://www.sciencedirect.com/science/article/pii/S1529943007010017
14. Likert R. A technique for the measurement of attitudes. Arch Psychol [Internet]. 1932 [cited 2018 Nov 22];22(140):55. Available from: http://psycnet.apa.org/record/1933-01885-001
15. Yin S, Du H, Yang W, Duan C, Feng C, Tao H. Prevalence of recurrent herniation following percutaneous endoscopic lumbar discectomy: a meta-analysis. Pain Physician [Internet]. 2018;21(4):337. Available from: https://www.painphysicianjournal.com/current/pdf?article=NTMwMw%3D%3D&journal=112
16. Park CH, Park ES, Lee SH, Lee KK, Kwon YK, Kang MS, et al. Risk factors for early recurrence after transforaminal endoscopic lumbar disc decompression. Pain Physician [Internet]. 2019;22(2):E133. Available from: https://www.painphysicianjournal.com/current/pdf?article=NjIxNw%3D%3D&journal=119
17. Tang T, Liu J, Cao J, He D, Cheng X, Xie S. Risk Factors and Causes of Reoperation in Lumbar Disc Herniation Patients after Percutaneous Endoscopic Lumbar Discectomy: A Retrospective Case Series with a Minimum 2-Year Follow-Up. Med Sci Monit [Internet]. 2023;29:e939844. Available from: http://www.ncbi.nlm.nih.gov/pubmed/37580900
18. Yang X, Zhang S, Su J, Guo S, Ibrahim Y, Zhang K, et al. Comparison of Clinical and Radiographic Outcomes Between Transforaminal Endoscopic Lumbar Discectomy and Microdiscectomy: A Follow-up Exceeding 5 Years. Neurospine [Internet]. 2024;21(1):303–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/38317550

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):66-72

RESEARCH ARTICLE

Influence of whey protein and creatine supplementation on liver and kidney function

Sabah Subhi Ismael Barani, Atyaf Talal Mahmood, Islam Khalid Kamal

Department of Clinical Laboratory Sciences, College of Pharmacy, University of Mosul, Iraq

Abstract

Background: Due to their availability and purported benefits for muscle growth and athletic performance, the use of whey protein and creatine supplements is widespread among recreational and amateur athletes. **Material and methods:** 600 gym-goers (24-52 years of age) were divided into 3 groups based on their dietary supplement use: none (group A), moderate amounts (group B) and high doses (group C). Biochemical analysis of kidney and liver function was performed. **Results:** High-dose supplementation significantly increased serum creatinine, serum urea and liver enzymes (AST, ALT) and bilirubin levels compared with the control and medium-dose groups. Group C showed the highest creatinine (1.33 ± 0.216 mg/dL) and serum urea (55.31 ± 6.49 mg/dL), suggesting a possible association with renal function biomarkers. Similarly, AST and ALT levels were significantly increased in supplement users (particularly in group C), suggesting increased transient variations in hepatic enzyme activity. **Conclusions:** Our findings highlight a dose-dependent effect of whey protein and creatine supplementation on liver and kidney function, and emphasize the importance of responsible dietary supplement use and periodic monitoring of biochemical markers.

Keywords: liver enzyme · kidney function · whey protein · creatine supplement · gym-goers

Citation

Barani SSI, Mahmood AT, Kamal IK. Influence of whey protein and creatine supplementation on liver and kidney function. Eur J Transl Clin Med. 2026;9(1):66-72.

DOI: 10.31373/ejtcm/219510

Corresponding author:
Sabah Subhi Ismael Barani, Department of Clinical Laboratory Sciences, College of Pharmacy, University of Mosul, Iraq
e-mail: sabah.barani@uomosul.edu.iq
Available online: ejtcm.gumed.edu.pl

Introduction

Whey protein (WP) in particular, a component of milk protein, has become popular for muscle building and weight management [1]. Despite the growing interest in nutritional supplementation among physically active people, data on the use of exercise supplements are limited [2]. It is estimated that 32-90% of athletes use dietary supplements [3].

Proteins such as β-lactoglobulin, α-lactalbumin, immunoglobulins and bovine serum albumin, which are obtained from the liquid by-product of milk following cheese production through enzymatic coagulation and subsequent purification processes [4]. These include many of the well-known branched-chain amino acids of WP. Studies show that WP is the most popular protein supplement among athletes, contributing to protein synthesis, increased lean muscle mass, and carbohydrate metabolism [5].

Many physically active people use WP without medical guidance and few studies examine the safety and adverse effects of such supplements. Excessive WP intake can lead to periodontal disease, diarrhea, microbial dysfunction and changes in kidney and liver metabolism [6]. It is necessary to differentiate the outcomes derived from animal studies from those observed in patients with pre-existing illnesses and in healthy people. Most reported adverse renal or hepatic effects from high protein or creatine intake come from animal studies or patients already having existing illnesses related to the kidneys or the liver, while there are inconsistent data from healthy recreational athletes or active individuals among whom no significant changes were observed [7-8].

Post-exercise protein intake is necessary to increase and balance muscle protein synthesis, supporting hypertrophy. Taking 40 g of casein before sleep improves nocturnal protein synthesis and recovery compared to carbohydrates, this process can increase muscle strength and growth over time [9]. High dietary protein intake can lead to kidney problems, e.g. hyperfiltration, glomerular injury, and proteinuria, which can lead to chronic kidney disease (CKD) Animal protein diet is the highest risk factor end-stage kidney disease (ESKD) is associated due to factors such as acid load and inflammation It should be the former, especially for those at risk [10].

Although though high protein intake has been associated with elevated glomerular filtration rate and a greater workload of the kidney, there is current evidence indicating this does not necessarily result in kidney injury among healthy individuals. This could be the case among those who already have kidney problems, though [11].

Creatine, a compound composed of the amino acids arginine methionine and glycine, is produced through diet and metabolism. Creatine supplements are a promising ergogenic adjuvant that enhance athletic performance during short-term intense exercise and are widely used by both professional athletes and the general public [12]. Consistent reports show that creatine supplementation increases muscle creatine and phosphocreatine levels [13]. This increases the availability of phosphocreatine, increases the cellular efficiency of the phosphogenic system and facilitates the transport of high-energy phosphate between the cytosol and the mitochondria through creatine phosphate interactions [14].

In some cases, people need to get enough creatine from their diet to meet a certain level of nutritional needs [15]. Dietary creatine is generally considered safe, although excessive levels can cause gastrointestinal discomfort. Some preclinical studies and data suggest that exogenous creatine may adversely affect liver function by increasing liver enzymes, exacerbating ethanol-induced alteration in liver enzyme markers, and potentially leading to changes in hepatic biomedical parameters [16]. However, the effect of dietary creatine on biomarkers of liver health in the general population remains unclear. The indiscriminate use of creatine supplements has raised safety concerns, particularly with respect to possible changes in the liver and kidneys [17].

Although WP or creatine supplementation at recommended dosages does not cause significant changes in the liver or kidney function of healthy subjects, there are evolving data implicating high doses of these supplements in detectable changes in serum creatinine, urea, and liver enzyme levels [18]. Accordingly, the purpose of our study was to assess the influence of WP and creatine supplementation on the chosen liver and kidney function parameters in healthy participants, especially focusing on the differences between physiological and clinically significant values.

Material and methods

This cross-sectional analytical study was carried out in Ninawa (Iraq), from September 2023 to April 2024. The participants regularly trained at gyms in different locations within the city. They were interviewed to respond to the questionnaires directly, supplement intake was assessed using a self-reported questionnaire. The questions focused on the quantity of taking supplement and other types of food taken daily. Information regarding diet was collected using a 24-hour recall procedure conducted on 3 non-consecutive days, consisting of 2 weekdays and 1 weekend day. Total daily protein intake was calculated by adding protein obtained from diet and protein supplements.

This was followed by biochemical tests to investigate the liver and kidney function. Ethical approval for this study was obtained from the Collegiate Committee for Medical Research Ethics at the University of Mosul. Informed consent was obtained from the participants.

Participants

All participants (600) in this study were male, 24 to 52 years of age who regularly trained at the gym 6 days/week, averaging 1 hour per day. Resistance training comprised the major part of their exercise. To avoid confounding by training, gym-goers who reported changes in training intensity, volume or type during the preceding month were not recruited. The rest of the exclusion criteria were as follows: females, coaches, professional athletes, hormone supplement use, chronic health conditions (e.g. diabetes, liver and kidney disease). The participants were divided into 3 equal groups. Group A included participants who did not consume WP and creatine supplements. Those in group B taking 30 grams (g) of WP and 5 g creatine, and group C taking 45 g of WP and 7.5 g creatine. The participants in groups B and C were requested to use WP and creatine supplements at least 8 weeks before blood sample collection. This ensured that the supplementation usage period was sufficient enough to allow any biochemical changes to occur.

Biochemical analysis

Consumption of total protein was determined using 24 hours recall combined with a meal replacement list. Body mass index (BMI) was calculated using the standard formula (weight (kg)/height (m^2)). A total of 5 ml of venous blood was collected, left to clot and centrifuged to obtain serum for biochemical analysis of liver function tests (AST, ALT, total bilirubin and direct bilirubin and kidney function tests (serum creatinine and urea). All participants were instructed to maintain their usual hydration status and to avoid vigorous exercise and alcohol consumption 24 hours prior to blood sampling.

Statistical analysis

The study data were analyzed using the SPSS software (version 24, IBM Corp., Armonk, USA). Normality for continuous variables was determined using the Shapiro-Wilk test. The homogeneity of variance assumption was checked by Levene's test. Variables within groups were compared using the chi-square test. Continuous variable of liver and kidney function served as depended variables and were compared by t-test. In situations requiring comparison of more than two groups, one-way ANOVA, combined with post hoc Tukey's test, was used to account for multiple comparison issues. Differences were considered statistically significant at $p < 0.05$. For continuous variables, the effect size in terms of Cohen's d was determined together with the 95% confidence interval.

Results

Age, weight, height and BMI of all participants are shown in Table 1 and no statistically significant differences between them were observed.

Kidney function

Our results demonstrated that the mean level of serum creatinine in the A, B and C groups were 0.965 ± 0.143 mg/dL, 1.011 ± 0.172 mg/Dl and 1.33 ± 0.216 mg/dL, respectively. This reveals that the participants in group A (who did not consume WP and creatine supplements) had normal serum creatinine. However, group B showed a significant increase in serum creatinine compared with group A. In addition, comparing with group A and B, those in group C had significantly higher levels of serum creatinine (Table 2). Such high serum creatinine levels may in part represent an association with muscle mass or creatine-produced creatinine, which are common in physically active people and not indicative of renal biomarker changes [7].

In addition, the mean level of serum urea in A, B and C groups were 41.52 ± 5.92 mg/dL, 43.87 ± 5.64 mg/dL and 55.31 ± 6.49 mg/dL, respectively. There was only a significant increase in serum urea in group C in comparison with both group A and B as shown in Table 3. Although there were significant differences in the levels of serum creatinine and urea, their results have been cautiously interpreted in the context of exercise, muscle mass and creatine supplements.

Liver function tests

The mean level of serum AST in groups B and C was higher (42.36 ± 1.82 U/L and 47.41 ± 1.58 U/L, respectively) than in group A (32.17 ± 2.12 U/L) (Table 4). In addition, the mean level of serum ALT was also elevated in groups B and C were (40.47 ± 1.23 U/L and 42.54 ± 1.64 U/L, respectively), compared to group A (33.65 ± 1.41 U/L). Groups B and C had significantly higher ALT than the group A, whilst no significant difference was found between groups B and C (Table 4). Furthermore, the mean levels of total bilirubin in groups A, B and C were 0.98 ± 0.07 mg/dL, 1.55 ± 0.1 mg/dL, 1.95 ± 0.12 mg/dL, respectively. The differences in total bilirubin between groups B and A as well as C and A were statistically significant. In addition, significant difference was also noted between groups B and C, as shown in the Table 5.

