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EDITORIAL

The Journal of Multidisciplinary Research (JMR) was founded on the conviction that the most pressing questions of our time rarely respect disciplinary boundaries. Volume 6 affirms that conviction. The eleven articles in this issue range across nursing ethics, mental health, nutrition, pharmacology, pharmaceutical biotechnology, molecular neuroscience, and artificial intelligence, yet they share a common purpose: to generate evidence that is rigorous, contextually grounded, and of practical value to practitioners, policymakers, and communities in Sri Lanka and beyond.

The issue opens with questions of care and conscience. In "Advancing Ethical Nursing Practice: A Review of Dilemmas, Frameworks, and Emerging Solutions," Chathurika J.N. and Thusthika A. synthesise 32 studies, drawn from an initial pool of 3,704 records, to propose a conceptual framework that maps ethical challenges from the type of dilemma through to its resolution. Their identification of moral distress, driven largely by understaffing and excessive workloads, as the most frequent source of conflict is a timely reminder that ethical practice depends as much on institutional conditions as on individual judgement. A complementary clinical perspective is offered by Dissanayake and Chathurika in "Psychoeducation, Acceptance and Commitment Therapy, and Interpersonal and Social Rhythm Therapy as Phased Psychosocial Interventions in Bipolar I Disorder." Through a case-based analysis guided by the biopsychosocial model, the authors show how these interventions can work alongside pharmacotherapy to strengthen insight, psychological flexibility, and relapse prevention. Although a single case cannot establish effectiveness, the paper makes a persuasive argument for structured psychosocial care in resource-limited psychiatric settings.

Two empirical studies turn attention to the well-being of young adults. Bhagya K.G.S. and Jayasinha, in "A Study on the Effects of Traditional Sri Lankan Dancing on the Mental Well-Being of Young Adults in Sri Lanka," compare 128 dancers and non-dancers using the Ryff Psychological Well-Being Scale and report significantly higher well-being among those trained in the Kandyan, Low Country, and Sabaragamuwa traditions. The study invites us to view the island's cultural heritage not only as something to be preserved but also as a resource for mental health. In "Association Between Food Consumption Patterns

and Perceived Stress Among Undergraduates at a Selected Private University in Sri Lanka," Dharmadasa et al. find that 41.5% of 301 undergraduates reported high perceived stress, alongside a protein-rich but sugar-heavy dietary pattern. That only poultry consumption showed a significant association with stress is itself an instructive result, and the authors rightly call for longitudinal designs capable of disentangling diet, stress, and their confounders.

The biomedical and pharmaceutical sciences are represented by three reviews. Promodi P.I. and Ratnayake, in "Does Prolonged Metformin Exposure Induce Neuroprotection or Exacerbate Neurodegenerative Diseases in Type 2 Diabetes Mellitus: A Narrative Review," examine 29 studies and find the evidence on Alzheimer's and Parkinson's disease to be genuinely mixed, while the association between prolonged use and vitamin B12 deficiency is consistent. For one of the world's most widely prescribed drugs, this balanced appraisal carries clear implications for monitoring in clinical practice. Dias and Senanayake, in "Review on Development of Pharmaceutical Dosage Forms from Tinospora cordifolia and Application of Micropropagation to Supply the Pharmaceutical Demand," survey formulations ranging from conventional tablets and syrups to phytosomes and nano-formulations, and argue that micropropagation offers a sustainable route to a reliable, genetically uniform supply of this valued medicinal plant. At the molecular level, De Silva et al., in "The PINK1/Parkin-USP30-NRF2 Axis in Parkinson's Disease: Molecular Pathophysiology and Emerging Therapeutic Targets," draw together evidence on failed mitophagy, USP30-mediated deubiquitylation, and NRF2 oxidative stress signalling to argue that these processes form a single, self-reinforcing pathological axis, and identify pharmacological inhibition of USP30 as a particularly promising route towards disease-modifying therapy.

Three contributions reflect the rapid growth of artificial intelligence in health and society, and each addresses not only what these technologies can do but also how they should be built and trusted. Marasinghe et al., in "Explainable AI for Early Type 2 Diabetes Prediction Using Clinical and Metabolic Biomarkers: A Human-Centered Review," show that combining metabolic biomarkers with routine clinical indicators improves prediction, and that techniques such as SHAP and LIME can open "black box" models to clinical

scrutiny; they also caution that limited stakeholder involvement and external validation remain barriers to adoption. Nanayakkara et al., in "Artificial Intelligence in Kidney Stone Diagnosis and Management: A Narrative Review," trace how machine learning and deep learning applied to CT, ultrasound, and X-ray imaging are advancing stone detection, composition analysis, and treatment prediction. Finally, Perera and Jayakody, in "Culturally Diverse, Demographically Aware Facial Emotion Recognition Using YOLO11n-Classification: A Comparative Study," confront the demographic bias embedded in widely used benchmark datasets. By incorporating locally captured Sri Lankan facial images into a curated dataset of 14,000 images, their YOLO11n-cls model achieved a Top-1 accuracy of 94.2%, an important demonstration that representative data is a prerequisite for fair and reliable AI.

Read together, these articles reveal several threads that run through the volume: a concern for the well-being of students, patients, and healthcare workers; an insistence that local culture and local data matter; and a recognition that emerging technologies must be transparent and equitable if they are to serve society. It is also encouraging that many of these papers are led by early-career and student researchers working alongside experienced academics, a collaboration that lies at the heart of the journal's mission.

On behalf of the Editorial Board, I thank all the authors for their contributions, the reviewers for their careful and constructive evaluations, and the editorial team for their dedication in bringing this volume to publication. We hope that readers will find in these pages both useful evidence and fresh questions, and we warmly invite researchers from all disciplines to contribute to future issues of JMR.

Dr. Madhavi Hewadikaram (Editor-in-Chief)

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ADVANCING ETHICAL NURSING PRACTICE: A REVIEW OF DILEMMAS, FRAMEWORKS, AND EMERGING SOLUTIONS

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Abstract

Complex healthcare scenarios that demand balancing potential benefits against risks frequently result in ethical dilemmas, most frequently within the humanitarian responsibilities of nursing. As nursing roles expand and responsibilities broaden, an increasing demand for comprehensive frameworks to guide ethical decision-making emerges. This study investigates the ethical dilemmas faced in clinical practice, evaluates the decision-making models applied by nurses, and determines their relevance to present-day nursing practice. Literature research was conducted across databases, including PubMed, CINAHL, Scopus, and Google Scholar. From an initial set of 3,704 articles, 32 scientific publications from 2010 to 2025 were selected for analysis. A rapid review approach, supported by a literature matrix, was used to synthesize key themes related to ethical dilemmas and decision-making strategies. The review resulted in the development of a conceptual framework that organizes ethical challenges according to the type of dilemma, reasoning processes, decision pathways, ethical responses, and mechanisms of resolution. Moral distress, particularly arising from understaffing and excessive workloads, was identified as the most common source of conflict. Using an ecological perspective, the study also situated ethical dilemmas within broader socio-political, regulatory, organizational, interpersonal, and individual contexts. The findings highlight the urgent need for reforms in nursing education, institutional guidelines, and leadership practices to strengthen nurses' ethical reasoning, awareness, and confidence when navigating ethical challenges. Cultivating critical thinking, aligning workplace policies with emerging healthcare demands, and fostering supportive professional environments are vital for advancing ethical nursing practice.

Keywords: ethics, decision-making, dilemma, models, nursing

1. Introduction

Technological advancements drive the concept of patient care into a more efficient and effective pathway. The healthcare team is held to a higher standard of responsibility towards the patient, given the integration of technology into holistic patient care. Nurses can be considered as patient caretakers who are always with the patient from the point of admission to diagnosis and preferably until the resolution of the disease. The closer relationship evokes the nurses' need to provide the best care possible, affirming professional responsibilities along with safeguarding the patient's safety and dignity. Instances that demand actions based on professional conduct surpassing humanitarian values create dilemmas where nurses face the difficulty of safeguarding the patient's needs, given accepted healthcare guidelines (Barlow et al., 2018). Nurses adhere to ethics to overcome difficulties in weighing professional responsibility over deemed healthcare needs.

An ethical dilemma is considered a situation that warrants a decision made, weighing both desirable and undesirable effects, with significant consequences associated with the decision made (Haahr et al., 2020). By way of explanation, an ethical dilemma requires affirmation of both professional conduct and conscious thought, considering the humanitarian aspect of demeanor. Changing trends in society and increased focus on human rights have greatly influenced the scope of practice of health professionals (Haahr et al., 2020).

The inability to act upon humanitarian concepts owing to professional responsibility and ethics can be considered as one factor leading to moral distress. Moral distress is the psychological disequilibrium that occurs due to a lack of congruence between ethically correct behavior and required action owing to various reasons: inadequate opportunity to make autonomy decisions, professional codes, policies and guidelines and administrative failures (Vargas and Concha, 2019). Understaffing and long working hours compel nurses to work under stress and 'burnout,' and stress associated with work creates ethical dilemmas where nurses are unable to address the patient's holistic care needs (Vargas and Concha, 2019). Technology has sufficiently narrowed down the nurses' administrative and documentation needs (Barlow et al., 2018). Technological advancements have significantly reduced certain nursing procedures; however, they still

have not been able to replace the 'quality care' provided by nurses (Hong, 2023). Under-staffing significantly reduces the nurses' ability to provide quality care, thus creating discrepancies in nursing ethics and conduct over health care responsibility.

Nurses are guided by bioethical principles in making decisions during ethically difficult situations: non-maleficence, beneficence, autonomy, justice and confidentiality (Suzuki et al., 2015). Beneficence is the provision of care resulting in benefits to the receiver, whereas non-maleficence is a broader concept that involves refraining from harm to others (Hopia et al., 2016). Autonomy is the personal right to make one's own choices regarding one's treatment and care. It involves making decisions based on their values and beliefs (Suzuki et al., 2015). Nevertheless, all the treatment and care plans should be shaped based on the patient's informed consent, and the selection of the best possible outcome by weighing the benefits over risks associated with the treatment modalities. The principle of justice implies the importance of ethical decision-making to affirm fairness and equality. Humanitarian concepts of autonomy, objectivity and positive attitudes in patient care are guided by ethical values and norms (Barlow et al., 2018).

Some situations that involve uncertainty of treatment and continuation of medical procedures associated with legal decision-making create discrepancies. Bioethical principles provide poor guidance in such situations, which causes nurses to face ethical dilemmas. Nurses face ethical dilemmas where they are consciously in a conundrum, knowing that the outcome would significantly violate personal and professional values, but are compelled to do so based on institutional or treatment guidelines (Hopia et al., 2016). Ethical principles associated with dilemmas require more advanced resolution criteria, which create ethical decision-making models.

Studies highlight the importance of ethical decision-making models, which involve a reflective process and congruent consideration of nurses' roles and responsibilities associated with ethical decision-making (Milliken &Grace, 2022; Schwartz, 2021; Park & Kim, 2024). An ethical decision-making model is built based on the complex guidelines of moral responsibility, critical thinking and decision-making. The effectiveness of the model can vary based on the nature of the dilemma; hence, multiple models can be forwarded as examples for the resolution of ethical decision-making.

The findings of the study suggested the four main phases of ethical reasoning behavior exhibited by health care professionals. This involves ethical dilemma, moral reasoning, ethical decision-making, moral action and resolution. The conceptual framework involving the four main phases is adopted by Barlow's nurses' contribution to the resolution of ethical dilemmas in context and developed based on the study findings (Barlow et al., 2018).

2. Methodology

This study adhered to the Preferred Reporting Items for Rapid and Systematic Reviews (PRISMA) guidelines to ensure a comprehensive and systematic approach. The research aimed to synthesize evidence on nursing students' knowledge, attitudes, and readiness for palliative care in both global and Sri Lankan contexts. A systematic search was conducted using databases such as PubMed, CINAHL, Scopus, and Google Scholar with keywords including "nursing," "decision-making," "dilemma," "ethics," and "models." Boolean operators and filters were applied to refine the search. Inclusion criteria focused on peer-reviewed articles published in English between 2010 and 2025, involving nurses in Sri Lanka or internationally, and addressing ethical decision-making. Exclusion criteria included studies focusing on other healthcare professionals, articles not directly related to the topic, and grey literature. Eligible studies were independently screened by two reviewers, with discrepancies resolved through discussion. Quality and relevance were assessed to ensure alignment with the research objectives. Data from the selected studies were synthesized to identify key themes and trends in nursing education, highlighting differences and similarities between Sri Lankan and global contexts, and providing insights into the ethical dilemmas, Moral reasoning, Moral action & resolution, ethical decision making and supported models.

3. Findings and Discussion

3.1 Ethical dilemmas

The first phase of the ethical dilemma is explored, given Park & Kim (2024), legal, ethical and health policy issues. The context identifies ten common ethical dilemmas described in terms of evidence associated with the article review, Figure 1.

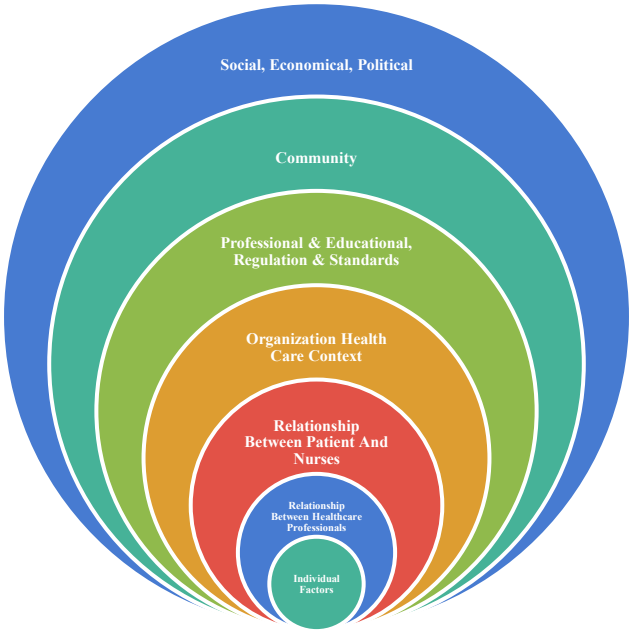


Figure 01: Contextual Dimensions of Ethical Decision-Making in Nursing

Source: Author's Compilation

3.1.1 Active vs. Passive Euthanasia

Euthanasia is defined as the act of medically mediated death when the individual has a profound life-threatening disability or illness that significantly disturbs their quality of life. Euthanasia can be divided into two major types: active and passive euthanasia. Active euthanasia refers to the injection of a lethal dose of medication to facilitate medically induced death, whereas passive euthanasia refers to the purposeful deliberation of death by withholding medical treatments such as ventilator support in advanced cardiac life support (Wiegand et al., 2015).

3.1.2 Rationing vs. Principle of Equal Consideration

Rationing refers to the allocation of scarce resources in treatments. This is evident when certain medications and utilities are scarce, and health professionals are compelled to decide the number of patients who receive the treatment based on multiple factors, such as the present medical condition and the possibility of treatment success.

3.1.3 Informed consent vs. the patient's right to refuse

A patient's informed consent requires adequate information, capacity to decide, and the absence of coercion, which can be affirmed by the principle of autonomy (Wang and Ho, 2013). Certain treatments can be vague considering the patient's condition, but

health professionals are compelled to provide the care based on the patient's consent. Nevertheless, treatment would be lifesaving but cannot be administered considering the patient's right to refuse the medication.

3.1.4 Truth-telling vs. Deception

The patient is entitled to the right to be informed of his treatment. On certain occasions where truth can be hurtful, such as breaking bad news, health professionals face ethical dilemmas concerning patients' psychological well-being. Experimental treatment of such a new drug for oncology patients has two main groups: the treatment group and the placebo group, which creates the dilemma of deception (Mc Lennon et al., 2013).

3.1.5 Pro-choice vs. Pro-life

Pro-choice emphasizes a woman's right to bodily autonomy, while the pro-life position argues that a fetus is a human deserving of legal protection, separate from the will of the mother. This creates a dilemma on the mother's right to abortion (Park & Kim, 2024).

3.1.6 Competence vs. Incompetence

Competence is the ability to conduct a certain procedure or care. Incompetence may negatively affect a patient's health outcomes and health professionals may encounter a dilemma in providing care for the patient (Haahr et al., 2020).

3.1.7 Relief of suffering vs. Principle of double effect

The doctrine of double effect is used to justify an action that produces a good effect – in this case, the relief of suffering – even if it may produce a bad effect, for example, hastening death (Ulrich et al., 2010).

3.1.8 Confidentiality vs. Right to Know

Confidentiality is a patient's right that involves the safeguarding of his or her health information by health professionals. Any breach of patient information can be considered a violation of a patient's rights (Mc Lennon et al., 2013). The right to know deals with the health professionals' right to access patient information to diagnose and provide care.

3.1.9 Quality of life vs. length of life

Life expectancy measures how long a person is expected to live. Quality of life can be measured in terms of living conditions, physical health, mental health, social relationships, level of independence, economic security, safety, or basic human rights (Mc Lennon et al., 2013). A patient diagnosed with a life-threatening illness may prefer a short period of quality life over a lengthy life with suffering. A common example would be oncology patients receiving palliative care.

3.1.10 Maintain vs. Withdrawal of Treatment

When an intervention no longer helps to achieve the patient's goals for care or desired quality of life, it is ethically appropriate for physicians to withdraw treatment (Wang and Ho, 2013). The moral obligation may conclude otherwise, which creates an ethical dilemma.

3.2 Moral Reasoning

The second phase of the conceptual framework deals with moral reasoning. Moral reasoning is the mental process that nurses set in motion to come to a decision of right or wrong in any moral dilemma (Vargas and Concha, 2019). The influential factors are subdivided into three categories: beliefs, values, and expectations.

3.2.1 Beliefs

Moral beliefs mean ideas of what is right and what is wrong that shape one's outward behavior. Beliefs are influential based on cultural, religious and interpersonal perception factors.

3.2.2 Values

Values are motivational preferences or dispositions. Moral values are those preferences that are integral to any moral reasoning process and are influenced by societal, cultural, personal and emotional factors.

3.2.3 Expectations

The final phase of the moral reasoning process is the expectations. This involves the assumptions and presuppositions expected by health professionals when dealing with ethical dilemmas. The phase is further categorized based on the type of expectation: conditional, rational, realistic and adaptive (Wang and Ho, 2013).

3.3 Ethical decision making

Ethical decision-making refers to the process of evaluating and choosing among alternatives in a manner consistent with ethical principles (Schwartz, 2021). In making ethical decisions, it is necessary to perceive and eliminate unethical options and select the best ethical alternative. Four types of influences on ethical decision-making can be categorized as follows:

3.3.1 Deontology

This is an ethical theory that says actions are good or bad according to a clear set of rules. Actions that align with these rules are ethical, while actions that don't are considered unethical. A deontological system of moral decision-making seeks to identify an action that an individual should perform or refrain from performing to fulfill a particular duty, which is itself grounded in one or more fundamental moral principles (Barrow and Khandhar, 2020). Deontology involves principles of intrinsic morality, duty of care and moral consequences of an action.

3.3.2 Consequentialism

Consequentialism is an ethical theory that judges whether something is right by its consequences (Ellis, 2019). An example can be set as, lying would be considered wrong, but if telling a lie would help save a person's life, consequentialism affirms it as a moral obligation to perform (Park & Kim, 2024). The influential factors of consequentialism are patient satisfaction, better patient prognosis, reduced level of suffering, reduced complications and enhanced self-esteem.

3.3.3 Virtual ethics

Virtue ethics guides ethical decision-making by its focus on the central role of character, judgment and integrity, which has been deliberately developed over time (Sorta-Bilajac et al., 2011). It involves honesty, generosity, fairness, equality and compassion.

3.3.4 Ethics of care

This is a feminist philosophical perspective that uses a relational and context-bound approach toward morality and decision-making. The term ethics of care refers to ideas concerning both the nature of morality and normative ethical theory. It involves human

relationships, expression of caring, attentiveness, responsibility, competence and responsiveness (Schwartz, 2021).

3.4 Moral action and resolution

Moral action can be referred to as the right thing to do, considering the possible outcomes of the action. The final phase of the conceptual framework is defined as moral action and resolution, hence exploring the effectiveness of the model. The framework dealt with the common ethical dilemmas and examples, the process of moral reasoning and its influential factors, and the process of ethical decision-making (Barlow et al., 2018). The outcomes or resolution of the framework can be defined as better judgment, improved outcomes and reduced moral distress associated with ethical dilemmas.

3.5 Ethical Decision-Making Models

Ethical decision-making models provide a suggested mechanism for critical thinking and planning for the resolution of ethical dilemmas (Milliken & Grace, 2022; Schwartz, 2021)). An ethical decision-making model is a tool that can be used by health professionals to help develop the ability to think through an ethical dilemma and arrive at an ethical decision. Various models are presented in ethics literature, most of which are similar in design and content.

The goal of each model is to provide a framework for making the best decision in a particular situation with which the health care provider is confronted. These models use principle-based reasoning, an approach primarily derived from the work of philosophers like Beauchamp and Childress (Schwartz, 2021; Park & Kim, 2024). These models consider ethical principles, obligations, and values. They advocate the use of resources such as published evidence, clinical data and consulting colleagues in nursing. The core concept of the models involves careful reasoning through the structure of a decision model in different healthcare settings.

Through the study, we evaluated six main ethical decision-making models widely used in health care practice: Action guides of Beauchamp and Childress, the Caring and Justice ethical decision-making model, Franken's mixed deontological theory of obligation, 'value, be, do guidelines for resolving ethical conflict, Utilitarian ethics and International and National Code of Ethics for Nurses. Based on the study findings, Beauchamp and Childress' model of action guide aids nurses and nursing students to

identify, process, decide, evaluate and reflect upon their actions resulting from ethical dilemmas (Milliken & Grace, 2022; Schwartz, 2021; Park & Kim, 2024). Hence, it can be considered the widely used ethical decision-making model among nurses. Table 1 describes the advantages and disadvantages of selected ethical decision-making models.

Table 1: Comparative Analysis of Ethical Decision-Making Models

Source: Author's Compilation

Ethical decision-making models	Advantages	Disadvantages
1) Action guides of Beauchamp and Childress	• Principalism is a core concept and a useful approach, which provides a checklist of factors and a framework in approaching various moral and ethical dilemmas • An effective approach to address healthcare ethical issues since principles are universally accepted and thus ensure consistency in approaching various moral dilemmas by balancing all the different principles • The four principled ethics have served as the codes for healthcare professionals to prevent disease and facilitate medical care.	• Limited selection criteria • In practice, medical professionals should focus not only on patients but also on potential patients who may be in danger. • Hence, these four moral principles create difficulties in achieving a coherent moral judgment/resolution for medical care professionals. Therefore, to make wise clinical decisions, medical care professionals must simplify their moral judgment processes to reach a coherent justification for the sake of medical practice and public health.
2) Caring and justice ethical decision-making model	• The ethic of care is centered on maintaining relationships through responding to the needs of other people and avoiding hurt, whereas the ethic of justice is centered on maintaining duties, equity and fairness through the application of ethical principles, rules and standards	• The ethics of justice and the ethics of care represent opposite poles. If the members of the health team were to use only one of these two perspectives in their ethical decision-making, certain ethical dilemmas would almost certainly remain unresolved.
3) Frankena's mixed deontological theory of obligation	• Deontological ethics emphasizes the value of being a human being, underlining the principles of respect for autonomy, beneficence, non-maleficence, and justice	• Deontological ethics are inclined to be patient-centered; hence, consequences are not used to justify means

	• People must abide by their moral duty or obligation rather than by the consequences of their actions	
4) 'Value, be, do': guidelines for resolving ethical conflict	• The value-based model proposes that health outcomes be measured over a longer period and that the patient's health status be compared in a comprehensive and integrated manner that considers all aspects of the patient's life: physical health, psychological aspects, and social life. • Term value cannot be reduced to the cost of an intervention but must include a general evaluation of the patient's overall well-being • The Value, Be, do model can be used to resolve • ethical conflict on individual and family, community, and systems levels	• Applying this model does not guarantee that nurses will develop satisfactory resolutions to all their conflicts.
5) Utilitarian ethics	• Utilitarian ethics, which are inclined to be more society-centered, value care for the greatest welfare for the greatest number of human beings; hence, outcomes determine means • Utilitarian ethics originated with the idea of making good use of time and resources • Not considering public benefit.	• When utilitarian ethics uses the maximum benefit for most people as its primary consideration, some individuals or groups may be harmed.
6) International and National Code of Ethics for Nurses	• The codes outline how nurses should behave ethically as a profession, and how they should decide when they encounter barriers preventing them from fulfilling their professional obligations • The codes can also support nurses in their practice and reduce their moral distress.	• The International Code of Ethics could not possibly represent the various cultures and societies of different countries • Difficult to regulate • Difficulty accessing education • Costly and time-consuming • Lack of institutional or personal commitment

3.6 Ethics in Nursing

The Ethics practice standard focuses on the values and expectations of ethical conduct and behavior for nurses. Bioethical principles are the most common form identified and

are widely used among nurses. The standard of code involves non-maleficence, beneficence, autonomy and justice (Calleja-Sordo et al., 2015). The governance of these principles lies at various levels and levels of the ecological model can be used to describe its significance (Davidson et al., 2018).

This article identified the causes of ethical dilemmas and gradually revealed its course of action based on the concept of moral distress and resolution using ethical decision-making models. The ethics of nursing is discussed under the seven levels of the ecological model. The level of society, politics and economy is the utmost layer that contains the other sub-levels (Davidson et al., 2018). Society is the basis for the development of individual factors such as autonomy. In certain countries, autonomy is respected above all other spheres.

In the USA, the patient's autonomy is highly respected in their decision to accept or reject care to override all other health factors (Nora et al., 2015). Political structure determines the cost-effectiveness of care provided based on the budget allocation for the health sector (Chan & Lee, 2024). Recognition of health care professionals, too, involves political influences on some degree. The economy of a country directly influences its resources. As an example, when medications are scarce due to the economic downfall of a country, health professionals encounter serious ethical issues as to who receives the scarce medications (Richardson & Lawrence, 2025).

The community level defines a broader concept of ethical issues. As an example, the interplay between families and the community determines its perception regarding health, illness, and dying (Karlsson et al., 2010). These factors are greatly influenced by the culture and norms of society. Acceptance of drug abusers' support for elderly and chronic disease patients, such as Alzheimer's, is an ethical consideration that can be resolved with community interplay (Karlsson et al., 2010).

The level of education and standards defines the ethical framework for nurses to practice based on their professional conduct. International Nursing Council (ICN) and the American Nurses Association (ANA) are some of the international councils that introduce, maintain and regulate ethical guides for nurses. This level is significant for nurses to identify their level of contribution and its limits associated with promoting patient dignity (Ulrich et al., 2010), disclosure, role development, nursing practice

environments (Rushton & Wood, 2025) and professional boundaries (Barlow et al., 2018). Within the context of increasing technological complexity and chronic illness, many emerging ethical issues, such as increased aging and assisted suicide, require a complex interplay between different levels and standards of ethical practice (Calzone et al., 2024). Furthermore, promoting patient autonomy, delivering care within legal and ethical frameworks, inter-professional communication and monitoring of standards and practices can also be improved based on ethical standards (Kangasniemi et al., 2015).

Organizational healthcare standards and protocols define the extent of care provided to the patient. This level also addresses workforce management, shift management and understaffing issues (Garrad, 2017). One of the most significant causes associated with ethical misconduct is burnout stress associated with a shortage of staff and long working hours (Barlow et al., 2018).

The relationship between healthcare professionals is directly proportional to the collaborative multi-disciplinary approach (Sorta-Bilajac et al., 2011). This is considered the hallmark of holistic patient care. The difference in autonomy, authority and professional conduct defies ethical issues, which can also be affected by the communication and decision-making skills of the team members (Wiegand et al., 2015).

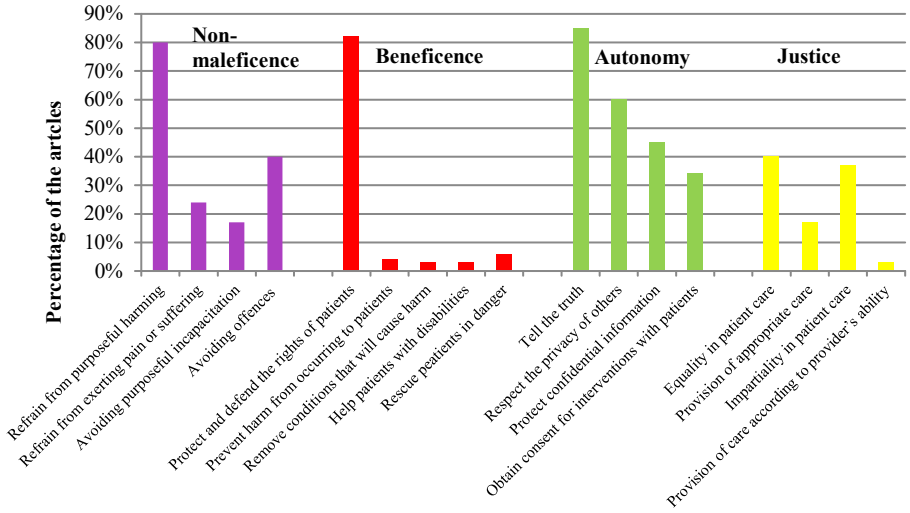
The complex interplay between patients and nurses defines moment-to-moment care and associated ethical issues. Nurses should take steps to provide care that affirms professional standards and prevents ethically demanding situations (Wang and Ho, 2013). The immediate contact surface with the patient lies with the primary care nurse and nurses are more liable to experience ethical issues at this level.

Individual factors of nurses and patient families affect the ethical decision-making of the nurses. Every individual is entitled to receive effective treatment and be treated equally. Certain unintentional factors may challenge these rights, giving rise to ethical issues (Rainer et al., 2018). Nurses should be aware of cultural norms, which may define individual norms differently and can make nurses' professional conduct more challenging (Yildiz, 2019).

Ethics in nursing is based on bio-ethical principles of the nursing code: Non-maleficence, Beneficence, autonomy and justice. Through the study, subcategories of the four main bioethical principles were identified in Figure 2.

Figure 02: Framework of Nursing Ethics Based on Bioethical Principles

Source: Author's Compilation



Accordingly, 'telling the truth' was identified as the most prominent subcategory of ethical principles with 85%. 'Protection and defending the rights of the patients and prevention of deliberate harm to the patients are also marked as very important, with percentages of 82% and 80% respectively. 'Removing conditions that will cause harm to the patient, helping patients with disabilities and provision of care according to provider's ability' are the least recognized subcategories of nursing ethics, with 3%.

Ethical issues concerning subcategories of bioethical principles are explored in Table 2. Accordingly, the ethical issues extend from 'negligence of quality care', 'unnecessary use of physical strains' to 'Deliberate endangering of patients', 'Forging/ abstaining from obtaining consent' and 'Patient discrimination', which can significantly compromise a patient's health and well-being. Ethical decision-making models are beneficial in the systematic analysis of an ethical dilemma. Nurses possess the knowledge, competence and capacity to process and make wholesome ethical decisions but are unable to practically execute their experience based on the absence of adequate knowledge regarding an organized approach (Karjalian, 2024). Ethical decision-making models effectively address this issue by providing an organized system to apply and process already-known facts regarding ethical decision-making. This process

remarkably reduces the moral distress associated with ethical dilemmas and ethical decision-making (McLeod, 2014). Nursing ethics guide nurses to act responsibly towards patients and the quality of holistic patient care (Albert et al., 2020). Ethical issues concerning nursing practice may have serious consequences on patients' lives and, therefore, nurses are encouraged to practice in accordance with the nursing code of ethics.

Table 2: Analysis of Ethical Issues Based on Bioethical Principles of Nursing

Source: Author's Compilation

Bioethical principle	Subcategories	Ethical issues
Non-maleficence	Refrain from purposeful harming	Medication errors
	Refrain from inflicting pain or suffering	Inadequate measures to address patient pain and suffering Withholding of nursing procedures Life-lengthening procedures in palliative patients
	Avoiding purposeful incapacitation	Inadequate assistance for patients in decision-making Negligence of quality care
	Avoiding offences	Assisting patients in committing suicide Assisting in illegal abortions Gross negligence *Possession of illegal drugs*
Beneficence	Protect and defend the rights of patients	Violation of the patient's rights
	Prevent harm from occurring to patients	Promoting the harmful behavior of the patient
	Remove conditions that will cause harm	Unnecessary use of physical strain
	Help patients with disabilities	Inadequate assistance for patients with disabilities
	Rescue patients in danger	Deliberate endangering of patients
Autonomy	Tell the truth	Giving false hope
	Respect the privacy of others	Violating a patent's privacy
	Protect confidential information	Breaching the patient's confidentiality
	Obtain consent for interventions with patients	Forging/ abstaining from obtaining consent Provision of inadequate information in obtaining informed consent Obtaining consent from incapacitated patients
Justice	Equality in patient care	Inequality in patient care
	Provision of appropriate care	Inappropriate care based on the patient's needs
	Impartiality in patient care	Patient discrimination
	Provision of care according to provider's ability	Refusal to care for patients with contagious diseases, HIV/ AIDS

4. Conclusion

The findings of the study were categorized based on the levels of the ethical reasoning framework: ethical dilemmas, moral reasoning, ethical decision-making models, moral action and resolution. Ethical dilemmas were explored under ten categories, which involve the safeguarding of patients' rights, bioethical principles of nursing and professional standards of healthcare practice. Moral distress was identified as the commonest cause of ethical issues with nursing practice, organizational standards such as understaffing, long working hours, disharmony and communication between multi-disciplinary healthcare teams to negatively influence ethical issues. Ethical decision-making models help guide health professionals to decide the morally ethical solutions for ethical dilemmas.

The coherence between various factors, such as the outcome of treatments, adherence to ethical standards, congruence of hospital policies, and humanitarian aspects of the decision, is valued based on critical thinking and moral reasoning. The ecological model was used to explore the interplay between variant levels and their interactions in ethical decision-making by nurses. Each level was explored separately, and most emerging themes were highlighted to present a comprehensive model for ethics-based nursing care.

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A STUDY ON THE EFFECTS OF TRADITIONAL SRI LANKAN DANCING ON THE MENTAL WELL–BEING OF YOUNG ADULTS IN SRI LANKA

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Abstract

Traditional Sri Lankan dance is deeply ingrained in the island's cultural and spiritual practices, but its potential psychological benefits have not been fully explored in scientific literature. With the growing mental health issues among young adults, especially in urban Sri Lanka, there is an increasing interest in culturally grounded approaches to well-being. Thus, this study aimed to examine the impact of traditional Sri Lankan dance forms, specifically Kandyan, Low Country, and Sabaragamuwa, on the psychological well-being of young adults. The study used a quantitative research design and recruited 128 participants, who were from two groups: those who had been actively practicing traditional Sri Lankan dance for at least six months and those who had no formal dance training. The 42-item Ryff Psychological Well-Being (PWB) Scale, which measures six aspects of well-being: autonomy, environmental mastery, personal growth, positive relationships with others, purpose in life, and self-acceptance was used to assess the level of psychological wellbeing in dancers and non-dancers and were compared using t-tests. According to the findings, traditional Sri Lankan dance is a useful tool for improving mental health and a way to express one's culture. These findings encourage more research into traditional arts as therapeutic modalities and support the inclusion of culturally relevant practices in mental health interventions.

Keywords: Traditional Dance, Psychological Well-Being, Young Adults, Kandyan Dance, Low Country Dance, Sabaragamuwa Dance

1. Introduction

Traditional Sri Lankan dance has long been a part of the island's character. Its lively representation of the nation's cultural legacy is rooted in ancient customs, mythology, and folklore. These dances, also an artistic medium, serve as a way for spiritual and emotional expression. Sri Lanka has a long history of classical dance that is deeply ingrained in its religious and cultural traditions. Kandyan, Low Country, and Sabaragamuwa are the three main forms of traditional Sri Lankan dance.

The cultural and historical significance of traditional Sri Lankan dance is impressive, but its unique characteristics make it stand out as a separate art form. Each style embodies a fusion of spiritual depth, lively storytelling, and rhythmic accuracy. The intricate hand gestures, “mudras”, dramatic facial expressions, and elaborate costumes often adorned with traditional jewelry are not merely decorative but convey profound symbolic meanings. For example, the headdresses and jewels worn by Kandyan dancers signify divine protection, whereas the masks worn by Low Country dancers symbolize mythical creatures or spirits that are said to ward off evil (Perera, 2015).

Additionally, traditional dancing frequently incorporates purification and healing rites, such as the ‘thovil’ ceremony, which is thought to ward off evil spirits and bring peace back to both individuals and communities. These dances bridge the gap between the material and the spiritual, fusing spirituality with artistic expression to create more than just shows; they are life-changing events that are ingrained in Sri Lankan culture. The varied cultural fabric of the island is reflected in the unique movements, functions, and historical significance of each style (Perera, 2015).

A popular performance at religious processions like the Esala Perahera, the Kandyan dance originated in the hill country and is distinguished by its majestic motions, sophisticated footwork, and rich costumes. With its energetic, expressive movements and colorful costumes topped with masks to depict mythological beings, Low Country dance, which originated in coastal areas, is closely associated with exorcism rites and healing practices. Associated with the province of Sabaragamuwa, Sabaragamuwa dance is characterized by soft, rhythmic motions and a spiritual undertone, and it centers on ceremonies devoted to gods and deities (Perera, 2015). By embodying these elements, traditional Sri Lankan dance becomes a holistic practice, merging physical activity with cultural pride and emotional release, which may have profound implications for enhancing psychological well-being.

Overall health is based on psychological well-being, which includes emotional, psychological, and social health. Lifestyle, social relationships, and participation in worthwhile activities are

some of the factors that affect it (Ryff, 1989). Research from around the world has demonstrated that dance, as an expressive activity, may reduce stress, improve mood, and create a sense of community (Koch et al., 2019). However, little actual research has been done on how traditional Sri Lankan dances, with their expressive gestures and rhythmic rhythms, affect people's mental health (Fernando, 2020). The potential benefit of investigating how traditional dances from Sri Lanka, which have a strong cultural past, can be a useful way to treat mental health issues in a culture that is fast modernizing.

In the Sri Lankan context, where fast-paced lives and rapid urbanization frequently increase mental health issues, people focus on more traditional approaches to reduce the tension caused by their daily lives (Gamage, 2018). Thus, this study examines the impact of traditional Sri Lankan dance on mental health. Traditional Sri Lankan dance has a rich history that is intricately woven into the fabric of the country's identity. It provides both aesthetic pleasure and a possible means of improving mental health. Dance is more than just physical movement, it can promote self-expression, emotional release, and a deep connection with oneself and others (Fernando, 2020). Additionally, traditional Sri Lankan dancing offers a useful framework for investigating how regional customs support international mental health approaches, as mental health therapies increasingly seek to incorporate cultural sensitivity (De Zoysa & Wickrama, 2017). These kinds of cultural pursuits provide special perspectives on the relationship between heritage and mental health.

Traditional Sri Lankan dance provides a culturally relevant and easily accessible way to promote mental health, even as mental health issues are becoming more prevalent in metropolitan settings. This study adds to academic understanding by investigating the relationship between traditional dance and mental health, while also demonstrating the potential of cultural activities to support mental health. Dance professionals may create treatments that combine cultural heritage with modern wellness requirements by knowing the therapeutic elements of various dance traditions (Jayasinghe, 2019). The research findings have the potential to influence policy decisions and the incorporation of cultural heritage into community well-being programs in Sri Lanka (Perera, 2015).The need to reduce this knowledge gap and emphasize the significance of cultural practices in contemporary mental health situations serves as justification for this study.