Discussion

Our findings provide an insight into the effects of WP and creatine supplements on kidney and liver function in

Table 1. Comparisons of mean age, height, weight and BMI among the 3 groups of participants

	Mean ± SD		
	Group A	Group B	Group C
Age (years)	29.34 ± 8.82	28.61 ± 7.93	27.88 ± 7.69
Height (cm)	171.64 ± 9.45	174.13 ± 6.58	172.96 ± 8.33
Weight (kg)	78.37 ± 11.24	81.67 ± 10.39	80.91 ± 12.73
BMI (kg/m²)	26.49 ± 3.97	26.97 ± 3.18	26.72 ± 4.11

Table 2. Renal function parameters in the 3 groups of participants

	Mean ± SD		
	Group A	Group B	Group C
Serum creatinine (mg/dl)	0.965 ± 0.143	1.011 ± 0.172	1.33 ± 0.216
Serum urea (mg/dl)	41.52 ± 5.92	43.87 ± 5.64	55.31 ± 6.49

Table 3. The p value for each pair ways tow sample t-test for each of the parameters (serum urea and serum creatinine)

	Groups		
	A vs. B	A vs. C	B vs. C
Serum urea	$p = 0.5227$	$p < 0.0000015$	$p < 0.0000216$
Serum creatinine	$p = 0.5875$	$p < 0.0001$	$p < 0.0001$

Table 4. Liver function parameters in the 3 groups of participants

Liver function test	Mean ± SD		
	Group A	Group B	Group C
AST (U/L)	32.17 ± 2.12	42.36 ± 1.82	47.41 ± 1.58
ALT (U/L)	33.65 ± 1.41	40.47 ± 1.23	42.54 ± 1.64
Total bilirubin (mg/dL)	0.98 ± 0.07	1.55 ± 0.1	1.95 ± 0.12
Direct bilirubin (mg/dL)	0.106 ± 0.015	0.322 ± 0.119	0.391 ± 0.029

Table 5. The p value for each paired two sample t-test for each of the parameters (AST, ALT, total bilirubin and direct bilirubin)

Parameter	Groups		
	A vs. B	A vs. C	B vs. C
AST	0.001084	0.00000365	0.050937
ALT	0.003858	0.0000567	0.321044
Total bilirubin	0.000077	0.000000855	0.016813
Direct bilirubin	0.000715	0.0000000162	0.00011

gym-goers. Although the consumption of WP and creatine and their effect on kidney and liver function are still controversial, same reports found that WP protects against hypertension and a wide range of metabolic disease (e.g. dyslipidemia, type 2 diabetes and obesity) [6]. Similarly to other studies, our data suggests that low dose supplementation of WP and creatine has no effect on the kidney and liver function [19].

The significantly increased levels in group C suggest that high-dose supplementation may increase creatinine production and retention, potentially due to increased muscle metabolism or decreased renal clearance. Higher creatinine level may also represent a higher muscle mass or creatinine production from creatine supplements and not necessarily renal functional biomarkers changes, particularly in people with high activity physical levels [7]. Elevated creatinine levels were associated with increased renal biomarkers due to high protein intake, which is consistent with findings in previous research demonstrating the potential effect of excessive WP supplementation on kidney function [20-21].

In addition, serum urea levels had a similar pattern in the groups. While there were no significant differences between groups A and B, group C showed significantly higher levels, indicating altered kidney function probably due to increased nitrogenous waste from higher protein metabolism, as confirmed by earlier studies. Urea (or blood urea nitrogen, BUN) is a nitrogen-containing substance produced in the liver during the urea cycle as a byproduct of protein metabolism. Around 85% of urea removed by the kidneys, while the remainder is excreted through the gastrointestinal system [20-22].

Elevations of liver enzymes (AST) and ALT levels were observed in groups B and C. These results suggest that WP and creatine use are associated with increased liver enzyme activity, indicating transient variations in liver biochemical markers. Previous reports associate WP intake with minor changes in liver enzymes, particularly in individuals with underlying disease or who excessively consume WP [1]. In addition, both total and direct bilirubin levels were significantly increased in groups B and C compared with group A, suggesting a dose-dependent effect of supplementation on bilirubin metabolism. Our findings are consistent with previous studies that caution against unsupervised supplement use due to possible disruption of liver detoxification pathways [22-23].

The liver plays a central role in protein metabolism, particularly in the deamination and transamination processes that break down amino acids. Supplementation with WP and creatine results in increased availability of amino acids in the bloodstream, requiring the liver to metabolize these excess substrates. This increased metabolic activity may increase the expression and release of liver enzymes such as AST and ALT into the bloodstream, as they are markers of hepatocyte activity or damage [24]. Furthermore, the elevation of transaminases secondary to exercise and/or WP and creatine supplementation also demands consideration, as these are not always indicative of hepatocellular damage in physically active populations [25]. Creatine, if consumed in excess, undergoes metabolism to form creatinine, primarily in the liver. This additional metabolic burden could lead to mild stress on the hepatocytes, causing enzymes to be released into the circulation. Studies have shown that prolonged or excessive creatine supplementation is associated with mild but reversible increases in liver enzyme levels [26].

Limitations of this study

Our study has several limitations. First and foremost, this was a the cross-sectional analysis without long-term data. Supplement intake was self-reported in our study. Furthermore, serum creatinine may not be a precise indicator of kidney function in physically active people. Unfortunately, we did not measure the participants' eGFR and other renal biomarkers. Finally, we did not analyze the possible role of dietary and exercise-related covariates. Therefore, our results cannot and should not be generalized to all physically active individuals.

Conclusions

This study demonstrated that high-dose WP and creatine supplementation is associated with elevations in several biochemical markers of liver and kidney function. Moderate supplementation, however, did not produce significant changes. Although these findings suggest potential physiological effects, they do not necessarily indicate organ damage or clinical dysfunction. Our results underscore the value of overseeing supplement intake by coach or dietician and interpreting changes in biochemical markers within the context of exercise, muscle mass, and creatine metabolism. Further longitudinal studies measuring additional renal and liver biomarkers are recommended to clarify the clinical relevance of these associations.

Acknowledgments

The authors would like to express their gratitude to the College of Pharmacy at the University of Mosul, for providing the support and facilities required to conduct this study. We extend our heartfelt appreciation to all laboratory Staff, whose support contributed to the completion of this study.

Funding

None.

Conflicts of interest

None to report.

References

1. Cava E, Padua E, Campaci D, Bernardi M, Muthanna FMS, Caprio M, et al. Investigating the Health Implications of Whey Protein Consumption: A Narrative Review of Risks, Adverse Effects, and Associated Health Issues. Healthcare [Internet]. 2024;12(2):246. Available from: https://www.mdpi.com/2227-9032/12/2/246
2. Ruano J, Teixeira VH. Prevalence of dietary supplement use by gym members in Portugal and associated factors. J Int Soc Sports Nutr [Internet]. 2020;17(1). Available from: https://www.tandfonline.com/doi/full/10.1186/s12970-020-00342-z
3. Giannopoulou I, Noutsos K, Apostolidis N, Bayios I, Nassis GP. Performance level affects the dietary supplement intake of both individual and team sports athletes. J Sports Sci Med [Internet]. 2013;12(1):190–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24149744
4. Minj S, Anand S. Whey Proteins and Its Derivatives: Bioactivity, Functionality, and Current Applications. Dairy [Internet]. 2020;1(3):233–58. Available from: https://www.mdpi.com/2624-862X/1/3/16
5. Pasiakos SM, McLellan TM, Lieberman HR. The Effects of Protein Supplements on Muscle Mass, Strength, and Aerobic and Anaerobic Power in Healthy Adults: A Systematic Review. Sport Med [Internet]. 2015;45(1):111–31. Available from: http://link.springer.com/10.1007/s40279-014-0242-2
6. Vasconcelos QDJS, Bachur TPR, Aragão GF. Whey protein supplementation and its potentially adverse effects on health: a systematic review. Appl Physiol Nutr Metab [Internet]. 2021;46(1):27–33. Available from: https://cdnsciencepub.com/doi/10.1139/apnm-2020-0370
7. Kreider RB, Kalman DS, Antonio J, Ziegenfuss TN, Wildman R, Collins R, et al. International Society of Sports Nutrition position stand: safety and efficacy of creatine supplementation in exercise, sport, and medicine. J Int Soc Sports Nutr [Internet]. 2017;14(1). Available from: https://www.tandfonline.com/doi/full/10.1186/s12970-017-0173-z
8. Poortmans JR, Dellalieux O. Do Regular High Protein Diets Have Potential Health Risks on Kidney Function in Athletes? Int J Sport Nutr Exerc Metab [Internet]. 2000;10(1):28–38. Available from: https://journals.humankinetics.com/view/journals/ijsnem/10/1/article-p28.xml
9. West D, Abou Sawan S, Mazzulla M, Williamson E, Moore D. Whey Protein Supplementation Enhances Whole Body Protein Metabolism and Performance Recovery after Resistance Exercise: A Double-Blind Crossover Study. Nutrients [Internet]. 2017;9(7):735. Available from: https://www.mdpi.com/2072-6643/9/7/735
10. Ko G-J, Rhee CM, Kalantar-Zadeh K, Joshi S. The Effects of High-Protein Diets on Kidney Health and Longevity. J Am Soc Nephrol [Internet]. 2020;31(8):1667–79. Available from: https://journals.lww.com/10.1681/ASN.2020010028
11. Martin WF, Armstrong LE, Rodriguez NR. Dietary protein intake and renal function. Nutr Metab (Lond) [Internet]. 2005;2(1):25. Available from: https://nutritionandmetabolism.biomedcentral.com/articles/10.1186/1743-7075-2-25

12. de Souza e Silva A, Pertille A, Reis Barbosa CG, Aparecida de Oliveira Silva J, de Jesus DV, Ribeiro AGSV, et al. Effects of Creatine Supplementation on Renal Function: A Systematic Review and Meta-Analysis. J Ren Nutr [Internet]. 2019;29(6):480–9. Available from: https://www.sciencedirect.com/science/article/pii/S1051227619302286
13. Heaton LE, Davis JK, Rawson ES, Nuccio RP, Witard OC, Stein KW, et al. Selected In-Season Nutritional Strategies to Enhance Recovery for Team Sport Athletes: A Practical Overview. Sport Med [Internet]. 2017;47(11):2201–18. Available from: https://doi.org/10.1007/s40279-017-0759-2
14. Wallimann T, Dolder M, Schlattner U, Eder M, Hornemann T, O'Gorman E, et al. Some new aspects of creatine kinase (CK): compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology. BioFactors [Internet]. 1998;8(3–4):229–34. Available from: https://doi.org/10.1002/biof.5520080310
15. Ostojic SM, Forbes SC. Perspective: Creatine, a Conditionally Essential Nutrient: Building the Case. Adv Nutr [Internet]. 2022;13(1):34–7. Available from: https://www.sciencedirect.com/science/article/pii/S2161831322005269
16. Marinello PC, Cella PS, Testa MTJ, Guirro PB, Brito WAS, Borges FH, et al. Creatine supplementation exacerbates ethanol-induced hepatic damage in mice. Nutrition [Internet]. 2019;66:122–30. Available from: https://www.sciencedirect.com/science/article/pii/S0899900719300693
17. Todorovic N, Korovljev D, Stajer V, Jorga J, Ostojic SM. Creatine consumption and liver disease manifestations in individuals aged 12 years and over. Food Sci Nutr [Internet]. 2023;11(2):1134–41. Available from: https://onlinelibrary.wiley.com/doi/10.1002/fsn3.3151
18. Pline KA, Smith CL. The Effect of Creatine Intake on Renal Function. Ann Pharmacother [Internet]. 2005;39(6):1093–6. Available from: https://journals.sagepub.com/doi/10.1345/aph.1E628
19. Wirunsawanya K, Upala S, Jaruvongvanich V, Sanguankeo A. Whey Protein Supplementation Improves Body Composition and Cardiovascular Risk Factors in Overweight and Obese Patients: A Systematic Review and Meta-Analysis. J Am Coll Nutr [Internet]. 2018;37(1):60–70. Available from: https://doi.org/10.1080/07315724.2017.1344591
20. Schlickmann DS, Molz P, Brand C, dos Santos C, da Silva TG, Rieger A, et al. Liver and kidney function markers among gym users: the role of dietary supplement usage. Br J Nutr [Internet]. 2021/09/13. 2022;128(4):704–11. Available from: https://www.cambridge.org/core/product/A5C351DDCDA7C1E5F69FD37D4394B425
21. Souza RA, Miranda H, Xavier M, Lazo-Osorio RA, Gouvea HA, Cogo JC, et al. Effects of high-dose creatine supplementation on kidney and liver responses in sedentary and exercised rats. J Sports Sci Med [Internet]. 2009;8(4):672–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24149610
22. Tawfik MS, Al-Badr N. Adverse Effects of Monosodium Glutamate on Liver and Kidney Functions in Adult Rats and Potential Protective Effect of Vitamins C and E. Food Nutr Sci [Internet]. 2012;03(05):651–9. Available from: https://www.scirp.org/journal/paperinformation?paperid=19078
23. D'Lugos A, Luden N, Faller J, Akers J, McKenzie A, Saunders M. Supplemental Protein during Heavy Cycling Training and Recovery Impacts Skeletal Muscle and Heart Rate Responses but Not Performance. Nutrients [Internet]. 2016;8(9):550. Available from: https://www.mdpi.com/2072-6643/8/9/550
24. Candow DG, Forbes SC, Chilibeck PD, Cornish SM, Antonio J, Kreider RB. Effectiveness of Creatine Supplementation on Aging Muscle and Bone: Focus on Falls Prevention and Inflammation. J Clin Med [Internet]. 2019;8(4):488. Available from: https://www.mdpi.com/2077-0383/8/4/488
25. Brancaccio P, Lippi G, Maffulli N. Biochemical markers of muscular damage. cclm [Internet]. 2010;48(6):757–67. Available from: https://www.degruyter.com/document/doi/10.1515/CCLM.2010.179/html
26. Ziegenfuss TN, Rogers M, Lowery L, Mullins N, Mendel R, Antonio J, et al. Effect of creatine loading on anaerobic performance and skeletal muscle volume in NCAA division I athletes. Nutrition [Internet]. 2002;18(5):397–402. Available from: https://www.sciencedirect.com/science/article/pii/S0899900701008024

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):73-86

META-ANALYSIS

Does dietary advice combined with complete dentures improve the nutrient intake and nutritional status of edentulous patients? A systematic review with meta-analysis

Sapna Rani, Neha Jain, Shipra Gupta, Balpreet Kaur, Khushboo Bhatia

School of Dental Sciences, Manav Rachna International Institute of Research and Studies, Faridabad, India

Abstract

Background: The purpose of this systematic review and meta-analysis was to assess the nutrient intake and nutritional status of edentulous patients who received dietary advice combined with complete dentures. **Material and methods:** Data were extracted from PubMed/Medline, Google Scholar, Scopus, and ScienceDirect from February 2002 to February 2025 by 2 independent reviewers. Ebscohost was searched for full-text articles from 1994 to 2025, in English. 1596 articles were excluded based on exclusion criteria and after removing 7 duplicates. Eight articles were included for qualitative analysis, followed by 3 articles for meta-analysis. **Results:** Non-significant improvements were seen in the intake of calcium ($p = 0.17$), iron ($p = 0.10$), protein ($p = 0.21$) and vitamin D ($p = 0.93$). Heterogeneity was low, indicating consistent results. GRADE analysis indicated a low level of evidence. **Conclusions:** Our results have not shown any significant improvement in nutrient intake after dietary advice and rehabilitation. In addition, the limited number of studies available for this meta-analysis highlights the need for more standardized, long-term clinical trials to achieve a higher level of evidence.

Keywords: systematic review · complete denture · nutritional deficiency · nutrient intake · dietary advice

Corresponding author:
Sapna Rani, School of Dental Sciences, Manav Rachna International Institute of Research and Studies, Faridabad, India
e-mail: drsapnadaksh@gmail.com
Available online: ejtcm.gumed.edu.pl

Citation

Rani S, Jain N, Gupta S, Kaur B. Does dietary advice combined with complete dentures improve the nutrient intake and nutritional status of edentulous patients? A systematic review with meta-analysis. Eur J Transl Clin Med. 2026;9(1):73-86.

DOI: 10.31373/ejtcm/222198

Introduction

The loss of teeth (edentulism) is considered an indication of poor oral cavity health [1-2]. Adequate nutrition is vital in maintaining a healthy life, especially in geriatric patients with complete removable prostheses [3]. Chen et al. reported that an estimated 0.35 billion people were affected by edentulism, accounting for 4.44% of the whole population [4]. This trend first increased in developed countries (e.g. UK and Japan) and later in developing countries such as India [5]. Literature has shown that dental status is closely linked to an individual's nutritional status. Hence, for improved human health, dentition status should be monitored for nutritional counselling, particularly in the elderly [6].

Diet and nutrition are often misunderstood as synonyms, but they differ in that diet constitutes the ingestion of food, whereast nutrition integrates macro and micronutrients for the building and repairing of tissues [7]. The European Society for Clinical Nutrition and Metabolism recommends 1.0-1.2 g/kg body weight/day for healthy older adults and 1.2-1.5 g/kg/day for older adults who are malnourished or have a chronic illness [8]. The nutritional status of elderly people is often impaired due to selective food intake, as they are more comfortable with certain food items that are easy to chew with their current prostheses [9]. In addition to this, elderly patients often swallow inadequately chewed food as a compensatory mechanism for reduced masticatory efficiency, which may also indirectly contribute to nutritional deficiencies.

Evidence from the literature shows that edentulism is associated with a high risk of malnutrition [10-11]. In developed countries, adults aged 60 years or older without functional dentition were found to be at a higher risk of malnutrition compared to those with functional dentition [10]. Authors also emphasized the importance of using validated tools to determine malnutrition in the elderly population. In two systematic reviews, nutritional assessment was measured using the Mini Nutritional Assessment (MNA) or its short form (MNA-SF) [10-11]. In another systematic review, McGowan et al. examined the nutritional status of adults after prosthetic rehabilitation (dentures) combined with dietary advice, dietary outcomes, as assessed by the Brief-Type Self-Administered Diet History Questionnaire (BDHQ), and serum marker levels reported in analyzed studies [12].

Several studies have demonstrated that tooth loss is associated with a reduced intake of fruits and vegetables, as well as proteins and dietary fiber [13-14]. Previous research by Hamada et al. and Moynihan et al. have shown that prosthetic rehabilitation alone leads to less predictable positive dietary behavior change [15-16]. The evidence remains inconclusive regarding whether complete rehabilitation of functional dentition alone leads to significant improvement in overall nutritional status. Additionally, the combined effect of removable complete dentures and dietary counseling was not comprehensively assessed in completely edentulous patients. Hence, we conducted a systematic review with meta-analysis in order to synthesize the data on the improvement in nutrient intake and nutritional status of patients who received dietary advice combined with complete removable dentures.

Material and methods

Review design and registration

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) checklist (2020) and the Cochrane Handbook for Systematic Reviews and Meta-analysis [17-18]. The PRISMA flowchart is shown in Figure 1. Our systematic review was registered in PROSPERO (registration number CRD42025633956) on January 4, 2025. Data extraction was updated in PROSPERO on 1st March 2025.

Research question and PICOS

The research question for this systematic review was: "does supplemental dietary advice along with complete rehabilitation enhance nutrient intake and nutritional status for completely edentulous patients?" The studied population consisted of completely edentulous patients with removable complete dentures. The intervention involved supplementary dietary advice in any form for individuals with complete

dentures. Control involved rehabilitation with complete dentures without dietary advice. The outcome measured was nutrient intake and nutritional status. We included full-text randomized clinical trials (RCTs), observational studies, and prospective clinical studies published in the English language. Exclusion criteria were: studies in which implant rehabilitation or partial rehabilitation was performed (the research question focused on complete denture therapy as the sole prosthetic rehabilitation), cross-sectional studies, case reports, theses, dissertations, pilot studies, studies published not in the English language, and unpublished data. Gray literature was not searched in this review.

Data extraction

Two authors (SR and NJ) independently extracted data from PubMed/Medline (as of April 2, 2025), Google Scholar (as of February 24, 2025), Scopus (as of February 10, 2025), and ScienceDirect (as of February 22, 2025), covering the period from February 2002 to February 2025. Ebscohost (as of February 7, 2025) was searched using a filter for full text, covering the years 1994-2025, in English. The search strategy for individual databases is presented in Table 1.

Review outcomes

Our systematic review focused on assessing the nutritional status of completely edentulous patients post-intervention. The nutrient intake was measured in terms of fruit and vegetable (F/V) intake, fiber, protein, fatty acids, energy, micronutrients, and macronutrients. At the same time, nutritional status was assessed using established questionnaires (MNA, MNA-SF, BDHQ) for the same or subsequent measurement of biochemical markers.

Meta-analysis

Two authors (SR and NJ) independently extracted data into a spreadsheet, detailing the authors, year of publication, country, demographic data, intervention details, and results at various time intervals. A meta-analysis was conducted using fixed-effects models to pool the quantitative data on patient nutrient intake, incorporating the mean and standard deviation values provided in the studies (Review Manager (Rev Man), v 5.4, Cochrane Collaboration). I^2 statistics were used to calculate the heterogeneity.

Risk of bias

The Cochrane tool was used to assess the risk of bias (RoB), including domains selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias. A revised JBI RoB tool was used for observational studies [19]. Two independent reviewers reported RoB for all included studies under the high-risk, low-risk, or unclear-risk categories.

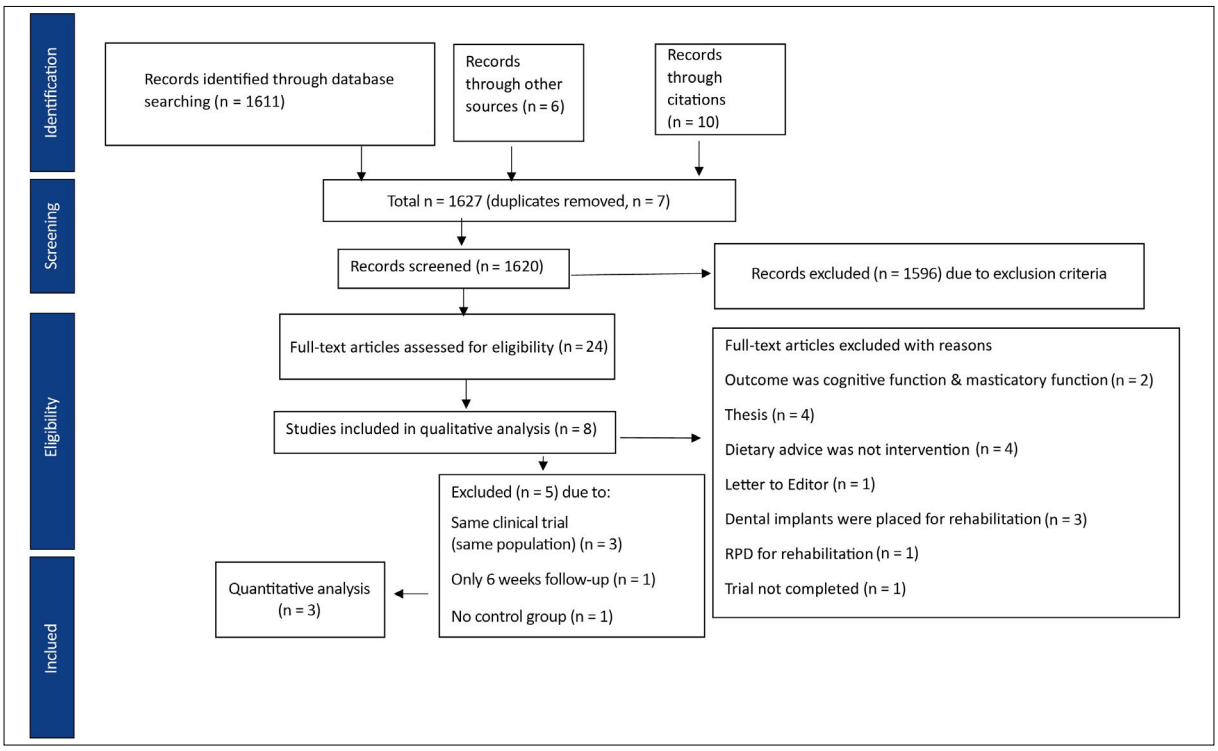


Figure 1. PRISMA flowchart of our systematic review

Level of evidence

The level of evidence was calculated using theGRADEpro GDT software (Evidence Prime Sp. z o.o., Kraków, Poland, available from gradepro.org). The quality was assessed as high, moderate, low or very low based on 5 criteria (risk of bias, inconsistency, indirectness, imprecision, other considerations).