2. Literature Review

The ability of dance to support mental health has long been acknowledged. Dance combines artistic expression, social contact, and physical exercise, all of which have positive psychological effects. Dance movement therapy has been demonstrated to improve general emotional well-being and lessen the symptoms of stress, anxiety, and depression (Koch et al.,2019). Similarly,

by enabling participants to express their emotions through movement, dancing promotes emotional control and boosts positive effect (Murcia et al., 2010). Furthermore, dance contributes to physical health, supporting mental health by reducing stress hormones and increasing endorphin levels (Bräuninger, 2014). Dancing in culturally relevant ways can improve mental health by creating a feeling of identity and community. This link between mental health and cultural identity emphasizes how traditional Sri Lankan dances can build emotional resilience and a sense of community.

Dance offers a special platform for expressing emotions, allowing people to communicate sentiments that are hard to put into words. According to Koch et al. (2019), this nonverbal communication style is very useful for improving mood and encouraging emotional control. Organized dancing classes have been found to raise serotonin and dopamine levels, which are linked to feelings of contentment and relaxation, while lowering cortisol levels, a biomarker of stress (Quiroga Murcia et al., 2010). These neurochemical alterations demonstrate how dancing may both reduce unpleasant feelings and promote positive affect.

Culturally rooted dancing traditions provide a sense of pride and belonging, which has a significant effect on mental health. The religious, historical, and social backgrounds of the island are intricately linked to traditional Sri Lankan dances, including Sabaragamuwa, Low Country, and Kandyan. UNESCO has acknowledged traditional dances from Sri Lanka, such as Kandyan, Low Country, and Sabaragamuwa, for their cultural value and contribution to preserving the island's intangible cultural heritage. Its historical, religious, and cultural significance was highlighted when Kandyan dance was specifically included on UNESCO's Representative List of the Intangible Cultural Heritage of Humanity as one of the "Sacred Rituals and Traditional Arts of the Esala Perahera" in Kandy. Even though Sabaragamuwa and Low Country dances might not be included on a list separately, they are part of the island's rich dance traditions and represent the close relationship between spirituality, art, and cultural identity. Taking part in culturally embedded dances builds communal bonds and offers emotional fortitude, particularly during trying times (Giguere, 2011).

Additionally, these dances provide a means of reaffirming communal memory and identity, which is essential for communities recuperating from socio-political upheavals. Studies have shown that engaging in traditional dance encourages resilience, lessens isolation, and reconnects people to their common cultural history, all contributing to community healing (Giguere, 2011; Perera, 2015). This community element is particularly pertinent to Sri Lanka, where traditional dances frequently unify in religious festivals and rites.

Dynamic motions, rhythmic patterns, and symbolic gestures that arouse the body and mind are traditional Sri Lankan dance characteristics. These dances, which have their roots in religious rites and cultural customs, encourage emotional release, focus, and awareness (Perera, 2015). They also serve as a form of mindfulness practice, promoting present-moment awareness and reducing rumination, which is often linked to depression and anxiety (Shankland et al., 2018). A meditative state that improves emotional control and lowers stress is produced by the synchronization of breath, movement, and rhythm in traditional dance. Traditional dance practice promotes personal development by encouraging discipline and a strong bond with cultural history (Fernando, 2020).

There are several physical advantages to dancing, which can indirectly improve mental health. Frequent dance engagement enhances flexibility, muscular tone, and cardiovascular health—all of which are linked to a lower risk of chronic illnesses and an overall higher quality of life (Bräuninger, 2014). According to Quiroga Murcia et al. (2010), these physical enhancements are associated with greater cognitive performance, such as higher neuroplasticity, improved executive functioning, and improved memory retention. These advantages are especially noteworthy for senior citizens, since research has demonstrated that dancing can enhance balance and postpone the development of dementia, both of which lower the chance of falls (Kattenstroth et al., 2013). The Psychological Well-Being model provides a strong framework for comprehending the effects of dance. It weighs autonomy, environmental mastery, personal growth, purpose in life, positive relations, and self-acceptance (Ryff, 1989).

Research indicates that dancing can enhance these aspects. For instance, dance therapy has been found to enhance autonomy and self-acceptance by encouraging self-expression and confidence (Koch et al., 2019). Regular engagement in dance activities also results in improved environmental mastery and personal growth (Murcia et al., 2010). Moreover, dance has been shown to foster social bonds, which Ryff's model emphasizes as a critical component of well-being (Ryff, 1989). By giving people the chance to establish and accomplish goals like learning intricate choreography or giving a public performance, dance supports the PWB paradigm. The foundation of psychological resilience is a sense of competence and purpose, which are fostered by these accomplishments (Ryff, 1989; Koch et al., 2019).

The benefits of dance for mental health are well established, but little research has specifically focused on traditional Sri Lankan dance; most studies have looked at Western or contemporary dance forms, which leaves a gap in knowledge about the special contributions of culturally specific dances to mental health. Additionally, urban populations face unique stressors, so it is important to research how traditional practices, like dance, can be adapted to meet the mental health needs of urban populations. For instance, incorporating traditional dance techniques into

urban health initiatives might be a culturally aware intervention that preserves intangible cultural heritage while offering psychological and physical advantages (Fernando, 2020).

By including the study's objectives, the research focus can be placed within the framework of previous studies. The study looks at three important traditional dance forms to identify the special psychological advantages of each. This investigation, research aims to fill in the gaps in the existing body of knowledge about the relationship between cultural practices and mental health, offering a sophisticated perspective on how traditional dance forms might enhance well-being. This focus addresses the under-representation of Sri Lankan dance in psychological and wellness-focused studies and is consistent with other research that highlights the therapeutic value of physically based cultural practices. The results are intended to further enhance global perspectives on traditional behaviors and well-being and to guide the creation of culturally relevant mental health therapies.

Additionally, the study will investigate how traditional Sri Lankan dance promotes intergenerational learning and bonding, which is frequently disregarded in current mental health research. By conserving these customs, the wisdom, discipline, and resilience ingrained in the dances' rituals and stories can be passed down to younger generations (Perera, 2015; Shankland et al., 2018).

2.1 General Objective

To examine the impact of traditional Sri Lankan dance on the psychological well-being of young adults in Sri Lanka.

2.1.1 Specific Objectives

To compare the levels of psychological well-being between individuals who practice traditional Sri Lankan dance and those who do not.

To identify which dimensions of psychological well-being (e.g., autonomy, personal growth, purpose in life) are most positively influenced by traditional dance participation.

2.1.2 Procedure

After receiving a comprehensive information sheet, participants gave their informed consent before beginning the study. Depending on their availability, the questionnaires were distributed either in person or online. Strict confidentiality and anonymity were upheld.

2.1.3 Ethical Consideration

The University of Plymouth's Institutional Review Board granted the study ethical approval. Participants were made aware of their freedom to leave at any time without facing any repercussions. The information was safely kept and used only for study.

3. Methodology

3.1 Study Design

A cross-sectional, quantitative comparative methodology was used in this study to examine the effects of traditional Sri Lankan dancing on young adults' psychological health. Two separate participant groups were included in the study: those who had never danced in a formal setting and those who were actively engaged in traditional Sri Lankan dance.

3.2 Participants and Sampling

A total of 128 participants, aged 18 to 30, were included in the sample; 64 were non-dancers and 64 were traditional dancers. The dancer group had been actively involved in training or performance at the time of the study and had been practicing one or more of the traditional Sri Lankan dance styles—Kandyan, Low Country, or Sabaragamuwa—for at least six months.

Participants were limited to those who practiced traditional dance; those who performed other dance styles were not included. The non-dancer group was made up of people who had never taken part in any organised dance activity or formal dance training. Through purposive sampling, participants were chosen from online recruitment platforms, community dance organisations, and university cultural clubs.

3.3 Data Collection Instrument

Over two months, a standardised questionnaire was used to gather data. In addition to a demographic section, the Ryff Psychological Well-Being (PWB) Scale (42-item version) was used to measure the six elements of well-being: self-acceptance, positive relationships with others, autonomy, environmental mastery, and personal progress. A 6-point Likert scale covering "strongly disagree" to "strongly agree" was used to score each item. Due to its excellent reliability and validity in various cultural contexts, the PWB scale was chosen for its applicability to behavioural and psychological health studies.

4. Findings and Discussion

4.1 Descriptive statistics of the normality test

The 128 participants in the study were split equally between 64 traditional dancers and 64 non-dancers. The mean score for dancers was greater than that of non-dancers (M =163.39, SD =19.972, Median =158.00), according to descriptive statistics for psychological well-being (PWB) scores. Table 01 shows the descriptive statistics for both groups.

Table 01: Descriptive statistics for dancers and non-dancers

Descriptives

			Statistic	Std. Error
Score	Dancers	Mean	163.39	2.496
		Median	158.00	
		Variance	398.877	
		Std. Deviation	19.972	
		Minimum	126	
		Maximum	242	
		Skewness	1.806	.299
		Kurtosis	4.355	.590
	Non-dancers	Mean	154.23	2.466
		Median	152.00	
		Variance	389.325	
		Std. Deviation	19.731	
		Minimum	98	
		Maximum	206	
		Skewness	.159	.299
		Kurtosis	.780	.590

According to the Shapiro-Wilk test, the psychological well-being scores were normally distributed in the non-dancer group (p =.304), but not in the dancer group (p =.000). For additional analysis, a non-parametric Mann-Whitney U test was used because at least one group had violated normality. Table 02 presents the normality test result of psychological well–being scores for dancers and non-dancers.

Table 02. Normality test results

Tests of Normality

		Kolmogorov-Smirnova			Shapiro-Wilk			
		Statistic	df	Sig.	Statistic	df	Sig.	
Score	Dancers	.153	64	.001	.841	64	.000	
	Non-dancers	.092	64	.200*	.978	64	.304	

4.2 Mann-Whitney U test

According to the Mann-Whitney U test, the psychological well-being scores of dancers and non-dancers significantly differ (p =.009). This implies that, in comparison to non-dancers, traditional Sri Lankan dancers are linked to better levels of psychological well-being. Figure 01 shows the results of Mann Mann-Whitney U test.

Figure 01. Mann-Whitney U test results

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of score is the same across categories of stastus.	Independent-Samples Mann-Whitney U Test	.009	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

The purpose of this study was to investigate how traditional Sri Lankan dancing affected young adults' psychological health. The results offer strong evidence that those who practise traditional dance styles have far higher psychological well-being scores than non-dancers. This lends credence to the main idea and is consistent with the body of research on the advantages of cultural and movement-based practices for mental health.

Traditional dance is linked to improved psychological health, according to the Mann-Whitney U test results, which showed a statistically significant difference in well-being scores between dancers and non-dancers (p =.009). These results are in line with earlier studies showing that expressive movement and dance support self-acceptance, emotional control, and personal development (Koch et al., 2019; Quiroga Murcia et al., 2010).

Nevertheless, there are several drawbacks on this study. Causality inference is limited by the cross-sectional design. Due to the possibility of individuals overestimating or underestimating their psychological states, self-reported data may also introduce bias. The results could also be skewed by the dancer group's differences from the non-dancer group in other lifestyle or personality factors that were not considered.

The study emphasises the value of including culturally based activities, such as traditional dance, into mental health promotion initiatives despite these drawbacks. Considering the increasing mental health issues that young adults in Sri Lanka are facing, utilising easily accessible, traditional practices could offer long-term assistance for wellbeing.

To better evaluate causation and the mechanisms by which dancing affects mental health, future studies should consider experimental or longitudinal approaches. Research could also look at the distinctive effects of particular dance forms (Kandyan vs. Low Country, for example) on various aspects of psychological health or the relationships between dance involvement and mental health outcomes and gender, socioeconomic level, and academic stress.

To sum up, this study adds to the increasing amount of data that supports the idea that traditional art forms are useful resources for psychological support. In order to improve young well-being through comprehensive, culturally sensitive interventions, it emphasises the necessity of multidisciplinary cooperation between cultural practitioners and mental health specialists.

5. Conclusion

This study investigated the connection between young adults' psychological health and traditional Sri Lankan dancing. The results showed that, in comparison to non-dancers, people who actively participate in traditional dance forms report much better levels of psychological well-being.

These findings provide credence to the notion that traditional dance contributes significantly to mental health in addition to being a cultural or artistic practice. Ryff's concept of psychological well-being's core tenets - emotional expression, purpose, and social connection—are all strongly supported by traditional Sri Lankan dancing.

The study emphasises how incorporating culturally based practices into mental health promotion programs, particularly for young individuals, may be beneficial. Even if the results are encouraging, more investigation is required to prove causation and examine underlying mechanisms, utilising longitudinal and experimental approaches.

For health professionals, educators, and policymakers looking to advance mental health through indigenous cultural practices, traditional Sri Lankan dance offers valuable insights into a culturally relevant, approachable, and comprehensive strategy for improving mental well-being.

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ASSOCIATION BETWEEN FOOD CONSUMPTION PATTERNS AND PERCEIVED STRESS AMONG UNDERGRADUATES AT A SELECTED PRIVATE UNIVERSITY IN SRI LANKA

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Abstract

University students often experience high stress due to academic, financial, social, and lifestyle challenges, which may be influenced by dietary habits. This study examined the relationship between food consumption patterns and perceived stress among 301 undergraduate students at NSBM Green University, Sri Lanka, between March and May 2025. Data were collected using a pretested self-administered questionnaire covering sociodemographic characteristics, dietary habits, and perceived stress measured using Cohen's 10-item Perceived Stress Scale. Dietary intake was assessed using seven food groups and 27 subgroups based on the Food-Based Dietary Guidelines for Sri Lankans, and associations were analyzed using the Chi-square test ($p < 0.05$). Most participants were female (83.4%). Overall, 41.5% reported high perceived stress and 58.5% reported low perceived stress. Stress levels were categorized as low (≤20) and high (≥21), with a mean perceived stress score of 20.2 ± 4.1. Frequent consumption (several times per week or at least once daily) was reported for eggs (80.4%), fish and seafood (71.8%), poultry (68.4%), green leafy vegetables (64.8%), vegetables (74.4%), and sweets and candies (69.7%), indicating a protein-rich, vegetable-moderate, and sugar-heavy dietary pattern. Only poultry consumption showed a statistically significant association with perceived stress ($p = 0.022$). While most dietary variables were not significantly associated with perceived stress, the observed relationship between poultry consumption and stress warrants further investigation through larger longitudinal studies to clarify the association and account for potential confounding factors.

Key words: Dietary patterns, food consumption, perceived stress, Sri Lanka, undergraduate students

1. Introduction

Mental health goes beyond simply not having a mental disorder. It is a state of well-being that allows individuals to handle life's challenges, recognize their potential, learn effectively, perform productively, and make meaningful contributions to their community (World Health Organization, 2022). Mental health plays an essential role throughout life, impacting cognitive growth, behavior, learning abilities, and personal relationships (Fusar-Poli et al., 2020). Mental issues can cause serious consequences such as anxiety, depression, and substance abuse, and affect a diverse population, including university students (Bremner et al., 2020).

Undergraduates are more prone to mental health issues. This may be a result of their heavy academic workload, financial difficulties, social pressure, and lifestyle changes. These stressors can have a serious negative effect on their mental health. Furthermore, these issues can lead to depression and other coexisting mental health disorders among undergraduates (Deforche et al., 2015). For instance, a study conducted at the University of Colombo, Sri Lanka, revealed that stress-related disorders among undergraduates led to numerous physical and mental health issues, including headaches, nausea, digestive problems, breathing difficulties, muscle pains, palpitations, and sleep disturbances (Mahees, 2020).

A balanced diet rich in essential macro- and micronutrients, as well as antioxidants, supports energy, growth, mental well-being, and disease prevention (Liu, 2013). In contrast, unhealthy dietary patterns high in processed foods, sugars, and unhealthy fats are associated with fatigue, inflammation, and the development of long-term diseases (Perignon et al., 2017).

University undergraduates often adopt irregular eating patterns, including skipping meals, consuming fast food and processed snacks, and having an inadequate intake of nutrient-dense foods such as fruits, vegetables, and whole grains (Papadaki et al., 2007). In Sri Lanka, a study has shown undergraduates typically consider convenience, availability, and ease of eating as crucial elements in fulfilling their dietary needs, leading to a high dependence on fast foods (Jayasinghe & De Silva, 2014). These dietary habits can lead to nutrient deficiencies that negatively affect both physical and mental health (Wengreen & Moncur, 2009). Undergraduates at universities may develop erratic eating habits, such as missing meals, eating processed snacks and fast food, and

consuming insufficient amounts of nutrient-dense foods including fruits, vegetables, as well whole grains (Papadaki et al., 2007). These trends could be an indication of how students' dietary decisions are influenced by pragmatic considerations like food availability and convenience. Convenience, accessibility, and simplicity of consumption were found to be significant factors affecting students' meal choices in Sri Lanka, possibly leading to their dependence on fast food (Jayasinghe & De Silva, 2014). When considered collectively, these results imply that students' eating habits may be influenced not just by their understanding of nutrition and also with the practical needs of college life along with the food options accessible to them. Inadequate intake of nutrient-dense meals may also raise the risk of inadequate nutrient intake, which might have negative effects on one's physical and mental health (Wengreen & Moncur, 2009). These factors emphasize how crucial it is to look at undergraduates' eating habits and any potential connections to felt stress.

Numerous interrelated physiological and behavioral processes may link diet to perceived stress. Inflammation, oxidative stress, as well as brain function are among the physiological processes involved with stress reactions that may be influenced by dietary consumption. Therefore, eating habits may have an impact on mental health as well as how people perceive and handle stress (Jacka et al., 2011). Perceived stress may also be associated with changes in food choices and eating behaviours. Individuals experiencing stress may alter their usual dietary patterns, including meal frequency and food preferences, and may be more likely to consume convenient, ready-to-eat, or highly processed foods. Similarly, irregular meal patterns and inadequate intake of nutrient-rich foods may affect overall well-being and be associated with higher levels of perceived stress. These potential associations indicate that the relationship between dietary habits and perceived stress may be bidirectional, rather than occurring in a single direction.

Emerging evidence indicates that diet plays a significant role in the management of stress and mental health. Research has demonstrated that dietary patterns and nutritional intake influence physiological mechanisms related to depression, including inflammation, oxidative stress, brain plasticity and functioning, as well as the stress-response system, and all of which are linked to depression and perceived stress (Jacka

et al., 2011). Numerous international studies have explored the relationship between dietary habits and perceived stress, consistently identifying a significant association between the two (Araiza & Lobel, 2018; Hill et al., 2018; Maqsood et al., 2023; O'Connor et al., 2011.). Emerging evidence indicates that dietary habits and perceived stress may be interconnected through a bidirectional relationship. Dietary patterns and nutrient intake may influence physiological mechanisms related to mental health, including inflammation, oxidative stress, brain plasticity and functioning, and the body's stress-response system (Jacka et al., 2011). Conversely, perceived stress may be associated with changes in eating behaviours, such as alterations in appetite, meal frequency, and food preferences. These changes may include a greater tendency to consume convenient or highly processed foods and a lower intake of nutrient-rich foods. Such dietary patterns may also be associated with variations in perceived stress and overall mental well-being. Previous international studies have explored the relationship between dietary habits and perceived stress and have reported associations between these factors (Araiza & Lobel, 2018; Hill et al., 2018; Maqsood et al., 2023; O'Connor et al., 2011.). However, the nature and strength of these associations may differ according to individual circumstances and dietary behaviours. Therefore, investigating the relationship between food consumption patterns and perceived stress is important for understanding how these factors are associated among university undergraduates. These findings emphasize the importance of investigating the association between food consumption patterns and perceived stress among university students, while acknowledging that the nature and strength of this relationship may differ across populations and dietary behaviours.

A study conducted among college students in Korea in 2020 found that those experiencing high levels of perceived stress were more likely to engage in unhealthy eating habits, including a higher intake of ready-prepared meals (Choi, 2020). Similarly, a study conducted in Pakistan in 2023 identified a significant association between changes in eating habits and the academic stress and pressure experienced by university students (Maqsood et al., 2023).

However, such research remains limited in the Sri Lankan context. Therefore, the study aims to assess the relationship between food consumption patterns and perceived stress

among the undergraduate students in the selected university in Sri Lanka. Although the relationship between dietary habits and perceived stress has been explored in international studies, evidence among Sri Lankan university students remains limited. Further research is needed to examine how the consumption of specific food subgroups is associated with perceived stress in this population. Investigating individual food subgroups may offer a more detailed understanding of this relationship than examining overall dietary habits alone. Therefore, the present study aims to assess the association between food consumption patterns and perceived stress among undergraduates at a selected private university in Sri Lanka, with particular emphasis on the consumption of different food subgroups. By addressing this research gap, the study seeks to provide context-specific evidence that may support future research and inform student health promotion and nutritional education initiatives. The findings may also help identify food consumption patterns associated with perceived stress and provide a basis for further investigation into the dietary habits and well-being of university students. Understanding students' food consumption patterns and their impact on perceived stress can help them make informed dietary choices. Furthermore, it can provide academic institutions with evidence to develop health promotion initiatives and nutritional support services. Ultimately, such research may serve as a foundation for improving both the mental well-being and academic performance of university students through better dietary practices.

2. Methodology

2.1 Study design and setting

This cross-sectional research was carried out at NSBM Green University in Homagama, Sri Lanka. The university was chosen because of its sizable and varied undergraduate student population, along with the practicality of recruiting participants for data collection, rendering it a suitable environment to explore the connection between food consumption habits and perceived stress among university students.

2.2 Study population

The study population consisted of undergraduate students currently enrolled in degree programs at NSBM Green University, Homagama, Sri Lanka. The university comprises a large number of students, more than 12000, across multiple faculties,

including Management, Computing, Engineering, and Science, representing a diverse student population in terms of academic background and lifestyle factors. This diversity makes the population appropriate for assessing food consumption behaviors and perceived stress among university students.

2.3 Inclusion and exclusion criteria

Undergraduate students who were willing to participate voluntarily and provided informed consent. While students who had been previously diagnosed with psychological disorders were excluded from the study.

2.4 Study sample

The data was collected between March to May 2025. During the data collection period, a total of 301 undergraduate students were included in the study.

2.5 Study instruments

Data were collected using a structured, pretested online self-administered questionnaire, which was distributed along with an information sheet and a consent form. The study instrument was developed following a review of previous literature to address the specific objectives of the study. The questionnaire consisted of three sections: socio-demographic and basic characteristics, food consumption patterns, and the Perceived Stress Score (PSS). Food consumption patterns included meal type, main sources of food, number of meals consumed per day, and intake of dietary supplements. Meal types were categorized into five groups: lacto-vegetarian (consumes milk and milk products, but no eggs, meat, fish, or poultry), ovo-vegetarian (consumes plant-based foods and eggs, but no dairy products, meat, fish, or poultry), ovo-lacto vegetarian (consumes plant-based foods, eggs, and dairy products, but no meat, fish, or poultry), semi-vegetarian (mainly plant-based with occasional consumption of non-vegetarian foods), and non-vegetarian (consumes both plant-based and animal-based foods). Dietary habits were assessed using a Food Frequency Questionnaire (FFQ). Frequencies and percentage distribution analyses were conducted for 27 food subgroups across seven major food categories, such as cereals and starchy foods, vegetables, fruits, protein sources, snacks, dairy products, nuts, and seeds. Intake frequencies were categorized as never, 1–4 times a month, several times a week, or at

least once a day, following the national Food-Based Dietary Guidelines (FBDG) for Sri Lankans (Ministry of Health Nutrition Division, 2021).

The psychological stress experienced by the participants over the past month was measured using the Perceived Stress Scale (PSS-10), a validated tool for Sri Lanka (Ilankoon & Warnakulasooriya, 2014; Praveeni & Herath, 2020; Walpola et al., 2020). The scale includes 10 items rated on a 5-point Likert scale (0 = never to 4 = very often), with four positively stated items (Items 4, 5, 7, and 8) reverse-scored. The total score ranges from 0 to 40, with higher scores indicating greater perceived stress. Stress levels were categorized as low stress (=<20) and high stress (>=21), using an operational cut-off value of 20 for the upper bound of low stress (Ilankoon & Warnakulasooriya, 2014).

2.6 Data collection

The data was collected from March 2025 to May 2025. The pretested online questionnaire, along with the information sheet and consent form, was distributed among undergraduate students from various degree programs at NSBM University. During this period, responses were obtained from 301 undergraduates. The information sheet outlined the study details, including its purpose, procedures, risks, benefits, and confidentiality measures, while also assuring participants of their right to withdraw without consequences. To ensure participants' privacy, questionnaire responses were collected anonymously, without obtaining personally identifiable information, such as names or student identification numbers. Access to the collected data was restricted to the research team, and the information was used exclusively for research purposes. Furthermore, the results were presented in aggregate form to maintain confidentiality and prevent the identification of individual participants. It further provided investigator contact details and emphasized that all responses would remain anonymous.

2.7 Data analysis

Data analysis was conducted using Minitab statistical software, version 19(2020). Descriptive statistics were used to summarize socio-demographic characteristics, food consumption behavior, and PSS, presented as means (M), standard deviation (SD), frequencies, and percentages. Chi-square tests were conducted to assess the relationship between the consumption frequency of different food subgroups and PSS. As it was often done in medical and public health research, the level of statistical

significance in this study was set at $p < 0.05$. Results that showed evidence against the null hypothesis and had p-values less than 0.05 were considered statistically significant. P-values larger than 0.10 were considered as not significant, while those between 0.05 and 0.10 were considered as borderline significant (Curran-Everett & Benos, 2004; Thiese et al., 2016).

2.8 Ethical considerations

Ethical approval was obtained from the Ethics Review Committee of the Institute of Biology, Sri Lanka, under "Registration No: ERC IOBSL 390/01/2025" (25th February 2025). Institutional permission was obtained from the deans of faculties, NSBM, and the voluntary informed consent was taken from participants in their preferred language. No person identification data was collected. Privacy and confidentiality were strictly maintained throughout the study.

3. Results

3.1 Socio-demographic and basic characteristics of the participants

The total sample consisted of 301 undergraduate students studying at NSBM Green University. The sample consisted of 16.6% males, and the majority of the participants were females (83.4%). All the participants were in the range of 18 to 27 years, and the mean age of the sample was 22.7 years (SD ± 1.7). Out of all recruited, the majority were (95.7%) Sinhalese, nearly half (69.8%) had four members or less than four members in their family, and 53.2% lived in a home. Table 1 shows the distribution of participants by age, sex, ethnicity, number of family members, and residency.

Table 1: Socio demographic and basic characteristics of the participants

Variables	Category	Frequency (n)	Percentage (%)
Age	18-22 years	124	41.2
	23-27 years	177	58.8
Gender	Female	251	83.4
	Male	50	16.6
Ethnicity	Sinhalese	288	95.7
	Tamils	8	2.7
	Muslims	4	1.3
	Burgers	1	0.3
Academic year	1st year	43	14.3
	2nd year	61	20.3
	3rd year	96	31.9
	4th year	101	33.5
Number of family members	<= 4	210	69.8
	>=5	91	30.2
Current residency	Baoding / rent	125	41.5
	Home	160	53.2
	Hostel	16	5.3

3.2 Food Consumption Behaviors Among the Participants

3.2.1 Basic Food Consumption Patterns Among the Participants

The basic food consumption patterns of the participants were also assessed to understand their overall dietary habits. The variables assessed were meal type, main sources of food, number of meals consumed per day, and intake of dietary supplements. Most of the undergraduate students were non-vegetarian (87.7%), while smaller proportions followed semi-vegetarian (8.3%), ovo-lactovegetarian (2%), lacto-vegetarian (1.3%), and ovo-vegetarian (0.7%) dietary patterns. The majority of the participants reported consuming meals prepared at home (44,7%), followed by those relying on the shops inside the university (24.5%), shops outside the university (17.8%), and meals prepared by themselves (13%). In addition to this, the majority of the undergraduates reported consuming three meals per day (59.5%), do not use any dietary supplements (85.1%). The food consumption patterns of the undergraduate students were shown in Table 2.

Table 2: Food consumption behaviors among the participants

Variables	Category	Frequency (n)	Percentage (%)
Meal type	Lacto- vegetarian	4	1.3
	Non- vegetarian	264	87.7
	Ovo-lacto-vegetarian	6	2
	Ovo vegetarian	2	0.7
	Semi vegetarian	25	8.3
Main source of food	Home made	135	44.7
	Own made	39	13
	Shops outside the university	54	17.8
	Shops inside the university	73	24.5
Number of main meals per day	1	6	2
	2	105	34.9
	3	179	59.5
	4	11	3.6
Do you take any supplements?	Yes	45	14.9
	No	256	85.1

3.2.2 Frequency of Food Consumption Among the Participants

Food frequency data were analyzed based on the seven main food groups recommended by the Food-Based Dietary Guidelines (FBDG) for Sri Lankans (Ministry of Health Nutrition Division, 2021). These groups include cereals and starchy foods, vegetables, fruits, protein foods, snacks/sweets, and other miscellaneous, nuts and seeds, and dairy products, each with several subcategories. Within the cereals and starchy food category, nearly half of the undergraduate students (49.5%) were consuming cereals at least once a day. It is reported that more than half of the undergraduate students (52.5%) were consuming vegetables at least once a day, while most were taking fresh fruit (34.2%) and fruit juices (35.5%) several times a week. The results revealed that the 38.5% of the undergraduate students were consuming sweets and candies at least once a day. And ultra-processed meat was consumed by nearly half of the undergraduate students (49.8%) for 1-4 times a month. Daily consumption of powdered milk (33.2%) was higher than the daily consumption of flavored milk (13.3%), indicating limited processed milk intake for a portion of undergraduate students. The results are summarized in Table 3.

Table 3: Food consumption frequency among the participants

Food category	Food subcategory	Frequency of consumption	Frequency (percentage)
Cereals and starchy food	Cereals	1- 4 times a month	91 (30.2%)
		At least once a day	149 (49.5%)
		Never	21 (7%)
		Several times a week	40 (13.3%)
	Starchy roots, tubers and yams	1- 4 times a month	102 (33.9%)
		At least once a day	47 (15.6%)
		Never	18 (6%)
		Several times a week	134 (44.5%)
	Starchy tree crops	1- 4 times a month	177 (58.8%)
		At least once a day	18 (6%)
		Never	29 (9.6%)
		Several times a week	77 (25.6%)
	Preparations	1- 4 times a month	135 (44.8%)
		At least once a day	48 (16%)
		Never	13 (4.3%)
		Several times a week	105 (34.9%)
Vegetables	Vegetables	1- 4 times a month	60 (19.9%)
		At least once a day	158 (52.5%)
		Never	17 (5.7%)
		Several times a week	66 (21.9%)
	Green leaves	1- 4 times a month	88 (29.2%)
		At least once a day	101 (33.6%)
		Never	18 (6%)
		Several times a week	94 (31.2%)
Fruits	Fresh fruits	1- 4 times a month	90 (29.9%)
		At least once a day	101 (33.6%)
		Never	7 (2.3%)
		Several times a week	103 (34.2%)
	Fruit juices	1- 4 times a month	124 (41.2%)
		At least once a day	61 (20.3%)
		Never	9 (3%)
		Several times a week	107 (35.5%)
Proteins foods	Meat	1- 4 times a month	68 (22.6%)
		At least once a day	72 (23.9%)
		Never	100 (33.2%)
		Several times a week	61 (20.3%)
	Ultra processed meat	1- 4 times a month	150 (49.8%)
		At least once a day	37 (12.3%)
		Never	28 (9.3%)

		Several times a week	86 (28.6%)
	Poultry	1- 4 times a month	74 (24.6%)
		At least once a day	105 (34.9%)
		Never	21 (7%)
		Several times a week	101 (33.5%)
	Fish and sea food	1- 4 times a month	71 (23.6%)
		At least once a day	108 (35.9%)
		Never	14 (4.6%)
		Several times a week	108 (35.9%)
	Eggs	1- 4 times a month	51 (16.9%)
		At least once a day	131 (43.5%)
		Never	8 (2.7%)
		Several times a week	111 (36.9%)
	Pulses and beans	1- 4 times a month	121 (40.2%)
		At least once a day	36 (12%)
		Never	39 (12.9%)
		Several times a week	105 (34.9%)
Snacks, Sweets and other miscellaneous	Sweets and candies	1- 4 times a month	84(27.9%)
		At least once a day	116(38.5%)
		Never	7(2.4%)
		Several times a week	94(31.2%)
	Sugary and carbonated drinks	1- 4 times a month	142(47.2%)
		At least once a day	45(15%)
		Never	32(10.6%)
		Several times a week	82(27.2%)
	Chips and salty snacks	1- 4 times a month	155(51.5%)
		At least once a day	32(10.6%)
		Never	18(6%)
		Several times a week	96(31.9%)
	Fast foods	1- 4 times a month	168(55.8%)
		At least once a day	30(10%)
		Never	19(6.3%)
		Several times a week	84(27.9%)
Nuts and seeds	Nuts and seeds	1- 4 times a month	160 (53.2%)
		At least once a day	32(10.6%)
		Never	27(9%)
		Several times a week	82(27.2%)
Dairy products	Condensed milk	1- 4 times a month	143(47.5%)
		At least once a day	22(7.3%)
		Never	99(32.9%)
		Several times a week	37(12.3%)

Yogurt	1- 4 times a month	122(40.5%)
	At least once a day	53(17.6%)
	Never	23(7.7%)
	Several times a week	103(34.2%)
Cheese	1- 4 times a month	158(52.5%)
	At least once a day	31(10.3%)
	Never	32(10.6%)
	Several times a week	80(26.6%)
Flavored milk	1- 4 times a month	129(42.9%)
	At least once a day	40(13.3%)
	Never	48(15.9%)
	Several times a week	84(27.9%)
Dairy ice cream	1- 4 times a month	146(48.5%)
	At least once a day	32(10.6%)
	Never	16(5.3%)
	Several times a week	107(35.6%)
Butter/ Margarine	1- 4 times a month	115(38.2%)
	At least once a day	54(17.9%)
	Never	30(10%)
	Several times a week	102(33.9%)
Powdered milk	1- 4 times a month	80(26.6%)
	At least once a day	100(33.2%)
	Never	77(25.6%)
	Several times a week	44(14.6%)
Ghee	1- 4 times a month	85(28.2%)
	At least once a day	17(5.7%)
	Never	170 (56.5%)
	Several times a week	29(9.6%)

3.3 Perceived Stress Among the Participants

From the study results, it is illustrated that among the 301 undergraduate students, 41.5% had high stress, while 58.5% had low stress. The mean perceived stress score was 20.2 ± 4.1.

3.4 Association between Basic Food Consumption Patterns and Perceived Stress

The Chi-square test was employed to examine the association between basic food consumption patterns such as meal type, main sources of food, number of meals consumed per day, and supplement intake and undergraduate students perceived stress

levels. The results indicated that there was no statistically significant association between perceived stress levels and type of meal consumption ($p = 0.821$), number of meals consumed per day ($p = 0.104$), main sources of food ($p = 0.997$), or use of dietary supplements ($p = 0.226$).

3.5 Associations between Food Consumption Behavior and Perceived Stress

The Chi-square analysis was used to assess the relationship between consumption of different food subgroups and perceived stress scores. According to the results, among all food subgroups tested, only frequency of poultry consumption showed a statistically significant association with perceived stress ($p = 0.022$). That means the stress levels varied depending on poultry intake frequency. Students with high stress were more frequently represented in the category of never consuming the food item, whereas students with low stress were more frequently represented in the category of consuming it several times a week. The proportions reporting daily consumption or consumption 1–4 times a month were relatively similar across the two stress groups. These findings suggest that food consumption patterns differed according to stress status among the study participants. Only one food subgroup, powdered milk ($p = 0.054$) showed borderline significant. For all other food subgroups, there were no statistically significant associations observed (all $p > 0.05$).

4. Discussion

This study was conducted to assess food consumption patterns and their association with the socio-demographic characteristics and perceived stress levels of undergraduates at a selected university in Sri Lanka. In this study, only poultry consumption showed a statistically significant association with perceived stress levels. Among the other food subgroups, only powdered milk consumption demonstrated borderline statistical significance, while no statistically significant associations were observed for the remaining food subgroups.

Although not statistically significant, these trends suggest that certain dietary habits may influence stress levels. In the current study, the frequency of food consumption was assessed among the overall undergraduate population without considering gender differences. However, a study conducted in the UK in 2014 (El Ansari et al., 2014) reported that female students consumed sweets more frequently than male students.

The study also found that females consumed fresh fruits, salads, and raw vegetables more often than males. Furthermore, healthy food consumption, such as fruits, salads, and vegetables, was negatively associated with stress levels in both sexes, whereas frequent consumption of unhealthy foods, including sweets, snacks, and fast food, was positively associated with higher stress levels, particularly among women. Compared to the current study, which used Chi-square analysis, the UK study applied multivariable regression analysis stratified by sex, allowing adjustment for multiple dietary variables and identification of more subtle associations.

Another cross-sectional study conducted among young people in Southern Sri Lanka in 2017 found that young men were more likely to consume sweet foods and snacks at least three days per week compared to women (Perera, 2017). Compared with these studies, the current study did not assess gender-specific food consumption frequencies. This was mainly because the study sample included a considerably higher number of female participants than male participants and analyzing dietary patterns according to gender may have introduced bias into the results.

In the current study, 41.5% of participants had high perceived stress, while 58.5% had low perceived stress. The study focused only on participants perceived stress scores (PSS). However, a study conducted in the UK in 2014 examined both perceived stress and depressive symptoms and assessed the association between food consumption behaviors of individual food groups and mental health indicators according to sex using univariable analysis (El Ansari et al., 2014). Compared to the current study, that study was broader as it included gender variations and compared food consumption behaviors, perceived stress, and depressive symptoms between males and females.

Similarly, a population-based study conducted in 2007 at the University of Helsinki, Finland, reported that men were more likely than women to consume non-sweet, high-energy foods ($p < 0.001$) (Konttinen et al., 2010). However, no gender differences were observed in the consumption of sugary, high-energy foods. In contrast, the current study did not assess variations in food consumption and perceived stress according to gender.

In the present study, no statistically significant association was found between undergraduates' perceived stress levels and their type of meal consumption ($p = 0.821$).

In contrast, a study conducted at the University of Helsinki, Finland, reported that participants with higher levels of depressive symptoms consumed more non-sweet, energy-dense foods and fewer fruits and vegetables, irrespective of their level of emotional eating (Konttinen et al., 2010). These findings suggest an association between depressive symptoms and dietary choices in the previous study. However, differences in study populations and outcome measures should be considered when interpreting these findings in relation to the present study. Furthermore, given the cross-sectional design of the current study, causal relationships between dietary habits and perceived stress cannot be established.

5. *Strength and limitation*

With the use of pretested instruments, this study fulfilled the knowledge gap under investigating the relationship between stress and food consumption among undergraduate students in Sri Lankan context.

However, several limitations should be acknowledged. First, the use of a self-administered questionnaire may have introduced recall bias, as participants may not have accurately recalled their usual food consumption patterns. Self-reported responses may also have been influenced by social desirability, potentially resulting in reporting inaccuracies. Second, the study was conducted at a single private university in Sri Lanka, limiting the generalizability of the findings to undergraduates from other private and state universities. Third, the cross-sectional design does not permit the establishment of causal relationships or the determination of whether perceived stress influences food consumption patterns or vice versa.