Results

Search results

A total of 1611 articles were retrieved from the database search: 12 articles in PubMed, 1470 in Google Scholar, 70 in Ebscohost, 7 in Scopus and 52 in ScienceDirect. A manual search of the included articles' reference lists identified 10 additional articles. 6 additional articles were selected from other sources (e.g., data registries, journals not indexed in databases). Google Scholar data was searched using MeSH keywords with Boolean operators AND and OR. Duplicates were removed manually by two independent reviewers (no software was used for removing duplicates in any database) (Table 1).

After removing 7 duplicates, the titles and abstracts were reviewed and 1596 articles were excluded based on the criteria listed earlier. If there was any confusion about an article, then the full text was read independently by 2 authors (SG and BK). To clarify any discrepancies regarding the inclusion of an article, a third reviewer was consulted until a clear consensus was reached. A total of 24 articles were assessed for eligibility and 8 were included for qualitative analysis (Figure 1). After excluding 5 more articles, 3 articles were included in the meta-analysis. In the event of any ambiguity, the decision was made by a third author (SR).

Included studies

A total of 7 RCTs and 1 observational study were included for systematic review; 1 study was conducted in the UK [20], 3 in India [21-23] and 4 in Japan [24-27]. In one of the studies (Kumar et al.) all of the participants were female [21]. Ethical approval and informed consent were reported in all of the analysed trials. In most of the included studies, dietary advice was provided to the patients in the form of written pamphlets either at the time of fitting or insertion of dentures [20, 22-27]. An exception is the study by Kumar et al., in which patients received dietary advice in the form of food supplements for 3 months [21]. The Brief Self-Administered Diet History Questionnaire (BDHQ) was used to assess the nutritional status of patients in 3 studies [24-26]. In 1 study the MNA tool was used to determine the participants' nourishment [23], while its short version (MNA-SF) was used in 2 others [22, 27]. Serum biomarkers (e.g. Vit D, Hb, serum Ca, BMI, BMD) were analysed in 3 studies [20-21, 23]. Masticatory performance was assessed in 2 studies [21, 25], 1 study also evaluated muscle activity using EMG [21] and one study assessed oral health-related quality of life [24] as secondary outcomes (Table 2). Baseline and follow-up mean values (along with standard deviation) for all included studies are provided in the Supplementary Table 1. The minimum follow-up time period in the included studies was 6 weeks, and the maximum follow-up period was 6 months. A summary of outcomes at different time periods is provided in Table 3.

Table 1. Search strategy used in this systematic review

Database	Search strategy	Articles
PubMed/Medline	(("Complete rehabilitation" [All Fields] AND "dietary counseling" [All Fields] AND "nutritional status" [All Fields] OR "nutritional biomarkers" [All Fields] AND "completely edentulous patients" [All Fields]	12
Google Scholar		1470
Scopus		7
EBSCOhost	TI Complete denture rehabilitation OR TX Completely edentulous AND TX Dietary supplements AND TX Dietary advice AND TX Nutritional status OR TI (dietary interventions or dietary advice or dietary recommendations) with filter (Full text 1994-2025, English)	70
ScienceDirect	Advanced research using keywords "edentulous", "complete denture", "dietary advice", "nutrient intake"	52

Table 2. Partcipant demographics and dietary advice described in the included studies

Author details, year [reference]	Country	Sample size, sex distribution	Mean age	Study type & duration	Dietary intervention	Time of dietary advice	Nutritional status assessment tools
Bradbury et al., 2006 [20]	UK	n = 32 (C), n = 34 (I)	45-80 years, C = 66.7 ± 6.7, I = 65.4 ± 8.9	RCT, Feb. 2000 – Jul. 2001	2 sessions, tailored written package	At baseline and 6 weeks of wearing dentures	BMI, mamicro- and macronutrients
Kumar et al., 2023 [21]	India	n = 50 (C), 56 (I) females only	CD = 49.6 ± 7.95, DWFS = 51.3 ± 7.9, DwoFS = 50.5 ± 8.85	non-RCT	Food supplement in lukewarm water	1 sachet/ day for 3 months	Albumin, hemoglobin, vitamin D, calcium
Amagai et al., 2017 [24]	Japan	n = 31 for each group, C = 16 (F) + 15 (M), I = 15 (F) + 16 (M)	Mean age C = 78.6, I = 75.3	RCT	1 session, 20 minutes of simple dietary advice in pamphlet form	At the time of denture insertion	BDHQ
Suzuki et al., 2018 [27]	Japan	n = 31 for each group, C = 15 (M) + 16 (F), I = 16 (M) + 15 (F)	Mean age C = 78.6, I = 75.3	RCT, Jun. 2015 – Sept. 2016	2 sessions, 20 minutes of simple dietary advice in pamphlet form	At the time of denture fitting and insertion	BDHQ
Kanazawa et al., 2019 [26]	Japan	n = 29 (C), n = 30 (I), C = 14 (F) + 15 (M), I = 15 (F), 15 (F)	Mean age C = 78.6, I = 74.8	RCT, Jun. 2015 – Dec. 2016	2 sessions, dietary advice in pamphlet form	At the time of denture fitting and insertion	BDHQ
Suzuki et al., 2019 [25]	Japan	n = 29 (C), n = 30 (I), C = 15 (M) + 14 (F), I = 15 (M) + 15 (F)	C = 78.6, I = 74.8	RCT, Jun. 2015 – Sept. 2016	2 sessions, dietary advice in pamphlet form	At the time of fitting and insertion	MNA-SF
Agarwal et al., 2023 [22]	India	n = 50	Not mentioned	obser-vational study, Apr. – Jul. 2023	2 sessions, dietary counseling in documented form	At the time of denture fitting and insertion	MNA-SF
Vijaya et al., 2024 [23]	India	n = 35 in each group, C = 17 (M) + 18 (F), I = 22 (M) + 13 (F)	I = 65.57, C = 65.77	non-RCT	Tailored dietary advice in pamphlet form	At the time of denture insertion	MNA, BMI

BDHQ – Brief-Type Self-Administered Diet History Questionnaire, BMI – body mass index, C – control group, CD – completely dentate, DWFS – complete dentures without food supplement, DwoFS – complete dentures without food supplement, F – female, I – intervention group, M – male, MNA – Mini Nutritional Assessment, MNA-SF – Mini Nutritional Assessment-Short Form, non-RCT – non-randomized controlled trial, RCT – randomized controlled trial

Table 3. Outcome assessment of included studies

Author details, year [reference]	Primary outcome (nutrient intake)	Secondary outcomes	Follow-up time period	Primary outcome at follow-up	Secondary outcomes at follow-up
Bradbury et al., 2006 [20]	F/V intake, energy, micro- and macronutrients	1) Nutritional status (BMI) 2) stage of change 3) chewing ability	6 weeks	1) F/V intake increased in Group (p = 0.001). 2) Only vitamin C (p < 0.0005) and β-carotene (p = 0.036) differed significantly.	1) BMI didn't increase significantly (p = 0.497) 2) significant movement from pre-action to action stages of change 3) chewing ability didn't differ significantly (p = 0.269) b/w groups
Kumar et al., 2023 [21]	nutritional status (Hb, serum calcium, serum albumin, vitamin D)	1) Bone mineral density 2) Masticatory performance 3) EMG	3 and 6 months	No statistical difference except for calcium	Statistical difference in BMD, masticatory performance and EMG
Agarwal et al., 2023 [22]	MNA-SF	–	Baseline, 1 month, 3 months	After 3 months, the nutritional status was significantly improved (p = 0.0331). Only 17.3% were at risk of malnutrition compared to 32 % at baseline.	–
Vijaya et al., 2024 [13]	Nutrient intake (protein, dietary fiber, iron, carbohydrates, calcium) energy	1) nutritional status (MNA) 2) BMI	3 months	Nutrient intake (e.g. protein, dietary fiber, total fat, calcium, carbohydrate, energy, iron, vitamin B12) was higher in the intervention group, though it was not statistically significant.	1) At 3 months there was an improvement in the MNA scores (p < 0.001) in both the groups, but the improvement was significantly higher in the intervention group than in the control group. 2) A statistically significant improvement in the BMI of participants in the intervention group (p = 0.030).
Amagai et al., 2017 [24]	Food intake (BDHQ)	OHRQoL (OHIP-Edent-J)	3 months	After 3 months, significantly greater intake of chicken (p = 0.013), fish with bones (p = 0.012), and carrots and pumpkins (p = 0.025)	Non-significant difference between the groups
Suzuki et al., 2018 [25]	Food intake (BDHQ)	Masticatory function	3 months	After 3 months, intake of protein (p = 0.001), lipid (p = 0.041), sodium (p = 0.013), potassium (p = 0.007), magnesium (p ≤ 0.001), phosphorus (p = 0.001), iron (p = 0.011), zinc (p = 0.001), vitamin B1 (p = 0.004), vitamin B2 (p = 0.027), vitamin B6 (p = 0.002), niacin (p = 0.002), folic acid (p = 0.031), and pantothenic acid (p ≤ 0.001) significantly increased in the intervention group	Non-significant difference between the control and intervention groups at post-treatment

BDHQ – Brief-Type Self-Administered Diet History Questionnaire, BMD – bone mineral density, BMI – body mass index, EMG – electromyography, F/V – food and vegetable intake, Hb – hemoglobin, MNA – Mini Nutritional Assessment, MNA-SF – Mini Nutritional Assessment-Short Form, OHIP-Edent-J – Oral Health Impact Profile for Edentulous Patients (Japanese version), OHRQoL – Oral-health-related quality of life

Table 3. Outcome assessment of included studies (continued)

Author details, year [reference]	Primary outcome (nutrient intake)	Secondary outcomes	Follow-up time period	Primary outcome at follow-up	Secondary outcomes at follow-up
Kanazawa et al., 2019 [26]	Food intake (BDHQ)	–	3 and 6 months	At 3 months post-treatment, the nutrient intake was significantly higher in the intervention group. At 6 months post-treatment, plant protein (p = 0.028) intake was significantly higher in the intervention group than the control group; on the contrary, a significantly higher intake of animal proteins (p = 0.049) and vitamin B12 (p = 0.028) was noted in the control group.	–
Suzuki et al., 2019 [27]	Nutritional Status (MNA-SF)	–	3 and v6 months	1) Non-significant at 3 months (p = 0.48) 2) Significantly higher than the control group by 6 months (p = 0.01)	–

BDHQ – Brief-Type Self-Administered Diet History Questionnaire, BMD – bone mineral density, BMI – body mass index, EMG – electromyography, F/V – food and vegetable intake, Hb – hemoglobin, MNA – Mini Nutritional Assessment, MNA-SF – Mini Nutritional Assessment-Short Form, OHIP-Edent-J – Oral Health Impact Profile for Edentulous Patients (Japanese version), OHRQoL – Oral-health-related quality of life

Bias assessment

According to Cochrane's tool for risk of bias assessment of RCTs, 6 of the included RCTs showed a low risk of bias and only one study had a high risk of bias [20]. Randomization and concealment of allocation were done adequately in 6 of the included studies. Reporting bias was also reduced by incorporating the appropriate measurement tool. In contrast, detection bias was high in 2 studies and unclear in 4 studies. The risk of bias in the 7 included RCTs is summarized in Figure 2. One of the included studies was observational and was found to have a low risk of bias, as calculated using the revised JBI tool [23].

Meta-analysis

It was not possible to conduct a meta-analysis of nutritional status because 1 of the studies in which the MNA-SF was used was an observational study with no control group of participants [22].

A qualitative analysis was conducted on a total of 8 studies, and a further quantitative analysis was performed on 3 of these studies for nutrient intake. We included 4 different articles published from the same single clinical trial, featuring a different outcome measure in one of the studies and differences in the follow-up [24-27]. To avoid double-counting data or introducing bias, in the meta-analysis, the same population with the same outcomes was considered only once across those 4 articles from the same clinical trial.

In the comparison of nutritional intake after a 3-month follow-up, 3 studies were included in the meta-analysis [21, 23, 26]. The heterogeneity across studies for the outcome 'calcium intake' was low (44%), so a fixed-effects model was used for analysis (Figure 3). The mean difference between the groups was -0.25 (95% confidence interval: -0.61 to 0.11). The pooled estimate did not show statistically significant differences (p = 0.17) in calcium intake between the intervention and control groups and the overall effect across studies showed results favouring the intervention group. Thus, it can be concluded that the mean calcium intake was better in the

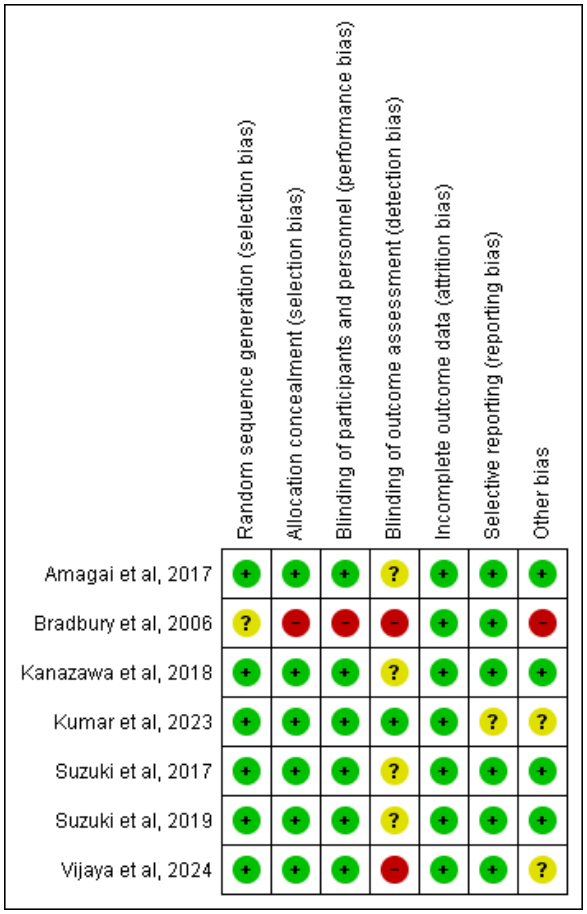


Figure 2. Risk of bias graph of the included RCTs using the Cochrane tool

supplement group across all studies, although that difference was not statistically significant.

For the outcome 'iron intake' the heterogeneity across the analysed studies was low (0%), so a fixed-effects model was used for analysis (Figure 4). The mean difference between the groups was 1.16 (95% confidence interval: -0.21 to 2.54). The pooled estimate did not show statistically significant differences (p-value = 0.10) in iron intake between the intervention and control groups and the overall effect across studies showed results favouring the control group. Thus, it can be concluded that the mean iron intake was not (statistically) significant different between the two groups, however in clinical terms the control group showed comparable results to the intervention group.

The heterogeneity across studies for the outcome 'protein intake' was low (0%), so a fixed-effects model was also used for analysis (Figure 5). The mean difference between the groups was 7.48 (95% confidence interval: -4.25 to 19.21). The pooled estimate showed no statistically significant differences (p-value = 0.21) in protein intake between the intervention and control groups, and the overall effect across studies showed results favouring the control group. Thus, it can be concluded that the mean protein intake was not (statistically) significantly different between the two groups, although clinically the control group showed comparable results to the test group.

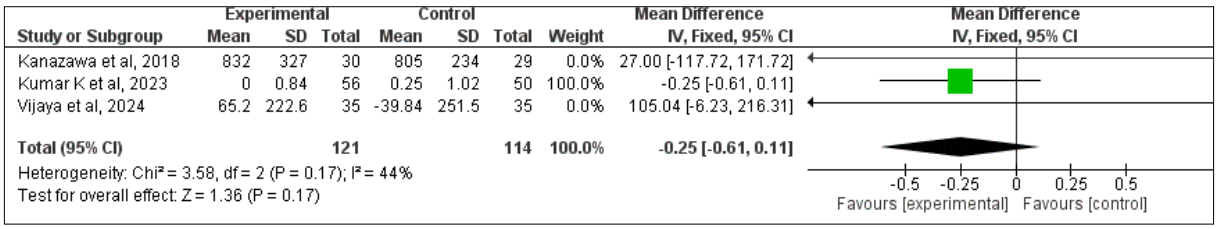


Figure 3. Forest plot distribution showing an outcome (calcium intake) with fixed effect model and 95% confidence interval

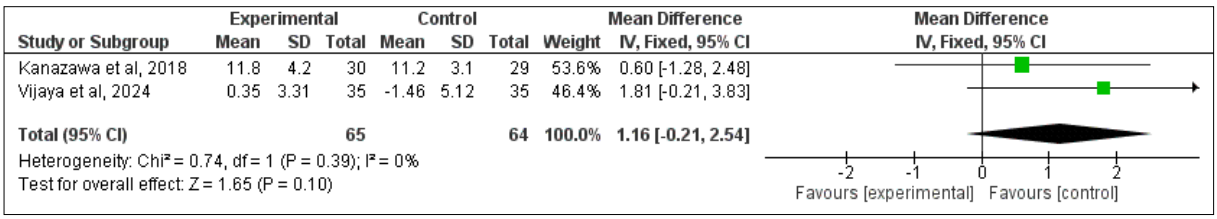


Figure 4. Forest plot distribution showing an outcome (iron intake) with fixed effect model and 95% confidence interval

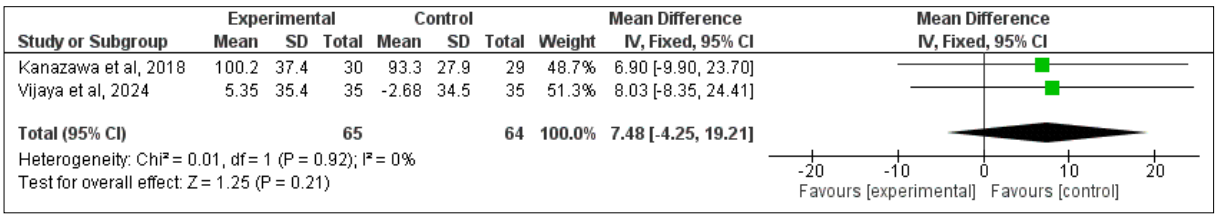


Figure 5. Forest plot distribution showing an outcome (protein intake) with fixed effect model and 95% confidence interval

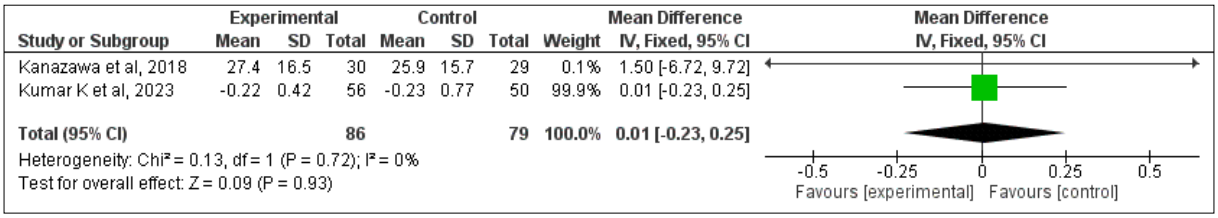


Figure 6. Forest plot distribution showing an outcome (vitamin D intake) with fixed effect model and 95% confidence interval

In terms of the outcome 'vitamin D intake', the heterogeneity across studies was low (0%), so a fixed-effects model was used for analysis (Figure 6). The mean difference between the groups was 0.01 (95% confidence interval: -0.23 to 0.25). The pooled estimate showed no statistically significant differences (p-value = 0.93) in vitamin D intake between the intervention and control groups, and the overall effect across studies showed results favoring both the intervention and control groups. Thus, it can be concluded that the mean vitamin D intake was similar results in the intervention and control groups. Publication bias was not assessed as only 3 studies were included in the meta-analysis.

GRADE analysis

According to GRADE, there was a low level of evidence for the outcomes of 'calcium intake', 'iron intake', 'protein intake', and 'vitamin D intake', respectively. The evidence was downgraded considering 2 domains: risk of bias and imprecision. One of the included RCTs was of high risk, whereas the other 2 studies showed low risk. Imprecision among the studies was also graded as serious, considering the wide uncertainty surrounding the estimates and the crossing of decision thresholds (Table 4).

Discussion

Complete tooth loss is directly related to dietary intake and nutritional status of individuals [28]. Completely edentulous patients with less functional occlusion tend to consume fewer fruits and vegetables and, hence, are more prone to systemic diseases [29-30]. In their systematic review, Hussein et al. indicated that people > 60 years of age who experience complete tooth loss face a 21% chance of malnutrition [11]. In another systematic review, the risk of malnutrition was found to be high in individuals who were partially/fully edentulous [12]. Findings from an umbrella review demonstrated that tooth loss (partial or complete) is a significant risk factor for malnutrition, indicating that compromised oral health can negatively impact nutritional status [31]. Although the above-cited systematic reviews demonstrated an association between nutritional status and the elderly population, we did not find a comprehensive review regarding the completely edentulous patients.

People with tooth loss tend to avoid chewing raw fruits and vegetables, which reduces their intake of protein and fiber, leading to low energy levels. Rehabilitation of functional units with complete dentures can empower patients to maintain a healthy nutrient intake [32]. However, studies have also shown that prosthetic rehabilitation alone may not be sufficient to enhance nutritional status [27, 33]. Simple dietary advice, supplemented with comprehensive rehabilitation, has been proven as an effective way to improve the nutritional outcomes of patients [25, 28].

Dietary advice can be provided in various forms (e.g. written pamphlets, individual consultations, tailored dietary regimens or specified food supplements), all of which can be customized according to age, sex, and individual requirements. However, providing tailored diet plans for patients in a dental setting is difficult as dentists lack specific knowledge of dietary supplements. Hence, in most of the included studies, simple dietary advice was provided to participants in written pamphlet form [22, 24-27].

There are various standardized tools for assessing nutritional status, while nutrient intake is evaluated through different components, including macronutrients and micronutrient intake. In our systematic review, the BDHQ questionnaire was administered in 3 studies [24-26]. Although the BDHQ is short (4 pages, can be completed in 10-15 minutes) and easy to understand, the drawback of this tool is that answers depend on the patients' recall of key details (e.g. the food they consumed, diet preparation, intake of beverages, including alcohol). This may result in recall bias during assessment of elderly participants.

A few studies measured components of fruit and vegetable intake, macronutrients, and micronutrients after intervention [20, 23-26]. In our review, it was found that the nutrient intake of calcium, iron, protein, and vitamin D was slightly improved after dietary advice, although the difference was non-significant. Our results were not consistent with other studies, as in a clinical trial conducted by Kanazawa et al., the intake of protein (p = 0.004), iron (p = 0.035) was statisticaly significant, but calcium (p = 0.281) and vitamin D (p = 0.295) were not statistically significant in the intervention group after 3 months of follow-up [26]. In a study by Vijaya

Table 4. Level of evidence assessed using the GRADE method

Certainty assessment	**№ of studies [reference]**	**3 [21, 23, 26]**	**№ of studies [reference]**	**2 [23, 26]**
	Risk of bias	not serious	**Risk of bias**	serious
	Inconsistency	not serious	**Inconsistency**	not serious
	Indirectness	not serious	**Indirectness**	not serious
	Imprecision	serious	**Imprecision**	not serious
	Other considerations	none	**Other considerations**	none
№ of patients (n)	**Calcium intake**	121	**Iron intake**	65
	Placebo	114	**Placebo**	64
Effect	**Relative**	–	**Relative**	–
	Absolute	MD **0.25 lower** (0.61 lower to 0.11 higher)	**Absolute**	MD **1.16 higher** (0.21 lower to 2.54 higher)
Certainty		⊕⊕⊕◯ moderate[a]		⊕⊕⊕◯ moderate[a]
Importance		IMPORTANT		IMPORTANT

[a] – wide uncertainty around the estimate and crossing decision thresholds, [b] – high risk of bias, CI – confidence interval, MD – mean difference

Table 4. Level of evidence assessed using the GRADE method (continued)

Certainty assessment	**№ of studies [reference]**	**2 [23, 26]**	**№ of studies [reference]**	**2 [21, 26]**
	Risk of bias	serious[b]	**Risk of bias**	not serious
	Inconsistency	not serious	**Inconsistency**	not serious
	Indirectness	not serious	**Indirectness**	not serious
	Imprecision	serious[a]	**Imprecision**	serious[a]
	Other considerations	none	**Other considerations**	none
№ of patients (n)	**Protein intake**	65	**Vitamin D**	86
	Placebo	64	**Placebo**	79
Effect	**Relative**	–	**Relative**	–
	Absolute	MD **7.48 higher** (4.25 lower to 19.21 higher)	**Absolute**	MD **0.01 higher** (0.23 lower to 0.25 higher)
Certainty		⊕⊕◯◯ low[a,b]		⊕⊕⊕◯ moderate[a]
Importance		IMPORTANT		IMPORTANT

[a] – wide uncertainty around the estimate and crossing decision thresholds, [b] – high risk of bias, CI – confidence interval, MD – mean difference

et al., the intervention group showed no statistically significant differences in protein (p = 0.30) or calcium intake (p = 0.196) after the same follow-up period [23]. In contrast, Kumar et al. found that calcium intake was significantly higher with supplements (p = 0.01), but not with vitamin D (p = 0.30). The differences in these results may be due to variations in the demographic distributions of the populations, as 1 of the analysed studies was conducted in Japan and the other 2 in India. Another variation was the exclusion of male participants in one of the analysed studies [23].