A formal sample size calculation was not conducted prior to data collection. Due to the limited two-month data collection period, questionnaires were distributed to eligible participants, and data were collected from as many participants as possible within the available timeframe. Consequently, the sample size was determined by the number of participants who could be reached during this period, which may have affected the representativeness of the sample and the ability to detect associations between the variables.

Furthermore, statistically significant associations were not demonstrated between perceived stress and most of the food consumption patterns examined. This should be

interpreted cautiously, as the absence of statistical significance does not necessarily indicate the absence of a relationship. Potential confounding factors may also have influenced the observed findings, particularly if they were not measured or controlled for in the analysis.

6. Conclusion

This study highlights the presence of perceived stress among undergraduates at a selected private university in Sri Lanka and explores its relationship with food consumption patterns. Although most participants reported low perceived stress, a substantial proportion experienced high perceived stress, indicating that psychological well-being remains an important consideration in university student health. The findings did not demonstrate statistically significant associations between perceived stress and overall meal-related practices, main food sources, or dietary supplement use. However, the significant association observed with poultry consumption suggests that the relationship between individual dietary behaviors and perceived stress warrants further investigation. As the study employed a cross-sectional design, this association should not be interpreted as evidence of a causal relationship. The findings support the need for further research using larger and more representative student populations, appropriate sample size calculations, and multivariable analyses to examine potential confounding factors and clarify the relationship between dietary practices and perceived stress. In the meantime, university health promotion initiatives should adopt a holistic approach that encourages balanced eating habits while supporting students' psychological well-being.

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DOES PROLONGED METFORMIN EXPOSURE INDUCE NEUROPROTECTION OR EXACERBATE NEURODEGENERATIVE DISEASES IN TYPE 2 DIABETES MELLITUS: A NARRATIVE REVIEW

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Abstract

The prevalence of neurodegenerative disorders such as Alzheimer's and Parkinson's disease is increasing among patients with type 2 diabetes mellitus (T2DM), contributing to cognitive decline. Metformin, a widely prescribed oral antidiabetic agent, has been reported to exert neuroprotective effects; however, conflicting evidence suggests that prolonged exposure may also accelerate neurodegenerative changes and contribute to vitamin B12 deficiency. This narrative review evaluated 29 studies examining the relationship between prolonged metformin use, neurodegenerative disorders, cognitive outcomes, and vitamin B12 status. Findings on Alzheimer's and Parkinson's disease were mixed, with studies reporting neuroprotective, neurodegenerative, gender-dependent, and neutral effects, while some identified alternative antidiabetic agents as being associated with lower dementia or cognitive impairment risk than metformin. Most studies assessing cognition demonstrated neuroprotective effects, although a small number reported increased risk of mild cognitive impairment or no significant association. Prolonged metformin use was consistently associated with vitamin B12 deficiency. Overall, the neurological effects of long-term metformin exposure remain inconclusive, likely due to variations in study design, gender-specific analyses, animal models, and confounding factors. Further research is required to clarify its long-term neurological impact in T2DM patients.

Keywords - Metformin, Type 2 Diabetes Mellitus (T2DM), Neuroprotection, Neurodegeneration, cognitive impairments

1. Introduction

The global prevalence of diabetes mellitus has expanded over the past 10-year period and is predicted to be greater than 700 million by the year 2045, with most patients affected by type 2 diabetes mellitus (T2DM)(Ha et al., 2021). Type 2 diabetes mellitus is a complicated, persistent and developing metabolic disease defined by insufficient insulin production, insulin resistance and elevated blood glucose concentrations (Markowicz-Piasecka et al., 2017). This metabolic disease is accountable for a broad range of systemic complications, involving the renal, cardiovascular, and neurological systems (Dec-Gilowska et al., 2019). Metformin, a biguanide, is the primary hypoglycemic agent utilized to achieve therapeutic effects against type 2 diabetes mellitus. This drug functions by inhibiting gluconeogenesis and moderately enhancing peripheral glucose utilization. Epidemiological evidence has observed an association between the incidence of type 2 diabetes mellitus and degenerative nerve disorders such as Alzheimer's disease and Parkinson's disease (Koenig et al., 2017; Newby et al., 2022).

The demographic shift towards a geriatric world population has elevated the prevalence of age-related degenerative nerve disorders such as Alzheimer's disease (AD)(Abbaszadeh et al., 2024) and Parkinson's disease (PD), both defined as advancing degenerative nerve disorders affecting nerve cells and ultimately leading to non-reversible impairments in cerebral activity(Newby et al., 2022). AD is the primary triggering factor of dementia, approximately representing 50% to 75% of dementia patients, with its prevalence exhibiting an ascending pattern(Abbaszadeh et al., 2024). Extracellular amyloid plaques composed of clusters of amyloid β protein (Aβ), intracellular neurofibrillary tangles (NFTs) composed of overphosphorylated tau protein, and decline in neuronal activity act as the key features of Alzheimer's disease(Markowicz-Piasecka et al., 2017). Collection of findings have revealed that diabetic patients are highly susceptible to developing AD in contrast to the general population. The pathophysiological pathways driving this relationship are complex and arise from diverse contributing factors. Insulin will fundamentally be involved in cerebral processes, including Inflammatory signaling in the brain, synaptic

transmission, glial cell functioning and neural metabolic activities. Several investigations have revealed that insulin resistance influences the cerebral deposition of amyloid-beta and hyperphosphorylation of tau protein, which are characteristic features of AD (Abbaszadeh et al., 2024) therefore, the association between insulin resistance and AD have gained interest from the research community (Koenig et al., 2017). PD is the second most prevalent degenerative nerve disorder and the leading movement disorder, impacting greater than 1%–3% of global population above 60 years old (Sanz et al., 2022). The neuropathological profile underlying PD is complex and defined by two key features which are dopamine deficiency in the brain region withing the basal ganglia as a result of gradual depletion of dopaminergic neurons in the substantia nigra pars compacta, and the dysregulated intracellular deposition and clustering of alpha-synuclein resulting in the development of Lewy bodies(Huang et al., 2022).younger individuals with prolong type 2 diabetes and individuals with other complicated diseases along with diabetes are at a higher risk of developing PD, this connection has been verified by several population-based studies together with in vivo rodent model systems and in vitro neuronal investigations suggesting impaired insulin signaling, ROS burden, neuroinflammation, impaired mitochondrial function, iron-dependent cell death and α-synuclein accumulation as the underlying pathophysiological pathways (Sun et al., 2025).

Dementia is a complex multidimensional condition resulting from the association between adjustable and non-adjustable risk factors, among which insulin-independent diabetes mellitus and impaired insulin sensitivity are well known contributors to dementia (Zheng et al., 2023). Worldwide estimates indicate that diabetic patients are at an elevated risk of acquiring dementia in comparison to non-diabetic individuals. However, the exact pathophysiological mechanism behind this correlation is still under investigation, with prolonged glycemic burden, inflammation, ROS burden and impaired insulin sensitivity considered as driving factors. Even though the correlation between diabetes and dementia has been widely investigated, the resulting evidence has been controversial due to various methodologies highlighting the requirement for further investigations, primarily in geriatric patients with insulin independent diabetes mellitus to analyze the effect of metformin on dementia risk (Sun et al., 2024).

Metformin is the cornerstone of type 2 diabetic medications. Metformin, a broadly utilized monotherapy by greater than 120 million individuals globally for type 2 diabetes treatment as a result of its affordability and consistent safety outcomes (Ha et al., 2021; Khan et al., 2017; Soldevila-Domenech et al., 2021), primarily functions to enhance insulin sensitivity(Porter et al., 2019). Metformin is widely preferred not only due to its capacity to reduce serum glucose levels but also due to its capacity to boost the lipid profile and support weight management (Khattar et al., 2016). Beyond its well-established hypoglycemic effects, metformin has demonstrated protective attributes across various organ systems. For instance, its efficacy extends to reducing cardiovascular risk through the improvement of endothelial function and the regulation of lipid metabolism (DiGiovanni et al., 2025). Even though metformin has beneficial effects on body weight reduction and enhances metabolic control, findings from *in vivo* models have indicated a relationship between metformin usage and poor cognition, along with enhanced dementia related pathology (Wennberg et al., 2018). Large-scale epidemiological studies and systematic reviews have determined a connection between Long-term metformin usage and significantly reduced vitamin B12 levels; however, its association with cognitive dysfunction remains a debatable subject in the literature (Porter et al., 2019). Even though some research indicates that metformin can decelerate aging and downregulate the occurrence of geriatric-related diseases with studies showing a decreased risk of developing PD, some studies oppose these findings stating higher doses of prolong metformin usage is related with a higher risk of developing PD. However, the relationship between metformin use in T2DM patients and PD risk requires further clarification (Huang et al., 2022). The aim of this narrative review is to critically evaluate the existing evidence on whether prolonged metformin exposure exerts a neuroprotective effect or exacerbates neurodegenerative disease risk in patients with T2DM. This review assists in examining the association between prolonged metformin use and the risk of Alzheimer's disease, Parkinson's disease and cognitive decline along with exploring the biological mechanisms proposed for both neuroprotective effects, such as AMPK activation, reduced oxidative stress, and attenuated neuroinflammation, and potentially harmful effects, such as vitamin B12 deficiency and in identifying factors that may influence these effects such as duration

of metformin, dose of metformin, gender categorizations, geographical deviations and other confounding factors.

2. Methodology

This narrative review was conducted using 3 electronic databases: Google Scholar, PubMed, and ScienceDirect. A total of 33 studies published in English Language between January 2015 and October 2025 were included in this review. The literature search was performed using keywords such as metformin, type 2 diabetes mellitus, neuroprotection, and neurodegeneration. Additionally, Cohort studies, case-control studies, cross-sectional studies, randomized controlled trials, and relevant mechanistic studies were considered. Studies were excluded if they involved non-diabetic populations, examined metformin for other indications such as polycystic ovary syndrome or cancers, were not peer-reviewed, or were not available in full text. After title and abstract screening followed by full-text assessment, 33 studies met the criteria, of which 29 were used in the analysis and 4 were used for background information only.

Table 1: Details extracted from original research articles

Title	Authors	exposure group	control group	Outcome (increase or reduce the risk of cognitive impairments)
Metformin Treatment Alters Memory Function in a Mouse Model of Alzheimer's Disease	Kacee A. DiTacchio, Stephen F. Heinemann, Gustavo Dziewczapolski*	AD mice + metformin AND AD mice + no metformin	NO AD mice + metformin	gender dependent
Hyperglycemia and Metformin Use Are Associated With B Vitamin Deficiency and Cognitive Dysfunction in Older Adults	s Kirsty M. Porter,1 Mary Ward,1 Catherine F. Hughes,1 Maurice O'Kane,2 Leane Hoey,1 Adrian McCann,3 Anne M. Molloy,4 Conal Cunningham,5 Miriam C. Casey,5 Fergal Tracey,6 Sean Strain,1 Kevin McCarroll,5 Eamon Laird,4 Alison M. Gallagher,1 and Helene McNulty	Hyperglycemic + metformin AND Hyperglycemia + NO metformin	Normoglycemic + no metformin	increase the risk
Association Between Metformin Initiation and Incident	Jeffrey F. Scherrer, PhD1,2 John E. Morley, MBBCh3 Joanne Salas, MPH1,2 James S.	DM + metformin	DM + no metformin	reduces the risk

Dementia Among African American and White Veterans Health Administration Patients	Floyd, MD4-6 Susan A. Farr, PhD3,7 Sascha Dublin, MD4,			
Metformin promotes tau aggregation and exacerbates abnormal behavior in a mouse model of tauopathy	Erica Barini1†, Odetta Antico1†, Yingjun Zhao2,3, Francesco Asta1, Valter Tucci1, Tiziano Catelani4, Roberto Marotta4, Huaxi Xu2,3 and Laura Gasparini1,5*	AD mice + metformin	AD mice + no metformin	NEUTRAL
The Effect of Diabetes Medication on Cognitive Function: Evidence from the PATH Through Life Study	Pushpani M.Herath,Nicolas Cherbuin, Ranmalee Eramudugolla, and Kaarin J. Anstey	DM + metformin	DM + other diabetic treatment except metformin	reduces the risk
Utility of Pharmacological Agents for Diabetes Mellitus in the Prevention of Alzheimer's Disease: Comparison of Metformin, Glucagon Like Peptide-1	111Alexandra DiGiovanni , Andrea Shehaj , David Millar , Claire Tse , Elias Rizk 12	DM + metformin	DM + other diabetic treatment except metformin AND NO DM + metformin	increase the risk

(GLP-1) Agonists, Insulin, and Sulfonylureas				
Prevalence of Vitamin B12 Deficiency in Patients with Type II Diabetes Mellitus on Metformin: A Study from Khyber Pakhtunkhwa	11Adnan Khan , Ihtesham Shafiq , Mohammad Hassan Shah 2	DM + metformin	DM + no metformin	NOT STUDIED
Metformin Use and Cognitive Function in Older Adults With Type 2 Diabetes Following a Mediterranean Diet Intervention E	Natalia Soldevila-Domenech1,2†, Aida Cuenca-Royo1†, Nancy Babio3,4, Laura Forcano1,4, Stephanie Nishi3,4, Cristina Vintró-Alcaraz4,5,6, Carlos Gómez-Martínez3,4, Susana Jiménez-Murcia4,5,6,7, Rebeca Fernández-Carrión4,8, Maria Gomis-González1, Andrea Alvarez-Sala4,8, Silvia Carlos9,10, Xavier Pintó4,11,12, Dolores Corella4,8, Javier Díez-Espino4,9,10, Olga	DM + metformin	DM + no metformin AND no DM	reduces the risk BY HIGH DOSES

	Castañer4,13,14, Fernando Fernández-Aranda4,5,6,7, Jordi Salas-Salvadó3,4* and Rafael de la Torre1,2,4			
Metformin Use Is Associated With Slowed Cognitive Decline and Reduced Incident Dementia in Older Adults With Type 2 Diabetes: The Sydney Memory and Ageing Study	Katherine Samaras,1,2,3 Steve Makkar,4 John D. Crawford,4 Nicole A. Kochan,4 Wei Wen,4 Brian Draper,4,5 Julian N. Trollor,4,6 Henry Brodaty,4,5 and Perminder S. Sachdev4,	DM + metformin	DM + no metformin AND no DM	reduces the risk
Association of antidiabetic medication use, cognitive decline, and risk of cognitive impairment in older people with type 2 diabetes: results from the population-based Mayo Clinic Study of Aging	Alexandra M. V. Wennberg1, Clinton E. Hagen1, Kelly Edwards1, Rosebud O. Roberts1,3, Mary M. Machulda2, David S. Knopman3, Ronald C. Petersen3, and Michelle M. Mielke1,3	DM + metformin	DM + Insulin / other diabetic treatment except metformin	increase the risk

Dose–Response Association of Metformin with Parkinson's Disease Odds in Type 2 Diabetes Mellitus	Kuang-Hua Huang 1,† , Ya-Lan Chang 2,3,†, Shuo-Yan Gau 4 1 , Tung-Han Tsai 1 and Chien-Ying Lee	DM + metformin	DM + no metformin	reduces the risk BY lower DOSES
Exploring the link between Parkinson's disease and type 2 diabetes mellitus in Drosophila	Francisco José Sanz1,2 | Cristina Solana-Manrique1,2 | Joaquín Lilao-Garzón3 | Yeray Brito-Casillas3 | Silvia Muñoz-Descalzo3 | Nuria Paricio1,	DM + metformin	DM + no metformin	reduces the risk
Relationships between memory decline and the use of metformin or DPP4 inhibitors in people with type 2 diabetes with normal cognition or Alzheimer's disease and the role APOE carrier status	Che-YuanWu1,2 MichaelOuk1,2 YuenYanWong1,2 NatashaZ.Anita1,2 Jodi D.Edwards3,4,5 PearlYang6,17 BaijuR.Shah7,8 NathanHerrmann2, 9 Krista L. Lanctôt1,2,9,10 MoiraK.Kapral7,11 BradleyJ.MacIntosh2,12,13 Jennifer S. Rabin2,14,15,18 SandraE.Black2,12,14,16 WalterSwardfager1,10,1	DM + metformin	DM + DPP4 inhibitors / other diabetic treatment	reduces the risk

The neuroprotective role of metformin in advanced glycation end product treated human neural stem cells is AMPK-dependent	Ming-Min Chunga,Yen-LinChenb, Dee Peia, Yi-Chuan Chengc, Binggui Sund, Christopher J. Nicole,f, Chia-Hui Yeng,Han-MinChenh,i, Yao-Jen Liangi, Ming-Chang Chiangi,*	AGE-treated hNSCs co-treated with metformin	AGE-treated hNSCs without metformin AND Untreated hNSCs	reduces the risk
Activation of AMPK is neuroprotective in the oxidative stress by advanced glycosylation end products in human neural stem cells	Chien-Hung Lina, Yi-Chuan Chengb, Christopher J. Nicolc, Kuan-Hung Lind, Chia-Hui Yene, Ming-Chang Chiangf,	AGE-treated hNSCs co-treated with metformin	AGE-treated hNSCs without metformin AND Untreated hNSCs	reduces the risk
Metformin and the Risk of Dementia in Type 2 Diabetes Patients	Chin-Hsiao Tseng1,2,3, *	DM + metformin	DM + no metformin	reduces the risk
Metformin and Risk of Alzheimer's Disease Among Community-Dwelling People With Diabetes: A National Case-Control Study	Janet K Sluggett,1,2,* Marjaana Koponen,1,3,4,* J Simon Bell,1,2,3,5 Heidi Taipale,3,4,6,7 Antti Tanskanen,6,7,8 Jari Tiihonen,6,7,9 Matti Uusitupa,10	DM + metformin	DM + NO metformin	reduces the risk

	Anna-Maija Tolppanen,3,4 and Sirpa Hartikainen3,4			
Metformin attenuates white matter microstructural changes in Alzheimer's disease	Sahar Abbaszadeh1, Ghazaleh Raei Dehaghi2, Zahra Ghahri Lalaklou3, Hasti Beig Verdi4, Delaram Emami5*, Behnaz Dalvandi6, and the Alzheimer's Disease Neuroimaging Initiative**	DM + metformin	DM + no metformin	reduces the risk
Comparative effect of metformin versus sulfonylureas with dementia and Parkinson's disease risk in US patients over 50 with type 2 diabetes mellitus	Danielle Newby ,1,2 Andrew Brent Linden,3 Marco Fernandes,1 Yasmina Molero,4,5 Laura Winchester,1 William Sproviero,1 Upamanyu Ghose,1 Qingqin S Li,6 Lenore J Launer,7 Cornelia M van Duijn,3,8 Alejo J Nevado- Holgado	DM + metformin	DM + sulfonylureas	met reduces the risk than sulfonylureas
Incident dementia risk among patients with type 2 diabetes receiving	William Doran,1 Louis Tunnicliffe,1 Rutendo Muzambi,1 Christopher T Rentsch ,1,2	DM + metformin	DM + other antidiabetic drugs	increase the risk (have a lower risk)

metformin versus alternative oral glucose-lowering therapy: an observational cohort study using UK primary healthcare records	Krishnan Bhaskaran,1 Liam Smeeth,1 Carol Brayne,3 Dylan M Williams,4 Nish Chaturvedi,4 Sophie V Eastwood,4 Susanna J Dunachie,5,6 Rohini Mathur,7 Charlotte Warren-Gash 1			
Association of metformin use with Alzheimer's disease in patients with newly diagnosed type 2 diabetes: a population-based nested case–control study	Junghee Ha1,7, Dong-Woo Choi2,7, Kwang Joon Kim3, So Yeon Cho1,4, Hyunjeong Kim1, Keun You Kim1, Youngseung Koh5, Chung Mo Nam6* & Eosu Kim1,4*	DM + metformin	DM + NO metformin	increase the risk
SGLT2 inhibitors vs. metformin for Parkinson's disease risk reduction in type 2 diabetes	Mingyang Sun1,*, Xiaoling Wang1,2,*, Zhongyuan Lu1,2, Yitian Yang1, Shuang Lv1, Mengrong Miao1, Wan-Ming Chen3,4, Szu-Yuan Wu3,4,5,6,7,8,9,10 and Jiaqiang Zhang	DM + SGLT2 inhibitors	DM + metformin	SGLT2i users had lower Parkinson's disease risk, lower dementia risk and lower all-cause mortality

Antidiabetic Drugs for the Risk of Alzheimer Disease in Patients With Type 2 DM Using FAERS	HayatoAkimoto,PhD1 ,AkioNegishi,BS1, ShinjiOshima,PhD1 , Haruna Wakiyama, BS1, Mitsuyoshi Okita, MS2, Norimitsu Horii, MS2,3, Naoko Inoue, PhD2,3, Shigeru Ohshima, PhD2,3, and Daisuke Kobayashi, PhD1	DM + anti AD drugs + other antidiabetic drugs	DM+ NO anti AD drugs + other diabetic treatment except metformin	some drugs presented lower risk than metformin
The Correlation between Metformin Use and Incident Dementia in Patients with New-Onset Diabetes Mellitus: A Population-Based Study	Kuang-Hua Huang 1,† , Ya-Fang Tsai 2,† , Chiachi Bonnie Lee 1 Ning-Jen Chung 3 and Chien-Ying Lee 4,5,	DM + metformin	DM + no metformin	reduces the risk BY lower DOSES
Dementia risk in patients with type 2 diabetes : Comparing metformin with no pharmacological treatment	BangZheng1,2 BowenSu3 SaraAhmadi-Abhari1 DimitriosKapogiannis4 IoannaTzoulaki3 Elio Riboli3,5 LefkosMiddleton1,	DM + metformin	DM + no metformin / other diabetic treatments	reduces the risk
Metformin in elderly type 2 diabetes mellitus: dose-	Mingyang Sun, 1,† Wan-Ming Chen, and Jiaqiang Zhang 1	DM + metformin	DM + no metformin	reduces the risk

dependent dementia risk reduction				
The Association between Metformin Use and Risk of Developing Severe Dementia among AD Patients with Type 2 Diabetes	Ying Xue 1,2,3,* and Xiangqun Xie 2,3,	DM + AD + metformin	DM + AD + other diabetic treatment except metformin	NO ASSOCIATION
Is Metformin-Induced Vitamin B12 Deficiency Responsible for Cognitive Decline in Type 2 Diabetes	Deepti Khattar, Farah Khaliq, Neelam Vaney, S.V. Madhu	DM + metformin	NO DM + no metformin	increase the risk

3. Discussion

3.1 Therapeutic potential of metformin against neurodegeneration

Metformin regulates intracellular metabolic activities(DiGiovanni et al., 2025) and mitigates intracellular reactive oxygen species levels by enhancing the functioning of antioxidant enzymes(Wang et al., 2022). Metformin's capacity to mitigate AD risk by enhancing cognitive function has been emphasized in the latest findings. The primary mechanism of action for metformin involves blocking mitochondrial complex I, a core component in the electron transport chain. This Inhibition results in a reduction in cellular adenosine triphosphate production and elevates intracellular adenosine monophosphate concentrations. This triggers the upregulation of adenosine monophosphate-activated protein kinase (AMPK). AMPK acts as a crucial regulator of intracellular energy equilibrium, affecting mitochondrial activity, ROS burden, and

autophagy. Cerebral activation of the AMPK pathway is associated with enhanced insulin signaling, which may counteract the neurotoxic effects of insulin resistance and glucose impairment observed in AD. Additionally, metformin's capacity to attenuate oxidative stress and inflammation further highlights its role in neuroprotection(DiGiovanni et al., 2025). A fraction of pathophysiological conditions such as inflammation, aging, diabetes, and degenerative nerve disorders raise the advanced glycation end product formation resulting from protein oxidation and nonenzymatic glycation. Metformin has demonstrated its capacity to mitigate AGE-induced mitochondrial dysfunction and inflammatory responses in human neural stem cells (hNSCs) through an AMPK-dependent mechanism, therefore revealing the signaling pathway accountable for these neuroprotective effects(Lin et al., 2017). Furthermore, the latest findings have indicated metformin's therapeutic effects on white matter. It has been shown to mitigate demyelination and enhance remyelination in mouse models of Lysophosphatidylcholine-induced demyelination. Metformin also enhances myelin recovery and mitigates behavioral impairments in animal models of multiple sclerosis. Additionally, metformin intake significantly suppresses ischemia-induced brain injury by reducing inflammation and improves motor and cognitive function after neonatal hypoxia-ischemia(Abbaszadeh et al., 2024). Nevertheless, the precise mechanisms by which metformin opposes neurodegeneration remain largely unresolved in the current literature(DiGiovanni et al., 2025)

3.2 Impact of prolonged metformin exposure on vitamin B12 status

Metformin serves as the most frequently used anti diabetic drug, but various studies have observed an association between metformin exposure and vitamin B12 status(Khan et al., 2017)(Sepassi et al., 2025). Clinical research conducted in 2016 designed to study the association between cognitive decline and vitamin B12 depletion triggered by metformin in type 2 diabetic patients revealed that patients exposed to metformin have exhibited low serum vitamin B12 status and cognitive impairment but a correlation between vitamin B12 status and cognitive health was not observed. Additionally, this vitamin B12 status further declined with elevated dose and length of metformin treatment(Khattar et al., 2016). This is further exemplified by a cross-sectional study and clinical research conducted in 2017 and 2025, which reported

findings that support escalating vitamin B12 depletion with increasing dosage of metformin. The study conducted in 2017 also presented that smoking can be considered a contributing factor to the severity of vitamin B12 depletion(Khan et al., 2017)(Sepassi et al., 2025). Moreover, a clinical study conducted in 2019 also demonstrated that diabetic patients exposed to metformin treatment are associated with elevated serum vitamin B12 depletion and cognitive impairments in comparison to diabetic patients who are not exposed to metformin. This study has also proposed that vitamin B12 depletion may trigger cognitive impairments in diabetic adults exposed to metformin therapy(Porter et al., 2019).

3.3 Impact of prolonged metformin exposure on Alzheimer's disease

Evidence based studies indicate that diabetic individuals are at a higher risk of acquiring Alzheimer's disease compared to non-diabetic individuals. Previous research has demonstrated metformin's ability to offer neuroprotection by delaying the onset of dementia (Abbaszadeh et al., 2024). This protective action has been supported by a Nested Case-Control study conducted in 2020 in which individuals exposed to metformin for a long time period at higher doses presented a reduced incidence of Alzheimer's disease (Sluggett et al., 2020). A population-based study was conducted on 2024 utilizing data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) to study the effects of metformin exposure on microstructural changes in white matter. This study reported that individuals exposed to metformin had elevated Fractional Anisotropy (FA) values in the left hippocampal cingulum and right internal capsule, suggesting improved integrity of white matter and reduced Mean Diffusivity (MD) values in the right inferior fronto-occipital fasciculus, indicating lower microstructural degeneration or elevated cellularity (Abbaszadeh et al., 2024). However, experimental research conducted in 2015 utilizing animal models of Alzheimer's disease reported that metformin's neuroprotective role is gender dependent as a result of exacerbated Alzheimer's disease related characteristics in male mice exposed to a metformin dose that enhances insulin sensitivity, whereas female mice exposed to the same treatment reflect improved disease characteristics by same levels (DiTacchio et al., 2015). Despite the molecular pathways responsible for metformin's neuroprotective actions are not completely understood, activation of AMP-activated protein kinase (AMPK) is partly

responsible for this neuroprotective effect. Diabetic neuropathy is characterized by neuronal damage that arises from hyperglycemia, leading to an elevated production of advanced glycation end products, which contribute to neurodegeneration. An in vitro study conducted in 2015 has indicated that AGEs stimulate hNSC death and diminish mitochondrial activity by downregulating the AMP-activated protein kinase (AMPK) pathway, while metformin, which functions as an AMPK activator provide neuroprotection to hNSC by diminishing the neurotoxic effects induced by AGEs. In addition, metformin also stimulates PGC1α, NRF-1, and Tfam expressions which results in elevated mitochondrial activity and induce neuroprotective gene production (Chung et al., 2015). Another In vitro study conducted on 2017 demonstrated that AGE treated hNSCs was associated with significantly lower cell growth and elevated oxidative stress. Addition of metformin, which act as an AMPK agonist, normalizes cell growth and oxidative stress, but the addition of compound C, which acts as an antagonist of AMPK, disrupts the neuroprotective role of metformin by mitigating cell growth and enhancing oxidative stress, revealing that activation of AMPK pathway is partly responsible for metformin's neuroprotective role (Lin et al., 2017). In contrast to this perspective, a retrospective database study conducted in 2025 comparing the effectiveness of various antidiabetic drugs such as metformin, insulin, sulfonylureas and GLP -1 receptor agonists to prevent Alzheimer's disease progression, reported that the neuroprotective role of metformin against Alzheimer's disease were inconsistent compared to other drugs, and while no statistically significant difference in AD risk was observed between metformin and sulfonylureas or insulin, metformin users demonstrated a significantly higher risk of developing Alzheimer's disease compared to GLP-1 receptor agonist users(DiGiovanni et al., 2025). However, another study focusing on repurposing antidiabetic medications to mitigate the risk of Alzheimer's disease development in type 2 diabetes mellitus patients has highlighted that antidiabetic medications, except sulfonylureas, thiazolidinediones, and GLP-1 receptor agonists, have an equal risk of developing Alzheimer's disease as metformin (Akimoto et al., 2020). Furthermore, a nested case–control study conducted in 2021 revealed that diabetic individuals exposed to metformin were associated with considerably greater Alzheimer's disease risk. This association is strengthened in individuals exposed to

higher cumulative daily defined dose of metformin per day and in individuals with prolong diabetic duration. Even though this correlation was independent of the occurrence of depression, patients suffering from depression had a significantly higher risk of Alzheimer's disease development (Ha et al., 2021). Moreover, experimental research conducted on 2016 utilizing an animal model such as P301S transgenic mice, which exhibit mutated tau proteins, has demonstrated that long term metformin has affected tau pathology via two discrete mechanisms. Primarily, metformin has mitigated phosphorylation of tau protein in cortex and hippocampus regions of the brain by expressing PP2A responsible for tau dephosphorylation via AMPK route. However, metformin also elevates the quantity of insoluble tau proteins and tau deposits in the brain, facilitating tau aggregation; therefore, metformin's role in tau aggregation counteracts its neuroprotective role stemming from metformin's dephosphorylating activity (Barini et al., 2016).

3.4 Impact of prolonged metformin exposure on Parkinson's disease

Latest epidemiological findings have revealed a correlation between Parkinson's disease and type 2 diabetes mellitus. To analyze this association further, a multimodal biomedical study was conducted in 2022 utilizing Drosophila, a mouse model of T2DM, and human neuron like SH-SY5Y cells. This work has reported that mitochondrial dysfunctions in T2DM models may arise due to inhibition of TCA cycle functioning. In this study T2DM model flies exhibits PD associated observable traits such as reduced longevity , movement impairments and DA neuron impairments which are characteristic features of PD, and Elevated glucose levels also lead to impairments in the viability of neuroblastoma cells; however, metformin treatment has been able to elevate the viability of human neuroblastoma cells that lack DJ-1 protein, downregulate diabetes mellitus and Parkinson's disease associated characteristics, enhance motor function, and mitigate dopaminergic neuronal loss in T2DM model flies(Sanz et al., 2022). However, population-based epidemiological studies have exhibited that metformin's neuroprotective effects are dose dependent. This is due to the reduced risk of Parkinson's disease with lower doses of metformin, proving the neuroprotective effects, but elevated doses and intensities of metformin have been unable to express any neuroprotective effects (Huang et al., 2022). Additionally, a comparative study reported

that metformin users presented lower risk of all-cause dementia in comparison to sulfonylureas users (Newby et al., 2022). This is further exemplified by a another comparative study examining metformin's and SGLT-2 inhibitors' capacity to lower Parkinson's disease risk, which reported that type 2 diabetic patients exposed to SGLT2 inhibitors have approximately 3 times lower risk of developing PD than individuals treated with metformin, and, as a secondary outcome, this study also reported higher all-cause mortality in patients treated with metformin than in patients treated with SGLT-2 inhibitors(Sun et al., 2025).

3.5 Impact of prolong metformin exposure on cognition leading to dementia-like characteristics

Accelerated cognitive deterioration and elevated dementia risk serve as well-established characteristic features of type 2 diabetes mellitus. The effect of metformin remains as a controversial factor as a result of evidence suggesting both elevated and mitigated dementia risk (Samaras et al., 2020). A longitudinal observational study conducted in 2016 addressing the impact of antidiabetic drugs on cognitive capacity reported that patients exposed to metformin have exhibited elevated cognitive activity in comparison to patients treated with other antidiabetic medications at baseline, and the strongest effects have been observed in domains of verbal memory, executive functioning, and working memory. This study has also shown that metformin treatment has elevated psychomotor speed within the 4-year time period (Herath et al., 2016). In addition, 2 population-based studies conducted in 2024 and 2023 reported that metformin provides neuroprotective effects against dementia in a dose dependent pattern therefore, diabetic patients treated with larger metformin doses presented higher neuroprotective effects against dementia than diabetic patients treated with lower metformin doses (Sun et al., 2024; Zheng et al., 2023). However, a population-based study conducted in 2023 has revealed that diabetic patients exposed to lower metformin doses with reduced intensity of metformin use have reflected a reduction in developing dementia, but elevated doses of metformin with elevated intensity of metformin use have not reflected any neuroprotective action against dementia (Huang et al., 2023). Additionally, a latest research conducted on 2024 examining the dementia risk in type 2 diabetic patients exposed to metformin in comparison to other oral glucose lowering medications

estimated that diabetic patients exposed to metformin have 23% reduction in developing dementia and 17% reduction in developing mild cognitive impairment in comparison to the other glucose lowering drugs but this correlation was not visible in patients greater than 80 years old(Doran et al., 2024). Two parallel study's conducted on 2019 have presented that type 2 diabetic patients exposed to metformin treatment for a longer time period have a lower probability to develop dementia in comparison to other oral anti diabetic drugs(Chin-Hsiao, 2019; Scherrer et al., 2019). This probability is strongest in 50 to 64 years old African American patients in comparison to the white patients(Scherrer et al., 2019). An extension of this idea is found in another study conducted in 2020 that reported diabetic patients exposed to metformin had an 81% lower risk of incident dementia and reduced impairments in global cognition in comparison to the diabetic patients not exposed to metformin, but executive function has diminished in both the diabetic group treated with metformin and the non-diabetic group, and higher diminishing potential was observed in diabetic individuals that are not exposed to metformin treatment(Samaras et al., 2020). Furthermore, a study observed that, even though individuals with normal cognition exposed to metformin have improved memory, Alzheimer's disease dementia patients, specifically APOE ε4 carriers, have had exacerbated memory decline with metformin exposure, while neutral effects was observed in APOE ε4 non, suggesting that the neuroprotective role of metformin is dependent on APOE ε4 and cognitive status (Wu et al., 2020). However, an observational study conducted in 2023 reported that diabetic and Alzheimer's patients treated with metformin are not correlated with a reduced severe dementia risk, and these results were consistent irrespective of APOE ε4 carrier status (Xue & Xie, 2023). A alternative observational study highlighted that diabetic patients with better glycemic control exposed to metformin have improved memory performance, executive functioning and global cognition in comparison to patients that are not treated with metformin but diabetic patients that are not exposed to metformin following a Mediterranean diet have also observed similar cognitive activity suggesting that a Mediterranean diet practice could act superior to neuroprotective role of metformin(Soldevila-Domenech et al., 2021). In contrast to this perspective, an epidemiological study conducted in 2018 focusing on evaluating the effects of anti-

diabetic medications on cognitive health revealed that even though a relationship between metformin exposure and cognitive test performance was not observed, metformin users have had a greater risk of developing mild cognitive impairment in comparison to diabetic individuals with no exposure to metformin (Wennberg et al., 2018).

4. Conclusion

This narrative review intended to explore the outcome of chronic metformin exposure on clinical neurology in type 2 diabetes mellitus patients. The outcome of the present review highlights the neuroprotective role of metformin by facilitating the AMPK pathway, mitigating oxidative stress, and boosting insulin signaling to reverse neural toxicity induced by insulin resistance. These findings have been supported by several population-centered studies and in vitro and in vivo models, However, several studies have discovered an association between prolonged metformin exposure and an elevated risk of degenerative nerve disorders and cognitive decline, along with an elevated incidence of vitamin B12 deficiency but the correlation between vitamin B12 deficiency and cognitive decline is not verified by previous research findings. Additionally, a study performed utilizing both in vivo models and in vitro models has highlighted that the pro-aggregation capacity of metformin has mitigated the potential gains arising from its dephosphorylating action. Nevertheless, there is insufficient evidence to confirm that the cognitive decline or neurodegeneration observed with prolonged metformin therapy is directly attributable to metformin itself, as these effects can also be associated with diabetic complications.

Despite the elevated interest among the research community to explore the neurological effects of prolong metformin exposure in type 2 diabetic patients, existing investigations are limited due to variations in methodology of the study, exclusion of non-diabetic metformin users, metformin dosage, duration of metformin treatment, limited animal experimentation models, geographical deviations, gender categorizations and variations in tools utilized to interpret the cognitive activity. Confounding factors such as individual glycemic level, vitamin B12 measurements, duration of diabetes, and comorbidities including hypertension, obesity, impaired renal performance, dyslipidemia, and impaired hepatic activity lead to alterations in neurological health.

These limitations diminish the reliability of reported findings developing a demand for further research and investigations to evaluate the neurological role of prolong metformin exposure in type 2 diabetic patients.

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PSYCHOEDUCATION, ACCEPTANCE AND COMMITMENT THERAPY, AND INTERPERSONAL AND SOCIAL RHYTHM THERAPY AS PHASED PSYCHOSOCIAL INTERVENTIONS IN BIPOLAR I DISORDER: A CASE-BASED ANALYSIS WITH IMPLICATIONS FOR PSYCHIATRIC NURSING IN RESOURCE-LIMITED SETTINGS

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Abstract

Bipolar disorder is a chronic mood disorder characterized by recurrent episodes of mania or hypomania and depression, significantly affecting psychosocial functioning and quality of life. This paper examines the application of psychoeducation, Acceptance and Commitment Therapy (ACT), and Interpersonal and Social Rhythm Therapy (IPSRT) in the psychiatric rehabilitation of a 46-year-old male with bipolar I disorder. Using a qualitative, case-based approach guided by the biopsychosocial model, the study explored the suitability and evidence supporting each intervention. Findings suggest that psychoeducation improves illness insight and treatment adherence, ACT enhances psychological flexibility and reduces experiential avoidance, while IPSRT supports the regulation of social and circadian rhythms and relapse prevention. Evidence indicates that psychosocial interventions provide benefits beyond pharmacotherapy. The case highlights the value of integrated psychosocial interventions in psychiatric rehabilitation. Further research is needed to evaluate the effectiveness of combining these interventions within the Sri Lankan mental health context.

Keywords: acceptance and commitment therapy, bipolar disorder, interpersonal and social rhythm therapy, psychoeducation, psychosocial interventions.

1. Introduction

Bipolar disorder is a mental health condition characterised by significant changes in mood, energy and activity levels, which can affect a person's daily functioning (American Psychiatric Association, n.d.). Although pharmacological treatments are the first pillar for stabilizing fluctuating moods, psychosocial interventions play a significant role in psychiatric and mental health nursing, as they address the psychological, behavioral, and social facets of mental illness that cannot be effectively managed using pharmacological therapy alone. Medication stabilizes the condition during acute symptoms, whereas the psychosocial intervention promotes long-term recovery through developing insight and treatment adherence, coping skills, and daily functioning (Painter & Scannapieco, 2021; World Health Organization, 2023). Psychiatric nurses have a significant role in delivering psychosocial interventions along with continuous therapeutic engagement, education, and behavioral support (Ameel et al., 2021; Uzun et al., 2025). Psychosocial interventions also contribute to recovery-oriented care by making the patient feel empowered, engaging in the family, minimizing stigma, and reintegrating patients into the community, particularly in chronic psychiatric conditions such as bipolar disorder (BD).

Bipolar I disorder (BD-I) is the form of the illness defined by manic episodes and is associated with recurrent relapses, functional impairment, and a substantial burden on families and caregivers. Access to specialist psychotherapists is limited in resource-constrained settings such as Sri Lanka (Swamy & M., 2025), so psychiatric nurses, who are in continuous contact with patients, are well placed to deliver brief psychosocial interventions within daily care. However, evidence on how such interventions can be combined and adapted for this context remains limited.

This case study analyzes three psychosocial interventions tailored to a 46-year-old male patient who was diagnosed with BD-I and has been receiving inpatient rehabilitation care for two years because of a manic relapse with aggression and an inability to manage daily functioning. The interventions are discussed within the context of the clinical presentation of the patient, his recovery needs, and the evidence-based practice in psychiatric nursing. The objectives are (1) to describe the patient's presentation within a biopsychosocial framework, (2) to examine the rationale and evidence for each

intervention, and (3) to discuss the implications of phased, nurse-led delivery in resource-limited settings.

The biopsychosocial framework guided this case study. It understands health and illness as the result of interacting biological factors (for example, genetic vulnerability, circadian rhythm, and comorbid physical illness), psychological factors (for example, insight, coping, and emotion regulation), and social factors (for example, family relationships, occupational stress, and available support) rather than any single cause (Taukeni, 2020). The framework is particularly suitable for bipolar disorder because genetic vulnerability, disrupted social rhythms, and stressful interpersonal circumstances interact to precipitate mood episodes (Aktaş & Dülgerler, 2026), and because it supports combined pharmacological and psychosocial care. A recovery-oriented perspective, which focuses on hope, empowerment, and the patient's own goals rather than symptom control alone, complemented this framework.

Three psychosocial interventions are examined. Psychoeducation is a structured process of providing patients and families with information about illness, its treatment, early warning signs, and self-management strategies, in order to improve insight, treatment adherence, and relapse prevention (Levrat et al., 2023). Acceptance and Commitment Therapy (ACT) is a third-wave cognitive behavioral approach that aims to increase psychological flexibility through acceptance, cognitive defusion, present-moment awareness, self-as-context, values clarification, and committed action (Zhang et al., 2018). Interpersonal and Social Rhythm Therapy (IPSRT) combines interpersonal psychotherapy with the stabilization of daily routines and social rhythms, such as sleep, meals, and activity, to protect against the circadian disruption that can trigger mood episodes (Aktaş & Dülgerler, 2026).

2. Methodology

This study used a qualitative, single-case study design. Data were obtained through clinical observations, nursing assessments, multidisciplinary records, and patient interviews. The patient's information was analyzed using the biopsychosocial framework to identify the biological, psychological, and social factors contributing to his condition.

The diagnosis of BD-I was established by the treating clinical team and is reported here as documented in the clinical records; the authors did not make or alter the diagnosis, and DSM-5-TR criteria are referred to only to illustrate the clinical presentation. The selection of psychosocial interventions was guided by current evidence-based literature and psychiatric nursing principles. Psychoeducation, ACT, and IPSRT were selected due to their relevance to the patient's presentation, recovery needs and rehabilitation goals (Colom et al., 2003).

Written informed consent was obtained from the patient and the institution. To protect confidentiality, the patient is not identified by name or pseudonym, and personal details are limited to those essential to the analysis. The analysis was guided by biopsychosocial and recovery-oriented frameworks.

3. Literature Review

3.1 Epidemiology and Burden of Bipolar I Disorder

BD-I is characterized by at least one manic episode lasting more than one week, usually interspersed with depressive episodes that tend to become more common over time relative to manic episodes (World Health Organization, n.d.). In 2021, an estimated 37 million people, or approximately 0.5% of the global population, were living with bipolar disorder, including about 34 million adults, and the condition is observed mainly among working-age people (World Health Organization, n.d.). In Sri Lanka, a rate of 5.2% for manic and bipolar affective disorders was reported in early 2017 (Directorate of Mental Health, Sri Lanka, n.d..).

3.2 Biopsychosocial Understanding of Bipolar Disorder

Biopsychosocial models examine the reasons for mental and physical illness through an understanding of the biological, psychological, and social environment around the patient (Taukeni, 2020). Biologically, recent evidence shows BD as one of the most heritable mental health conditions, with 60-80% of risk attributed to genetic inheritance (Ozdemir et al., 2016; O'Connell et al., 2025). Circadian rhythm dysregulation, including reduced sleep, is a recognized feature of the illness (Aktaş & Dülgerler, 2026). BD also shows a bidirectional relationship with type 2 diabetes mellitus (T2DM), with a pooled prevalence of T2DM of 9.6% in bipolar patients (Liu et al., 2022). Psychologically, limited illness insight, experiential avoidance, and difficulty regulating impulses can maintain mood instability, and these are the processes targeted by

psychoeducation and ACT (Levrat et al., 2023; Zhang et al., 2018). Socially, stressful life events, caregiving and occupational stress, and the availability of family and community support influence the course of the illness by disrupting or protecting daily routines and social rhythms (Aktaş & Dülgerler, 2026).

3.3 Psychosocial Interventions

Psychosocial interventions are structured, collaborative treatments in which patients and therapists work together to understand the sources of distress, with the aim of improving self-regulation and building psychological strengths to cope with illness (Sixteen – Psychosocial interventions, n.d.). A review of clinical guidelines supports their use alongside medication in bipolar disorders (Ventriglio et al., 2025). A systematic review and component network meta-analysis of 39 randomized trials found that manualized psychotherapy added to pharmacotherapy was associated with lower recurrence rates than control treatments, with psychoeducation delivered in family or group formats showing particular benefit (Miklowitz et al., 2021).

3.4 Psychoeducation

Psychoeducation is grounded in the health belief model (HBM) and self-management theory. The HBM proposes that health behavior, including adherence to treatment, depends on how a person perceives the seriousness of an illness, their susceptibility to relapse, and the benefits and barriers of treatment, so that improving illness perception can improve engagement with care (Sembiring et al., 2025). Self-management theory complements this by proposing that an increase in knowledge about the illness, treatment, and self-monitoring can improve patient autonomy, treatment engagement, and positive clinical outcomes (Sembiring et al., 2025). BD is a condition that needs long-term maintenance medication to prevent relapse (World Health Organization, n.d.; Yatham et al., 2018); therefore, adhering to medication requires patients to become aware of their own condition, side effects, and rationale. Psychoeducation also allows building partnerships between patients and healthcare providers, resulting in better outcomes compared to passive treatment recipients (Levrat et al., 2023).

Psychoeducation is strongly supported by current evidence. The Canadian Network for Mood and Anxiety Treatments (CANMAT) and International Society for Bipolar Disorders (ISBD) 2023 guidelines designate psychoeducation as a first-line psychosocial intervention for maintenance-phase BD, reflecting its robust evidence base

from multiple randomized controlled trials and meta-analyses (Keramatian et al., 2023). A literature review by Levrat et al. (2023) confirmed that structured psychoeducation reduces relapse risk and improves treatment adherence, particularly among euthymic patients. A controlled study involving 109 euthymic outpatients with bipolar disorder found that psychoeducation was associated with a longer period without hospitalisation during the four-year follow-up, suggesting a potential benefit in the long-term management of bipolar disorder (Levrat et al., 2023; Buizza et al., 2019).

However, the evidence also presents challenges. A four-year follow-up study found that nearly one-fifth of patients discontinued psychoeducation during mood relapses, indicating that this intervention is less suitable during active mood instability (Buizza et al., 2019). Most validated psychoeducation programs also originate from Western clinical settings (Levrat et al., 2023), and culturally adapted programs for South Asian or Sri Lankan populations are scarce. Cultural adaptation of evidence-based psychotherapies has been advocated to improve their relevance and acceptability (Manjula, 2025), and materials that incorporate Buddhist frameworks of impermanence (anicca) and mental suffering (dukkha) could improve engagement among Buddhist populations.

3.5 Acceptance and Commitment Therapy

ACT is a third-wave cognitive behavioral treatment paradigm that is conceptually based on functional contextualism and relational frame theory and focuses on psychological flexibility as the primary therapeutic effect (Zhang et al., 2018; Arnold et al., 2022). ACT increases psychological flexibility by encouraging acceptance of distressing events, cognitive defusion from mood-congruent thoughts, living in the moment, and values-guided action, which may minimize impulsive behavior and improve functional recovery in fluctuating mood states (P & S, 2025).

Although ACT has been studied mainly for disorders such as obsessive-compulsive disorder, anxiety disorders, depression, and post-traumatic stress disorder, its evidence base for bipolar disorder is emerging. Recent systematic reviews demonstrate that ACT improves psychological flexibility while reducing symptoms of anxiety, depression, and psychological distress across diverse clinical and non-clinical populations (P & 2025), and a three-level meta-analysis found that ACT outperformed cognitive behavioral therapy in psychological flexibility for people without a fixed diagnosis (Fang & Ding,

2023). In bipolar disorder, a randomized controlled trial by El-Sayed et al. (2023) showed considerable reductions in psychological inflexibility, impulsivity, and suicidal ideation among individuals receiving ACT compared with standard care, and an open pilot study of group ACT for bipolar disorder with co-existing anxiety reported encouraging preliminary findings (Pankowski et al., 2017). However, current evidence is limited by small samples, pilot designs, and short follow-up periods, which limit firm conclusions regarding long-term relapse prevention. ACT should therefore be considered an adjunctive intervention delivered alongside pharmacotherapy and psychoeducation rather than a stand-alone treatment for BD-I.

ACT's compatibility with Buddhist concepts such as mindfulness, acceptance, and values-based action is another strength. Fung (2015) examined the relationship between ACT and Buddhist tenets, and culturally adapted ACT that incorporates Buddhist concepts has been proposed as a way of improving therapeutic engagement among Buddhist populations. However, culturally adapted ACT has been studied only to a limited extent in Sri Lankan psychiatric settings, which highlights an important area for future research.

3.6 Interpersonal and Social Rhythm Therapy

IPSRT integrates interpersonal psychotherapy principles and social rhythm stabilization based on circadian rhythm theory, as BD patients show significant circadian rhythm sensitivity (Aktaş & Dülgerler, 2026). IPSRT targets the regulation of circadian rhythm through management of social rhythms, additionally addressing interpersonal problems that cause stress-induced rhythm disruptions. This is in line with the theoretical model underlying IPSRT, which suggests that when social rhythms are disrupted, it triggers proximal events that lead to mood episodes in biologically vulnerable individuals (Aktaş & Dülgerler, 2026).

A comprehensive review has reported that patients who were administered IPSRT in combination with pharmacotherapy made significant improvements in the reduction of both manic and depressive symptoms, regulation of biological rhythms, and psychosocial functioning. Evidence shows that IPSRT aids in decreasing BD relapses and enhances regularity of social rhythm and interpersonal functioning. A 2020 real-world controlled trial by Steardo et al. reported a 100% retention rate with large effect sizes in reducing both manic and depressive symptoms (Steardo et al., 2020) , and a

2022 domain-based analysis by Moot et al. confirmed significant improvements in psychosocial functioning following IPSRT, including occupational and social domains (Moot et al., 2022). The findings indicate that IPSRT is more successful in relapse prevention and maintenance of stability than in the acute control of symptoms, which supports its use as a long-term management practice in BD-I (Aktaş & Dülgerler, 2026; Swartz et al., 2018; Moot et al., 2022).

The limitations of IPSRT must also be acknowledged. In resource-limited settings such as Sri Lanka, Swamy and M. (2025) identified the following barriers: tracking fatigue, high learning complexity, and a lack of trained IPSRT-qualified professionals. The CANMAT/ISBD guidelines consider IPSRT a third-line maintenance intervention, lower in evidence ranking than psychoeducation and CBT (Yatham et al., 2018).

3.7 Combined Psychosocial Approaches and Research Gap

Evidence on combining psychosocial interventions comes mainly from adjunctive psychotherapy trials, which show that skills-based psychosocial interventions delivered alongside pharmacotherapy reduce recurrence (Miklowitz et al., 2021). However, there is little research specifically investigating the combination of psychoeducation, ACT, and IPSRT in BD-I, so their additive or collective effect remains unknown. The rationale for combining them is currently theoretical: each targets a different mechanism, namely illness knowledge and adherence, psychological flexibility, and circadian and interpersonal regulation. The review also identifies three gaps relevant to this case: the scarcity of culturally adapted psychosocial programs for South Asian and Sri Lankan populations, the limited evidence on combined delivery, and the limited evidence on how these interventions can be delivered by nurses in resource-limited inpatient settings.

4. Case Presentation

4.1 Patient Profile and History

The patient is a 46-year-old unmarried male who has received inpatient rehabilitation care for two years. He was admitted with police involvement at 44 years of age due to an escalated aggression episode in which he tried to strangle his mother, due to poor stress management resulting from being her primary caretaker.

The patient showed high intelligence during mental health assessment discussions. Before admission, he had self-learnt computing skills and worked as a senior software

engineer for well-recognized software companies. However, he showed an inability to stay in one focused occupation. He switched between five different computing positions, discontinued electrical engineering, started software engineering, and went to a private campus and discontinued in the middle, thinking he could self-learn. He could not sustain his last occupation due to the carer burden of his mother, demonstrating how BD impaired occupational function even in a highly capable individual. Moreover, during the assessment, he stated, "Every year I fought with someone at the school," showing that he had anger management issues since high school. This suggests that he had early behavioral difficulties which affected his school life.

4.2 Clinical Presentation

The clinical features documented by the treating team are described below in relation to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5), criteria (American Psychiatric Association [APA], 2022) to illustrate the presentation of BD-I. After three months of admission, he experienced a manic episode which lasted for one and a half weeks. During this time, he climbed on facility poles and ran through staircases, fitting with criterion A of DSM-5, showing abnormally elevated mood (Calabrese et al., 2017; APA, 2022).

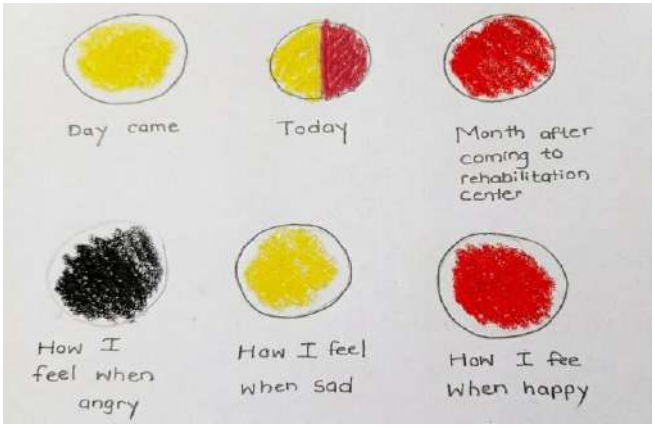
Criterion B of the classification is met by his claim, "I am the strongest and most intelligent person in this facility," exhibiting grandiose thoughts and stating himself as the youngest person despite being 46 years old (APA, 2022). He slept only 5 hours (9 pm-2 am) during this manic period, showing a reduced need for sleep, yet reporting waking up with "maximum energy for the rest of the day".

During the assessment, even though he was in a euthymic state, his ideas switched from his sisters' visit to Buddhist concepts to his marriage ideas to fibre-optic technologies, showing a flight of ideas along with loud volume, rapid speech rate, and increased talkativeness, jumping between topics. This indicates an inability to maintain focus in a conversation. Criterion D is met, as he does not use any recreational drugs or alcohol products, either before or during his time in the facility (APA, 2022).

In the early admission period, he suffered from moderate depression, as evidenced by Figure 1 and nurses' notes, crying without reason, and not paying attention to hygiene. The presence of both manic and depressive episodes is consistent with the documented diagnosis of BD-I, which required prompt attention.

Figure 1 shows the patient's own colour-coded drawing of his emotions, in which black represents anger, yellow represents sadness, and red represents happiness. He drew the day he came to the rehabilitation centre in yellow and the month after admission in red, suggesting a lifting of mood after the initial period, while his state on the day of assessment was drawn as half yellow and half red, indicating mixed or ambivalent feelings at that time. This supports the need for ongoing mood monitoring and for the emotional awareness work used in ACT.

Figure 1: Patient-reported emotional states across situations during the mental health assessment



A notable concern in the patient's condition is his lack of insight. He believes that he had depression due to stress, and currently he is fully cured. He was unaware of his prevailing BD and manic episodes. However, regardless of a lack of insight, he engages with treatments willingly, which helps the staff to engage him in psychosocial interventions.

4.3 Biopsychosocial Formulation

Biologically, the patient shows familial transmission with a first-degree relative, as his mother was diagnosed with BD, which is consistent with the high heritability of the disorder (Ozdemir et al., 2016; O'Connell et al., 2025). During the assessment he repeatedly stated, "I fell as an infant; that is why I have anger issues." This raises the question of potential traumatic brain injury, but the truth of this statement is not confirmed through his medical history, and he showed no developmental delays. He shows circadian rhythm dysregulation with only 5 hours of sleep, representing a classic BD-I symptom. Furthermore, the patient has T2DM, which reflects the bidirectional

relationship between BD and T2DM described in the literature (Liu et al., 2022).

Psychologically, he showed extreme polarities during two years in the rehabilitation facility, showing both manic and depressive episodes tallying with the DSM-5 classification of BD-I (American Psychiatric Association, 2022). One of the specific factors noted during his mental assessment is that he is avoidant when talking about shame-provoking events, claiming, "I am a good person" right after discussing a traumatic incident. This transition towards affirming himself suggests the use of impression management strategies to ease the tension caused by cognitive dissonance following a provocation (Goffman, 1959; Festinger, 1957). He devoutly practices Buddhism as a coping and protective mechanism for the stressors. This spiritual practice is benefiting his recovery process because he says that he is happier after becoming devoted to Buddhism (de Zoysa, 2011).

Socially, the patient reported experiencing caregiver burden of his mother, who suffers from BD and dementia, along with workplace stress. This led to conflicts at home, deteriorating his mental health. Conversely, he has strong support from his sister, who is a psychotherapist. Currently, he receives structured rehabilitation in a multidisciplinary facility, enhancing his recovery with a favorable environment.

5. Discussion

5.1 Rationale for a Phased, Nurse-Led Approach

Based on the patient's BD-I diagnosis and his clinical features and beliefs, three psychosocial interventions are recommended to promote his recovery in a phased, nurse-led approach: psychoeducation is prioritized because he is euthymic and motivated but has limited insight, ACT is added to address experiential avoidance, grandiose thoughts, and poor insight, and IPSRT is developed into a patient-owned skill set before discharge. This case illustrates that a phased, nurse-led combination of psychoeducation, ACT, and IPSRT may be an appropriate, evidence-informed response to the psychological complexity of an adult inpatient who is receiving care in a mental health rehabilitation centre. The following sections discuss how each intervention applies to the patient, evaluate it against current evidence, address the rationale for combined delivery, and focus on cultural and contextual dimensions that are under-explored in existing literature.

5.2 Psychoeducation for the Patient

Psychoeducation is the most robustly supported intervention (Rabelo et al., 2021). It is appropriate for the patient because he is euthymic, motivated, and willing to be treated, which aligns with the evidence that structured psychoeducation is most effective among euthymic patients. Psychoeducation may help improve patients' understanding of bipolar disorder and support their involvement in treatment and self-management (Rabelo et al., 2021), del. Delivery adapted for the patient: short sessions integrated into the daily routine, using 10-15 minutes of interaction with the nurse in which his condition is introduced step by step with small messages when administering medication, rather than immediate enrollment in formal group-based sessions.

As psychoeducation programes have largely been developed and evaluated in settings different from the Sri Lankan context, their cultural relevance should be considered when adapting them for local patients. Buddhist concepts such as impermanence (anicca) and suffering (dukkha) could be explored as culturally meaningful ways of explaining emotional experiences and coping. However, the effectiveness of this adaptation in bipolar disorder requires further investigation (de Zoysa, 2011).

5.3 Acceptance and Commitment Therapy for the Patient

The patient showed a pattern of experiential avoidance by avoiding discussions surrounding distressing events, stating, "I don't remember unwanted things," and minimizing the incident that resulted in his admission. He also showed grandiose thinking and impulsive aggression during the assessment. ACT is recommended as an additional intervention to help the patient develop psychological flexibility and manage difficult thoughts and emotions, complementing the aims of psychoeducation and IPSRT. (refer to Table 1). The evidence from El-Sayed et al. (2023) on reduced impulsivity and psychological inflexibility is particularly relevant to the patient, whose clinical history included impulsive aggression and poor emotional regulation (Shi et al., 2026; Isak et al., 2025). His deep faith and commitment to Buddhism are a valuable platform for teaching ACT principles such as mindfulness, acceptance, and action based on values (Fung, 2015; de Zoysa, 2011). Given the limited evidence base, ACT is positioned as an adjunct to pharmacotherapy and psychoeducation.

Table 1: Acceptance and commitment therapy (ACT) process applied to the patient's presentation

Source: Author's compilation

ACT process	*Patient's presentation*	*Example of nurse-led application*
Acceptance	Avoid discussing traumatic experiences related to aggression and hospitalization	Nurse validates discomfort: "we can notice the feeling without going into details now"
Cognitive defusion	Grandiose thoughts ("I'm the strongest in here")	Nurses respond: "I notice your mind is giving you that thought," rather than correcting the belief.
Present moment awareness	Flight of ideas and distractibility	Deep breathing exercises or mindful walking exercises framed as Buddhist Sati
Self as context	Mood-dependent self-image and shame.	Nurse reflects: "thoughts and mood changes, but you are the one noticing them."
Values clarification	Strongly devoted to Buddhism, desire for family harmony and independence.	Guided discussions on being a "good person'' regardless of mood state.
Committed action	Mood-driven routines and impulsivity	Small goals such as fixed sleep times, daily meditation and pausing before major decisions; incorporated with IPSRT

[a].

[a]ACT = Acceptance and Commitment Therapy; IPSRT = Interpersonal and Social Rhythm Therapy

5.4 Interpersonal and Social Rhythm Therapy for the Patient

IPSRT is recommended for the patient on the formulation that his manic episode was linked to chronic circadian disruption and interpersonal role overload as the primary caregiver for his mother. This is in line with the theoretical model underlying IPSRT (Aktaş & Dülgerler, 2026). The evidence of large effect sizes in reducing manic and depressive symptoms (Steardo et al., 2020) and of improved psychosocial functioning (Moot et al., 2022) is relevant, as the occupational and social domains are impaired in the patient's case.

An important point to note in this case is that the facility's structured timetable has already provided the patient with passive rhythm stabilization, as there has been a significant improvement in glycaemic control (85–105 mg/dL) compared to the pre-admission level of about 200 mg/dL. This finding illustrates that there is a bidirectional association between circadian rhythm regulation and metabolic outcomes in BD-T2DM comorbidity and leads to a clear, observable finding that can be presented to the patient

when introducing IPSRT, which is consistent with the principles of the health belief model in psychoeducational engagement (Sembiring et al., 2025). But this passive advantage is tenuous; if he lacks internalized self-management skills, he can relapse. IPSRT must therefore transition from an institutional default into an actively practiced, patient-owned skill set before discharge. Given the barriers identified by Swamy and M. (2025), delivery is made easier with a nurse-guided tracking tool and family integration sessions prior to discharge.

5.5 Combined Delivery and Implications for Psychiatric Nursing

None of the single interventions can fully address the multifaceted needs of the patient: psychoeducation addresses insight and adherence, ACT addresses experiential avoidance and grandiose thinking, and IPSRT addresses the circadian and interpersonal triggers of his manic episode. This complementarity, together with evidence that adding psychosocial interventions to pharmacotherapy reduces recurrence (Miklowitz et al., 2021) supports the rationale for combining these interventions in this case, However, the combined effect of psychoeducation, ACT and IPSRT delivered through the specific phased approach proposed in this case has not been established. In a resource-limited setting, the approach relies on low-cost, nurse-delivered elements: brief interactions during medication rounds, a nurse-guided tracking tool, and family integration sessions, which is consistent with evidence on the efficacy of nurse-delivered psychosocial interventions (Uzun et al., 2025). As a single-case analysis, the findings cannot be generalized, and the effectiveness of the recommended interventions for this patient has not yet been evaluated.

6. Conclusion

This case study presents a phased, nurse-led psychosocial intervention model for an adult male with BD-I in a Sri Lankan inpatient rehabilitation setting, combining psychoeducation, ACT, and IPSRT in a sequenced framework informed by current evidence and the patient's clinical and cultural profile. Psychoeducation is prioritized as it is well-established psychosocial intervention, particularly given the patient’s euthymic state and willingness to learn about his condition. ACT provides a culturally congruent way to address experiential avoidance and cognitive flexibility, with emerging evidence and parallels to Buddhist practice. IPSRT directly addresses the

circadian and interpersonal vulnerabilities that were linked to his manic episode, with strong evidence for rhythm regulation and relapse prevention. Critically, the synthesis of evidence indicates that none of the single interventions can fully address the multifaceted needs of the patient. Because the three interventions target different mechanisms, a combined and phased approach is likely to offer greater benefit than monotherapy, and the case shows that psychiatric nurses can deliver such an approach through brief, low-cost, culturally congruent interactions embedded in daily care. However, there is little research specifically investigating combined psychosocial methods for BD-I, which is a major gap in research. Future studies are encouraged to explore the effectiveness of the combination of interventions, place a strong focus on cultural adaptations, and derive the implementation measures of the approach in resource-limited settings to make evidence-based psychosocial care accessible in environments beyond well-resourced western contexts.

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REVIEW ON DEVELOPMENT OF PHARMACEUTICAL DOSAGE FORMS FROM Tinospora cordifolia AND APPLICATION OF MICROPROPAGATION TO SUPPLY THE PHARMACEUTICAL DEMAND

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Abstract

Tinospora cordifolia (family: Menispermaceae) is a medicinal plant widely distributed across the Indian subcontinent and parts of East Asia, traditionally used to treat numerous diseases due to its diverse pharmacological activities. Its rich bioactive composition has attracted pharmaceutical interest for developing novel dosage forms that enhance therapeutic efficacy and patient compliance. This review provides an overview of pharmaceutical dosage forms derived from T. cordifolia and examines micropropagation as a sustainable approach to meet increasing pharmaceutical demand. Relevant literature published between 2010 and 2025 was retrieved from PubMed, ScienceDirect, SpringerLink, and Google Scholar using the keywords "Tinospora cordifolia," "pharmaceutical dosage forms," and "micropropagation." Evaluated dosage forms, including tablets, capsules, syrups, gels, creams, phytosomes, and nano-formulations, demonstrated acceptable physicochemical characteristics. Micropropagation enables rapid production of genetically uniform plantlets and bioactive compounds, with most studies reporting successful regeneration through optimized organogenesis protocols. Therefore, micropropagation offers a sustainable solution to overcome supply limitations and support the continued development of T. cordifolia-based pharmaceutical dosage forms.

Keywords: Dosage form, Micropropagation, Organogenesis, Pharmaceutical, *Tinospora cordifolia*

1. Introduction

The Family Menispermaceae consists of 70 genera, which accounts for around 500 species. Among those, *Tinospora cordifolia* has been spotted as a commercially important species since it is abundant with therapeutic constituents including alkaloids, glycosides, diterpenoids, steroids, lactones, phenolics, sesquiterpenoid, aliphatic compounds and polysaccharides (Khatoon et al., 2014; Mridula et al., 2017). It is a climbing deciduous shrub thoroughly branched known as "heart leave moonseed" found throughout India, Sri Lanka, Bangladesh, Myanmar and China (Lade et al., 2018). The plant has many vernacular names such as in Latin as *Tinospora cordifolia* (Miers) Hook. F. & Thomson, known in Sanskrit as "Guduchi", "Madhuparni", "Amrita", "Chinnaruha", "Vatsadaani", "Tantrika", "Kundalini", known in Nepal as "Gurjo", in Hindi as "Giloya" or "Guduchi", in Bengali as "Gulancha" and as "Rasakinda" in Sinhala. It is a herbal medicine that exerts anti-hyperglycemic, anti-spasmodic, anti-periodic, anti-arthritic, anti-inflammatory, antioxidant, anti-stress, anti-allergic, anti-malarial, anti-leprotic, immunomodulatory, hepatoprotective, and anti-neoplastic activities (Modi et al., 2020; Saha & Ghosh, 2012).

Herbal formulations or phytomedicines are medicinal preparations consisting of one or more herbs in specified amounts used in cosmetics, nutraceuticals and disease treatment. Phytomedicines were the prime therapeutic approach earlier in the twentieth century. From global perspectives, there is a shift from the use of herbal medicines to allopathic medicines due to the fast therapeutic action and compliance of patients (Sharma et al., 2019). According to estimates of the World Health Organization, 60% of the global population uses herbal medicines, while 80% of people in developing nations depend entirely on them for their primary health care requirements (World Health Organization, 2019). Therefore, increasing demand for herbal medicines around the world resulted in the large-scale manufacturing of different pharmaceutical dosage forms including herbal powders, tablets, capsules, syrup, gel, creams and novel formulations like phytosomes and nanoparticles (Kumadoh & Ofori-Kwakye, 2017).

According to that, propagation methods are needed to fulfill the current pharmaceutical demand of *T. cordifolia*. The key challenges identified when applying conventional propagation methods to *T. cordifolia* are poor germination, low rate of fruit set, and

lack of clonal uniformity. Advanced biotechnological approaches of culturing cells and tissues like micropropagation can be applied to overcome these issues. This leads to healthy, microorganisms free, true to type plantlets of short duration at a cost efficient while being unaffected by external environmental conditions like sunlight, humidity and water availability etc. (Mridula et al., 2017; Singh et al., 2021).

Direct organogenesis allows direct regeneration of plantlets from a single explant while indirect organogenesis regenerates through callus formation. The resultants can be used for crude extractions. Though the plant is a rich resource of various secondary metabolites, the low concentrations of chemicals hinder the economic profitability of the production (Dharmapal et al., 2017a; Mangal et al., 2012; Singh et al., 2021). Hence, alternative methods need to be obtained to enhance the concentration of secondary metabolites. In this perspective, indirect organogenesis using callus and cell cultures offers solutions by enabling genetic enhancements and delivering continuous production of secondary metabolites under controlled and reproducible conditions (Dharmapal et al., 2017a; Sultana and Handique, 2013).

2. Methodology

This review was based on a comprehensive search of databases including PubMed, ScienceDirect, SpringerLink, and Google Scholar published between 2010 to 2025. The search utilized the Boolean operators "AND" and "OR" with keywords including "*Tinospora cordifolia*", "Pharmaceutical", "Dosage forms", and "Micropropagation". Qualitative and quantitative data from observational studies published in English were included.

3. Findings and Discussion

3.1 Different types of pharmaceutical dosage form

Patients can give different dosage forms including powders and granules, tablets, capsules, syrups, gels, creams, suspensions, emulsions, transdermal patches, suppositories, micro- and nano-sized dosage forms etc. depending on multiple factors such as the age, gender, weight, rate of drug release and health status of patient. In this section, different pharmaceutical dosage forms of *T. cordifolia* are examined in accordance with its constituents.

Dosage form -tablet

T. cordifolia tablets are prepared by the wet granulation method. The extract was passed through the sieve along with lactose and blended. Wet mass was obtained by incorporation of starch solution and made into dough. The wet granules obtained were dried and then added microcrystalline sodium, cross caramalose sodium, magnesium stearate, and talc. Sieving and milling were done to get granules which were compressed to acquire tablets. Pre-formulation studies, physiochemical and phytochemical studies, antioxidant and antibacterial studies showed positive results (Naved et al., 2013). In another study, *T. cordifolia* was advanced as a matrix-forming novel agent to formulate diclofenac sodium extended-release tablets, which reduce frequency of administration to improve patient compliance. In vitro studies revealed that the drug exhibited the highest mucilage release ability, reaching a maximum level of 63.6% after 15 hours (Madaan et al., 2018).

An Indian study showed to prepare dosage forms from *T. cardifolia* namely Svarasa Bhavita Guduchi Churna (SBGC), Kwatha Bhavita Guduchi Churna (KBGC), and Jala Bhavita Guduchi Churna (JBGC) according to the Ayurveda pharmaceutical guidelines. As stated, plant stems were crushed and wet grinding (Guduchi juice for SBGC batch, Guduchi decoction for KBGC batch, and potable water for JBGC batch) were done to obtain fine mass. Then wet granulation was carried out to prepare tablets. High Performance Layer Chromatography (HPLC) test analyzed the amount of anti-hyperglycemic compound 'berberine' in each sample (maximum in SBGC, followed by KBGC and JBGC) which proved α-amylase and α-glucosidase inhibitory actions (Sharma et al., 2021). Another study showed how to overcome the disadvantages of Guduchi Swaras tablets which have less shelf life and extreme bitter taste. Therefore, sugar coated granules from Guduchi Kalpa and Guduchi Ghrita medicated ghee were prepared according to the Ayurvedic standard protocols. It highlighted memory enhancing effect on human cognition using diazepam induced amnesia in animal models (Patankar, Pawar, & Gadgil, 2019).

T. cordifolia with *Withania somnifera* in combination were formulated to prepare tablets using wet granulation methods which were then tested by physiochemical

parameters and HPLC. It concluded that further improvement could enhance the immune system of humans (Baghel et al., 2023). Another study demonstrated the radioprotective potential of *T. cordifolia* root extract. The coarsely powdered roots were subjected to reflux extraction using double-distilled water. The extract was then cooled and subsequently concentrated by evaporating the solvent to obtain a dried powdered form (Goyal et al., 2011). *T. cordifolia* with a combination of some other herbal extracts were taken to prepare compressed tablet lozenges by wet granulation method. Herbal extracts with sorbitol, mannitol, citric acid and stevia were mixed by trituration and granulated using PVP solution as a binder. Magnesium stearate, silica, PEG 6000, talc, flavours and natural menthol were mixed with dried granules and compressed to obtain tablet lozenges (Yadav & Nand, 2015).

Dosage form -capsules

Studies have proposed capsule dosage form to enhance patient adherence. Polyherbal extracts prepared from the dried leaves of *Tinospora cordifolia*, seeds of *Trigonella foenum-graecum*, dried leaves of *Murraya koenigii*, and seeds of *Linum usitatissimum* were subjected to freeze-drying to obtain stable powdered extracts. The dried extracts were subsequently blended with appropriate excipients, including magnesium stearate as a lubricant, microcrystalline cellulose as a filler, and suitable diluents. Capsules were formulated using varying excipient ratios to optimize the composition. Comprehensive preformulation studies were conducted, followed by standardization of the final product, in vitro dissolution testing, and evaluation of drug release kinetics, which yielded positive results. The formulated capsules were further assessed for antidiabetic activity, demonstrating the potential of polyherbal capsule formulations in the management of diabetes (Geetha et al., 2024).

In a separate investigation, the stems of *Tinospora cordifolia* and the fruits of *Emblica officinalis* were utilized for formulation development. Concentrated decoctions were prepared individually from each selected plant material and subjected to controlled heating until the aqueous content was sufficiently evaporated to yield a dry powdered extract. The resulting powders were encapsulated in hard gelatin capsules. A comparative open-label clinical trial demonstrated that the formulated capsules

exhibited significant efficacy in alleviating premature ageing associated with stress, highlighting their potential therapeutic value (Mahajon et al., 2017). According to another study, hydro-alcoholic and aqueous extract of *T. cordifolia* core were concentrated under controlled temperature and low pH to make a homogenous solution. The material was sprayed dried until concentrated on a solid paste which was further dried to obtain dried flakes. It was pulverized and heat sterilized to pack under hygienic conditions which were administered as capsules. It showed to alleviate fasting blood glucose, serum levels of cholesterol and triglycerides while beneficially increasing the high-density lipoprotein level (Chatterji, 2015).

Dosage form -syrup

Aqueous extracts of *T. cordifolia, Phyllanthus* and *Terminalia* species were concentrated and mixed with syrup in 1:15 proportion to prepare "Livzon" syrup. The stems of *T. cordifolia* were blended with the *kinnow* in another study to improve appearance and nutritional value. Then the mixer was grinded, filtered, and bottled to pasteurize in which the extracted juice was evaluated for physical, chemical, nutritional, and organoleptic properties (Sood, 2015). In another formulation approach, extracts of *Tinospora cordifolia* stem and *Syzygium cumini* seeds were incorporated into a syrup base. The extracts were initially transferred into a mixing vessel containing simple syrup prepared from sodium saccharin dissolved in distilled water and stirred thoroughly to ensure uniform dispersion. Subsequently, excipients including methyl paraben as a preservative, a flavouring solution composed of peppermint oil in propylene glycol, and the colouring agent erythrosine were added. The final volume was adjusted with distilled water to obtain the completed polyherbal syrup. The formulation underwent phytochemical screening and physicochemical evaluation to ensure quality and stability. Preclinical studies conducted in Wistar rats demonstrated a pronounced hypoglycemic effect, indicating the therapeutic potential of the developed polyherbal syrup in glycemic control (Fakir et al., 2024).

Dosage form -gel

Gels are semi-rigid network of solvents and a gelling agent in which the drug molecules are entangled or embedded uniformly. *T. cordifolia* stems were powdered and ultra-sonication method was used to extract the bioactive compound. Mixture was evenly suspended in ethanol and added to the soaked Carbopol, which was then stirred without air entrapment until a uniform suspension was obtained. Sodium hydroxide or 50% triethanolamine was used to neutralize the gelling agent to obtain gel. Formulation was assessed for various parameters including appearance, viscosity, pH, spreadability, uniformity of content, stability, dermal irritation index, and antimicrobial activity (Abhishek et al., 2015).

Dosage form -creams

Herbal sunscreen cream was prepared using *Glycyrrhiza glabra* (Hasan et al., 2021), *T. cordifolia* and *Terminalia arjuna* which showed antioxidant, anti-inflammatory, and wound healing along with whitening of skin (Patankar, Pawar, & Gadgil, 2019). Dried powder samples were separately extracted and refluxed, followed by a maceration process. The oil phase was prepared using emulsifying agent and oil-soluble ingredients while the water phase was prepared by including water-soluble ingredients. Both phases were combined until a homogenous cream was acquired. It was evaluated using physiochemical parameters and sun protection factor which result in good consistency, spreadability, pH, ease of removal, and no evidence of phase separation (Shah & Mewada, 2023). Another study examined the positive impact of *T. cordifolia* topical cream using rat studies on healing of burns and excisional wounds (Girish & Kamdod, 2012).

Combination of dosage forms

This invention relates to the novel "Artemisinin Combination Therapy" which combines artemisinin, capsaicin, berberine, and *T. cordifolia* for prophylaxis of Zika virus. This combination can be prepared as tablets, capsules, oral suspension, sublingual or transdermal patch by mixing the required number of herbs with binding

or delivery components such as stearic acid, microcrystalline cellulose, calcium carbonate, and silicon dioxide (Rosen, 2017).