In their meta-analysis Bezerra et al. concluded that in completely edentulous patients the bioavailability of the nutrients remained stable with implant-supported dentures. They also emphasized the role of the nutritional specialist for the outcomes of rehabilitation [34]. Additionally, nutrient intake is influenced by multiple factors, particularly in edentulous patients, therefore the systemic health condition of the included population in trials should be taken into consideration. The meta-analysis also reveals low heterogeneity, as indicated by the forest plots, with consistent results and a low level of evidence. In one of the analysed studies, Kumar et al. presented calcium and Vitamin D intake were presented as median and IQR [21]. By using the formula described by Wan et al., we converted these values into mean and SD for inclusion in our meta-analysis [35].

For, The MNA [23], MNA-SF [22, 27], or more informative assessments, such as BMI [20, 23], and serum biomarkers (Hb, Ca, Fe) [21], have been used by the authors for assessment of the participants' nutritional status in the included studies. The MNA-SF determines the population at risk of malnutrition via only 6 questions, compared to the MNA form which includes screening followed by a detailed assessment. In a study, after a 3-month follow-up, nutrient intake was significantly better in the intervention group (p = 0.0331) [22], compared to another study in which significant improvement was found after 6 months (p = 0.01) [25]. Vijaya et al. reported that MNA scores were significantly improved in both the control and intervention groups after 3 months (p < 0.001), while there was only a slight improvement in the intervention group when inter-group comparison was carried out [23]. This result was supported by a more reliable marker (BMI), which significantly improved in that study's intervention group [23].

A limitation of our analysis is that most of the included studies were conducted in Japan, India, and the UK, therefore the dietary advice provided in these studies cannot be generalized to other populations. The dietary advice was limited to only 1 or 2 consultations during the study period. There was a paucity of literature on the assessment of nutritional status using the same tool for meta-analysis with a long follow-up. More RCTs are needed using a standard tool to determine the nutrient intake and nutritional status of edentulous patients when supplemented with dietary advice and complete denture therapy. Long-term follow-up and regular reinforcement of dietary advice are required in future studies to assess whether the outcomes are due to prostheses or dietary advice.

Conclusions

Within the limitations of our analysis, it can be concluded that there was no significant difference in the nutrient intake of calcium, iron, protein, and vitamin D in people who received dietary advice along with complete dentures. Furthermore, nutritional status was improved in the intervention group, but additional standardized long-term RCTs are required to obtain conclusive results.

Funding

Self funded.

Conflict of interest

None.

References

1. Chalub LLFH, Ferreira RC, Vargas AMD. Influence of functional dentition on satisfaction with oral health and impacts on daily performance among Brazilian adults: a population-based cross-sectional study. BMC Oral Health [Internet]. 2017;17(1):112. Available from: http://bmcoralhealth.biomedcentral.com/articles/10.1186/s12903-017-0402-5
2. Lamster IB, Asadourian L, Del Carmen T, Friedman PK. The aging mouth: differentiating normal aging from disease. Periodontol 2000 [Internet]. 2016;72(1):96–107. Available from: https://onlinelibrary.wiley.com/doi/10.1111/prd.12131
3. Kazemi S, Savabi G, Khazaei S, Savabi O, Esmaillzadeh A, Keshteli AH, et al. Association between food intake and oral health in elderly: SEPAHAN systematic review no. 8. Dent Res J (Isfahan) [Internet]. 2011;8(Suppl 1):S15-20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23372590

4. Chen HM, Shen K, Ji L, McGrath C, Chen H. Global and Regional Patterns in Edentulism (1990-2021) With Predictions to 2040. Int Dent J [Internet]. 2025;75(2):735–43. Available from: https://www.sciencedirect.com/science/article/pii/S0020653924016162
5. Noto S. Perspectives on Aging and Quality of Life. Healthcare [Internet]. 2023;11(15):2131. Available from: https://www.mdpi.com/2227-9032/11/15/2131
6. Sahyoun NR, Lin C-L, Krall E. Nutritional status of the older adult is associated with dentition status. J Am Diet Assoc [Internet]. 2003;103(1):61–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S000282230200007X
7. Perry C. Nutrition for senescent denture patients. J Prosthet Dent [Internet]. 1961;11(1):73–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/0022391361901123
8. Deutz NEP, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, et al. Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. Clin Nutr [Internet]. 2014;33(6):929–36. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0261561414001113
9. Joshipura KJ, Willett WC, Douglass CW. The impact of edentulousness on food and nutrient intake. J Am Dent Assoc [Internet]. 1996;127(4):459–67. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002817715613939
10. Zelig R., Goldstein S., Touger-Decker R., Firestone E., Golden A., Johnson Z., et al. Tooth Loss and Nutritional Status in Older Adults: A Systematic Review and Meta-analysis. JDR Clin Transl Res [Internet]. 2022;7(1):4–15. Available from: https://doi.org/10.1177/2380084420981016
11. Hussein S, Kantawalla RF, Dickie S, Suarez-Durall P, Enciso R, Mulligan R. Association of Oral Health and Mini Nutritional Assessment in Older Adults: A Systematic Review with Meta-analyses. J Prosthodont Res [Internet]. 2022;66(2):JPR_D_20_00207. Available from: https://www.jstage.jst.go.jp/article/jpr/66/2/66_JPR_D_20_00207/_article
12. McGowan L, McCrum L-A, Watson S, Cardwell C, McGuinness B, Rutherford H, et al. The impact of oral rehabilitation coupled with healthy dietary advice on the nutritional status of adults: A systematic review and meta-analysis. Crit Rev Food Sci Nutr [Internet]. 2020;60(13):2127–47. Available from: https://www.tandfonline.com/doi/full/10.1080/10408398.2019.1630600
13. Nakamura M, Ojima T, Nagahata T, Kondo I, Ninomiya T, Yoshita K, et al. Having few remaining teeth is associated with a low nutrient intake and low serum albumin levels in middle-aged and older Japanese individuals: findings from the NIPPON DATA2010. Environ Health Prev Med [Internet]. 2019;24(1):1. Available from: https://environhealthprevmed.biomedcentral.com/articles/10.1186/s12199-018-0752-x
14. Iwasaki M, Yoshihara A, Ogawa H, Sato M, Muramatsu K, Watanabe R, et al. Longitudinal association of dentition status with dietary intake in Japanese adults aged 75 to 80 years. J Oral Rehabil [Internet]. 2016;43(10):737–44. Available from: https://onlinelibrary.wiley.com/doi/10.1111/joor.12427
15. Hamada MO, Garrett NR, Roumanas ED, Kapur KK, Freymiller E, Han T, et al. A randomized clinical trial comparing the efficacy of mandibular implant-supported overdentures and conventional dentures in diabetic patients. Part IV: Comparisons of dietary intake. J Prosthet Dent [Internet]. 2001;85(1):53–60. Available from: https://www.sciencedirect.com/science/article/pii/S0022391301634589
16. Moynihan P., Butler T., Thomason J., Jepson NJ. Nutrient intake in partially dentate patients: the effect of prosthetic rehabilitation. J Dent [Internet]. 2000;28(8):557–63. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0300571200000440
17. 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 [Internet]. 2021;n71. Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.n71
18. Higgins J, Thomas J. Cochrane handbook for systematic reviews of interventions [Internet]. 6.5. Cochrane. 2024 [cited 2026 May 22]. Available from: https://www.cochrane.org/authors/handbooks-and-manuals/handbook
19. Barker TH, Habibi N, Aromataris E, Stone JC, Leonardi-Bee J, Sears K, et al. The revised JBI critical appraisal tool for the assessment of risk of bias for quasi-experimental studies. JBI Evid Synth [Internet]. 2024;22(3). Available from: https://journals.lww.com/jbisrir/fulltext/2024/03000/the_revised_jbi_critical_appraisal_tool_for_the.4.aspx
20. Bradbury J, Thomason JM, Jepson NJA, Walls AWG, Allen PF, Moynihan PJ. Nutrition Counseling Increases Fruit and Vegetable Intake in the Edentulous. J Dent Res [Internet]. 2006;85(5):463–8. Available from: https://journals.sagepub.com/doi/10.1177/154405910608500513
21. Kumar K, Kumar S, Khandpur M, Singh N, Singh BP, Garg RK. The effect of food supplements on completely edentulous women rehabilitated with complete dentures: A randomized controlled trial. J Indian Prosthodont Soc [Internet]. 2023;23(4):347–55. Available from: https://journals.lww.com/10.4103/jips.jips_237_23
22. Agrawal S, Sathe S, Paul P, Doshi K, Agrawal A, Rathi N. Evaluation of the Role of Dentures & Dietary Advice on Nutritional Status of Complete Edentulous Patients Using MNA®-SF: An Observational Study. Cureus [Internet]. 2023; Available from:

https://www.cureus.com/articles/196426-evaluation-of-the-role-of-dentures--dietary-advice-on-nutritional-status-of-complete-edentulous-patients-using-mna-sf-an-observational-study

23. Vijaya S, Rodrigues A, Garg M, Vijaya S, Shetty MJ, Dhanania S. Effectiveness of dietary intervention in geriatric patients receiving new complete dentures: A randomized controlled trial. J Indian Prosthodont Soc [Internet]. 2024;24(4):329–35. Available from: https://journals.lww.com/10.4103/jips.jips_166_24
24. Amagai N, Komagamine Y, Kanazawa M, Iwaki M, Jo A, Suzuki H, et al. The effect of prosthetic rehabilitation and simple dietary counseling on food intake and oral health related quality of life among the edentulous individuals: A randomized controlled trial. J Dent [Internet]. 2017;65:89–94. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0300571217301793
25. Suzuki H, Kanazawa M, Komagamine Y, Iwaki M, Amagai N, Minakuchi S. Changes in the nutritional statuses of edentulous elderly patients after new denture fabrication with and without providing simple dietary advice. J Prosthodont Res [Internet]. 2019;63(3):288–92. Available from: https://linkinghub.elsevier.com/retrieve/pii/S188319581830152X
26. Kanazawa M, Suzuki H, Komagamine Y, Iwaki M, Amagai N, Minakuchi S. Combined effects of new complete denture fabrication and simplified dietary advice on nutrient intake in edentulous elderly patients for 6 months. Clin Oral Investig [Internet]. 2019;23(5):2245–52. Available from: http://link.springer.com/10.1007/s00784-018-2669-6
27. Suzuki H, Kanazawa M, Komagamine Y, Iwaki M, Jo A, Amagai N, et al. The effect of new complete denture fabrication and simplified dietary advice on nutrient intake and masticatory function of edentulous elderly: A randomized-controlled trial. Clin Nutr [Internet]. 2018;37(5):1441–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0261561417302637
28. Yoshida M, Kikutani T, Yoshikawa M, Tsuga K, Kimura M, Akagawa Y. Correlation between dental and nutritional status in community-dwelling elderly Japanese. Geriatr Gerontol Int [Internet]. 2011;11(3):315–9. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1447-0594.2010.00688.x
29. Crowe FL, Roddam AW, Key TJ, Appleby PN, Overvad K, Jakobsen MU, et al. Fruit and vegetable intake and mortality from ischaemic heart disease: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heart study. Eur Heart J [Internet]. 2011;32(10):1235–43. Available from: https://academic.oup.com/eurheartj/article-lookup/doi/10.1093/eurheartj/ehq465
30. Li M, Fan Y, Zhang X, Hou W, Tang Z. Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies. BMJ Open [Internet]. 2014;4(11):e005497. Available from: http://dx.doi.org/10.1136/bmjopen-2014-005497
31. Kaurani P, Kakodkar P, Bhowmick A, Samra RK, Bansal V. Association of tooth loss and nutritional status in adults: an overview of systematic reviews. BMC Oral Health [Internet]. 2024;24(1):838. Available from: https://bmcoralhealth.biomedcentral.com/articles/10.1186/s12903-024-04602-1
32. Sysal P, Veronese N, Arik F, Kalan U, Smith L, ISIK AT. Mini Nutritional Assessment Scale-Short Form can be useful for frailty screening in older adults. Clin Interv Aging [Internet]. 2019;Volume 14:693–9. Available from: https://www.dovepress.com/mini-nutritional-assessment-scale-short-form-can-be-useful-for-frailty-peer-reviewed-article-CIA
33. Brígido JA, de Oliveira da Rosa WL, Lund RG. The effect of prosthetic rehabilitation with or without dietary advice on nutritional status in elderly patients: a systematic review. Aging Clin Exp Res [Internet]. 2023;35(11):2399–411. Available from: https://link.springer.com/10.1007/s40520-023-02578-6
34. Bezerra AP, Gama LT, Pereira LJ, van der Bilt A, Peyron M-A, Rodrigues Garcia RCM, et al. Do implant-supported prostheses affect bioavailability of nutrients of complete and partially edentulous patients? A systematic review with meta-analysis. Clin Nutr [Internet]. 2021;40(5):3235–49. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0261561421000923
35. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol [Internet]. 2014;14(1):135. Available from: https://bmcmedresmethodol.biomedcentral.com/articles/10.1186/1471-2288-14-135

EUROPEAN JOURNAL OF TRANSLATIONAL
AND CLINICAL MEDICINE 2026;9(1):87-92

STUDY PROTOCOL

Medication errors in anti-retroviral therapy among people living with HIV and the impact of stewardship interventions: study protocol for a systematic review and meta-analysis

Carlos de Andrés David[1], Jose Ignacio Mateo-González[2]

[1] Hospital Universitario de Dénia, Alicante, Spain
[2] Consorci Hospital General Universitari de València, Spain

Abstract

Background: Medication errors involving antiretroviral therapy (ART) in people living with HIV (PLHIV) are frequent, particularly in general hospitals and during care transitions, and may lead to preventable toxicity, virological failure and drug resistance. Several stewardship interventions have been implemented to reduce these errors, but the available evidence remains fragmented and heterogeneous. **Material and methods:** We will conduct a systematic review and meta-analysis of observational studies and intervention studies reporting ART-related medication errors in adults (≥ 18 years) with HIV in any clinical setting. We will search MEDLINE, Embase, CINAHL, Web of Science, CENTRAL and grey literature without language or date restrictions. Two reviewers will independently screen records, extract data and assess risk of bias using design-specific tools (RoB-2, ROBINS-I, Newcastle-Ottawa Scale and EPOC). We will perform random-effects meta-analyses when appropriate and synthesize implementation outcomes narratively. Certainty of evidence for key outcomes will be assessed with GRADE. **Conclusions:** This review will provide up-to-date, quantitatively synthesized estimates of the burden and clinical impact of ART-related medication errors and will evaluate the effectiveness of antiretroviral stewardship and related interventions. Its findings will inform HIV units, antimicrobial stewardship programmes and policy-makers aiming to improve the safety of ART. **Study registration:** PROSPERO CRD420251233303.

Keywords: HIV · patient safety · medication errors · antiretroviral therapy · stewardship

Citation

De Andrés David C, Mateo-González JI. Medication errors in anti-retroviral therapy among people living with HIV and the impact of stewardship interventions: study protocol for a systematic review and meta-analysis. Eur J Transl Clin Med. 2026;9(1):87-92.

DOI: 10.31373/ejtcm/225160

Corresponding author:
Carlos de Andrés David, Hospital Universitario de Dénia, Alicante, Spain
e-mail: c.andres@outlook.com
Available online: ejtcm.gumed.edu.pl

Introduction

Medication errors involving anti-retroviral therapy (ART) in people living with HIV represent a persistent patient-safety issue that is often underestimated in routine clinical care. Although current ART regimens are highly effective, they remain pharmacologically complex. Fixed-dose combinations, strong inhibition (or induction) of metabolic pathways, relevant transporter effects and the need for dose adjustment in renal or hepatic impairment all increase the potential for error. In clinical practice, small prescribing or administration changes can have disproportionate clinical consequences [1-5].

These problems are particularly visible in hospital settings. During inpatient care, ART is frequently prescribed or modified by clinicians who do not routinely manage HIV treatment, often under time pressure and with limited access to specialist advice. The reported prevalence of ART-related medication errors varies widely, but rates above 20% (and in some settings substantially higher) are repeatedly described [1, 3, 6-8]. Typical errors include incomplete regimens, unintended treatment interruptions, inappropriate substitutions between non-equivalent agents, dosing mistakes and clinically significant drug-drug interactions, particularly at admission and discharge.

The clinical impact of such errors goes beyond theoretical concern. Unplanned interruptions or incomplete ART regimens may lead to virological rebound and facilitate the selection of resistance mutations. Conversely, failure to account for organ dysfunction or drug interactions can result in avoidable toxicity and adverse drug events [1-2, 4 ,6]. At a population level, these individual failures matter because sustained viral suppression is central not only to individual prognosis but also to public-health objectives, including the UNAIDS 95-95-95 targets [9].

Several factors contribute to the occurrence of ART-related medication errors. Many reports highlight limited familiarity with ART among non-specialist prescribers, electronic prescribing systems that inadequately reflect ART-specific constraints, variable involvement of clinical pharmacists, and the absence of standardised processes to validate or reconcile ART prescriptions [2, 4, 8, 10]. In parallel, the demographic profile of people living with HIV is changing. Increasing age, multimorbidity and polypharmacy further amplify the risk of errors and drug–drug interactions in everyday practice.

In addition to prescriber- and system-level determinants, patient-related and structural factors may modify the risk and consequences of ART-related medication errors. Substance use disorders, mental health comorbidity, unstable housing, stigma, privacy concerns and avoidance of healthcare services can impair adherence to ART and clinic attendance, and may increase vulnerability to treatment interruptions, incomplete medication reconciliation or delayed recognition of prescribing errors. These factors are not medication errors, but they may influence both the likelihood that errors occur and their clinical impact, particularly during hospital admission, discharge and transitions of care.

In response to these challenges, a range of interventions has been developed over the last decade. These include pharmacist-led medication reviews, dedicated antiretroviral stewardship programmes, predefined order sets, structured reconciliation workflows and electronic decision-support tools. Many single-centre studies report reductions in error rates or faster correction of errors after implementation of such strategies. However, interpretation is difficult because definitions of “medication error,” outcome measures and study designs differ substantially across reports [1, 3-4, 6-7, 11].

For these reasons, a comprehensive and methodologically rigorous synthesis of the available evidence is warranted. This protocol describes a systematic review and meta-analysis designed to quantify the burden and nature of ART-related medication errors, explore associated clinical consequences and assess the observed impact of stewardship-type interventions. It describes applying design-specific risk-of-bias tools and grading the certainty of evidence using the GRADE methodology. In this systematic review and meta-analysis we aim to provide results that are both transparent and directly applicable to clinical practice and healthcare organisation.

Material and methods

Study design and registration

This manuscript describes the protocol for a prospectively registered systematic review and meta-analysis. The protocol was developed in accordance with PRISMA-P recommendations and registered in the PROSPERO registry (CRD4202512-33303) before study selection began. The completed review will be reported in accordance with the PRISMA 2020 guidance [12].

From the outset, this systematic review was planned to address 2 closely related questions. First, how frequent are medication errors involving antiretroviral therapy in adults living with HIV and how do these errors differ across various healthcare settings. Second, whether antiretroviral stewardship interventions are associated with measurable reductions in such errors. Key methodological decisions, including eligibility criteria, outcomes of interest and analytical approaches, were specified a priori to minimise selective reporting and analytical flexibility.

Objectives

The primary objective of this systematic review is to identify and characterise the full spectrum of medication errors related to antiretroviral therapy and HIV treatment stewardship in adults living with HIV, across different clinical contexts. This will include errors during prescribing, medication reconciliation, dispensing, administration, treatment interruption, regimen substitution and transitions of care. A second primary objective is to evaluate the effect of interventions designed to prevent or correct these errors, including pharmacist-led programmes, stewardship initiatives and system-level strategies.

Secondary objectives include summarising reported clinical consequences of ART-related medication errors (e.g. virological outcomes, drug-related toxicity and preventable adverse events) as well as organisational outcomes when available (e.g. length of hospital stay or time to error correction). Where data permit, this review will also explore sources of heterogeneity, including healthcare setting, study design and type of intervention implemented.

Eligibility criteria

We will include studies that report medication errors related to antiretroviral therapy in adults (≥ 18 years) living with HIV, as well as studies evaluating interventions intended to reduce such errors. Eligible study designs will comprise observational studies (cross-sectional, cohort and case-control) and interventional studies (including randomised trials), quasi-experimental designs and before-after evaluations. Case reports and case series with < 10 participants will be considered when they provide sufficient methodological and clinical detail to identify uncommon, severe or potentially preventable ART-related medication errors. These reports will be included only in the qualitative synthesis and will not contribute to pooled prevalence estimates or quantitative analyses of intervention effects. Larger case series, observational studies and interventional studies will be considered for quantitative synthesis when appropriate.

We will include studies conducted in any healthcare setting, including inpatient wards, emergency departments, outpatient clinics and transitional care environments. No restrictions will be applied based on country, healthcare system or language of publication. Studies focusing exclusively on paediatric populations will be excluded because paediatric ART formulations, dosing strategies and prescribing processes differ substantially from those used in adults. We will also exclude case reports, narrative reviews, editorials and conference abstracts that do not provide sufficient methodological detail.

The primary outcomes of interest are the prevalence and types of ART-related medication errors and for interventional studies, also changes in error frequency following implementation of a stewardship-type intervention. Secondary outcomes include reported clinical consequences (e.g. virological failure, drug toxicity or preventable adverse events) and organisational or process-related outcomes (e.g. time to error detection or length of hospital stay), when these data are available.

Information sources and search strategy

We will perform comprehensive searches in MEDLINE (via PubMed), Embase, CINAHL, Web of Science Core Collection and the Cochrane Central Register of Controlled Trials (CENTRAL). Searches will not be restricted by language or publication date (from database inception to the present). Grey literature will be explicitly included in the search strategy in order to minimise publication bias and capture relevant unpublished or difficult-to-index evidence. Sources will include OpenGrey, ProQuest Dissertations and Theses, conference proceedings and other relevant institutional or professional sources when sufficient information is available to assess eligibility. In addition, we will screen reference lists of included studies and perform forward citation tracking using Google Scholar. If clarification or additional data are required, we will contact study authors.

Search strategies will combine controlled vocabulary terms (MeSH and Emtree) with free-text keywords related to HIV, antiretroviral therapy, medication errors and stewardship-type interventions. Strategies will be adapted for each database and developed with input from an experienced medical librarian. Where possible, search strategies will be peer reviewed using the PRESS framework. Full search strings for all databases will be provided in the supplementary materials.

Study selection

All records retrieved from the searches will be imported into Rayyan for de-duplication and screening [13]. Two reviewers will independently screen titles and abstracts against the predefined eligibility criteria. Full texts of potentially relevant articles will then be assessed independently by the same reviewers. Any disagreements will be resolved through discussion and, when necessary, consultation with a third reviewer.

Reasons for exclusion at the full-text stage will be documented, and the overall study selection process will be summarised in a PRISMA flow diagram. If multiple publications report data from the same underlying study population, these reports will be linked and treated as a single study, with data extracted from the most complete and informative source.