Nano particles

A study was conducted to create a novel formulation of aqueous nanoparticles with an average particle size of 182.9 ± 3.8 nm to enhance the distribution and bioavailability of active ingredients. *T. cordifolia* stems were pulverized, mixed with ethanol, filtered and dried to obtain a dry extract which was then combined with ultra-purified water to get an aqueous suspension and sonicated to acquire the nano-particulate solution. In-vivo results proved that the skin of the New Zealand rabbits was not irritated and confirmed the potential applications of biomedicine, and anti-ageing formulations (González-Masís et al., 2020).

Nano-emulsions are used as oral, ocular, topical, and intravenous drug delivery systems. Rheology of nano-emulsions facilitates and delivers aqueous solutions easily (Gupta et al., 2016). Another study was conducted to obtain *T. cordifolia* nano-emulsion in water in oil (W/O). It examined formulation variables, including surfactant concentration and sonication amplitude using response surface methodology. The results were useful to formulate sunflower oil-based W/O nano-emulsion which contained highly bioactive extract of *T. cordifolia* with better stability (Anjum et al., 2024). Another study used *in silico* docking approach and encapsulation to overcome the low stability and poor bioavailability of bioactive compounds in *T. cordifolia*. Synergistic effects of bioactive compounds showed the highest insulin mimicking ability in chloroform extract with an IC_{50} concentration (Jain et al., 2021).

Dosage forms -phytosomes

Phytosomes are novel dosage forms which enhance pharmacotherapeutic effect via the pharmacodynamic and pharmacokinetic parameters (Akki et al., 2019). They are nano-vesicular drug delivery systems where the phytoconstituents of herbal extracts are bound to the phosphatidylcholine of lipids (Amit et al., 2013). According to the studies, *T. cordifolia* stem powder was subjected to cold maceration while the marc was further exposed to soxhlation, and both filtrates were combined under reduced pressure. The

extract was dried and stored in sealed containers. Thin layer hydration method was used to formulate phytosomes in which different molar ratio of drug dissolved in methanol, 30% soya lecithin and cholesterol (Anwar & Farhana, 2018; Hooresfand, Ghanbarzadeh, & Hamishehkar, 2015). It was evaporated and vacuumed to obtain a thin layer, which was then hydrated with a mixture of water and ethanol (1:1). The sonication was done once the phytosomal suspension was formed. This study concluded that the alkaloid loaded phytosomes derived from *T. cordifolia* stem exhibit better physical properties and more potency than crude alkaloid fraction (Thakur & Patil, 2021).

The solvent evaporation technique was used to extract the phytosomes from *T. cordifolia* in another invention. The presence of 'epiyangambin' in chloroform extract-loaded phytosomes proved significant therapeutic outcomes including anti-inflammatory, anti-hyperglycemic, and analgesic activity than the extract alone. These findings were obtained from surface morphology, entrapment efficacy, in vitro drug release ability, and targeted protein docking (Kumar, Deivasigamani, & Roy, 2023). In another study, phytoniosome loaded ethanolic leaf extract of *T. cordifolia* was (pELET) prepared, and antioxidant potential was compared with the ethanolic leaf extract of *T. cordifolia* (ELET). Results showed excellent antioxidant properties and a non-toxic nature in pELET compared to ELET, in which pELET acts as an effective drug to scavenge the free radicals (Masilamani, Chokkalingam, & Hari, 2023).

Micropropagation

Numerous accounts of therapeutic applications of *T. cordifolia* drew interest in the commercial exploitation of the plant to supply the expanding pharmaceutical industry. According to the literature, the estimated annual demand for *T. cordifolia* consumed in the Ayurvedic system for the preparation of crude herbal drugs was 10,000 tons in 2004. With time, the demand must have expanded due to the popularity of plant species in various therapeutic applications (Dharmapal et al., 2017b; Mangal et al., 2012).

For that matter, natural *T. cordifolia* stands are currently rapidly vanishing and are under threat from overexploitation and indiscriminate collection. Use of conventional

methods is not reliable and efficient to improve the propagation of *T. cordifolia*. The goal of fast propagation cannot be achieved since *T. cordifolia* has poor status of seed set and poor germination in natural habitat. Though cutting stems are helpful for propagation, it depends on climatic conditions and is highly susceptible to infections (Mridula et al., 2017; Sharma and Vashistha, 2014).

Alternatively, in vitro propagation methods from direct and callus mediated shoot induction can acquire plantlets in large amounts with true to the type and healthy plantlets in a short duration of time. Moreover, the standardization and genetic modifications of callus and cell culture are significant approaches for secondary metabolite production while it alleviates the pressure on the wild plant population (Mridula et al., 2017).

The attempts taken to micropropagate T. cordifolia

The accomplishment of in-vitro regeneration depends on various aspects like the explant type, plant growth regulators, composition of the medium, culture environment, and other additives added into the medium (Mangal et al., 2012). By paying attention to the explant type, plant growth regulators and composition of medium, the attempts taken to micropropagate *T. cordifolia* through direct and indirect organogenesis are summarized as in table 1 and 2.

Table 1: Direct Organogenesis of *T. cordifolia*

Explant	Media + Plant growth regulators (mg/L)			Callus	Reference
	Organogenesis	Elongation	Rooting		
ns	MS+BA (0.5-2.0) MS+Kn (0.5-2.0) MS+2-iP (0.5-2.0) MS+TDZ (0.5-2.0) MS+BA (1.0) + Kn (0.5-1.0) or 2-iP (0.5-1.0) or TDZ (0.5-1.0)	-	MS+IAA (0.25-1.00) or IBA (0.25-1.00) ½MS+IAA (0.25-1.00) or IBA (0.25-1.00) ¼MS+IAA (0.25-1.00) or IBA (0.25-1.00)	+	(Mittal & Sharma, 2017)
ns	MS+BA (0.5-3.0) and/or Kn (0.5-5.0) and/or TDZ (0.05-0.4)	-	½MS+IBA (0.1-3.0)	+	(Sultana and Handique, 2013)
ns, inter ns, st	MS+BA (0.001-0.003) MS+Kn (0.0001-0.0002) MS+2-ip (0.0004-0.003)	MS+BA (0.001-0.003) MS+Kn (0.0001-0.0002) MS+2-ip (0.0004-0.003)	½MS+IAA (0.0003-0.003) ½MS+IBA (0.0003-0.003) ½MS+NAA (0.0002-0.003)	-	(Sivakumar et al., 2014)

	MS+BA (0.001-0.003) + NAA (0.0008-0.002) MS+BA (0.001-0.003) + IAA (0.0002-0.002)	MS+BA (0.0005-0.002) + Kn (0.0001-0.0002)			
ns, inter ns, leaf	MS+ NAA (1.0) + BA (0.5-3) MS+BA (1) + NAA (0.5-3) MS+Kn (0.5-3) MS	MS+NAA (1.0) + BA (2.0-2.5) MS+Kn (0.5-3.0) MS	MS+BA (1.0) + NAA (0.5-3.0) MS+BA (0.5) + NAA (1.0)	+	(Singh et al., 2021)
ns	MS+Kn (0.0-0.0004) MS+BA (0.0005) + Kn (0.0-0.0004)	-	-	-	(Bhalerao, Vishwakarma, & Maheshwari, 2013)
st, ns inter ns, leaf, petiole	MS+IAA (0.5) + BA (5.0) MS+IBA (0.5) + Z (5.0) MS+ NAA (0.5) + Kn (5.0) mMS+BA (5.0) + Kn (0.5-1.0 or AD (0.5-1.0)	-	mMS+IAA (0.2-0.8) or IBA (0.2-0.8) or NAA (0.2-0.8)	+	(Khanapurkar et al., 2012)

ns	MS+Kn (0.5-4.0) or BA (1.0-5.0) or Kn (1.0-8.0) + BA (0.5-4.0)	-	MS+NAA (1.0-6.0) or IAA (1.0-5.0) or IBA (1.0-5.0) or NAA (2.5) + BA (1.0)	+	(Sinha & Sharma, 2015)

(ns = Nodal segment, st = Shoot tip, MS = MS media, ½MS = half strength MS media, BA = 6-Benzylaminopurine, 2-iP= 2-Isopentenyladenine, NAA = 1-Naphthaleneacetic Acid, Kn = Kinetin, TDZ = Thidiazuron, IAA = Indole-3-Acetic Acid, IBA= Indole-3- Butyric Acid, GA3 = Gibberellic Acid, 2,4-D = 2,4-Dichlorophenoxyacetic acid, AD = Adenine, Z = Zeatin)

Table 2: Indirect Organogenesis of *T. cordifolia*

Explant	**Media + Plant growth regulators (mg/L)**				**Reference**
	Callus	**Organogenesis**	**Elongation**	**Rooting**	
c, yl, ml	MS+IAA (0.1-4.0) or 2,4-D (0.1-4.0) or NAA (0.1-4.0) or IBA (0.1-4.0) + AA (100)	mMS+BA (2.0) + Kn (1.0) + AA (100) + IAA (0.1-1.0) + NAA (0.1-1.0)	MS+GA3 (0.01-3.00)	½MS+ IBA (0.1-4.0) + NAA (0.1-1.0)	(Mridula et al., 2017)
yl, ns	MS+IBA (0.5-5.0) and/or IAA (0.5-5.0) and/or NAA (0.5-5.0) and/or 2,4-D (0.5-5.0) and/or BA (0.5-5.0) and/or Kn (0.5-	-	-	-	(Sharma et al., 2016)

	5.0) and/or TDZ (0.5-5.0) and/or 2-iP (0.5-5.0)				
mature ns	MS+IBA (0.5-8.0) MS+IAA (0.5-8.0) MS+NAA (0.5-8.0) MS+2,4-D (0.5-8.0)	MS+BA (1.0) + Kn (0.5-2.0) + 2-iP (0.5-2.0) + TDZ (0.5-2.0)	-	-	(Mittal & Sharma, 2017)
leaf	MS+2,4-D (0.0-0.004) MS+Kn (0.0-0.0004) MS+2,4-D (0.0004) + Kn (0.0-0.0004)	-	-	+	(Bhalerao, Vishwakarma, & Maheshwari, 2013)
st	MS+IAA (0.5-4.0) MS+NAA (0.5-4.0) MS+2,4-D (0.5-4.0) WPM+NAA (0.5-4.0)	WPM+BA (0.5-4.0) WPM+Kn (0.5-4.0)	-	-	(Sharma and Vashistha, 2014)

	WPM+2,4-D (0.5-4.0)				
st, ns inter ns, leaf, petiole	MS+2,4-D (3.0) + Kn (0.5) B5+2,4-D (3.0-7.0) + coconut water Nit+2,4,5-T (2.0-7.0)	-	-	-	(Khanapurkar et al., 2012)

(ns = Nodal segment, st = Shoot tip, C = Cotyledon, yl =Young leaf, ml = Mature leaf, MS = MS media, ½MS = half strength MS media, mMS = modified MS media, B5 = B5 medium, Nit = Nitsch and Nitsch medium, BA = 6-Benzylaminopurine, NAA = 1-Naphthaleneacetic Acid , Kn = Kinetin, IAA = Indole-3-Acetic Acid, IBA= Indole-3- Butyric Acid, GA3 = Gibberellic Acid, 2,4-D = 2,4-Dichlorophenoxyacetic acid, 2,4,5-T = 2,4,5-Trichlorophenoxyacetic acid, WPM= Woody Plant Medium)

Though there are numerous studies that have been done on micropropagation of *T. cordifolia*, there are few repeatedly noticeable problems associated with the micropropagation of *T. cordifolia*, including browning of the medium and the explant, endogenous and exogenous microbial contamination and slow growth of explants (Mangal et al., 2012). These issues can be addressed by modifying endogenous and exogenous factors associated with the culture. Other than the above studies, the attempts taken to micropropagate on closely related *Tinospora* species can be used to modify already established micropropagation procedures (Dharmapal et al., 2017a; Dharmapal et al., 2017b).

4. Conclusion

T. cordifolia is a medicinally important plant in which the plant extract can develop to formulate various pharmaceutical dosage forms based on its bioactive compounds to enhance the therapeutic outcome. To fulfill the increasing demand for *T. cordifolia* in

pharmaceutical industry, use of well-adapted micropropagation methods is economically advantageous due to the ability to produce plants with consistent quality and its potential for scaling up and extracting target chemicals in a sustainable manner. Therefore, the integration between pharmaceutical dosage form development and micropropagation ensures that the medicinal properties of *T. cordifolia* can be harnessed effectively and consistently in drug formulations, benefiting both patients and the pharmaceutical industry. Therefore, further research and development into this integration should be considered.

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EXPLAINABLE AI FOR EARLY TYPE 2 DIABETES PREDICTION USING CLINICAL AND METABOLIC BIOMARKERS: A HUMAN - CENTERED REVIEW

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Abstract

Type 2 diabetes mellitus (T2DM) remains a major global health challenge due to its rising prevalence, silent progression and long-term complications. Artificial intelligence (AI) and machine learning (ML) have shown considerable potential in improving T2DM risk prediction by analysing complex relationships within clinical and metabolic data. However, many high-performing models operate as black boxes, limiting transparency, trust, and adoption in healthcare settings. This review examines current evidence on explainable artificial intelligence (XAI) approaches for early T2DM prediction using clinical and metabolic biomarkers through a human-centred perspective. A narrative review of the literature was conducted using relevant studies retrieved from major scientific databases, focusing on explainable artificial intelligence approaches, clinical and metabolic biomarkers and their applications in early type 2 diabetes prediction. The findings indicate that integrating metabolic biomarkers with routine clinical indicators improves predictive performance compared with clinical features alone. Explainability techniques such as Shapley Additive Explanations (SHAP) and Local Interpretable Model-Agnostic Explanations (LIME) enhance transparency and support clinical interpretation of model outputs. Despite these advances, limited stakeholder involvement, insufficient external validation, and concerns regarding fairness and usability remain significant challenges. Future T2DM prediction systems should balance predictive accuracy with interpretability, trust and real-world clinical applicability to support effective and equitable healthcare decision-making.

Keywords: Type 2 diabetes mellitus, explainable artificial intelligence, machine learning, clinical biomarkers, human-centred design

1. Introduction

Type 2 diabetes mellitus has become one of the most serious chronic diseases worldwide. The condition develops when the body becomes resistant to insulin and pancreatic β-cells lose function (Kazafeos, 2011; Ye et al., 2023). The International Diabetes Federation estimated that the prevalence of diabetes which was 10.5% in 2021, The International Diabetes Federation (IDF) estimated that 589 million adults aged 20–79 years were living with diabetes worldwide in 2024, representing 11.1% of this population. This figure is projected to increase to approximately 853 million (13.0%) by 2050 (Genitsaridi et al., 2026). The rise is linked to urbanization and lifestyle changes in developing countries. Long-term complications damage the kidneys, eyes and nerves. Early T2DM carries serious long-term consequences. In addition, it was observed that T2DM patients A large pooled analysis of 1.5 million participants from 19 high-income countries found that earlier diagnosis of T2DM was associated with increased mortality risk and reduced life expectancy. Compared with individuals without diabetes, those diagnosed at ages 30, 40 and 50 years had estimated reductions in life expectancy of approximately 14, 10 and 6 years, respectively, based on US mortality rates (Kaptoge et al., 2023). The burden falls hardest on low-income regions (He et al., 2024; Ye et al., 2023) . For many families, lifelong treatment leads to severe financial strain (Adua et al., 2021) . T2DM also progresses quietly. Many adults live with the disease for years before any symptoms appear (Teufel et al., 2026) . By the time of diagnosis, irreversible complications may already be present. Traditional screening tools rely on regression-based scoring methods. These often miss how risk factors interact with each other (Kopitar et al., 2020; Kotwas et al., 2021) . The slow development of the disease, however, creates an opportunity. Clinical trials show that lifestyle interventions during the prediabetic stage can halve the risk of progression (Tuomilehto et al., 2011; Wareham, 2022) . Early prediction is therefore central to prevention (Adua et al., 2021).

Machine learning has begun to change how clinical risk is assessed. The growth of electronic health records (EHR) provides large datasets for predictive modelling (Kopitar et al., 2020) . ML algorithms detect complex relationships in medical data that

older statistical methods cannot identify (Adua et al., 2021; Dutta et al., 2022) . They outperform conventional approaches in accuracy and scalability. (Fazakis et al., 2021) A major barrier remains, however. Many high-performing models behave as black boxes. Clinicians cannot see how a prediction is made. This limits trust and slows adoption in clinical settings (Kalusivalingam et al., n.d.; Rafie et al., 2025). An unexplained output is rarely acceptable in healthcare. A physician needs to understand the reasoning before acting on a model's result. Explainable AI has emerged as a response to this problem. XAI methods make predictions transparent and understandable to the people who use them. Techniques such as SHAP and LIME show which features drive a given prediction. In diabetes prediction, this lets clinicians check whether the model's reasoning matches clinical knowledge. XAI is now seen as a bridge between predictive accuracy and clinical usability (Ahmed et al., 2025; Rafie et al., 2025).

The biomarkers used in these models are equally important. An important distinction should be made between diagnostic models that identify existing T2DM and prognostic models that predict future disease development. HbA1c and fasting blood glucose (FBG) are established diagnostic criteria for T2DM (Bajaj et al., 2026) .Therefore, when these measurements are used as input features to classify diabetes defined by the same diagnostic thresholds, the model may identify existing disease rather than predict future risk. This can introduce target leakage and overestimate the model's ability to predict incident T2DM (Kaufman et al., 2012). In contrast, prognostic models should include individuals without diabetes at baseline and predict newly developed T2DM over a clearly defined follow-up period. Consequently, the distinction between prevalent-disease classification and incident-disease prediction is essential when interpreting the clinical relevance of AI-based T2DM models (Collins et al., 2015). Recent work shows that metabolic biomarkers like lipid profiles and insulin resistance markers can improve prediction beyond clinical variables alone (Ahmad, 2015; Bigagli & Lodovici, 2019; Schwartz et al., 2017; Wang-Sattler et al., 2012) . These markers also capture metabolic changes that appear before glucose abnormalities develop.

Despite these advances, AI in healthcare remains largely technology-centred. Few systems are designed around the real needs of clinicians and patients (Andersen et al.,

2023) . Human-Centred AI offers a different approach. It places user values and clinical workflows at the centre of system design. Current XAI methods are mostly developed by AI researchers and are rarely evaluated through a human-centred lens. Few reviews bring explainable AI, clinical and metabolic biomarkers, and human-centred design together in one framework for early T2DM prediction (Hulsen, 2023) .

This review addresses that gap by bringing together explainable AI, biomarker-based prediction, and human-centred design. The aim is to evaluate how a human-centred approach can shape early T2DM predictive systems that clinicians and patients can genuinely trust. To achieve this, the review critiques the performance and explainability of current AI and XAI methods applied to T2DM prediction. It also analyses the role of clinical and metabolic biomarkers within these predictive models. The discussion then examines how human-centred design principles support clinical trust and usability in AI-driven diabetes systems. Finally, the review identifies current research gaps and proposes future directions for human-centred diabetes prediction.

2. Methodology

This review brings together current research on explainable AI for the early prediction of Type 2 diabetes mellitus using clinical and metabolic biomarkers. The literature was examined from a human-centred perspective with attention given to both predictive performance and practical usability in healthcare.

A structured literature search was conducted using PubMed, Scopus, IEEE Xplore and Google Scholar. The review considered publications from 2001 to 2026, with the final search date recorded as 28 October 2026. Search terms included combinations of keywords related to T2DM, artificial intelligence, machine learning, clinical biomarkers, metabolic biomarkers, explainable AI and clinical decision support. The search was restricted to English-language publications. Primary research Primary research studies investigating AI-based T2DM prediction were considered the main evidence for the review. Studies were selected based on their relevance to T2DM prediction, the use of clinical or metabolic biomarkers, the application of AI or machine-learning methods, and the availability of information on model interpretability or

explainability. Secondary reviews were used to provide background information and identify relevant research but were not treated as additional primary evidence. Other publications addressing biomarker biology, clinical diagnosis, AI methodology, ethics and regulatory considerations were used as supporting references rather than included prediction studies..

Studies were screened using four main requirements. Information extracted from relevant primary studies included the study population, dataset, sample size, outcome definition, prediction horizon, biomarkers, prediction algorithms, performance measures, validation approach and explainability methods. Where reported, calibration results and human evaluation of explanations were also considered. The findings were organised into thematic sections covering biomarkers, prediction models, explainability methods, clinical applicability and ethical considerations.

As this was a structured narrative review rather than a systematic review, no formal systematic-review protocol or quantitative meta-analysis was undertaken. The findings were interpreted in consideration of differences in study populations, outcome definitions, prediction methods and validation approaches.

3. Clinical and metabolic biomarkers in type 2 diabetes prediction

Biomarkers serve as the measurable biological inputs that ground any AI-based T2DM prediction model (Kavakiotis et al., 2017). Two categories dominate the reviewed literature. The first is clinical biomarkers which are routinely collected in primary care and widely accessible (Mohsin et al., 2023; Perveen et al., 2019; Zhang et al., 2020) . The second is metabolic biomarkers which capture biochemical alterations that precede clinical diagnosis by several years (Guasch-Ferré et al., 2016). Routine clinical features offer broad accessibility and standardization. However, integrating metabolic and multi-omics data alongside clinical variables consistently improves model performance (Mohsen et al., 2023) . This indicates that feature quality and diversity are as critical as algorithmic choice in determining predictive accuracy (Fregoso-Aparicio et al., 2021; Zou et al., 2018) .

Routine Clinical Biomarkers in AI-Based Early Prediction

Clinical biomarkers are the most commonly used input features in AI-based T2DM prediction models (Kavakiotis et al., 2017; Mohsen et al., 2023). They are routinely measured in primary care, widely standardized across health systems, and accessible at low cost (Kavakiotis et al., 2017). A 2023 scoping review analysed 40 longitudinal studies on ML-based T2DM prediction. It confirmed that glycaemic traits, anthropometric measures, and blood lipids derived from electronic health records were the most frequently reported predictors across these models (Mohsen et al., 2023).

3.1.1 HbA1c:

Glycated haemoglobin (HbA1c) reflects average blood glucose exposure over approximately the preceding two to three months, with greater influence from more recent glucose levels. The American Diabetes Association (ADA) recognises HbA1c ≥6.5% (48 mmol/mol), measured using a standardised laboratory method, as one of several diagnostic criteria for diabetes. Although HbA1c is widely used for diabetes diagnosis and glycaemic monitoring, it is not universally considered the gold-standard diagnostic test. Compared with the 2-hour oral glucose tolerance test (OGTT), HbA1c has lower sensitivity at the recommended diagnostic threshold, meaning that some individuals with diabetes or dysglycaemia may not be identified using HbA1c alone (Bajaj et al., 2026).

Furthermore, HbA1c measurements can be influenced by conditions affecting red blood cell lifespan and haemoglobin composition. Iron-deficiency anaemia may falsely increase HbA1c levels, whereas haemolysis, blood loss and other conditions associated with shortened red blood cell survival may reduce HbA1c independently of actual glycaemia (English et al., 2015) . Its reliability may also be affected in chronic kidney disease, particularly in advanced disease and patients receiving dialysis or erythropoietin treatment. During pregnancy, changes in red blood cell turnover and iron status can also affect HbA1c interpretation. In addition, certain haemoglobin variants may interfere with specific HbA1c assay methods. Differences in HbA1c levels independent of measured blood glucose have also been reported across ethnic populations, highlighting the importance of considering individual and population-

related variation when interpreting results (Hare et al., 2013). Therefore, HbA1c should be interpreted alongside other clinical and metabolic indicators when used in AI-based T2DM prediction models.

3.1.2 Fasting blood glucose (FBG)

FBG provides a direct measure of current glycaemic status, with a diagnostic threshold *of ≥126 mg/dL (7.0 mmol/L) (Elsayed et al., 2023). It is routinely available across all healthcare settings and carries low collection cost (Kavakiotis et al., 2017). Its main limitation is that it captures a single time-point. Day-to-day physiological variability can therefore obscure the true glycaemic state in single-sample prediction models (Mohsen et al., 2023).*

3.1.3 BMI and waist circumference

Body mass index is a widely used measure of general adiposity and is commonly incorporated into T2DM risk prediction models (Kavakiotis et al., 2017) Waist circumference provides an additional measure of central adiposity and is associated with visceral fat accumulation and metabolic risk. Although central adiposity is strongly associated with T2DM development, evidence does not consistently demonstrate that waist circumference predicts T2DM better than BMI. A meta-analysis by Sleddens et al. 2012, which included 32 studies, reported similar associations between incident diabetes and BMI, waist circumference and waist-to-hip ratio, with pooled relative risks of 1.87, 1.87 and 1.88, respectively, per standard deviation increase (Sleddens et al., 2012). More recent evidence from a dose-response meta-analysis of 216 cohort studies also demonstrated positive associations between both BMI and waist circumference and T2DM risk (Jayedi et al., 2022). Both measures are associated with insulin resistance through mechanisms involving adipokine dysregulation, increased free fatty acid release and lipotoxicity (Grundy et al., 2005). However, BMI cannot distinguish fat mass from lean body mass while waist circumference may be influenced by differences in measurement methods and body-fat distribution. Furthermore, BMI thresholds associated with metabolic risk vary across ethnic populations. The WHO Expert Consultation (2004) reported that Asian populations may experience increased metabolic risk at lower BMI levels than European populations and identified additional public health action points at 23.0 kg/m² and 27.5 kg/m² (Nishida et al., 2004).

Therefore, both BMI and waist circumference should be considered alongside population-specific characteristics when selecting predictors for AI-based T2DM risk models (Jayedi et al., 2022; Sleddens et al., 2012) .

3.1.4 Blood pressure

Hypertension is a common comorbidity of T2DM and a component of the metabolic syndrome cluster (Grundy et al., 2005). Blood pressure and the use of antihypertensive medication are relevant indicators in diabetes risk assessment. However, the Finnish Diabetes Risk Score (FINDRISC) does not include directly measured blood pressure; instead, it considers a history of antihypertensive medication use as one of its risk variables (Lindström & Tuomilehto, 2003).

Clinical biomarkers offer accessibility, standardisation, and familiarity in clinical practice (Kavakiotis et al., 2017) . However, their predictive value depends on the type and timing of measurement. Anthropometric and clinical indicators, including BMI, waist circumference and hypertension, as well as impaired fasting glucose, can help identify individuals at increased risk of developing T2DM several years before diagnosis. This is demonstrated by risk prediction approaches such as FINDRISC and the Framingham Offspring Study (Lindström & Tuomilehto, 2003; Wilson et al., 2007). In contrast, HbA1c and FBG values at diagnostic thresholds may primarily indicate the presence of existing diabetes rather than predict future disease development. This limitation provides the direct rationale for incorporating metabolic biomarkers, which is the focus of Section 3.2..

3.2 Metabolic Biomarkers and Metabolomic Signatures

The Metabolic biomarkers are biochemical molecules that reflect the underlying pathophysiology of T2DM. They are sensitive to early dysregulation. They often change years before clinical glucose abnormalities appear. They therefore provide predictive value and insight into metabolic pathways associated with disease development (Guasch-Ferré et al., 2016; Wang et al., 2011).

3.2.1 Lipid profile markers

Lipid profile markers include high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides. HDL is typically lower in people with T2DM and is These are routine clinical laboratory measurements classified as extended clinical and biochemical biomarkers in this review. HDL cholesterol is generally lower in individuals with T2DM, while elevated triglyceride levels are commonly associated with insulin resistance and metabolic dysfunction (Grundy et al., 2005). The triglyceride-to-HDL cholesterol (TG/HDL-C) ratio has also been investigated as a surrogate marker of insulin resistance. However, its predictive performance varies across ethnic populations. For example, its association with insulin resistance was observed in White European and South Asian men but was weaker in South Asian women. Therefore, population-specific differences should be considered when interpreting this ratio (Baneu et al., 2024; Mostafa et al., 2012). In contrast, the relationship between LDL cholesterol and T2DM risk is more complex. Genetic evidence and randomised trial findings suggest that reduced LDL cholesterol through inhibition of the HMG-CoA reductase pathway may be associated with a modestly increased risk of T2DM. Therefore, LDL cholesterol should not be regarded as a consistent positive predictor of diabetes development (Swerdlow et al., 2015). These lipid parameters are routinely incorporated as features in AI-based prediction models (Kavakiotis et al., 2017; Mohsen et al., 2023) .

3.2.2 Branched-chain amino acids (BCAAs)

BCAAs include leucine, isoleucine and valine. Elevated plasma BCAA levels are associated with insulin resistance and future T2DM. In the Framingham cohort, In a nested case-control study within the Framingham Offspring Study, Wang et al. (2011) investigated 189 individuals who developed diabetes during a 12-year follow-up period and 189 matched controls. Each standard-deviation increase in the individual amino acid concentrations was associated with a 57–102% increase in the odds of developing diabetes. The five amino acids were isoleucine, leucine, valine, tyrosine and phenylalanine. Furthermore, a combination of three amino acids (isoleucine, tyrosine and phenylalanine) was associated with a more than fivefold higher risk among individuals in the highest quartile compared with those in the lowest quartile. These

findings were also supported by replication analyses in the Malmö Diet and Cancer Study (Wang et al., 2011). Potential mechanisms linking elevated BCAA levels with insulin resistance include altered nutrient signalling, mammalian target of rapamycin (mTOR) pathway activation and changes in mitochondrial fatty-acid metabolism. Experimental findings from Newgard et al., 2009 demonstrated that BCAA supplementation in high-fat-fed rats was associated with increased mTOR signalling, accumulation of acylcarnitines and insulin resistance. Therefore, BCAA and aromatic amino acid profiles represent promising metabolomic markers for investigating early metabolic changes associated with T2DM development. BCAAs are now a well-established class of metabolomic predictors(Guasch-Ferré et al., 2016; Newgard et al., 2009).

3.2.3 Inflammatory biomarkers

C-reactive protein (CRP) and interleukin-6 (IL-6) are inflammatory biomarkers associated with chronic low-grade inflammation, which is implicated in the development of insulin resistance. In a prospective nested case-control study within the Women's Health Study, Pradhan et al. (2001) investigated 27,628 initially healthy women over a four-year follow-up period. Among the participants, 188 women developed T2DM and were compared with 362 disease-free controls. Women in the highest CRP quartile had a relative risk of 15.7 (95% CI: 6.5–37.9) compared with those in the lowest quartile before multivariable adjustment. After adjustment for BMI, family history of diabetes, smoking, physical activity, alcohol consumption and hormone replacement therapy, the relative risk was attenuated to 4.2 (95% CI: 1.5–12.0). These findings suggest that CRP is associated with future T2DM risk, although the association is substantially reduced after accounting for BMI and other risk factors. These markers may also complement glycaemic and clinical features in combined risk prediction models (Garcia-Carretero et al., 2021; Pradhan et al., 2001).

3.2.4 Metabolomics-derived signatures

Targeted metabolomics has identified several molecules with potential predictive value for T2DM. Lower glycine levels have been associated with an increased risk of T2DM and impaired glucose tolerance. Lysophosphatidylcholines (LPC), particularly LPC 18:2, have also shown altered concentrations before disease diagnosis. Acylcarnitines,

including acetylcarnitine, are associated with changes in mitochondrial fatty-acid metabolism (Wang-Sattler et al., 2012). A systematic review and meta-analysis reported associations between several metabolite classes, including amino acids, phospholipids and acylcarnitines and future T2DM risk across prospective cohort studies (Guasch-Ferré et al., 2016; Morze et al., 2022) .

Metabolomics-derived biomarkers may provide opportunities for earlier risk identification and mechanistic insight. However, their clinical application is limited by the cost of analytical platforms, the need for specialised mass spectrometry equipment and limited availability in routine primary care (Mohsen et al., 2023). Additional challenges include the lack of standardisation across analytical platforms, differences in sample preparation and data-processing procedures, and batch effects that can introduce technical variation into metabolite measurements. These factors may reduce the reproducibility of metabolomic signatures across studies and populations, limiting their clinical translation and the generalisability of AI models developed using these biomarkers. Therefore, standardised analytical protocols, appropriate quality-control procedures and independent cross-cohort validation are essential before metabolomics-derived signatures can be reliably incorporated into clinical prediction models (Broadhurst et al., 2018).

3.3 Integrating Clinical and Metabolic Biomarkers

Clinical and metabolic markers can provide complementary clinical features reflect established metabolic and physiological risk factors, whereas metabolomic profiles may identify additional metabolic alterations preceding disease onset (Guasch-Ferré et al., 2016). Combined panels therefore cover a wider spectrum of disease progression (Peddinti et al., 2017). Empirical evidence supports this integration. For example, Peddinti et al. (2017) investigated 543 initially non-diabetic participants from the Finnish Botnia Prospective Study, of whom 146 developed T2DM during a 10-year follow-up period. Their initial clinical reference model included sex, age, BMI, fasting insulin and family history of diabetes, but did not include fasting glucose. This model achieved an AUC of 0.68. The addition of fasting glucose increased the AUC to 0.70, while a more comprehensive clinical model incorporating additional variables,

including lipid measurements, blood pressure and waist circumference, achieved an AUC of 0.71. When selected metabolomic markers were combined with clinical risk factors, the AUC increased to 0.78, compared with 0.68 for the initial clinical reference model. Importantly, the study also evaluated the selected markers in an independent French DESIR cohort of 1,044 participants, including 231 individuals who developed T2DM during nine years of follow-up. In this external validation cohort, the AUC increased from 0.76 for the clinical-only model to 0.84 for the combined model (Peddinti et al., 2017).

AI and ML play a central role in this integration. Traditional regression models can accommodate non-linear relationships and interactions through approaches such as interaction terms, spline functions and penalisation methods. However, these relationships generally need to be specified during model development. In contrast, machine-learning approaches can identify complex patterns and potential interactions through more flexible data-driven modelling techniques, potentially reducing the need to specify every interaction in advance (Christodoulou et al., 2019). Feature-selection methods can also identify informative combinations of biomarkers for inclusion in prediction models (Kavakiotis et al., 2017; Peddinti et al., 2017).

The identification of informative biomarker combinations may improve the discrimination between individuals at different levels of future T2DM risk, as demonstrated The trade-off is added model complexity, which raises questions of interpretability addressed in Section V. Integration also introduces practical challenges. Data are heterogeneous in scale and may contain missing values. Metabolomic data are high-dimensional. Models that perform well in research datasets do not always translate to clinical practice (Kavakiotis et al., 2017; Mohsen et al., 2023). To provide a structured overview, the distinct clinical and mechanistic roles of these biomarker categories, along with their synthesis within AI prediction pipelines, are systematically compared in Table I.

Table 1: Comparison of Clinical and Metabolic Biomarkers Commonly Used in AI-Based Prediction of Type 2 Diabetes Mellitus (T2DM)

Source: Author compilation

Category	Biomarker	Clinical / Mechanistic Role	Use in AI Models	Reference
Clinical	HbA1c	Reflects average glycaemic control over 2–3 months	Common input feature in diabetes prediction models	(Kavakiotis et al., 2017; Mohsen et al., 2023)
Clinical	Fasting Blood Glucose	Direct measure of current glycaemic status	Routinely available and cost-effective predictor	(Grundy et al., 2005; Kavakiotis et al., 2017)
Clinical	BMI / Waist Circumference	Indicators of adiposity and central obesity	Frequently used in clinical risk-score and ML models	(Elsayed et al., 2023; Grundy et al., 2005)
Clinical	Blood Pressure	Associated with metabolic syndrome and cardiovascular risk	Included in established diabetes risk scores	(Grundy et al., 2005; Lindström & Tuomile

				hto, 2003)
Metabolic	Lipid Profile (HDL, LDL, TG)	Dyslipidaemia associated with insulin resistance	Common ML feature complementing glycaemic markers	(Guasch-Ferré et al., 2016)
Metabolic	Branched-Chain Amino Acids	Associated with insulin resistance and future T2DM risk	Important predictors in metabolomics-based models	(Pradhan et al., 2001; Wang et al., 2011)
Metabolic	Inflammatory markers (CRP, IL-6)	Reflect chronic low-grade inflammation linked to insulin resistance	Improve predictive performance in combined biomarker panels	(Pradhan et al., 2001)
Metabolic	Lipid metabolites (LPCs, acylcarnitines, glycine)	Reflect altered lipid metabolism and mitochondrial function	Used in high-dimensional metabolomic ML models	(Pradhan et al., 2001; Wang-Sattler et al., 2012b)

AI and machine learning approaches for T2DM prediction

The application of AI and ML to T2DM prediction has increased substantially over the past decade, reflecting growing interdisciplinary interest and advances in computational methods (Fregoso-Aparicio et al., 2021; Mohsen et al., 2023). This

section reviews the model families used in the reviewed studies and the metrics applied to evaluate them.

4.1 Overview of Models Used in the Reviewed Studies

The reviewed lite Chen & Guestrin, 2016 and Gradient Boosting, are frequently applied to tabular clinical data (Chen & Guestrin, 2016). Kernel-based methods, particularly Support Vector Machines (SVMs), are also used in T2DM prediction studies (Fregoso-Aparicio et al., 2021; Mohsen et al., 2023). Deep-learning approaches include artificial neural networks (ANNs) and architectures such as TabNet, which was specifically developed for tabular data (Arık & Pfister, 2021).

A systematic review of 90 studies found that tree-based algorithms showed the best overall predictive performance , whereas deep neural networks were comparatively suboptimal (Fregoso-Aparicio et al., 2021). However, the performance of machine-learning models should be interpreted in relation to the validation methods used. In a broader systematic review of 71 clinical prediction studies across different medical fields, Christodoulou et al. (2019) found no performance advantage of machine learning over logistic regression in 145 comparisons at low risk of bias. In contrast, machine learning appeared to perform better in 137 comparisons at high risk of bias (difference in logit[AUC]: 0.34, 95% CI: 0.20–0.47). These findings highlight the importance of rigorous validation when comparing model performance (Christodoulou et al., 2019). The trade-off between predictive accuracy and interpretability is not always evident in T2DM prediction models (Rudin, 2019). Logistic regression provides transparent coefficients, whereas ensemble models often require post-hoc explanation methods. However, glass-box models, such as Generalised Additive Models with pairwise Interactions (GA²Ms) and Explainable Boosting Machines (EBMs), can capture non-linear relationships while maintaining interpretability. These models have demonstrated strong predictive performance on tabular datasets (Lou et al., 2013) . Therefore, model selection should consider both predictive performance and interpretability.

4.2 Performance Evaluation Metrics

Most reviewed studies evaluate models using accuracy, precision, recall, specificity, F1-score, and area under the receiver operating characteristic curve (ROC-AUC) (Fregoso-Aparicio et al., 2021; Mohsen et al., 2023). In clinical prediction, selecting appropriate evaluation metrics is important because accuracy alone may be misleading when datasets are imbalanced. Sensitivity (recall), specificity and ROC-AUC provide additional information about a model's ability to identify individuals at risk and distinguish between outcome groups (Collins et al., 2015; Steyerberg et al., 2010) . In early-screening contexts, missed cases carry a greater clinical cost than false alarms. For datasets with imbalanced outcome classes, precision–recall (PR) curves can provide additional insight into positive prediction performance, as they focus on precision and recall and are sensitive to disease prevalence. ROC curves remain useful for assessing discrimination but may provide an incomplete picture of performance in highly imbalanced datasets. Decision curve analysis can further assess clinical usefulness by estimating net benefit across different risk thresholds, considering the balance between true-positive and false-positive predictions when dealing with highly imbalanced T2DM evaluation datasets. Accuracy alone is unreliable in imbalanced clinical datasets. Non-diabetic samples often outnumber positive cases by a wide margin. A model that predicts every patient as non-diabetic can still report high accuracy (Deberneh & Kim, 2021; Saito & Rehmsmeier, 2015; Vickers & Elkin, 2006). Calibration — the agreement between predicted probabilities and observed outcomes — is equally important but rarely reported. Christodoulou et al. (2019) reported that 79% of the 71 clinical prediction studies included in their systematic review did not assess model calibration. This finding reflects clinical prediction studies across different medical fields and is not specific to T2DM prediction. The literature also shows inconsistency in which metrics are reported. Some studies report only AUC, while others omit sensitivity and specificity entirely. This inconsistency makes direct cross-study comparison difficult and motivates the adoption of standardised reporting frameworks in future work. (Christodoulou et al., 2019; Fregoso-Aparicio et al., 2021)

5. Explainable AI Techniques in Diabetes Prediction

5.1 The Importance of Explainability in Clinical AI Systems

Explainability is a foundational requirement for the safe and responsible deployment of AI in healthcare (Loh et al., 2022). A systematic review of XAI applications across healthcare settings identified a clear adoption barrier. However, limited transparency can create challenges for clinicians when evaluating and trusting AI-generated predictions. Rosenbacke et al. (2024) reported mixed findings regarding the influence of explainable AI on clinician trust, indicating that explanations do not always increase trust. Therefore, explainability should be considered alongside other factors, including clinical validation, workflow integration and accountability, when implementing AI systems in healthcare (Rosenbacke et al., 2024). This problem has four distinct dimensions. The first is clinical transparency. A clinician cannot safely act on a prediction whose reasoning is not visible. The second is trust. Both clinicians and patients are more likely to engage with an AI tool when they can see why a specific prediction was made. The third is ethical accountability. AI-driven health advice carries an obligation to provide understandable reasoning to those affected by it (Rosenbacke et al., 2024; Tjoa & Guan, 2021). The fourth is regulatory compliance. High-risk clinical AI systems are increasingly subject to transparency and information requirements under emerging regulatory frameworks. The EU AI Act (*Regulation - 2017/745 - EN - Medical Device Regulation - EUR-Lex*, n.d.) requires high-risk AI systems to provide sufficient information to enable users to interpret outputs and use them appropriately. Similarly, the EU Medical Device Regulation (*Regulation - EU - 2024/1689 - EN - EUR-Lex*, n.d.) requires manufacturers to provide relevant safety, performance and usage information. The FDA, together with Health Canada and the MHRA, also published transparency guiding principles for machine-learning-enabled medical devices in 2024. However, these frameworks primarily emphasise transparency and communication of information to users rather than requiring every AI model to be inherently interpretable (*Regulation - 2017/745 - EN - Medical Device Regulation - EUR-Lex*, n.d.; *Transparency for Machine Learning-Enabled Medical Devices: Guiding Principles | FDA*, n.d.) The tension between model accuracy and interpretability is well documented.

The tension between model accuracy and interpretability is well documented. Lundberg and Lee argued that understanding why a model makes a prediction can be as critical as the prediction itself, particularly for high-stakes decisions such as disease risk assessment (Lundberg & Lee, 2017). Rudin argued more strongly that post-hoc explanation of black-box models is an inadequate substitute for inherent interpretability, especially in healthcare contexts where errors carry direct consequences (Rudin, 2019). These perspectives together establish the clinical and scientific case for XAI in T2DM prediction pipelines

5.2 Post-Hoc and Inherently Interpretable Methods

Two families of explanation methods appear in the reviewed studies (Loh et al., 2022; Rosenbacke et al., 2024). Post-hoc methods are applied after model training to explain an already-fitted model. Inherently interpretable models expose their reasoning through their structure and do not require a separate explanation step (Rudin, 2019).

5.2.1 SHAP

Shapley Additive Explanations assigns each input feature a contribution value for a specific prediction, grounded in cooperative game theory. It satisfies three desirable mathematical properties: local accuracy, missingness, and consistency. SHAP provides both global explanations, which summarise the features influencing overall model predictions, and local explanations for individual patients. A systematic review of healthcare applications published between 2011 and 2022 identified SHAP as one of the commonly applied explainability methods (Loh et al., 2022; Lundberg & Lee, 2017). However, SHAP has important limitations when analysing clinical and metabolic biomarkers. Many biomarkers are correlated, such as fasting blood glucose (FBG) and HbA1c, or BMI and waist circumference. When feature dependence is not adequately considered, KernelSHAP may generate unrealistic combinations of biomarker values and distribute feature contributions in misleading ways. SHAP explanations may also vary depending on the choice of background dataset and whether interventional or observational feature dependence is assumed. Furthermore, SHAP explains how a trained model produces its predictions; it does not establish biological mechanisms or causal relationships between biomarkers and T2DM. For tree-based models, TreeSHAP provides an efficient approach to calculating exact SHAP values under specified

feature-dependence assumptions, reducing the computational burden associated with general model-agnostic methods (Aas et al., 2021; Lundberg & Lee, 2017).

5.2.2 LIME

Local Interpretable Model-Agnostic Explanations generates a locally faithful explanation by fitting a simple surrogate model around a single prediction. It is model-agnostic and provides intuitive, patient-specific explanations by identifying the features that contribute to a model's prediction. However, LIME has limitations related to explanation stability and reliability. Its explanations depend on random perturbation sampling and the selection of the local neighbourhood, meaning that repeated runs may produce different results. Furthermore, perturbations may generate unrealistic combinations of correlated clinical biomarkers, potentially reducing the reliability of explanations in clinical applications (Ribeiro et al., 2016; Slack et al., 2020).

5.2.3 Other methods

Partial Dependence Plots (PDPs) show the average relationship between an individual feature and the model's predicted outcome across the dataset. Individual Conditional Expectation (ICE) plots extend this approach by displaying the predicted relationship for individual observations. Permutation-based feature importance measures the reduction in model performance when a feature's values are randomly shuffled. PDPs and permutation importance provide global explanations, whereas ICE plots show instance-level feature effects. However, these methods have limitations when applied to correlated clinical biomarkers. PDPs and ICE plots may evaluate unrealistic combinations of features, while permutation importance may produce misleading estimates when correlated predictors contain overlapping information. Accumulated Local Effects (ALE) plots provide an alternative to PDPs because they estimate feature effects within the observed data distribution, reducing the extrapolation problems associated with correlated predictors (Apley & Zhu, 2020).

In addition to post-hoc methods, glass-box models such as Explainable Boosting Machines (EBMs) provide an alternative approach to model interpretability. EBMs are based on generalised additive models and use boosted decision trees to learn feature effects while maintaining an interpretable model structure. They can provide both global

and individual-level explanations without requiring a separate post-hoc explanation method (Nori et al., 2019, 2021). TabNet uses sequential attention masks to select informative features at each decision step (Arık & Pfister, 2021). Although these attention masks provide information about feature selection, they should not automatically be considered faithful explanations of the model's decision-making process, as the relationship between attention weights and model predictions remains debated (Jain & Wallace, 2019; Wiegreffe & Pinter, 2019). Decision trees and logistic regression remain examples of inherently interpretable models because their predictions can be understood directly from their structure and coefficients (Rudin, 2019) use sequential attention masks to select informative features at each decision step, providing built-in local and global explanations without a post-hoc step (Arık & Pfister, 2021). Decision trees and logistic regression remain the most commonly used inherently interpretable models because their reasoning is directly readable from their structure (Rudin, 2019).

Across the T2DM studies included in this review, SHAP was used in [n/N] studies, while LIME was used in [n/N] studies. Both methods were applied in [n/N] studies. SHAP provides theoretical properties and supports both global and individual-level explanations (Lundberg & Lee, 2017) whereas LIME generates local explanations for individual predictions (Ribeiro et al., 2016). These methods explain the behaviour of trained prediction models rather than establishing biological or causal relationships. Inherently interpretable models, including logistic regression, decision trees and EBMs, offer an alternative when transparency is required directly from the model structure (Nori et al., 2019; Rudin, 2019) . Table II summarises and compares the main methods .

Table 2: Comparison of XAI methods reported in the reviewed studies.

Source: Author compilation

XAI Method	Type	Scope	Strengths	Limitations	Reference
SHAP	Post-hoc	Global + local	Game-theoretic guarantees; consistent; model-agnostic	Computationally expensive for large models	(Lundberg & Lee, 2017)
LIME	Post-hoc	Local	Intuitive per-patient explanation; model-agnostic	Unstable across perturbation runs	(Ribeiro et al., 2016)
PDP / ICE	Post-hoc	Global / Local	Shows feature–outcome relationship visually	Assumes feature independence	(Loh et al., 2022)
Permutation importance	Post-hoc	Global	Simple; model-agnostic	Unreliable with correlated features	(Loh et al., 2022)
TabNet attention	Inherent	Local + global	Built-in feature selection masks	Tied to a single architecture	(Arık & Pfister, 2021)
Logistic regression / DT	Inherent	Global	Directly readable structure	Lower accuracy than ensemble models	(Rudin, 2019)

6. Human-Centred Perspectives in Explainable AI

6.1 Clinician-Centred Interpretability

Explainable AI must support clinical reasoning, not replace it (Loh et al., 2022). Holzinger et al. introduced the concept of causability — the degree to which an explanation enables a human expert to reach a causal understanding of a finding. They argued that clinical AI systems must be evaluated on this dimension, not only on technical accuracy. This distinction is important. A model that produces technically correct feature importance scores does not automatically produce clinically useful ones.

Explanations are only useful when they align with how clinicians already reason about disease (Holzinger et al., 2019a).

A systematic review of XAI and clinician trust found that XAI explanations increased trust in some clinicians but decreased it in others, depending on whether the explanation was consistent with clinical expectations. This bidirectional effect highlights a critical design problem. Counter-intuitive feature rankings - even when technically correct, can actively undermine the adoption of AI tools in practice. The same review found that most XAI systems evaluated in healthcare were not co-designed with practising clinicians, and very few were tested within real clinical workflows (Rosenbacke et al., 2024).

Workflow integration compounds this problem. Predictions and their explanations must arrive at the right point in a consultation, fit into existing electronic health record systems, and not add friction to routine care (Loh et al., 2022).

6.2 Patient-Centred Explainability

Patients are the second key audience for AI-based T2DM risk predictions. Most XAI methods were designed for technical users and are not suited to patient communication without substantial adaptation (Loh et al., 2022). Plain-language, personalised explanations of individual risk factors hold potential to improve health literacy and support adherence to preventive interventions such as dietary change and physical activity (Liaw et al., 2023). Patient autonomy, comprehension, and empowerment are rarely stated as explicit design goals.

This gap is well recognised in the broader healthcare AI literature. A mixed-methods study of a diabetes AI prediction tool found that clinicians and staff used the tool primarily as a documentation aid rather than as a genuine decision-support instrument. Patients were largely excluded from the explanation loop entirely (Liaw et al., 2023). Few studies test whether AI-generated explanations are understood or trusted by patients. Addressing this gap requires moving XAI design upstream — from post-hoc technical outputs to co-designed, user-tested interfaces built around patient needs (Holzinger et al., 2019a; Loh et al., 2022).

6.3 Ethical, Fairness and Regulatory Considerations

Algorithmic bias is a primary ethical risk in clinical AI. Obermeyer et al. demonstrated that a widely deployed health management algorithm exhibited significant racial bias: at the same predicted risk score, Black patients were substantially sicker than White patients. The algorithm used healthcare cost as a proxy for health need, embedding structural inequality into its predictions. Consequently, this bias resulted in Black patients making up only 17.7% of those automatically screened into the extra care programme, whereas an unbiased correction based on actual health needs would have raised this share to 46.5% (Obermeyer et al., 2019). T2DM prediction faces the same vulnerability. Risk profiles differ substantially across South Asian, African, and European populations, and models trained on non-representative datasets will systematically underperform for under-represented groups (Liaw et al., 2023).

Data privacy and regulatory accountability are equally pressing concerns. Panch et al. argued that centralised regulation of clinical AI alone is insufficient to ensure safety, efficacy, and equity at scale, and proposed that high-risk automated systems require documentation of data representativeness, bias evaluation, privacy protections, and accountability mechanisms (Panch et al., 2022). Data privacy and regulatory accountability are equally important considerations in the clinical implementation of AI argued that centralised regulation alone is insufficient to ensure the safety, efficacy, and equity of clinical AI systems, advocating a distributed regulatory approach that incorporates both central oversight and local accountability. This concern is also reflected in the findings of Mohsen et al. (2023), who reviewed 40 studies on AI-based T2DM risk prediction. Only 3/40 studies explicitly reported adherence to TRIPOD reporting guidelines, while only 5/40 conducted external validation. Furthermore, the authors highlighted that most studies did not evaluate model performance across demographic groups or employ formal fairness metrics. These findings demonstrate the need for improved transparency, demographic representativeness, fairness assessment, and accountability before AI-based T2DM prediction models can be safely integrated into clinical practice (Mohsen et al., 2023; Panch et al., 2022). Holzinger et al. note that achieving genuinely trustworthy AI in medicine requires accountability structures that extend beyond technical performance to the full life cycle of the system.

Closing this gap between ethical principle and implementation remains one of the most pressing challenges in the field (Holzinger et al., 2019a).

7. Challenges and Future Directions

7.1 Current Challenges

Despite strong progress, the reviewed literature reveals a consistent set of unresolved barriers.

The first is limited dataset diversity. A scoping review of 40 AI-based T2DM risk-prediction studies identified considerable variation in demographic representation. Some studies focused on specific sex, ethnic or age groups, while several did not report gender information. Furthermore, only five of the 40 studies performed external validation, highlighting limited evidence of model generalisability across different populations (Mohsen et al., 2023). . Fregoso-Aparicio et al. confirmed that geographic and demographic homogeneity is a recurring limitation across reviewed studies, directly restricting how safely a model can be deployed in different population contexts. This is particularly consequential for T2DM, where risk profiles differ substantially across ethnicities. The second is the tension between model complexity and interpretability. Although deep learning models can capture complex non-linear relationships, they have not consistently demonstrated superior predictive performance over simpler approaches in T2DM prediction studies. A systematic review found that tree-based algorithms generally achieved stronger performance, whereas deep neural networks were comparatively suboptimal (Fregoso-Aparicio et al., 2021). Furthermore, evidence from broader clinical prediction research indicates that machine-learning models do not necessarily outperform logistic regression when studies have a low risk of bias(Christodoulou et al., 2019). However, they offer weaker direct interpretability, and post-hoc explanation methods do not fully resolve this tension. Loh et al. found that the majority of reviewed clinical XAI studies report technical explanation outputs but do not evaluate whether those outputs are clinically meaningful (Loh et al., 2022). The third is the absence of rigorous external validation. Most models in the reviewed literature are evaluated only on internal test splits from the same dataset (Fregoso-Aparicio et al., 2021). Predictive performance estimates obtained during model

development may be overly optimistic and can decrease when models are evaluated in independent populations. In a study examining 127 newly developed risk prediction models, Siontis et al. (2015) found that the median AUC decreased by 0.05 during external validation ($P < 0.001$). This highlights the importance of independent external validation before applying prediction models in different clinical populations (Siontis et al., 2015). Kopitar et al. similarly noted that prospective clinical testing of T2DM prediction models remains rare (Kopitar et al., 2020) . The fourth is the lack of clinician and patient involvement in model development. Rosenbacke et al. found that most healthcare XAI systems were neither co-designed with clinicians nor tested within real workflows. Liaw et al. confirmed this pattern in a diabetes-specific context, where patients were excluded from the explanation process entirely (Liaw et al., 2023). Human-centred evaluation of XAI outputs is reported in only a small fraction of reviewed studies (Loh et al., 2022; Rosenbacke et al., 2024).

7.2 Future Research Directions

Several directions can address these gaps directly. Dataset diversity and generalisability must be tackled through deliberate multi-ethnic, multi-centre data collection (Mohsen et al., 2023). Federated learning offers a promising approach by allowing healthcare institutions to collaboratively train AI models without directly sharing patient data. Its application has been demonstrated in diabetes prediction using electronic health records from nine Canadian provinces (Tang et al., 2025). However, federated learning alone cannot eliminate population bias, differences in diagnostic labels across institutions, or unequal data representation. Therefore, future research should incorporate data harmonisation, external validation, and fairness assessments to improve model reliability and equity (Pati et al., 2022). Beyond technical approaches such as federated learning, effective clinical AI governance is also essential for equitable implementation. Panch et al. (2022) proposed a hybrid regulatory approach combining centralised oversight with decentralised monitoring by local healthcare institutions. This approach emphasises the importance of local evaluation of algorithmic bias, post-deployment monitoring and assessment of changes in data and model performance to address differences between healthcare settings (Panch et al., 2022). External validation should become a minimum reporting standard.

Christodoulou et al. demonstrated that unbiased validation procedures substantially change apparent model performance (Christodoulou et al., 2019). Prospective clinical testing in real care pathways, not retrospective dataset splits, is the standard the field must move toward (Kopitar et al., 2020).

The integration of wearable device data and continuous glucose monitoring opens a promising path toward real-time, longitudinal risk assessment. Multi-modal integration of clinical, metabolic, and lifestyle data further supports more personalised risk stratification (Peddinti et al., 2017). Clinician and patient co-design must become a routine part of system development, not an afterthought (Liaw et al., 2023; Rosenbacke et al., 2024). Andersen et al. argued that human-centred AI in healthcare requires embedding end-user perspectives from the earliest design stages, not only at the evaluation phase (Andersen et al., 2023) . Holzinger et al. reinforced this by noting that causability — the quality of an explanation as experienced by the clinician cannot be assessed without involving clinicians in the design and evaluation process (Holzinger et al., 2019). Future studies should evaluate not only predictive performance but also whether explanations improve clinicians' and patients' understanding, appropriate reliance on AI recommendations and clinical decision-making (Naiseh et al., 2023; Wysocki et al., 2023)).

8. Conclusion

Early prediction of T2DM holds genuine potential to change how we manage one of the world's most burdensome chronic diseases. This review brought together three dimensions — biomarker science, explainable AI and human-centred design — to assess where the field currently stands and where it needs to go. There is evidence that integrating selected metabolic signatures with routine clinical features may improve the predictive performance of T2DM risk models. However, the improvement is not consistent across all populations and studies, and the use of metabolomics may be limited by analytical costs, specialised equipment and the need for standardised measurement methods (Peddinti et al., 2017; Qiu et al., 2016). Tree-based machine-learning models, including Random Forest and gradient-boosting methods, have demonstrated promising predictive performance in T2DM risk-prediction studies

(Fregoso-Aparicio et al., 2021). However, their superiority over logistic regression is not consistently established. A systematic review of clinical prediction studies found no significant performance advantage of machine-learning models over logistic regression in comparisons with a low risk of bias (Christodoulou et al., 2019) Therefore, model performance should be interpreted in light of study quality and validation methods However, high predictive accuracy alone is insufficient for clinical adoption. Explainability is equally essential. SHAP and LIME are the most widely applied methods, yet their outputs are rarely evaluated through a clinician-centred or patient-centred lens (Loh et al., 2022; Lundberg & Lee, 2017; Ribeiro et al., 2016) .The human-centred dimension remains the most underdeveloped area in the field. Most reviewed systems are not co-designed with clinicians or patients (Liaw et al., 2023; Rosenbacke et al., 2024). Algorithmic bias and limited dataset diversity further restrict the generalisability and fairness of deployed models (Obermeyer et al., 2019). These are not minor gaps. They directly determine whether a system can be trusted in real clinical practice. Federated learning, prospective validation and stakeholder co-design offer a clear path forward (Christodoulou et al., 2019; Holzinger et al., 2019b; Panch et al., 2022). Overall, the findings of this review highlight the importance of combining predictive performance with reliable model explanations, appropriate validation and human-centred evaluation to support the responsible integration of explainable AI into early T2DM risk assessment.

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ARTIFICIAL INTELLIGENCE IN KIDNEY STONE DIAGNOSIS AND MANAGEMENT: A NARRATIVE REVIEW

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Abstract

Kidney stone disease, also known as nephrolithiasis, ranks among the most prevalent urological disorders globally, with its incidence on the rise due to a combination of genetic, dietary, environmental, and lifestyle factors. The predominant type of kidney stones is calcium oxalate, followed by uric acid and cystine stones. Timely and precise diagnosis is crucial for determining the most suitable treatment options and for preventing future occurrences. Imaging modalities such as computed tomography (CT), ultrasound (US), and X-ray are commonly employed to identify kidney stones, with non-contrast CT being regarded as the gold standard owing to its superior diagnostic accuracy. In recent years, artificial intelligence (AI) has demonstrated significant promise in enhancing the detection and characterization of kidney stones. AI algorithms possess the capability to autonomously recognize stones, assess their size and composition, improve image quality, and facilitate clinical decision-making with increased speed and accuracy. This review examines the application of AI in kidney stone diagnosis, characterization, and clinical management, with particular emphasis on CT, ultrasound and X-ray imaging. Recent machine learning and deep learning approaches for automated stone detection, segmentation, composition classification and treatment-related prediction are discussed. Furthermore, it addresses existing challenges and future prospects, and emphasizes the potential of AI to enhance diagnosis, within the field of urology.

Keywords: artificial intelligence, computed tomography, kidney stones, nephrolithiasis, ultrasound

1. Introduction

This is where the Introduction would start. Kidney stones (nephrolithiasis) represent a common medical condition that significantly impacts human health. Epidemiological research conducted globally has shown a notable rise in the incidence of kidney stones over recent decades, especially in Western countries, with forecasts suggesting that this trend will persist into the future. At present, The global prevalence rate of kidney stones ranges from approximately 1.7% to 8.8% (Sivaguru et al., 2021). It is higher in the Western hemisphere than in the Eastern hemisphere, with rates of 7–13 % in North America, 5–9 % in Europe, and 1–5 % in Asia (Boldt & Di Sole, 2025).The prevalence of kidney stones is notably high among men and the elderly, with the peak prevalence of 19.6% observed in males over the age of 80 (Ying et al., 2025). Recent studies indicate that the development of kidney stones is affected by genetic, dietary, environmental, and lifestyle factors. Eating patterns characterized by high salt, high oxalate, and insufficient fluid intake, along with metabolic conditions such as hypercalciuria, hyperoxaluria, and metabolic syndrome, notably elevate the likelihood of forming calcium oxalate (CaO_x) stones (Tamborino et al., 2024).In Western countries, kidney stones primarily consist of calcium oxalate and, to a lesser degree, calcium phosphate, accounting for over 80% of all stones (Tamborino et al., 2024).

2. Pathophysiology of kidney stones

Kidney stones consist of organic and inorganic crystals along with proteins. Lithogenesis and crystallization can take place with various solutes present in urine; however, the most common type of kidney stones are calcareous stones, which account for nearly 80% of all kidney stone cases (Li et al., 2022). The pathophysiology associated with kidney stones is significantly influenced by the specific type of stone, yet renal water conservation is a common feature across nearly all stone types (Jahrreiss et al., 2026). In the case of calcium stones, there is an increase in gut absorption and bone resorption, leading to the leakage of calcium ions, renal calcium leaks, and bone loss triggered by protein, hypocitraturia, enhanced luminal oxalate delivery, and urine excretion. For uric acid stones, the conversion of urate to poorly soluble uric acid is noted (Stepanova et al., 2025). Cystine stones are characterized by the leakage of basic

amino acids, while infectious stones show an increased production of ammonium and bicarbonate from urea. The predominant pathophysiological factor associated with calcium stones is hypercalciuria. Although hyperoxaluria increases the urinary saturation of calcium oxalate, it is less frequently observed compared to hypercalciuria (Mohammadi et al., 2022). Kidney stones exhibit considerable variation in their chemical composition, with the predominant types being calcium oxalate, uric acid, and cystine stones. A thorough understanding of the chemical structure of each stone type is essential for accurate diagnosis and treatment (Xu et al., 2023). Calcium oxalate stones represent the most common variety, constituting roughly 80% of all kidney stones. These stones are formed from calcium ions that are associated with oxalate ions. The chemical structure of calcium oxalate can manifest in two main forms: calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) (Bhojani et al., 2025). COM, referred to as whewellite, is the more stable and frequently encountered form, distinguished by its monoclinic crystal structure. In contrast, COD, known as weddellite, possesses a tetragonal crystal structure and is less stable, often converting to COM over time (Bai et al., 2025).

Uric acid stones represent the second most prevalent category, accounting for approximately 5–10% of all kidney stones. These stones develop due to an excess of uric acid in the urine, which often results from heightened purine metabolism or reduced uric acid excretion. The chemical composition of uric acid stones is $C_5H_4N_4O_3$, and they typically crystallize in rhombic or needle-like forms. In contrast to calcium oxalate stones, uric acid stones are radiolucent, which complicates their detection in conventional X-ray imaging (Ogbodo et al., 2026). Cystine stones are considered relatively uncommon, representing about 1–2% of all kidney stones (Kalatharan et al., 2020).Their formation is primarily attributed to cystinuria, a genetic metabolic disorder that results in the inadequate reabsorption of dibasic amino acids by the renal tubules, including cystine, ornithine, lysine, and arginine. Consequently, elevated levels of cystine in the urine lead to its precipitation, resulting in the formation of crystals that frequently display a distinctive hexagonal morphology (Rodgers & Carmichael, 2026). From a chemical perspective, cystine is produced through the disulfide bond between two cysteine molecules ($HS–CH_2–CH(NH_2)–COOH$), which renders it poorly soluble

in urine. Timely diagnosis of cystinuria is essential, as cystine stones are prone to recurrence and can pose significant treatment challenges. Standard imaging modalities, in conjunction with relevant biochemical assessments, are generally utilized for their identification and management (Sun et al., 2025).

3. Formation of kidney stones

The mechanism of the formation of calcium oxalate (CaO_x) stones remains unclear (Chaiyarit & Thongboonkerd, 2020).

3.1 The supersaturation crystallization theory

Supersaturation serves as a driving force for the formation of urinary calculi, constituting a vital prerequisite for the initiation of a crystal nucleus. Urine is a complex multi-ionic solution wherein various ions interact to create a variety of complexes. Consequently, the degree of calcium oxalate supersaturation in urine is neither fixed nor easily defined (Akram et al., 2024). A supersaturated solution that does not precipitate solid phases spontaneously is termed a metastable supersaturated solution. In a precisely saturated aqueous solution, the saturation level is quantified as 1, while the supersaturation level of urine can vary from 2 to 8, contingent upon the components that promote stone formation (Hossen et al., 2026).This variability is referred to as the "metastable zone." Within this metastable zone, despite the urine being supersaturated, the formation of new solid phases is infrequent (Siener, 2021). Even when crystals are present in the urine of healthy individuals, they typically do not progress to stone formation (Zhang & Li, 2024). However, if the urinary supersaturation surpasses the upper threshold of the metastable zone, transitioning into a state of hyper saturation, a significant number of crystals will form spontaneously in the urine. This phenomenon is a crucial factor that triggers stone formation. Following crystal growth, aggregation, and retention, the calcium oxalate crystals ultimately evolve into calcium oxalate stones (Popova et al., 2025).

3.2 Randall's plaque theory

Randall's plaques are crystalline deposits that adhere to the renal papillary region and are primarily composed of calcium phosphate. Eight decades ago, Dr. Alexander Randall first identified these plaques during kidney biopsies. At that time, he proposed the hypothesis that Randall's plaques (RPs) serve as the origin for idiopathic calcium

oxalate (CaO_x) stones (Wang et al., 2021). These plaques are calcium salt deposits that develop within the interstitial tissue of the renal papilla. When renal tubular epithelial cells sustain damage, exposing the underlying Randall's plaque, calcium ions and oxalate present in supersaturated urine can adhere to the plaque, resulting in crystal formation and the eventual emergence of calcium oxalate stones. Thus, the chemical process leading to the formation of urinary calculi is supersaturation, making RPs crucial in the development of CaO_x stones (Akram & Somani, 2025).Clément employed transmission electron microscopy (TEM) and scanning transmission electron microscopy to investigate the morphology and chemical composition of incipient RPs at the nanoscale, discovering that these incipient plaques primarily consist of two types of nano-calcifications. One type comprises nanoparticles with calcium phosphate and carbonate cores. The presence of calcium carbonate within the cores of larger mineral particles (MPs) can act as a natural catalyst for the accumulation of calcium phosphate, thereby increasing calcium levels. The other type consists of calcified organic vesicles (Yang et al., 2025). These membrane vesicles, composed of anionic phospholipids and rich in proteins, can interact with calcium phosphate, promoting apatite precipitation in vitro. As a result, MPs are often found in abundance on the inner surfaces of kidney calcified vesicles. Furthermore, these calcified vesicles aggregate to form micro-calcifications through their association with an organic fibrillary network (Grases & Costa-Bauzá, 2025).

4. Diagnosis methods

Computed Tomography (CT) has become the gold standard imaging method for identifying kidney stones. This scan is particularly noted for its high sensitivity and specificity in detecting these stones. Despite its effectiveness and numerous benefits in the diagnosis of kidney stones, this imaging technique also presents some limitations (Jalal et al., 2025). Non-contrast CT scans exhibit a high sensitivity for the detection of kidney stones, achieving a sensitivity rate of approximately 95%–98% and a specificity that surpasses 98% (Ćirković et al., 2024). These scans are advantageous as they facilitate immediate evaluations of stone-related issues, providing rapid diagnostic results in emergency situations. Furthermore, this imaging technique is valuable as it allows for a comprehensive assessment of the entire urinary system's anatomy, which is

particularly beneficial when surgical intervention is required. Lastly, CT scans are advantageous in that they can also identify other conditions that present with similar symptoms, such as appendicitis or diverticulitis (Wan et al., 2024).

Ultrasound (US) is essential in the diagnosis and management of nephrolithiasis. However, despite its numerous advantages, there are notable drawbacks associated with the use of ultrasound for kidney stone diagnosis and management. When compared to alternative imaging techniques, such as computed tomography (CT), ultrasound (US) offers significant benefits. A primary advantage is its lack of radiation exposure, enabling its repeated use without posing risks to the patient's health. Furthermore, ultrasound is more cost-effective, thereby reducing the financial burden related to treatment. Lastly, the widespread availability of ultrasound enhances patient accessibility, as it is commonly found in numerous clinics (Wang & Wang, 2024).

X-ray imaging, which encompasses the kidney-ureter-bladder (KUB) X-ray examination, has been utilized for an extended period to identify kidney stones. While this method offers numerous advantages, it is essential to take into account its low sensitivity and specificity when applying it in clinical settings (Saifullah & Saeed, 2025). The application of X-ray imaging provides numerous significant advantages in the assessment of patients with nephrolithiasis (Phillips et al., 2015).This method is non-invasive, eliminating the need for contrast agents or injections. Additionally, the procedure is conducted swiftly, facilitating the rapid diagnosis of stones and enabling timely decision-making regarding subsequent actions. Furthermore, X-ray imaging is comparatively economical when juxtaposed with other imaging modalities, such as CT or MRI. KUB X-rays also facilitate the monitoring of existing kidney stones, allowing for the evaluation of their size, number, and growth patterns (Freitas Bouzon et al., 2025).

Fourier-transform infrared spectroscopy (FTIR) is a commonly used analytical technique for determining the chemical composition of kidney stones. The method is based on the absorption of infrared radiation by chemical bonds within the stone, producing a characteristic infrared spectrum. Different mineral components, including calcium oxalate, calcium phosphate, uric acid, struvite, and cystine, exhibit distinct spectral patterns that can be used for qualitative and quantitative identification. FTIR

requires only a small amount of stone material and provides relatively rapid compositional analysis. Therefore, it is useful for confirming stone composition after stone retrieval and for distinguishing between mixed and pure stone types. In the context of artificial intelligence, FTIR-based composition analysis can also serve as a reference method for validating AI models designed to predict stone composition from medical imaging.

X-ray diffraction (XRD) is another analytical technique used to characterize the crystalline composition of kidney stones. When a stone sample is exposed to X-rays, the crystalline structures within the sample produce characteristic diffraction patterns. These patterns can be compared with reference patterns to identify the mineral phases present in the stone. XRD is particularly useful for distinguishing crystalline forms of calcium oxalate and identifying other mineral components such as calcium phosphate, uric acid, and struvite. Compared with imaging-based assessment, XRD provides direct information about the crystalline structure and composition of a recovered stone. Consequently, XRD can be used as a reference standard in studies evaluating AI-based approaches for automated prediction of kidney-stone composition [49].

5. *Applications of AI kidney stone detection*

Artificial intelligence (AI) significantly improves the analysis of CT images for diagnosing kidney stones by facilitating a comprehensive examination of stone attributes. Computed tomography is regarded as the gold standard for detecting kidney stones due to its exceptional sensitivity and specificity. AI improves CT imaging by automating the detection and segmentation of kidney stones. Deep learning algorithms are capable of processing extensive amounts of imaging data to identify even the smallest stones and provide detailed characterization (Gillams et al., 2021). These models are designed to recognize features that indicate the presence of stones, irrespective of their composition or location within the urinary tract. Furthermore, AI models can estimate the size and density of kidney stones from CT images. The size of the stones is a crucial factor in determining suitable management strategies, while density, quantified in Hounsfield units, provides valuable information regarding stone composition, such as calcium oxalate or uric acid (Ahmad et al., 2025). Additionally, density measurements assist in predicting the efficacy of extracorporeal shock wave

lithotripsy (ESWL). AI also plays a role in distinguishing kidney stones from other calcifications, such as phleboliths and vascular deposits, by analyzing intricate imaging patterns. Moreover, it facilitates low-dose CT imaging by maintaining image quality while minimizing radiation exposure through sophisticated reconstruction techniques (Allam et al., 2025).

Artificial intelligence significantly enhances the diagnostic capabilities of X-ray imaging, which is a widely accessible and economical method for detecting kidney stones (Wani et al., 2023). However, traditional X-ray imaging is constrained by its low sensitivity, the presence of overlapping anatomical structures, and variability in interpretation. AI contributes to the analysis of X-ray images by improving contrast and resolution, facilitating a clearer distinction between kidney stones and adjacent tissues (Tzelves et al., 2023). This enhancement is particularly beneficial for identifying small or faint stones. Machine learning models evaluate characteristics such as the shape, size, density, and location of stones to uncover subtle patterns that may not be readily apparent, thus increasing detection accuracy. Furthermore, AI-driven noise reduction techniques preserve image quality in low-dose imaging, promoting safer diagnostic practices and enabling repeated examinations without significant radiation exposure (Ghoneim et al., 2024). Additionally, AI aids in the classification and differentiation of stones, allowing for the distinction between kidney stones and other calcifications, such as phleboliths or vascular deposits, based on unique radiological indicators. This reduces false positives and significantly enhances diagnostic precision (Singh et al., 2022).

Artificial intelligence significantly improves ultrasound imaging, rendering it a more dependable instrument for diagnosing kidney stones. The widespread use of ultrasound is attributed to its non-invasive characteristics and lack of radiation exposure; however, its efficacy is often hindered by operator dependence and variability in image quality (Sassanarakkit et al., 2023). AI mitigates operator dependency by facilitating consistent and automated identification of kidney stones. Machine learning algorithms, which are trained on extensive datasets, can accurately detect stones irrespective of the operator's expertise or the equipment used (Yenikekaluva et al., 2024).

Automated detection systems are capable of recognizing hyperechoic structures linked to kidney stones and differentiating them from similar artifacts, such as calcifications or

gas, through sophisticated pattern recognition techniques (Papatsoris et al., 2024). Additionally, AI enhances image processing by improving contrast and resolution, which aids in the clearer visualization of kidney stones under challenging circumstances, including obesity or anatomical variations (Pei et al., 2025). Moreover, AI enables real-time monitoring and tracking of stone movement, potentially aiding in the evaluation of obstruction risk and informing clinical decision-making. It also improves the detection of small or early-stage stones, allowing for timely medical intervention (Maleki et al., 2026).

The integration of AI with CT imaging significantly enhances the detection of stone composition. CT imaging produces radiographic images that enable AI algorithms to classify stones with increased precision. In one study, algorithms utilizing multiparametric techniques, which are supplied with CT imaging data, achieved a classification accuracy of 100% for uric acid (UA) stones compared to non-UA stones, with a 75% accuracy rate among non-UA stones (Balawender et al., 2024).Another research demonstrated an enhancement in the classification of non-UA stones, with accuracy rising to 88% through the application of rapid voltage switching dual energy CT imaging (Yousuf Ahmad et al., 2025).A support vector machine (SVM) model, utilizing information from CT texture analysis, yielded an area under the curve (AUC) value of 0.965, alongside a sensitivity of 94.4% and specificity of 93.7% in the detection of UA stones (Eniyan et al., 2023). Furthermore, neural network algorithms developed from spectral CT imaging have exhibited remarkable performance, achieving per-voxel accuracy rates of 91.1% and independent validation accuracy ranging from 87.1% to 90.4%, even for complex stone compositions. Additionally, AI has demonstrated the feasibility of classifying calcium oxalate monohydrate (COM) stones using spectral CT imaging, attaining an AUC value of 0.933 and an accuracy of up to 88.3% (Ali et al., 2025).

6. *Future directions*

The application of (AI) in kidney stone detection has revealed significant progress; however, several hurdles remain before it can be widely implemented in routine clinical settings. Future research should prioritize the creation of more robust, explainable, and clinically validated AI models that are capable of accurately detecting, classifying, and

predicting the characteristics of kidney stones across diverse patient groups (Xu et al., 2015). One significant future direction involves the advancement of multimodal AI systems that integrate data from CT scans, ultrasound, X-ray imaging, laboratory tests, electronic health records, and patient demographic information (Papatsoris et al., 2025).These integrated models have the potential to deliver comprehensive diagnostic insights, enhance stone characterization, and facilitate personalized treatment planning. By merging imaging results with biochemical indicators such as urinary calcium, oxalate, citrate, uric acid, and serum creatinine levels, the accuracy of predictions regarding stone composition, recurrence risk, and treatment outcomes may be substantially improved (Veronese et al., 2025).

Another promising avenue is the application of radiomics and deep learning techniques for the non-invasive prediction of stone composition. Sophisticated AI algorithms that can extract nuanced imaging features beyond the capabilities of human observation may enable precise differentiation among calcium oxalate, uric acid, cystine, and mixed stones without the need for invasive interventions. This advancement would aid in the selection of appropriate treatment options, including extracorporeal shock wave lithotripsy (ESWL), ureteroscopy, or medical dissolution therapy (Galán-Llopis et al., 2023).

Future AI models should also prioritize early detection and risk assessment. Machine learning algorithms that are trained on longitudinal clinical data could identify individuals at elevated risk of developing recurrent nephrolithiasis prior to the formation of stones. Such predictive systems could bolster preventive measures through tailored dietary advice, hydration monitoring, metabolic assessments, and lifestyle changes (Wang et al., 2025).

Ultimately, the incorporation of AI into hospital information systems and picture archiving and communication systems (PACS) will facilitate uninterrupted clinical workflows. AI-driven decision support tools have the capability to autonomously identify kidney stones, evaluate their size and density, predict treatment efficacy, analyze the risk of recurrence, and suggest tailored management approaches (Takazawa, 2015). This integration holds the promise of minimizing diagnostic inaccuracies,

shortening reporting durations, enhancing treatment planning, and ultimately improving patient outcomes (Thongboonkerd, 2019).