Data extraction

Data extraction will be performed independently by 2 reviewers using a standardised extraction form that will be piloted on a small sample of included studies and refined if necessary. For each study, we will collect information on study design, setting, country, study period and participant characteristics. Definitions and methods of detecting medication errors vary widely across the literature, therefore we will also record how they were defined and identified in the included studies.

For descriptive studies, we will extract data on the number and types of ART-related medication errors, prevalence estimates and any reported clinical or organisational consequences. For interventional studies, we will additionally collect detailed information on the intervention itself (e.g. its main components, duration, implementation context and comparator conditions). If outcome data are incomplete or unclear, we will attempt to contact the corresponding authors for clarification. If numerical results are presented only graphically, we will do our best to extract approximate values using digital measurement tools. Equivocal situations will be resolved through discussion or by involving a third reviewer.

When reported, we will also extract patient-related and structural risk modifiers that may influence ART safety, e.g. substance use disorders, mental health comorbidity, housing instability, stigma/privacy concerns, history of poor adherence, retention in care and use of long-acting injectable antiretroviral agents. These variables will be summarised descriptively and, if sufficiently reported across studies, explored as potential sources of heterogeneity or effect modification.

Risk of bias assessment

The risk of bias will be assessed independently by two reviewers, using tools appropriate for each study design. Randomized controlled trials will be assessed using the Cochrane Risk of Bias 2 (RoB 2) tool [14]. Non-randomized interventional studies will be assessed using ROBINS-I, paying particular attention to confounding factors and deviations from planned interventions [15]. Observational studies will be assessed using the Newcastle-Ottawa scale, adapted pragmatically where necessary to reflect the specific challenges of medication error research [16].

For studies evaluating healthcare system or educational interventions, the criteria of the Cochrane Effective Practice and Organization of Care (EPOC) group will also be considered. Disagreements in risk of bias assessments will be discussed until consensus is reached, with the involvement of a third reviewer when necessary. Risk of bias assessments will inform sensitivity analyses and interpretation of results, but studies will not be excluded solely on the basis of these assessments.

Data synthesis and statistical analysis

First, we will summarise the characteristics and results of the included studies descriptively, grouping them by study design, context, and outcome. When studies present with enough comparable results, we will perform a meta-analysis using random-effects models to account for the expected clinical and methodological heterogeneity. For prevalence estimates, appropriate transformations will be applied when necessary to stabilize variances prior to pooling.

In interventional studies, effect estimates will be synthesized using risk ratios, odds ratios, or mean differences, depending on the type of outcome and the form of reporting. Statistical heterogeneity will be quantified using the I^2 statistic and explored using prespecified subgroup analyses, such as healthcare setting, type of intervention, and study design, provided that an adequate number of studies are available. Sensitivity analyses will be performed by excluding studies considered to be at high risk of bias. When at least 10 studies contribute to a given meta-analysis, we will assess potential publication bias using funnel plots and formal statistical tests. All statistical analyses will be performed using Stata (StataCorp LLC, College Station, TX, USA).

Certainty of evidence

The certainty of evidence for key outcomes will be evaluated using the GRADE framework [17]. For each outcome, we will consider risk of bias, inconsistency, diffuseness, imprecision and potential publication bias. When the systematic review is feasible, GRADE assessments will be presented in table format. If quantitative synthesis is not possible, the same principles will be applied in a narrative review in order to ensure transparency in the reliability with which evidence is assessed. These assessments are intended to help readers distinguish between outcomes supported by evidence and those where conclusions remain uncertain.

Use of artificial intelligence

Artificial intelligence (AI) tools were used to assist with language review during writing of the manuscript and the translation to English. Decisions regarding study design, eligibility criteria, data extraction, risk-of-bias assessment, statistical analysis and interpretation were made by the authors. The final version of the manuscript was reviewed and edited by the authors to ensure accuracy, consistency and alignment with the study objectives.

AI tools will not replace independent human judgement while conducting this systematic review. Study selection, data extraction, risk-of-bias assessment, statistical analysis and interpretation will be performed and verified by the authors according to the methods described above.

Results

This article describes the study protocol, therefore results are not yet available and are not presented here. This document highlights the methods that will be used in study selection, data collection and synthesis. The systematic review will be conducted after the publication of this protocol, following the procedure described earlier.

Discussion

Despite the availability of well-established clinical guidelines and simplified therapeutic regimes, medication errors involving antiretroviral therapy (ART) continue to happen in standard hospital practices. The problem is more frequent during admissions and care transitions, when ART is usually managed by a non-specialist practitioner outside office hours. Multiple studies have addressed this issue, but the literature lacks in quality and is difficult to translate across healthcare settings [1, 3-4, 6-7, 11]. This protocol describes a structured approach via systematic review of the published evidence surrounding this issue with the objective of assessing the burden of ART-related medication errors and the observed impact of stewardship-type interventions.

The key strength of this systematic review is the inclusion of observational and interventional studies within a single analytical framework. Descriptive studies inform on how often this errors occur and what kind of them are, while interventional studies address whether the efficacy of targeted strategies in reducing the error frequency. Previous data suggest that reviews led by pharmacists, stewardship programmes and computer-level interventions can lead to significant reduction in the quantity and quality of errors, although the extent to which these reduction can be extrapolated to different healthcare settings remains unknown [1, 3-4, 7]. By integrating risk-of-bias reductions tools in the design and implementation of a grading system to measure the certainty of evidence (GRADE), we aim to clarify how much confidence can be placed in these estimates.

Several challenges are anticipated. There is not a consensus on the definition of “medication error” and the methods used to identify and categorize those errors are considerably different in the literature. These differences are likely to account for part of the expected heterogeneity of data. Interventions described as “stewardship” also differ greatly in method and type, raging form single-component pharmacist review to multidisciplinary and multi-approach programmes that combines education, restrictions of prescription and computer-supported decision-making [3-4, 6-7]. Such variability can be a limitation of this study, via diminishing the precision of the quantitative pooling for particular outcomes and confound direct comparison between different strategies.

The emergence of long-acting injectable ART also deserves specific consideration in the context of medication safety. Long-acting cabotegravir + rilpivirine may reduce some risks associated with daily oral dosing, pill burden, dispensing complexity and inadvertent treatment interruptions, particularly in selected people with adherence challenges. However, these regimens do not eliminate medication-safety risks, but rather they shift part of the risk toward scheduling of injections, management of missed or delayed doses, oral bridging strategies, documentation across care settings and prevention of functional monotherapy or resistance in the setting of prolonged subtherapeutic drug exposure. For this reason, stewardship interventions may need to adapt to long-acting ART by incorporating injection tracking, structured recall systems and clear protocols for missed doses or hospital admissions.

Despite these limitations, the planned systematic review and meta-analysis is expected to be clinically informative. By quantifying the frequency and nature of ART-related medication errors and summarising which intervention components appear most consistently associated with error reduction, this review can support decision-making in infectious diseases units, hospitals and antimicrobial stewardship programmes. Ultimately, improving the safety and continuity of ART prescriptions requires interventions that are not only effective but also feasible in real-world clinical environments, especially outside specialist HIV units.

Conclusions

This protocol describes the methodology of a systematic review and meta-analysis on medication errors associated with ART and its impact of stewardship interventions. The review will take into consideration how often these mistakes happen, how they are reported across different healthcare settings and whether structured stewardship interventions can be associated with a reduction in error frequency. It is expected that our results can potentially guide efforts to improve safety and ensure continuity of the ART prescription in both specialist and general care.

References

1. Daniels LM, Raasch RH, Corbett AH. Implementation of targeted interventions to decrease antiretroviral-related errors in hospitalized patients. Am J Heal Pharm [Internet]. 2012;69(5):422–30. Available from: https://academic.oup.com/ajhp/article/69/5/422/5112862
2. Liedtke M, Tomlin C, Skrepnek G, Farmer K, Johnson P, Rathbun R. HIV Pharmacist's Impact on Inpatient Antiretroviral Errors. HIV Med [Internet]. 2016;17(10):717–23. Available from: https://onlinelibrary.wiley.com/doi/10.1111/hiv.12375
3. Brizzi MB, Burgos RM, Chiampas TD, Michienzi SM, Smith R, Yanful PK, et al. Impact of Pharmacist-Driven Antiretroviral Stewardship and Transitions of Care Interventions on Persons With Human Immunodeficiency Virus. Open Forum Infect Dis [Internet]. 2020;7(8). Available from: https://academic.oup.com/ofid/article/doi/10.1093/ofid/ofaa073/5896552
4. Billedo JAS, Berkowitz LB, Cha A. Evaluating the Impact of a Pharmacist-Led Antiretroviral Stewardship Program on Reducing Drug Interactions in HIV-Infected Patients. J Int Assoc Provid AIDS Care [Internet]. 2016;15(1):84–8. Available from: https://journals.sagepub.com/doi/10.1177/2325957415600700
5. Agu KA, Oqua D, Adeyanju Z, Isah MA, Adesina A, Ohiaeri SI, et al. The Incidence and Types of Medication Errors in Patients Receiving Antiretroviral Therapy in Resource-Constrained Settings. Ensoli B, editor. PLoS One [Internet]. 2014;9(1):e87338. Available from: https://dx.plos.org/10.1371/journal.pone.0087338
6. Sanders J, Pallotta A, Bauer S, Sekeres J, Davis R, Taege A, et al. Antimicrobial Stewardship Program to Reduce Antiretroviral Medication Errors in Hospitalized Patients with Human Immunodeficiency Virus Infection. Infect Control Hosp Epidemiol [Internet]. 2014;35(3):272–7. Available from: https://www.cambridge.org/core/product/identifier/S0899823X00191196/type/journal_article
7. Chiampas TD, Biagi MJ, Badowski ME. Impact of an HIV-trained clinical pharmacist intervention on error rates of antiretroviral and opportunistic infection medications in the inpatient setting. Pharm Pract (Granada) [Internet]. 2019;17(3):1543. Available from: https://www.pharmacypractice.org/index.php/pp/article/view/1543
8. Carcelero E, Tuset M, Martin M, De Lazzari E, Codina C, Miró J, et al. Evaluation of antiretroviral-related errors and interventions by the clinical pharmacist in hospitalized HIV-infected patients. HIV Med [Internet]. 2011;12(8):494–9. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1468-1293.2011.00915.x
9. Understanding Fast-Track: accelerating action to end the AIDS epidemic by 2030. Joint United Nations Programme on HIV/AIDS (UNAIDS) [Internet]. 2014. Available from: https://www.unaids.org/sites/default/files/media_asset/JC2686_WAD2014report_en.pdf
10. Arshad S, Rothberg M, Rastegar DA, Spooner LM, Skiest D. Survey of physician knowledge regarding antiretroviral medications in hospitalized HIV-infected patients. J Int AIDS Soc [Internet]. 2009;12(1):1–1. Available from: https://onlinelibrary.wiley.com/doi/10.1186/1758-2652-12-1
11. Koren DE, Scarsi KK, Farmer EK, Cha A, Adams JL, Pandit NS, et al. A Call to Action: The Role of Antiretroviral Stewardship in Inpatient Practice, a Joint Policy Paper of the Infectious Diseases Society of America, HIV Medicine Association, and American Academy of HIV Medicine. Clin Infect Dis [Internet]. 2020;70(11):2241–6. Available from: https://academic.oup.com/cid/article/70/11/2241/5564335
12. 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 [Internet]. 2021;n71. Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.n71
13. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan — a web and mobile app for systematic reviews. Syst Rev [Internet]. 2016;5(1):210. Available from: http://systematicreviewsjournal.biomedcentral.com/articles/10.1186/s13643-016-0384-4
14. Sterne JAC, 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 [Internet]. 2019;l4898. Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.l4898
15. Risk of bias tools. ROBINS-I V2 tool [Internet]. Medical Research Council. 2025 [cited 2026 Jul 1]. Available from: https://sites.google.com/site/riskofbiastool/welcome/robins-i-v2
16. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses [Internet]. The Ottawa Hospital Research Institute. 2000 [cited 2026 Jul 1]. Available from: https://ohri.ca/en/who-we-are/core-facilities-and-platforms/ottawa-methods-centre/newcastle-ottawa-scale
17. Grading quality of evidence and strength of recommendations. BMJ [Internet]. 2004;328(7454):1490. Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.328.7454.1490