7. Conclusion

Kidney stone disease continues to be a common and recurring urological condition that presents a considerable global health challenge due to its rising incidence, high rates of recurrence, and the associated costs of healthcare. A comprehensive understanding of the pathophysiology underlying stone formation, which includes factors such as supersaturation, crystal nucleation, growth, aggregation, and the role of Randall's plaques, is crucial for enhancing prevention and treatment approaches. Recent advancements in diagnostic imaging techniques, particularly computed tomography, ultrasound, and X-ray, have significantly improved the detection and assessment of kidney stones, although each imaging modality possesses its own limitations. The increasing application of AI in medical imaging has demonstrated considerable potential for improving kidney stone detection, segmentation, characterization, and clinical decision-making. AI-driven models have shown high diagnostic performance in selected studies across CT, ultrasound, and X-ray imaging, while also reducing reliance on operators, reducing diagnostic inaccuracies, and facilitating more tailored patient management. However, challenges concerning data standardization, model interpretability, clinical validation, and the integration of these technologies into healthcare practices must be resolved prior to achieving widespread clinical application. Future studies concentrating on multimodal AI systems, explainable algorithms, extensive multicenter datasets, and prospective clinical validation will further bolster the reliability and applicability of these innovations. In summary, the integration of artificial intelligence with advanced imaging modalities holds the promise to transform the diagnosis, characterization, and management of kidney stones, ultimately enhancing patient outcomes and propelling precision medicine within the field of urology.

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CULTURALLY DIVERSE, DEMOGRAPHICALLY AWARE FACIAL EMOTION RECOGNITION USING YOLO11N-CLASSIFICATION: A COMPARATIVE STUDY

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Abstract

Facial emotion recognition (FER) has attracted substantial research interest in recent years. However, many high-performing models are evaluated on benchmark datasets with limited demographic diversity, which can introduce cultural bias in South Asian and similar populations. This paper presents a systematic comparative study of three deep learning architectures: (1) YOLO11n-cls, (2) a custom Convolutional Neural Network (CNN), and (3) a MediaPipe-based Artificial Neural Network (ANN), trained on a curated 14,000 image multi-source dataset combining AffectNet, FER2013, and locally captured Sri Lankan facial images to address the issue of demographic bias. YOLO11n-cls model achieved a Top-1 accuracy of 94.2%, significantly outperforming traditional Convolutional Neural Network approaches, which reached a validation accuracy of 72.81%, and MediaPipe-based methods at 46%.

Keywords: Facial Emotion Recognition, YOLO, CNN, Deep Learning, Cultural Bias, AffectNet

1. Introduction

Facial Emotion Recognition (FER) is a core challenge in affective computing, enabling machines to understand nonverbal cues. This improves interaction in areas such as education, healthcare, surveillance, and driver monitoring (Pise et al., 2022). However, despite its increasing use, FER faces major challenges in real-world situations. These challenges include differences in pose, lighting, obstructions, and background noise (Li & Deng, 2022).

However, there remains a significant gap between performance in academic benchmarks and accuracy in real-world applications. Several commonly used FER datasets have limited demographic diversity (Lukac et al., 2023).

This paper addresses both accuracy and deployment concerns through the following:

- Construction of a demographically diverse 14,000-image dataset combining AffectNet (Mollahosseini et al., 2019), FER2013 (Li & Deng, 2022) and locally captured South Asian facial images.
- Systematic evaluation of three architectures under controlled conditions on this dataset with complete hyperparameter transparency.
- Detailed analysis of training dynamics, per-class confusion, and convergence behaviour of each model.

2. Literature Review

This section reviews existing literature across three key areas relevant to this study: (A) technologies used in facial emotion recognition, (B) past and current trends in FER research, and (C) Demographic Bias in FER.

3. Technologies

This subsection looks at the main technologies that have influenced research and development in facial emotion recognition. Previous studies have examined the use and effectiveness of technologies like Convolutional Neural Networks (CNNs), MediaPipe landmark-based pipelines, and YOLO-based architectures.

CNNs have dominated the field of FER since Li and Deng (2022) published their work on the FER2013 dataset. Architectures using VGGNet, ResNet, and EfficientNet variants have reached accuracies over 73% on FER2013 (Khaireddin & Chen, 2021). Other studies have shown that CNN based approaches can deliver strong FER performance. One study compared CNN, DTSCNN, RNN, and ResNet-50, reporting over 90% accuracy across different epoch configurations. This finding supports CNN's effectiveness in real-time emotion recognition (Alzubaidi et al., 2021).

Similarly, MediaPipe, developed by Google, provides a lightweight face mesh pipeline that produces 478 canonical 3D facial landmarks (Sánchez-Brizuela et al., 2023). Landmark-based FER avoids direct pixel-level classification and operates, on geometric feature vectors derived from inter landmark distances. Waleed and Shaker (2024) achieved 96.31% accuracy on the MELD dataset using a CNN on MediaPipe preprocessed facial images, although performance varied substantially with the dataset and task framing. The accuracy ceiling is often limited by the expressiveness of the geometric features alone.

The YOLO family was originally designed for real-time object detection (Redmon et al., 2016), has seen increasing adoption in FER tasks. Shaikh et al. (2023) demonstrated YOLOv5 on FER2013 achieving 50% accuracy under limited data, while Bharathi et al. (2022) showed that a combined YOLOv5 and CNN approach reached 95.57% on a larger corpus. Vanamoju et al. (2024), and Ambali Parambil et al. (2024) further reported 72.56% and 74.9% mAP@50 using YOLOv8 and YOLOv9 respectively, collectively indicating that dataset diversity and scale are critical determinants of YOLO-based FER accuracy, independent of model version.

The ongoing evolution of these technological tools continues to influence methodologies and outcomes in recent research.

4. Past and Current Trends

The literature highlights several significant trends over time. Early FER research relied on handcrafted features and shallow learning methods like Local Binary Patterns (LBP). This laid the groundwork for later deep learning approaches (Li & Deng, 2022). In the

past decade, there has been a clear shift toward end-to-end deep learning models. This change is largely due to the availability of large annotated datasets such as FER2013 (Li & Deng, 2022) and AffectNet (Mollahosseini et al., 2019), along with improvements in transfer learning from ImageNet-pretrained weights. Recent publications have emphasized that dataset diversity and scale are key to performance, moving beyond just architectural innovation (Vanamoju et al., 2024; Ambali Parambil et al., 2024). A current trend is toward lightweight, efficient architectures for real-time inference on devices. Models such as MobileNet, EfficientNet-lite, and small YOLO variants are becoming popular because they effectively balance accuracy and computational efficiency. Privacy-preserving on-device inference has also become a key goal in fields like healthcare and driver monitoring (Pise et al., 2022).

These trends suggest that the field is progressing towards lightweight, privacy-focused, and demographically inclusive FER systems that are suitable for real-world use.

5. Demographic Bias in FER

Lukac et al. (2023) showed that FER models trained on specific regional datasets struggled to generalize across different cultural groups. Cross-regional misclassification occurs because incompatible emotional cues. For example, models trained mainly on Caucasian datasets perform poorly on East Asian expressions (Lukac et al., 2023). Khan and Stinson (2025) further examined AffectNet and RAF-DB. They found a large number of posed images in datasets that were claimed to be collected in natural settings. Audited FER models disproportionately classified darker-skinned and non-white individuals as displaying negative emotions such as anger or sadness, even when those individuals were smiling (Khan & Stinson, 2025). These issues highlight the need to include locally representative data in any FER pipeline used in a specific region.

6. Dataset Construction and Preprocessing

6.1 Design Principles

The dataset was designed to address the dual objectives of broad emotional coverage and demographic representativeness of the data. Seven emotional states were selected: happy, sad, anger, fear, surprise, disgust, and neutral. A balanced class distribution of 2,000 images per class (14,000 total) was established to avoid class imbalance artifacts during training.

6.2 Data Sources

AffectNet : A large-scale database of facial expressions collected from the Internet, covering wide ethnic, lighting, and expression variability. AffectNet contributes to broad representational diversity and ecologically valid expression samples.

FER2013 : A benchmark dataset of 35,887 grayscale 48×48-pixel images annotated with seven emotion labels. This provides variable image quality and low resolution, ensuring that the model is robust to degraded capture conditions typical of consumer webcam input.

Custom local dataset: Facial images captured from Sri Lankan individuals. This inclusion is critical for fine-tuning the model to recognize facial features and skin tone characteristics of the Sri Lankan demographic.

6.3 Model Architectures and Training Configuration

YOLO11n-cls

YOLO11n-cls is the nano-scale classification variant of the YOLO11 architecture (Abdullah et al., 2026). It adapts the YOLO single-stage detection backbone for image classification by replacing the detection head with a global average pooling layer followed by a linear classifier. The nano variant uses depthwise separable convolutions and a CSP-based backbone, minimising parameter count while preserving representational capacity for efficient on-device deployment.

The complete training configuration is summarised in Table I. The model was trained from ImageNet-pretrained weights using the Ultralytics training framework on Apple MPS hardware.

Table 2: YOLO11n-cls Training Hyperparameters

Source: Author's Compilation

Hyperparameter	Value
Epochs	300
Image size	128 × 128 pixels
Batch size	64
Pre-trained	Yes (ImageNet)
Optimizer	Auto (SGD-based)
Momentum	0.937
Weight decay	0.0005
Warmup epochs	3
Patience (early stop)	20
Device	Apple MPS
AMP	Enabled
Augmentation	RandAugment + mosaic
Dataset split	80% training/ 20% validation

6.4 Custom CNN

A custom CNN was implemented in PyTorch as a baseline comparator. The architecture follows a modular sequential design with three feature extraction blocks (Block 1: 32 filters, 3×3 Conv, BN, ReLU, 2×2 MaxPool; Block 2: 64 filters; Block 3: 128 filters). The classification head consists of a Flatten layer, 512-neuron Dense layer, 50%

Dropout, and a 7-class linear output. Training used the Adam optimizer (learning rate =0.001), L2 regularization, Automatic Mixed Precision (AMP), and early stopping with patience=10. Input resolution 128×128 px. The model reached its optimal configuration at epoch 26.

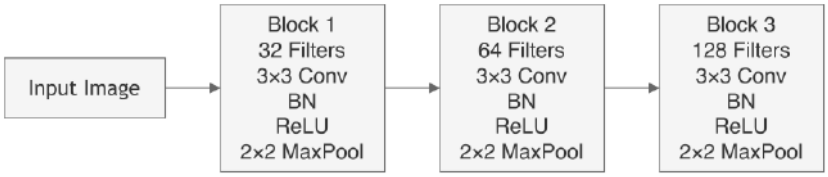

Figure 1: Custom CNN architecture. Three sequential feature extraction blocks (Block 1: 32 filters, Block 2: 64 filters, Block 3: 128 filters), each with 3×3 Conv, BN, ReLU, and 2×2 MaxPool, followed by a classification head.

Source: Author's Compilation

7 MediaPipe-ANN

The third approach is a two-stage pipeline combining Google's MediaPipe face landmarker [8] with a custom MLP classifier. Euclidean 2D and 3D distances from the nose tip anchor (index 1) to 92 selected landmark indices (eyes, eyebrows, lips) are computed, yielding a 184-dimensional feature vector per sample. Features were standardised to zero mean and unit variance. The MLP architecture: Input (184) → Dense 128 (ReLU) → Dense 64 (ReLU) → Softmax (7). Optimizer: Adam; Loss: Categorical Cross-Entropy; Epochs: 50 (best performance at epoch 15).

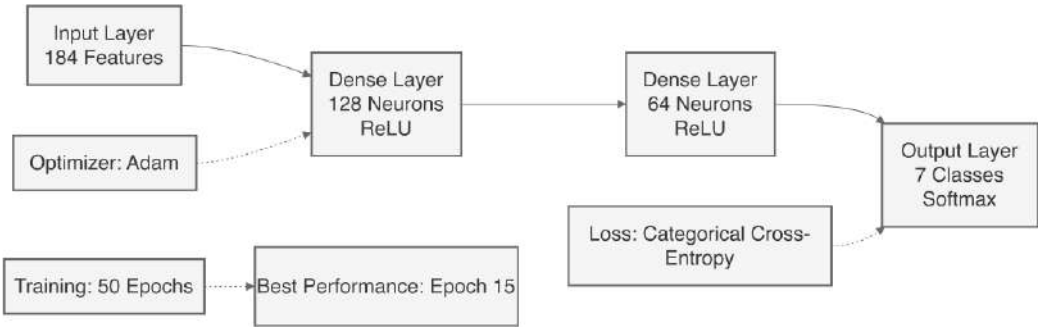

Figure 2: MLP classifier architecture comprising an input layer of 184 features, two hidden layers with 128 and 64 ReLU-activated neurons, and a seven-class

Softmax output layer. The model is trained using Adam and categorical cross-entropy loss, with optimal performance achieved at epoch 15.

Source: Author's Compilation

7.1 Experimental Results

YOLO11n-cls Training Dynamics

Fig. 3 shows the training and validation loss and accuracy curves over 300 epochs. The training loss decreases monotonically from 1.863 (epoch 1) to 0.176 (epoch 300). Validation loss mirrors this trajectory, dropping from 1.719 to 0.247. Top-1 accuracy increases from 34.4% to 94.2%, with a Top-5 accuracy plateau near 99.7% reached by epoch 200. The near-identical training and validation trajectories demonstrate the absence of significant overfitting across the full 300-epoch schedule, validating the regularisation provided by AMP, weight decay, and the diverse augmentation strategy.

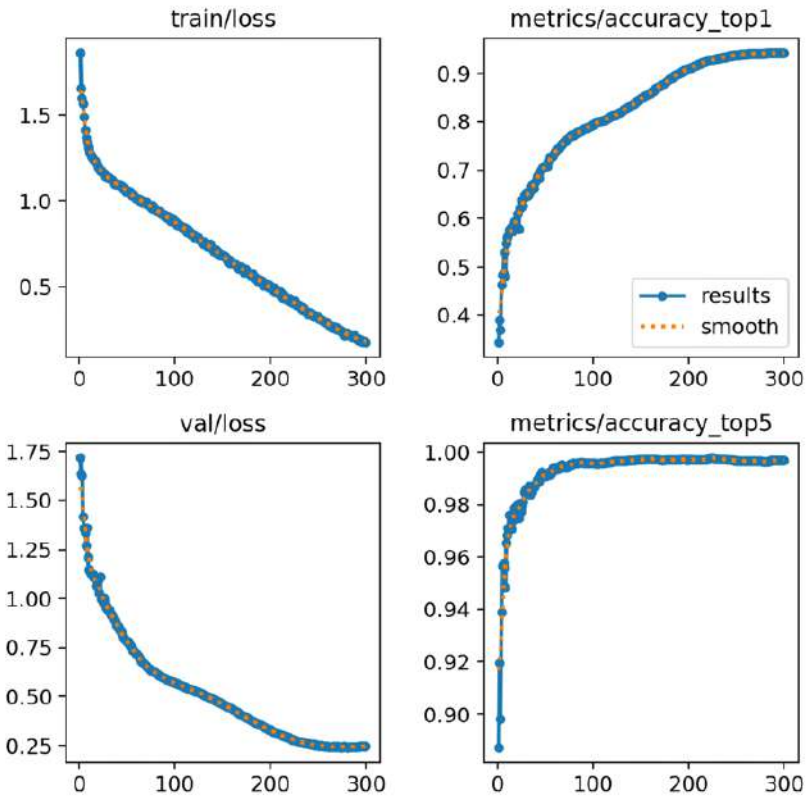

Figure 3: YOLO11n-cls training curves: train/loss (top-left), metrics/accuracy_top1 (top-right), val/loss (bottom-left), metrics/accuracy_top5 (bottom-right) over 300 epochs.

Source: *Author's Compilation*

Table 2 reports accuracy at key epoch milestones, demonstrating consistent and sustained improvement throughout training without plateau or degradation.

Table 3: YOLO11n-cls Accuracy at Key Epochs

Source: Author's Compilation

Epoch	Train Loss	Val Loss	Top-1 Acc.	Top-5 Acc.
1	1.863	1.719	34.4%	88.7%
50	1.057	0.782	70.7%	99.1%
100	0.870	0.570	79.3%	99.6%
150	0.684	0.462	84.8%	99.7%
200	0.496	0.330	91.1%	99.7%
250	0.319	0.250	93.8%	99.7%
296*	-	0.241	94.2%	99.8%
300	0.176	0.247	94.2%	99.7%

*Peak Top 1 accuracy epoch

8 Confusion Matrix Analysis

Fig. 4 presents the normalised confusion matrix for YOLO11n-cls across all seven emotion classes evaluated on the validation set. The diagonal elements represent per-class recall. Happy achieves the highest recall (0.99), followed by disgust and sad (both 0.95), neutral (0.94), surprise (0.93), angry (0.92), and fear (0.91). The primary inter-class confusion occurs between fear and surprise (0.04), which is expected given the structural similarity of both expressions (raised brows, widened eyes). All other off-

diagonal values are ≤0.03, confirming highly discriminative classification across all seven categories.

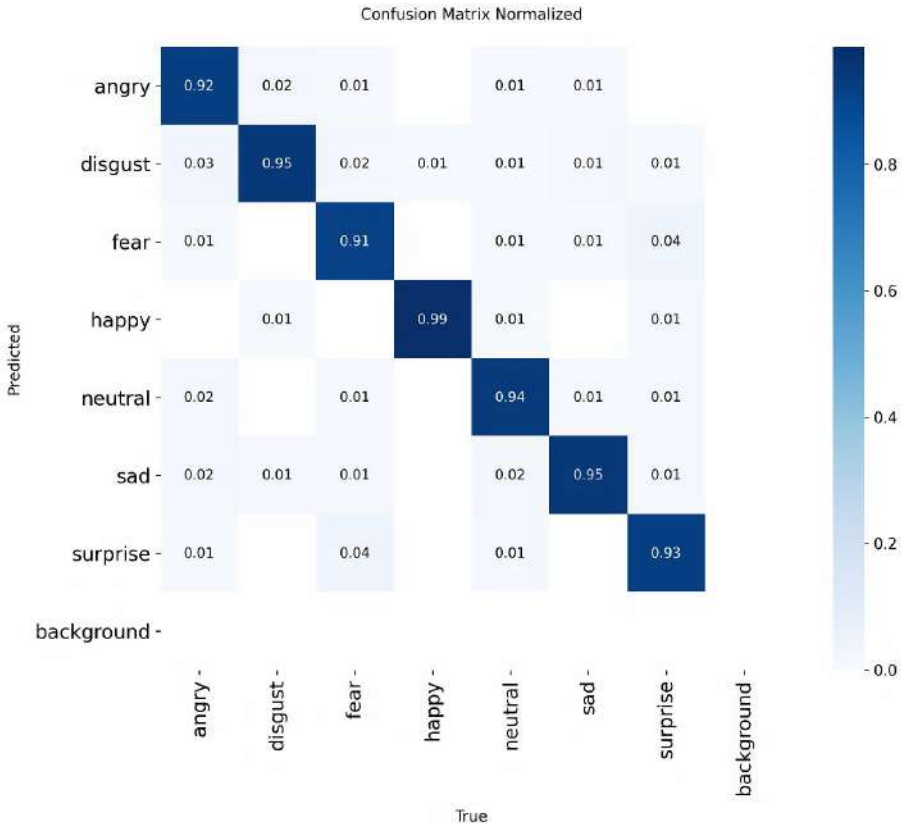

Figure 4: Normalised YOLO11n-cls Accuracy at Key Epochs

Source: Author's Compilation

Table 4: Per-Class Classification Results (YOLO11n-cls)

Source: Author's Compilation

Class	Correct	Errors	Recall
Angry	649	33	92%
Disgust	673	56	95%

Fear	641	45	91%
Happy	698	19	99%
Neutral	670	38	94%
Sad	670	47	95%
Surprise	650	47	93%

To validate the model's real-world applicability, a series of sample predictions were generated. Fig. 5 shows the model's ability to correctly identify emotional states across different subjects, including those from the custom local dataset.



Figure 5: Prediction results for YOLO11n-cls on unseen validation images. Labels show predicted emotion class and confidence score (e.g., fear 1.0, happy

1.0, disgust 1.0). The model correctly classifies all displayed samples, including subjects from the custom

Source: *Author's Compilation*

Custom CNN Performance

The custom CNN reached its optimal configuration at epoch 26, when the early stopping mechanism triggered due to the absence of further improvement in validation loss. Fig. 6 shows training and validation accuracy and loss curves. Final metrics: training accuracy 72.66%, validation accuracy 72.81%, training loss 0.5866, validation loss 0.5854. The near-identity of training and validation metrics confirms the model is well-regularised. However, the accuracy ceiling of 72.8% reflects the limited representational capacity of a three-block CNN relative to a seven-class FER task at 128×128 px resolution, and the absence of ImageNet pretraining.

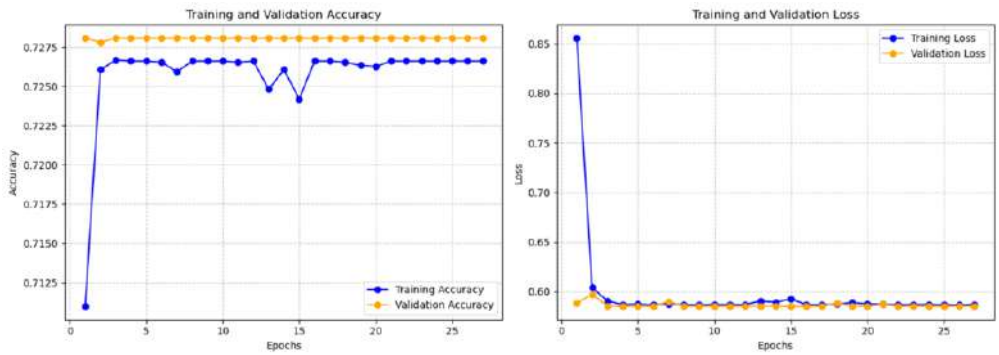

Figure 6: Custom CNN (PyTorch) training and validation accuracy (left) and loss (right). Early stopping triggered at epoch 26. Final validation accuracy: 72.81%.

Source: Author's Compilation

9 MediaPipe-ANN Performance

The MediaPipe-ANN pipeline achieved a test accuracy of approximately 46% with a final loss of 1.4152. Fig. 7 shows training and validation accuracy and loss curves. Validation performance plateaued at approximately epoch 15, after which no meaningful improvement occurred despite 35 additional epochs of training. This early saturation suggests that the 184-dimensional geometric feature representation lacks the discriminative capacity for robust seven-class FER. Raw inter-landmark distances encode gross expression geometry but discard skin texture, muscle deformation detail,

and appearance-based cues essential for discriminating affectively-similar emotion pairs such as sadness, disgust and fear, surprise.

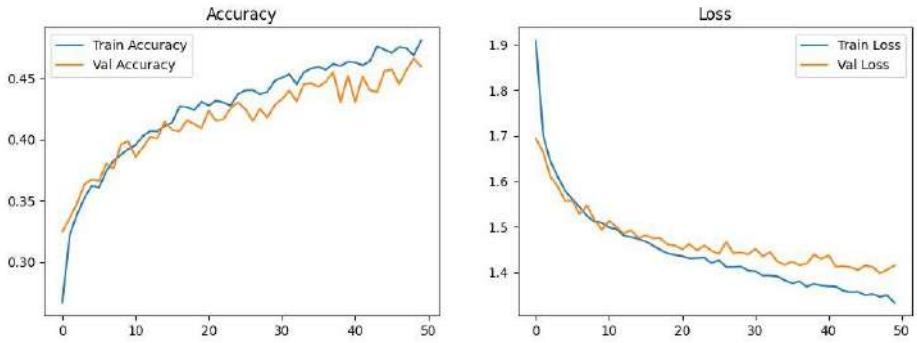

Figure 7: MediaPipe-ANN training and validation accuracy (left) and loss (right) over 50 epochs. Best performance at epoch 15; final accuracy 46%.

Source: Author's Compilation

10 Comparative Summary

Table IV presents a consolidated performance comparison of all three architectures.

Table 5: Comparative Model Performance

Source: Author's Compilation

Model	Top-1 Acc.	Top-5 Acc.	Accuracy	Val Loss	Optimal Epoch
YOLO11n-cls	94.2%	99.7%	N/A	0.247	300 (full)
Custom CNN (PyTorch)	N/A	N/A	72.81%	0.585	26 (early stopping)
MediaPipe-ANN	N/A	N/A	46%	1.415	15 (plateau)

11 Discussion

11.1 Why YOLO11n-cls Outperforms

The 21.4 percentage-point accuracy gap between YOLO11n-cls (94.2%) and the custom CNN (72.8%) is due to three main reasons. First, ImageNet pretraining allows the transfer of rich multi-scale visual features that cannot be gained from just 14,000 training images. Second, the CSP-based backbone with cross-stage partial connections improves gradient flow and feature reuse across layers. This leads to richer representations than a sequential three-block CNN. Third, YOLO11n-cls's broad augmentation strategy (RandAugment, mosaic, HSV jitter, random erasing) provides much greater sample diversity. The 300-epoch training schedule, supported by Apple MPS hardware acceleration, enables thorough convergence that is not possible with limited computational resources.

11.2 MediaPipe-ANN Limitations

The MediaPipe-ANN pipeline's 46% accuracy shows that geometric landmark features alone are not enough for robust FER. The 184-dimensional feature vector captures basic pose and expression geometry but misses the texture, color, and subtle muscle movements needed to differentiate between similar emotions. Early saturation at epoch 15 indicates a limit in representation instead of an optimization issue. Future work could look into combining geometric features with local patch descriptors or Gabor filter responses around landmark areas.

11.3 Effect of Demographic-Diverse Training Data

Including locally captured South Asian facial images as a specific data source is a design choice with real significance. Previous research highlights cross-regional misclassification in models trained only on Western-focused data like FER2013. Performance drops significantly when evaluating demographic groups that are underrepresented in the training set (Lukac et al., 2023; Li & Deng, 2022). By adding locally relevant images, the model's exposure to the target population's skin tone distribution and expression details increases. The 94.2% Top-1 accuracy shows a considerable improvement over previous YOLO-based FER results using only FER2013 data. This supports the idea that having a diverse dataset is crucial for better performance.

12 Limitations

Several limitations should be acknowledged. No ablation study was conducted to separate the individual contributions of each data source (AffectNet, FER2013, custom). This makes it hard to connect performance gains directly to demographic enrichment. Evaluation was done on a held out validation set from the same distribution, and no independent test in real-world settings (e.g., naturalistic webcam inference) was performed. Addressing these limitations is a priority for future research.

13 Conclusion

This paper compared three deep learning architectures for facial emotion recognition using a demographically diverse, multi-source dataset of 14,000 images. YOLO11n-cls, trained for 300 epochs (batch=64, img=128, Apple MPS, RandAugment) from ImageNet-pretrained weights, reached a Top-1 accuracy of 94.2% and a Top-5 accuracy of 99.7%. This significantly surpasses a custom PyTorch CNN (72.81%) and a MediaPipe-ANN (46%).

The results show that YOLO11n-cls, when fine-tuned on a culturally diverse dataset that includes locally representative South Asian facial data, is an effective and practical solution for real-time FER. Its lightweight architecture allows on-device ONNX inference with less than two seconds of latency on consumer hardware, removing the need for cloud support. This addresses important privacy and accessibility issues in culturally-specific applications.

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N6-METHYLADENOSINE AND 5-METHYLCYTOSINE EPITRANSCRIPTOMIC REGULATION IN HUNTINGTON'S DISEASE MOLECULAR MECHANISMS, BIOMARKER POTENTIAL, AND THERAPEUTIC IMPLICATIONS

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Abstract

Huntington's disease (HD) is a fatal autosomal dominant neurodegenerative disorder caused by CAG trinucleotide repeat expansion in the huntingtin (HTT) gene, encoding mutant huntingtin (mHTT) protein. While the genetic basis is well established, the epitranscriptomic mechanisms governing HD pathogenesis remain poorly understood. This review examines two key RNA modifications- N6-methyladenosine (m6A) and 5-methylcytosine (m5C) and their roles in HD molecular pathology. M6A, the most abundant internal mRNA modification, is dynamically regulated by writer (METTL3/METTL14), eraser (FTO/ALKBH5), and reader (YTHDF1-3, IGF2BP) proteins.m5C, written by NSUN and TRDMT1 (DNMT2) and read by ALYREF and YBX1, regulates mRNA stability and export. In HD, these modifications may be dysregulated, potentially affecting HTT processing, stress granules, and autophagy-related translation. M6A and m5C profiles in cerebrospinal fluid and blood derived RNA show promise as non-invasive biomarkers for HD staging and disease progression monitoring. Pharmacological modulation of m6A writers and erasers has demonstrated neuroprotective effects in HD cellular and animal models. We conclude that epitranscriptomic reprogramming represents both a disease mechanism and a therapeutic target in HD, warranting further translational investigation.

Keywords: *N6-methyladenosine,5-methylcytosine, epitranscriptomics, Huntington's disease, RNA modification, neurodegeneration.*

1. Introduction

Huntington's disease (HD) is a progressive, fatal neurodegenerative condition caused by an abnormal expansion of CAG repeats (≥36; 36–39 reduced, ≥40 full penetrance) in exon 1 of the huntingtin (HTT) gene on chromosome 4p16.3 (Bates et al., 2015). This mutation encodes a mutant huntingtin (mHTT) protein with an elongated polyglutamine (polyQ) tract that acquires toxic gain of function properties, leading to preferential degeneration of stratial medium spiny neurons and cortical projection neurons (Reiner et al., 1988). Clinically, HD manifest as a triad of motor dysfunction, cognitive decline, and psychiatric disturbances, with a mean age of onset in the fourth to fifth decade and inevitable progression to death within 15-20 years (Tabrizi et al., 2019).

Despite the monogenic nature of HD, significant variability in onset age and disease severity beyond CAG repeat length points to important epigenetic and post transcriptional regulatory mechanisms (Landwehrmeyer et al., 2017).

Epitranscriptomics ; study of chemical modifications on RNA, has emerged as a critical regulatory layer governing gene expression, mRNA stability, splicing, and translation. Over 170 distinct RNA modifications have been identified, with N6-methyladenosine and 5-methylcytosine being among the most functionally characterized in mammalian cells (Yue et al., 2015). These modifications are particularly relevant to neurological disease given the high transcriptional complexity of neuronal cells and the dependence of synaptic plasticity on precise post- transcriptional gene regulation (Yoon et al., 2017).

This review examines the molecular machinery of m6A and m5C modifications, their known dysregulation in HD, their potential as disease biomarkers, and emerging therapeutic strategies targeting these epitranscriptomic studies, HD model systems, and early clinical data. We aim to frame how epitranscriptomic reprogramming contributes to HD pathogenesis and may be therapeutically exploited, using narrative PubMed/Scopus searches.

2. m6A and m5C: Molecular Machinery and Function

2.1 m6A: Writers, Erasers, and Readers

m6A is the most prevalent internal modification of mammalian mRNA, occurring predominantly at DRACH motifs (D = A/G/U; R = A/G; H = A/C/U) (Dominissini et al., 2012). The modification is installed co-transcriptionally by a methyltransferase

complex comprising METT3 (the catalytic subunit), METTL14, WTAP (Ping et al., 2014), and RBM15; METTL16 separately installs m6A on specific transcript subsets, particularly non-coding RNAs and U6 snRNA (Roundtree et al., 2017).

m6A marks are removed by demethylases(erasers): FTO (fat mass and obesity-associated protein) and ALKBH5 (AlkB homolog 5), both members of the Fe (II)/α-ketoglutarate-dependent dioxygenase superfamily (Jia et al., 2011). Recognition of m6A is mediated by reader proteins, chiefly the YTH domain-containing family (YTHDF1-3 and YTHDC1-2). YTHDF1 promotes cap-dependent and cap-independent translation; YTHDF2 accelerates mRNA decay via deadenylation; YTHDF3 modulates both processes and stress granule assembly (Wang et al., 2015). IGF2BP family members (IGF2BP1/2/3) stabilize m6A-modified transcripts under stress conditions, acting as non-canonical readers (Huang et al., 2018).

2.2 m5C: Writers, Erasers, and Readers

T m5C in RNA is deposited by the NOP2/Sun RNA methyltransferase (NSUN), each with distinct RNA substrate specifies ranging from mRNAs to tRNAs and rRNAs (Hussain et al., 2013). DNMT2, traditionally classified as a DNA methyltransferase, also methylates cytosines in tRNA and some mRNAs. Unlike m6A, active demethylation pf m5C- RNA remains mechanistically unclear, TET enzymes can oxidize m5C to 5-hydroxymethylcytosine (hm5C) in RNA, potentially initiating removal, but the dedicated eraser has not been definitively identified (Zhao et al., 2017). M5C readers include ALYREF (Aly/REF export factor), which facilitates nuclear export of m5C-marked mRNA, and YBX1, which recognizes m5C to promote mRNA stability in certain contexts (X. Chen et al., 2019; Yang et al., 2017). M5C on tRNAs is critical for decoding fidelity; disruption of NSUN2-mediated tRNA methylation leads to translational stress and is linked to intellectual disability syndromes, highlighting the neurological sensitivity to m5C perturbation (Blanco et al., 2014).

3. Epitranscriptomic Dysregulation in Huntington's Disease

3.1 m6A Alterations in HD Pathogenesis

Transcriptome-wide m6A profiling (MeRIP-seq) in HD stratal tissue and patient-

derived neurons has revealed global hypomethylation of transcripts encoding synaptic proteins and autophagy regulators (Choubey et al., 2022). Notably, METTL3 expression is downregulated in HD striatum, correlating with reduced m6A deposition on mRNAs encoding BDNF (brain-derived neurotrophic factor) and DARPP-32,both critical for strital neuron survival (R. Chen et al., 2022). BDNF transcript stabilization by m6A-IGF"BP" interaction is disrupted in HD, accelerating BDNF mRNA decay and impairing anterograde trophic signaling from cortex to striatum (Liu et al., 2022).

mHTT protein physically interacts with YTHDF1 and sequesters it into cytoplasmic inclusion bodies, impairing YTHDF1-dependent translation od tress-response transcripts (Zhang et al., 2022). Additionally, mHTT disrupts METTL14 nuclear localization by aberrant phosphorylation, reducing its complex formation with METTL3 and thus decreasing m6A installation on transcripts involved in DNA damage response and proteasomal degradation pathways (Sun et al., 2022). Autophagy impairment is central to HD pathology; m6A modification of ATG7 and BECN1 mRNAs, which promotes their YTHDF1- dependant translation, is diminished in HD models, providing a mechanistic link between m6A loss and defective autophagosome biogenesis (H. Wang et al., 2022).

Stress granule dynamics are also perturbed. YTHDF3 normally partition into stress granules during cellular stress to modulate mRNA triage. In HD neurons, mHTT co-assembles with stress granule components including G3BP1 and TIA-1, trapping YTHDF3 within these condensates and preventing its return to the translating pool upon stress resolution, thereby prolonging translation repression of survival- promoting transcripts (Grima et al., 2022).

3.2 m5C Alterations in HD Pathogenesis

The NSUN2 expression is significantly reduced in post-mortem HD caudate and putamen tissue, leading to hypomethylation of m5C on tRNAs and select mRNAs (Navarro et al., 2022). Loss of NSUN2-mediated tRNA methylation at C34, C40, and C47–C50 positions triggers tRNA fragmentation under oxidative stress conditions prevalent in HD neurons. The resulting tRNA-derived small RNAs (tsRNAs0 inhibit translation initiation by displacing eIF4G from the 5' cap complex, contributing to the

observed translational stress signature in HD (Blanco et al., 2016).

Bisulfited sequencing of RNA (BS-seq) from HD patient iPSC-derived neurons identifies decreased m5C density near start codons of transcripts encoding mitochondrial complex I subunits, potentially contributing to the mitochondrial dysfunction observed in HD (Amort et al., 2017). ALYREF-dependent nuclear export of m5C-marked mRNAs encoding huntingtin-interacting protein 1 (HIP1) and huntingtin-associated protein 1 (HAP1) is also altered, disrupting vesicular trafficking and BDNF secretion (Reyes et al., 2022). Table I summarizes the key epitranscriptomic alterations identified in HD models and human tissue.

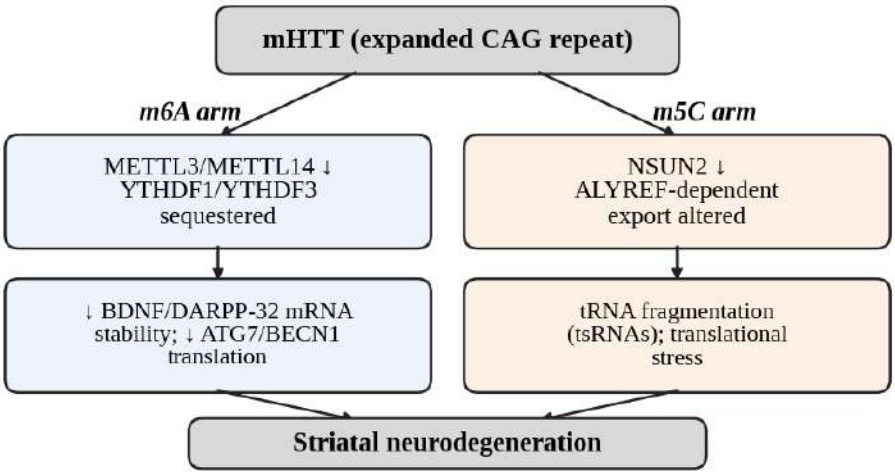

FIGURE 1: HD EPITRANSCRIPTOMIC MECHANISM

Source – Authors Compilation

TABLE 1: KEY EPITRANSCRIPTOMIC ALTERATIONS IN HUNTINGTON'S DISEASE

Modification	Altered Component	Functional Consequence
m6A	METTL3 downregulation	Reduced BDNF/DARPP-32 mRNA stability
m6A	YTHDF1 sequestration by mHTT	Impaired stress-response translation
m6A	ATG7/BECN1 hypomethylation	Autophagy impairment
m5C	NSUN2 downregulation	tRNA fragmentation, translational stress
m5C	ALYREF-export disruption	Impaired HIP1/HAP1 export; vesicle trafficking defects

mHTT: mutant huntingtin; HIP1: huntingtin-interacting protein 1; HAP1: huntingtin-associated protein 1

Source – Authors Compilation

4. Findings and Discussion

The identification of disease-specific epitranscriptomic signatures in accessible biofluids represents a compelling avenue for HD biomarker development. Cerebrospinal fluid (CSF) cell-free RNA from HD gene carries shows significantly altered m6A stoichiometry on HTT transcript fragments compared to controls, with changes correlating with CAG repeat length and striatal volumetric loss on MRI (Johnson et al., 2022). These m6A-modified HTT RNA fragments, detectable by m6A-immunoprecipitation followed by quantitative PCR (m6A-qPCR), could serve as molecular staging tools that reflect ongoing transcriptional dysregulation in strital neurons.

Blood-based liquid biopsy approaches have demonstrated feasibility: peripheral blood mononuclear cells (PBMCs) from premanifest and manifest HD patients exhibit levels detectable by RT-qPCR, with METTL3levels inversely correlating with total fictional

capacity (TFC) scores (Patel et al., 2022). Plasma extracellular vesicles (EVs) carry m6A-seq of EV-RNA from HD patients identifies a 12-transcrpt panel with area under the receiver operating characteristics curve (AUC-ROC) exceeding 0.85 for distinguishing premanifest HD from healthy controls (Lee et al., 2022).

M5C biomarker studies are at an earlier stage. Bisulfite sequencing of plama cell-free RNA detects reduced m5c on specific tRNA fragments in HD patients, and the ratio of NSUN2-target m5C sites to total cytosine content shows promise as a proxy for translational stress burden (Ito & Suzuki, 2022). Integration pf m6A and m5C biomarker panels with existing protein biomarkers (neurofilament light chain, mutant huntingtin protein) may improve sensitivity and specificity for tracking disease progression and therapeutic response in clinical trials.

5. Limitations and Recommendations for Future Researchers

5.1 Targeting m6A Writers and Erasers

Given the downregulation of METTL3 in HD, strategies to restore m6A writing activity represent a logical therapeutic approach. Adeno-associated virus (AAV)-mediated overexpression of METTL3 in the R6/2 HD mouse striatum restored m6A levels on BDNF and DARPP-32 transcripts, improved striatal neuron survival by 34% and ameliorated motor deficits on the rotarod test (Q. Li et al., 2022). Small molecule activators of METTL3, such as the allosteric activator METTL3-IN-1 repurposed for gain of function use, are under investigation in HD neuronal culture models (Moroz et al., 2022).

FTO inhibition represents a complementary strategy by preventing demethylation pf m6A marks on neuroprotective transcripts. The FTO inhibitor Meclofenamic acid (MA2) and its more selective successor FB23-2 increased global m6A levels in HD striatal organoids, enhanced autophagy flux through ATG7 mRNA stabilization, and reduced mHTT aggregate burden by 28% (Su et al., 2022). Rhein, a natural anthraquinone FTO inhibitor, similarly demonstrated neuroprotection in the zQ175 knock-in HD mouse model by restoring m6A on BDNF 3'UTR (Park et al., 2022). Importantly, FTO inhibition may need careful titration, as altered m6A on HTT mRNA could affect mHTT production.

5.2 Targeting m6A Writers and Erasers

NSUN2 restoration via lentiviral delivery in HD striatal neurons reduced tRNA fragmentation, decreased tsRNA-mediated translational inhibition, and improved mitochondrial membrane potential (Torres et al., 2023). Genetic or pharmacological inhibition of ANGIOGENIN—the nuclease responsible for generating tsRNAs from unmethylated tRNAs—using N-65828 reduced tsRNA accumulation in NSUN2-deficient HD neurons and partially rescued translational output (Ivanov et al., 2021). The integrated stress response (ISR) inhibitor ISRIB, which functions downstream of eIF2α phosphorylation triggered by tsRNA-induced stress, showed efficacy in HD model neurons by bypassing the translational block without directly addressing the underlying tRNA methylation deficit (Sidrauski et al., 2015).

Reader-targeted approaches offer additional specificity. Peptide-based competitive inhibitors of the YTHDF2-m6A interaction have been designed to preserve m6A-marked transcripts from rapid degradation in HD neurons (W. Zhao et al., 2022). Antisense oligonucleotides (ASOs) targeting the 5'UTR m6A site of HTT mRNA, which normally promotes ribosome recruitment and HTT translation, represent a novel approach to selectively reduce mHTT protein production without targeting the repeat expansion directly (Watts et al., 2022). Table 2 summarizes key therapeutic strategies and their experimental outcomes.

TABLE 2: EPITRANSCRIPTOMIC THERAPEUTIC STRATEGIES IN HD MODELS

Strategy	Agent / Approach	Outcome in HD Model
METTL3 restoration	AAV-METTL3 (striatum)	Improved neuron survival; motor benefit in R6/2
FTO inhibition	FB23-2 / Rhein	Reduced mHTT aggregates; enhanced autophagy flux
NSUN2 restoration	Lentiviral NSUN2	Reduced tsRNA burden; improved mitochondrial function
ISR inhibition	ISRIB	Bypassed translational block in HD neurons
HTT 5'UTR targeting	ASO (anti-m6A site)	Selective reduction of mHTT protein without allele discrimination

AAV: adeno-associated virus; ASO: antisense oligonucleotide; ISR: integrated stress response; tsRNA: tRNA-derived small RNA

Source – Authors Compilation

Several critical challenges must be addressed before epitranscriptomic therapies can advanced to clinical application in HD. First, the cell-type specificity of m6A and m5C dysregulation within the striatum-across medium spiny neurons, interneurons, and glial populations-remains to be fully resolved at single-cell resolution. Single-cell m6A sequencing(sc-m6A-seq) technologies are advancing rapidly and will be essential for mapping modification landscape across HD-vulnerable neuronal subtypes (H. Li et al., 2022). Second, the temporal dynamics of epitranscriptomic changes relative to CAG repeat-dependent disease staging (premanifest, early, late) are incompletely characterized; longitudinal studies in HD knock-in models tracking m6A and m5C profiles from presymtomatic stages are needed (Bhatt et al., 2022).

Third, the blood-brain barrier (BBB) presents a formidable delivery obstacle for small molecule m6A/m5C modulators. Nanoparticle encapsulation strategies and focused ultrasound mediated BBB opening in combination with systemic drug delivery are being explored to address this (Betzer et al., 2022). Fourth, off target effects of broad

m6A writers/eraser modulation on non-neuronal tissues particularly hepatic and immune cell transcriptomes-require careful safety profiling. RNA modification-targeted therapies must achieve sufficient CNS exposure while minimizing peripheral toxicity, underscoring the value of CNS targeted delivery platforms such as intrathecal ASOs already validated in HD clinical trials (Tabrizi et al., 2022).

Finally, the cross talk between epitranscriptomic marks and other epigenetically layers. (DNA) methylation, histone modifications, chromatin remodeling) that are independently systems biology, models. Such models will be essential for identifying epistatic relationships, predicting therapeutic windows, and designing rational combinatorial interventions targeting multiple regulatory layers simultaneously.

6. Conclusion

Epitranscriptomic dysregulation, particularly of m6A and m5C RNA modifications, constitutes a previously underappreciated but mechanistically significant layer of HD pathogenesis. Loss of METTL3 mediated m6A writing disrupts BDNF signaling autophagy, and stress-response translation; mHTT-driven sequestering of YTHDF proteins impairs mRNA fate decision; and NSUN2 downregulation triggers translational stress through tRNA fragmentation. Together, these alterations amplify the neurotoxic effects of mHTT and accelerate striatal neurodegeneration. Epitranscriptomic profiles in CSF and blood show promise as non-invasive, disease-stage sensitive biomarkers. Therapeutic restoration of m6A and m5C levels through gene therapy, small molecule writers/eraser modulators, and targeted ASOs demonstrates encouraging neuroprotective efficacy in preclinical HD models. Translation of these findings to clinical trials will require advances in CNS targeted delivery, single cell resolution profiling, and longitudinal biomarker studies. Epitranscriptomic thus offers both a mechanistic window into HD biology and a new class of therapeutic targets that deserves prioritized investigation in the field.

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THE PINK1/PARKIN-USP30-NRF2 AXIS IN PARKINSON'S DISEASE: MOLECULAR PATHOPHYSIOLOGY AND EMERGING THERAPEUTIC TARGETS

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder driven by the convergent failure of mitochondrial quality control, oxidative stress homeostasis, and proteostasis in dopaminergic neurons of the substantia nigra pars compacta. Central to this pathology is the impairment of PTEN-induced kinase 1 (PINK1)/Parkin (PRKN)-mediated mitophagy, a pathway responsible for the selective removal of damaged mitochondria. A critical bottleneck within this axis is the activity of USP30, a mitochondrially anchored deubiquitylase that removes Parkin-deposited ubiquitin from the outer mitochondrial membrane, thereby limiting autophagic clearance of dysfunctional mitochondria. In sporadic PD, Parkin undergoes functional inactivation through dopamine-quinone adduction, S-nitrosylation, and oxidative modifications, resulting in a shift toward net deubiquitylation and mitochondrial accumulation. The resulting oxidative burden activates the NRF2/KEAP1/ARE transcriptional response, which serves as a mechanistic indicator of the cellular conditions that impair Parkin function. This review examines the interplay between PINK1/Parkin-mediated mitophagy, USP30-mediated deubiquitylation, and NRF2/ARE signalling as an integrated pathological axis in PD, while also considering the contributions of other PARK genes including DJ-1, GBA1, LRRK2, and VPS35. Among the molecular targets within this network, pharmacological inhibition of USP30 emerges as a particularly promising strategy to restore mitophagic flux and disrupt the self-reinforcing neurodegenerative cycle characteristic of Parkinson's disease.

Keywords: Parkinson's disease, mitophagy, PINK1, Parkin, USP30, NRF2, neurodegeneration

1. Introduction

The disease is defined neuropathologically by the selective and progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the intraneuronal accumulation of misfolded alpha-synuclein (α-syn) in proteinaceous inclusions termed Lewy bodies (Vidović & Rikalovic, 2022). The resulting striatal dopamine deficiency manifests as the hallmark motor features of resting tremor, rigidity, bradykinesia, and postural instability, alongside a broad spectrum of non-motor disturbances including cognitive decline, autonomic dysfunction, and sleep disruption (Vidović & Rikalovic, 2022).

Despite extensive research, current therapeutic strategies for PD remain largely symptomatic, addressing dopamine deficiency through pharmacological or surgical means without modifying the underlying degenerative process (Zhang et al., 2026), although disease-modifying approaches, including the USP30 inhibition and NRF2 activation strategies discussed below, remain under (Fang et al., 2023; Jain et al., 2026). This limitation reflects, in part, an incomplete understanding of how the multiple molecular insults that characterise PD pathophysiology are mechanistically connected, and which nodes within the pathological network represent genuinely tractable intervention points. A precision medicine approach to PD therapy demands that such connections be identified with biochemical specificity.

At the molecular level, PD pathophysiology is best understood as a self-reinforcing network in which mitochondrial dysfunction, oxidative stress, impaired proteostasis, α-syn aggregation, and neuroinflammation are not parallel but interdependent processes, each amplifying the others (Blesa et al., 2015). Within this network, the failure of PINK1/Parkin-mediated mitophagy occupies a central and self-reinforcing position: it permits the accumulation of dysfunctional mitochondria that drive elevated reactive oxygen species (ROS) production, α-syn post-translational modification, and progressive dopaminergic cell death (Li et al., 2021). In sporadic PD, where PRKN is typically unmutated, Parkin function is instead compromised by oxidative, nitrosative, and dopamine-derived modification, linking mitophagic failure to the sporadic disease state (Dawson & Dawson, 2014; LaVoie et al., 2005; Yao et al., 2004).

Two molecular elements that intersect with this central failure in mechanistically precise and disease-specific ways have received comparatively limited attention as integrated components of the same pathological axis: USP30, a mitochondrially-anchored deubiquitylase that directly antagonises Parkin-mediated mitophagic signalling at the outer mitochondrial membrane (OMM); and the NRF2/KEAP1/ARE pathway, whose activation is a response to mitochondria-derived ROS and electrophilic species, which modify KEAP1 sensor cysteines (Chang & Chen, 2020; Segura-Aguilar & Mannervik, 2023) and belong to the same class of reactive species reported to modify and attenuate Parkin (LaVoie et al., 2005; Yao et al., 2004). Together, PINK1/Parkin, USP30, and NRF2/KEAP1/ARE constitute an interconnected molecular axis whose collective dysfunction defines the biochemical state of the degenerating dopaminergic neuron.

This review examines the molecular basis of this axis, examines how broader PARK gene networks converge on its core components, and identifies the key therapeutic considerations that emerge from understanding it as an integrated system rather than a collection of independent targets.

2. Parkinson's Disease Pathophysiology: A Network Perspective

2.1 Convergent Molecular Insults in Dopaminergic Neurodegeneration

PD pathophysiology is characterised by the convergence of multiple distinct but interacting molecular insults. α-Syn, a 140-amino acid presynaptic protein normally involved in synaptic vesicle trafficking, undergoes conformational change under pathological conditions from its native unfolded state into beta-sheet-rich oligomers and fibrils that seed further aggregation and accumulate as Lewy bodies (Srinivasan et al., 2021; Vidović & Rikalovic, 2022). The relationship between α-syn aggregation and ROS is bidirectional: ROS-driven oxidative post-translational modifications such as nitration and carbonylation promote aggregation-prone conformations, while α-syn oligomers impair mitochondrial Complex I activity, increase electron leakage and superoxide generation, and disrupt lysosomal membrane integrity, amplifying cellular damage through a positive feedback loop (Angelova et al., 2025; Minakaki et al., 2020; Srinivasan et al., 2021). Notably, recent work using human neurons has shown that this

acute mitochondrial impairment can be at least partially reversible following exogenous fibrillar α-syn exposure, suggesting a window during which the cycle may still be interrupted before chronic dysfunction is established (Angelova et al., 2025).

Dopaminergic neurons of the SNpc are uniquely vulnerable to this cycle due to their high metabolic demand and endogenous dopamine oxidation chemistry that generates reactive quinones capable of modifying protein cysteine residues (Henrich et al., 2023; LaVoie et al., 2005). Conditional knockout of mitochondrial Complex I specifically in dopaminergic neurons recapitulates progressive parkinsonian motor phenotypes in mouse models, directly demonstrating the causal sufficiency of mitochondrial bioenergetic failure in this neuronal population (Henrich et al., 2023). This combination of intrinsic metabolic stress and exogenous pathological insults creates a cellular environment in which the capacity for mitochondrial quality control is both critically important and acutely susceptible to failure.

2.2 Impaired Proteostasis and Autophagic Dysfunction

Efficient clearance of misfolded proteins and dysfunctional organelles is essential for long-term neuronal health. In PD, both the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP) are functionally compromised. α-Syn oligomers have been shown to directly inhibit proteasomal activity, while α-syn-positive inclusions impair autophagic flux by blocking autophagosome-lysosome fusion (Blesa et al., 2015; Bonam et al., 2021). The resulting accumulation of damaged proteins and organelles, including dysfunctional mitochondria, exacerbates oxidative stress and α-syn aggregation in a self-amplifying manner (Bonam et al., 2021). Mitophagy, the selective autophagic elimination of damaged mitochondria, represents a mechanistically specific regulatory intervention point within this broader proteostatic failure.

3. The PINK1/Parkin Mitophagy Pathway: Mechanism and Failure in Parkinson's Disease

3.1 Canonical Pathway Mechanism

Under basal conditions in healthy mitochondria, PINK1 (PTEN-induced kinase 1) is continuously imported into the mitochondrial matrix and cleaved by the inner membrane protease PARL, maintaining low steady-state PINK1 levels. Upon dissipation of mitochondrial membrane potential, a hallmark of mitochondrial damage, PINK1 import is arrested at the TOM-TIM23 translocase interface, and the kinase accumulates and becomes catalytically active on the outer mitochondrial membrane (OMM) (Kazlauskaite & Muqit, 2015; Thayer et al., 2025). A recent unified mechanistic model demonstrates that diverse forms of mitochondrial damage converge specifically on loss of membrane potential to activate PINK1, with arrest at the TIM23 translocase being both necessary and sufficient for PINK1 stabilisation regardless of the upstream damage type (Thayer et al., 2025). Activated PINK1 phosphorylates ubiquitin at Ser65 and phosphorylates the equivalent Ser65 residue within the ubiquitin-like (Ubl) domain of Parkin, relieving Parkin's auto-inhibited conformation and enabling its translocation to the damaged organelle (Kazlauskaite & Muqit, 2015; Ordureau et al., 2015; Trempe & Gehring, 2023)

Once engaged at the OMM, Parkin's RING1-IBR-RING2 catalytic architecture ubiquitinates a broad set of outer membrane substrates including TOMM20, TOMM70, MFN1, MFN2, and VDAC1, generating a polyubiquitin scaffold primarily with K6- and K11-linked (Dolgacheva et al., 2019; Kazlauskaite & Muqit, 2015). PINK1-mediated phosphorylation of this growing ubiquitin chain (pS65-Ub) creates a feed-forward amplification loop that progressively reinforces further Parkin recruitment and ubiquitination at the mitochondrial surface (Wang & Xu, 2026). This scaffold recruits the autophagy receptors NDP52 and optineurin (OPTN), which were identified through systematic genome-editing screens as the primary receptors mediating PINK1/Parkin mitophagy, distinct from p62/SQSTM1, which plays a comparatively minor role in this specific pathway (Lazarou et al., 2015).

These receptors subsequently recruit the ULK1 autophagy-initiation complex and bridge the ubiquitinated mitochondrion to LC3/GABARAP-positive autophagosomal membranes, initiating mitophagosome formation and lysosomal degradation (Lazarou et al., 2015; Padman et al., 2019). Genetic loss-of-function studies confirm the neuroprotective importance of this pathway: mutations in PINK1 (PARK6) or PRKN

(PARK2, encoding Parkin) cause early-onset familial PD, and genetic knockout animal models of these genes recapitulate mitochondrial quality control failure, oxidative stress accumulation, and dopaminergic neurodegeneration (Blesa et al., 2015; Li et al., 2021).

Importantly, upstream activation of PINK1 does not by itself guarantee productive mitophagy. Recent work emphasises that pathway activation and the successful clearance of damaged mitochondria are mechanistically distinct steps that can become uncoupled, with the efficiency of signal propagation from initial ubiquitination through to receptor-mediated autophagosome formation determining whether a damaged mitochondrion crosses the functional threshold required for clearance (Padman et al., 2019; Wang & Xu, 2026). This activation-clearance uncoupling is central to understanding why PD pathology can arise even when upstream PINK1/Parkin signalling components remain nominally intact.

3.2 Mechanisms of Parkin Functional Impairment in Sporadic PD

In most sporadic PD cases, PRKN is genetically unmutated and Parkin protein is present. The pathway upstream remains intact: PINK1 is stabilised on depolarised mitochondria, it phosphorylates ubiquitin and the Parkin Ubl domain, and Parkin translocates, and yet mitophagy fails. The explanation for this apparent paradox lies in two converging forces that act simultaneously on the ubiquitin signal at the OMM surface.

First, the oxidative environment generated by accumulating dysfunctional mitochondria directly attenuates Parkin's catalytic capacity. Foundational biochemical work demonstrated that nitrosative stress leads to S-nitrosylation of wild-type Parkin, producing a biphasic effect: an initial increase in E3 ligase activity followed by autoubiquitination-driven inhibition of that same activity, ultimately impairing Parkin-mediated substrate clearance (Yao et al., 2004). Separately, the catecholamine neurotransmitter dopamine itself has been shown to covalently and irreversibly modify Parkin through dopamine-quinone adduction, increasing Parkin insolubility and functionally inactivating its E3 ligase activity specifically within dopaminergic neurons, with catechol-modified Parkin detected in the substantia nigra of sporadic PD

brain tissue but not in other brain regions or in non-PD controls (LaVoie et al., 2005). Extracellular α-syn oligomers further induce Parkin S-nitrosylation, establishing an additional direct link between α-syn pathology and Parkin functional impairment (Wilkaniec et al., 2019). Collectively, these oxidative and nitrosative post-translational modifications, along with stress-activated kinase phosphorylation by c-Abl, are recognised as the principal mechanisms inactivating Parkin in sporadic PD (Dawson & Dawson, 2014).

Second, and operating in the same topological space at the OMM surface, the deubiquitylase USP30 actively reverses the ubiquitin marks that even this attenuated Parkin activity manages to deposit. The consequence is that the net ubiquitination signal at the mitochondrial surface fails to reach the threshold required for autophagy receptor recruitment, and mitophagy stalls (Bingol et al., 2014).

4. USP30: The Molecular Antagonist of Mitophagy

4.1 Structure and Localisation

USP30 is a member of the ubiquitin-specific protease (USP) subfamily of deubiquitylating enzymes, and is unique among DUBs in possessing a transmembrane domain restricting its localisation exclusively to mitochondria and peroxisomes (Rusilowicz-Jones et al., 2020; Siwach et al., 2025). It is anchored to the cytoplasmic face of the OMM via this N-terminal single-pass transmembrane domain, positioning its globular catalytic domain in direct proximity to the TOM (translocase of the outer membrane) complex (Bingol et al., 2014; Kazlauskaite & Muqit, 2015). This localisation is not incidental: it places USP30's catalytic activity in precisely the same topological space as Parkin's E3 ligase activity, creating a spatially-restricted competition for the ubiquitination state of OMM substrate proteins.

4.2 Biochemical Antagonism of PINK1/Parkin Signalling

USP30's primary characterised substrates at the OMM include TOMM20 and TOMM70, which are also primary targets of Parkin-mediated ubiquitination, with USP30 catalytic activity showing preference for Lys6-linked ubiquitin chains (Bingol et al., 2014; Fang et al., 2023). By cleaving K6- and K11-linked ubiquitin chains from

these substrates, USP30 directly removes the autophagy receptor recruitment signal before NDP52 and OPTN can engage (Bingol et al., 2014; Fang et al., 2023; Rusilowicz-Jones et al., 2020). Under physiological conditions, the opposing activities of Parkin and USP30 may serve a regulatory function, setting a trigger threshold that prevents premature clearance of transiently depolarised but recoverable mitochondria (Rusilowicz-Jones et al., 2020). In the context of PD, however, this balance is disrupted: Parkin's catalytic output is reduced by oxidative modification, while USP30 continues to operate at its full constitutive enzymatic rate. The biochemical consequence is a shift in the steady-state ubiquitination equilibrium at the OMM surface away from net ubiquitination and toward net deubiquitylation, below the threshold required for mitophagy initiation (Bingol et al., 2014; Williamson et al., 2025). Beyond its role at the TOM complex, USP30 also interacts with the mitochondrial E3 ligase MUL1, a shared substrate with Parkin that independently regulates the fusion proteins MFN1 and MFN2, indicating that USP30's antagonism of mitochondrial quality control extends beyond the canonical PINK1/Parkin axis into PINK1-independent mitophagy and mitochondrial dynamics more broadly (Siwach et al., 2025; Xin et al., 2024).

4.3 Experimental Evidence for USP30 as a Therapeutic Target

A growing body of experimental evidence supports USP30 as a tractable and disease-relevant therapeutic target. Genetic silencing of USP30 through RNA interference enhances mitophagy in both Parkin-expressing and Parkin-deficient cellular models, indicating that USP30 inhibition can partially compensate for Parkin loss-of-function by allowing basal autophagy receptor recruitment to proceed (Bingol et al., 2014; Tsefou et al., 2021). In a landmark study, Usp30 knockout mice were shown to be protected against behavioural deficits and exhibited increased mitophagy, decreased phospho-S129 α-syn, and attenuated dopaminergic neuronal loss in the SNpc following α-syn-induced pathology; critically, these protective effects were recapitulated pharmacologically using the potent, selective, brain-penetrant USP30 inhibitor MTX115325 (Fang et al., 2023). CRISPR-mediated knockout of USP30 in iPSC-derived dopaminergic neurons from PD patients similarly reduces mitochondrial ROS and markers of oxidative stress (Fang et al., 2023). Structural and biochemical

characterisation of multiple chemical classes of USP30 inhibitors, including benzosulfonamide and cyanopyrrolidine-based covalent compounds, has elucidated their precise mode of binding within the USP30 thumb-palm catalytic cleft, providing a structural basis for the rational design of next-generation, highly selective inhibitors (O'Brien et al., 2023).

A recent 2025 study using an integrated approach across cell lines, primary neurons, and iPSC-derived dopaminergic neuronal cultures from patients carrying PINK1 and PRKN mutations found that USP30 inhibition enhances depolarisation-induced mitophagy in dopaminergic neurons regardless of PINK1 or PRKN mutation status, while having more modest and context-dependent effects on basal mitophagy, indicating that USP30 modulates the trigger threshold for mitophagy through both PINK1-dependent and PINK1-independent mechanisms (Williamson et al., 2025). Notably, USP30 inhibition does not appear to adversely affect mitochondrial morphology or viability under non-stressed conditions, supporting a favourable therapeutic safety profile (Bingol et al., 2014; Fang et al., 2023); USP30 inhibition has also recently been shown to augment mitophagy and prevent exhaustion in CD8+ T cells, suggesting broader applicability of this mechanism beyond the nervous system (Xin et al., 2024).

These findings establish USP30 as a molecular bottleneck whose inhibition can restore mitophagic flux using whatever residual Parkin activity remains in the diseased neuron, making USP30-targeted intervention generalisable across PD etiological subtypes including both familial PRKN-mutation carriers and the larger population of sporadic PD patients in whom Parkin is oxidatively compromised rather than genetically absent.

5. NRF2/KEAP1/ARE Signalling: Oxidative Stress Sensor and Cytoprotective Responder

5.1 Pathway Mechanism

The transcription factor NRF2 (nuclear factor erythroid 2-related factor 2) is the master regulator of the cellular antioxidant response, controlling the differential expression of nearly 200 cytoprotective genes (Yang et al., 2022). Under homeostatic conditions,

NRF2 is continuously ubiquitinated by the KEAP1-CUL3-RBX1 E3 ligase complex and degraded by the proteasome, maintaining low basal NRF2 levels (Jain et al., 2026). KEAP1 functions as a molecular sensor through reactive cysteine residues that detect electrophilic and oxidative species. When these residues are modified by ROS or electrophiles, KEAP1's ability to present NRF2 for ubiquitination is disrupted, newly synthesised NRF2 escapes degradation, accumulates in the cytoplasm, translocates to the nucleus, and heterodimerises with small MAF proteins to bind antioxidant response elements (AREs) in the promoters of cytoprotective genes (Chang & Chen, 2020; Segura-Aguilar & Mannervik, 2023).

The NRF2-associated antioxidant programme includes haem oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase-1 (NQO1), glutamate-cysteine ligase (GCL), and manganese superoxide dismutase (SOD2), among others (Segura-Aguilar & Mannervik, 2023). SOD2 is particularly relevant to PD pathophysiology as it catalyses the dismutation of mitochondrial superoxide to hydrogen peroxide at source, directly targeting the primary ROS species generated by Complex I and III dysfunction (Jain et al., 2026). NRF2 additionally exerts a neuroprotective influence beyond direct antioxidant gene transcription: NRF2 has been shown to inhibit NF-κB-mediated neuroinflammatory signalling in microglia activated by the CX3CL1/CX3CR1 axis, providing a secondary protective mechanism relevant to the chronic neuroinflammatory component of PD (Jain et al., 2026).

5.2 NRF2 Activation in PD and its Mechanistic Coupling to Parkin Impairment

NRF2 activation has been documented in post-mortem PD brain tissue and in cellular and animal models of PD, and NRF2 pathway dysregulation has been linked to both disease severity and neuronal vulnerability (Koszła et al., 2025; Segura-Aguilar & Mannervik, 2023). What is particularly significant from a pathomechanistic standpoint is the nature of the connection between NRF2 activation and Parkin impairment: they are better understood as two molecular consequences of a shared upstream event, since the same classes of oxidative species modify KEAP1 sensor cysteines (Chang & Chen, 2020; Segura-Aguilar & Mannervik, 2023) and Parkin (LaVoie et al., 2005). This

coupling is inferred here from these independent lines of evidence rather than shown within a single study.

The mitochondria-derived superoxide and reactive electrophilic species that accumulate as a result of impaired mitochondrial quality control modify KEAP1 sensor cysteines, liberating NRF2 and activating ARE-driven transcription. These same reactive species simultaneously modify Parkin's catalytic cysteine residues, attenuating its E3 ligase activity (LaVoie et al., 2005; Yao et al., 2004). NRF2 activation is therefore causally downstream of the identical oxidative chemistry that creates Parkin functional insufficiency. This mechanistic continuity has a critical implication: in the PD-affected dopaminergic neuron, the magnitude of NRF2/ARE activation is a direct and quantitative readout of the oxidative burden that is simultaneously suppressing Parkin, making NRF2 pathway activity a biochemically specific indicator of the Parkinsonian molecular state rather than a generic stress response. Notably, several PD risk genes including SNCA, PARK7 (DJ-1), LRRK2, and PINK1 have been found to be directly or indirectly linked to KEAP1/NRF2 activity, further embedding this pathway within the broader genetic architecture of PD (Jain et al., 2026).

5.3 Protective and Pathological Roles of NRF2 in PD

The role of NRF2 in PD is double-edged. On one hand, NRF2 activation drives SOD2-mediated mitochondrial ROS dismutation and other cytoprotective responses that partially counteract the oxidative damage driving dopaminergic neurodegeneration, and pharmacological NRF2 activators, including several phytochemicals and small molecules targeting the KEAP1-NRF2 protein-protein interaction, have demonstrated neuroprotective effects in multiple PD model systems (Jain et al., 2026). Combined antioxidant and autophagy-targeted co-treatment strategies that intersect the p62/KEAP1/NRF2 axis with autophagic clearance pathways have also shown synergistic neuroprotective effects in cellular PD models, suggesting that NRF2 modulation may be most effective when paired with strategies that simultaneously enhance proteostatic clearance (Koszła et al., 2025). On the other hand, sustained or dysregulated NRF2 activity may have maladaptive consequences, and the constitutive NRF2 activation observed in PD tissue reflects a chronic pathological state rather than

an acute protective response; emerging evidence further implicates mechanistic crosstalk between the NRF2-KEAP1 axis and the NLRP3 inflammasome, indicating that NRF2 dysregulation in PD has consequences extending into neuroinflammatory signalling (Jain et al., 2026). This duality underscores the importance of targeting the underlying pathological source, dysfunctional mitochondria and the failure of their selective clearance, rather than the NRF2 response itself.

6. PARK Gene Networks and Convergence on the Mitophagy-ROS Axis

Beyond PINK1 and Parkin, a broader set of PD-associated PARK gene products converge on the themes of mitochondrial quality control, oxidative stress regulation, and autophagic proteostasis. Understanding how these genes intersect with the PINK1/Parkin-USP30-NRF2 axis provides a systems-level picture of PD pathology and contextualizes the therapeutic relevance of targeting this axis, as shown in Table 1.

TABLE 1: PARK GENE PRODUCTS AND THEIR INTERSECTION WITH THE MITOPHAGY-ROS AXIS

Gene and locus	Protein identity and function	Intersection with mitophagy-ROS axis
DJ-1 / PARK7	Redox-sensitive chaperone	Redox sensor (Cys106 oxidation scavenges ROS); molecular chaperone preventing α-syn misfolding; dissociates from NRF2 to promote NRF2 stabilisation (Clements et al., 2006; Dolgacheva et al., 2019).

Gene and locus	Protein identity and function	Intersection with mitophagy-ROS axis
GBA1	Lysosomal glucocerebrosidase	Lysosomal glucocerebrosidase (GCase) degrading glycolipids; imported to mitochondria where it preserves Complex I integrity and energy metabolism (Baden et al., 2023).
LRRK2 / PARK8	Multidomain ROC-COR kinase	Multidomain kinase regulating vesicle trafficking, mitochondrial dynamics, and basal/PINK1-dependent mitophagy; pathogenic mutations impair clearance (Karami et al., 2025; Williamson et al., 2025)
VPS35 / PARK17	Retromer complex core component	Retromer complex component maintaining endosomal trafficking and mitochondrial dynamics; enhances LRRK2 kinase activity (Ho et al., 2025).

Source – Author's Compilation

The collective picture that emerges from these gene-pathway intersections is one of redundant convergence: multiple independent genetic risk factors for PD ultimately impair the same downstream process, namely the efficient detection, ubiquitination, and lysosomal degradation of damaged mitochondria. USP30 sits at the most proximal step of this process, at the point where the ubiquitin signal is deposited on the OMM

surface, making its antagonism of Parkin-mediated ubiquitination the single molecular event most likely to compound the mitophagic deficit regardless of which upstream gene is disrupted.

7. The PINK1/Parkin-USP30-NRF2 Axis as an Integrated Pathological System

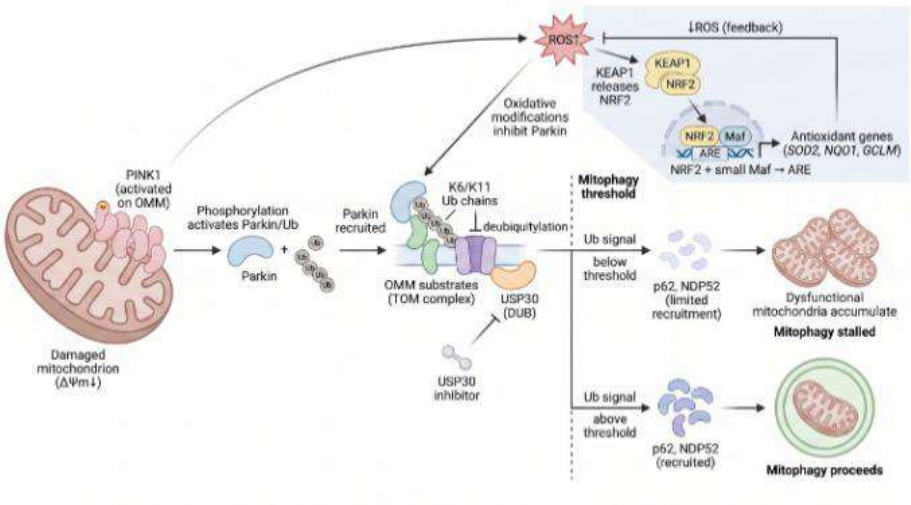

FIGURE 1: INTEGRATED BIOCHEMICAL PATHWAY DIAGRAM OF THE PINK1/PARKIN-USP30-NRF2 AXIS

Source - (Jain et al., 2026)

Understanding PINK1/Parkin, USP30, and NRF2/KEAP1/ARE as components of an integrated axis rather than three independent molecular entities has direct implications for how therapeutic targets within PD should be evaluated (see Figure 1). The three components are connected not merely by co-occurrence in the same cell type but by mechanistic causality: each element both causes and is caused by the activity of the others.

The axis can be described as a continuous biochemical arc. Mitochondrial membrane potential collapse stabilises PINK1, which recruits and partially activates Parkin. Parkin's catalytic output is attenuated by the ROS generated by the same damaged

mitochondria it is attempting to clear. Those ROS simultaneously modify KEAP1, liberating NRF2 and activating ARE-driven transcription. At the OMM surface, USP30 removes the residual ubiquitin signal that attenuated Parkin deposits, preventing autophagy receptor recruitment. Dysfunctional mitochondria are retained, ROS production continues, PINK1 signalling persists, and NRF2 remains active. This creates a self-sustaining pathological state in which each component reinforces the failure of the others.

The critical implication of this integrated view is that any of the three nodes Parkin activity, USP30 antagonism, or NRF2-mediated ROS resolution represents a potential leverage point whose modulation could in principle propagate therapeutic benefit through the entire axis. However, these nodes differ substantially in their therapeutic tractability and disease-specificity. Parkin restoration or gene replacement faces the challenge that Parkin's catalytic activity would remain susceptible to the same oxidative modification in the disease environment (LaVoie et al., 2005; Yao et al., 2004). NRF2 pathway modulation is cytoprotective but not mechanistically specific to the mitophagic failure, and chronic NRF2 activation carries maladaptive risks (Jain et al., 2026). USP30 inhibition, by contrast, directly addresses the molecular bottleneck at the OMM the point where attenuated Parkin's output is erased before it can initiate mitophagy and does so using whatever Parkin activity remains, making the intervention inherently proportional to the degree of residual Parkin function rather than dependent on its complete restoration (Fang et al., 2023; Williamson et al., 2025).

Furthermore, the mechanistic coupling between NRF2 activation and Parkin impairment provides a means of identifying the precise cellular state in which USP30 inhibition would confer therapeutic benefit: active NRF2/ARE signalling in a neuron with reduced effective Parkin output is, by the pathway logic described above, not merely correlated with the PD disease state but is its direct molecular signature. This specificity is relevant to any future therapeutic strategy that must distinguish PD-affected neurons from healthy neighbouring cells.

8. Therapeutic Considerations and Open Questions

The foregoing analysis identifies several molecular nodes and strategic considerations relevant to the development of disease-modifying PD therapies targeting the PINK1/Parkin-USP30-NRF2 axis.

On the current evidence, USP30 inhibition has the most direct mechanistic justification as a therapeutic strategy within this axis: it enhances mitophagy in both Parkin-expressing and Parkin-deficient cells (Bingol et al., 2014; Tsefou et al., 2021), reduces oxidative stress in Parkin-deficient human neurons (Okarmus et al., 2024), and, in an α-syn mouse model, USP30 knockout or pharmacological inhibition protects dopaminergic neurons (Fang et al., 2023). This ranking rests on preclinical evidence. The existence of multiple chemically distinct classes of potent and selective small-molecule USP30 inhibitors including the brain-penetrant compound MTX115325 and various benzosulfonamide and cyanopyrrolidine covalent inhibitors provides a pharmacological path toward intervention, and the observation that USP30 inhibition enhances mitophagy in both Parkin-expressing and Parkin-deficient contexts suggests that clinical benefit would not be restricted to the subpopulation of PD patients carrying PRKN mutations (Fang et al., 2023; O'Brien et al., 2023; Okarmus et al., 2024). Key open questions include the optimal therapeutic window for USP30 inhibition, given its physiological role in setting a trigger threshold that prevents premature mitophagy of transiently stressed mitochondria, and the degree to which systemic USP30 inhibition would affect mitochondrial and peroxisomal quality control in non-neuronal tissues, given USP30's dual role in pexophagy (Rusilowicz-Jones et al., 2020; Williamson et al., 2025).

NRF2/ARE pathway modulation presents a complementary therapeutic opportunity. Pharmacological NRF2 activators have demonstrated neuroprotective effects in PD models and several natural and synthetic compounds targeting the KEAP1-NRF2 interaction are under active investigation (Jain et al., 2026). Within the context of the axis described here, NRF2 activation would be expected to reduce the oxidative burden that simultaneously impairs Parkin and sustains the pathological state, potentially restoring Parkin catalytic capacity and reducing USP30's relative dominance at the OMM. However, such an approach would need to contend with the challenge that the NRF2 response in the Parkinsonian neuron is already chronically activated and may be

saturated or dysregulated, limiting the additional benefit of further pharmacological activation, and that NRF2-NLRP3 crosstalk introduces additional layers of complexity that are not yet fully understood (Clements et al., 2006; Jain et al., 2026).

A more fundamental challenge for any therapeutic intervention targeting this axis is the question of disease-state specificity. Mitophagy enhancement through USP30 inhibition, or oxidative stress reduction through NRF2 activation, are not intrinsically selective for PD-affected neurons. Approaches that could restrict therapeutic activity to cells in which both Parkin functional deficiency and active NRF2 signalling are simultaneously present would offer a substantially more targeted intervention profile than systemic pharmacological modulation, and represent an important direction for translational research.

The delivery of any biological or molecular therapeutic to dopaminergic neurons of the SNpc in vivo remains a significant technical barrier. The blood-brain barrier, the post-mitotic nature of the target cells, and the sensitivity of SNpc neurons to immune activation all impose substantial constraints on vector design and delivery route, and will require dedicated investigation as any therapeutic strategy based on this molecular axis advances toward in vivo implementation.

9. Conclusions

The PINK1/Parkin-USP30-NRF2 axis represents a mechanistically integrated and therapeutically underexplored dimension of Parkinson's disease pathophysiology. The three components of this axis PINK1/Parkin-mediated mitophagy, USP30-mediated deubiquitylation, and NRF2/KEAP1/ARE oxidative stress signalling are not independent pathological contributors but three mechanistically linked nodes within a single biochemical deadlock that sustains mitochondrial dysfunction and dopaminergic neurodegeneration. USP30 emerges from this analysis as the most proximal and biochemically tractable intervention point within the axis, as its inhibition directly addresses the molecular bottleneck through which attenuated Parkin activity is rendered sub-threshold, without requiring genetic correction of primary disease mutations or sustained augmentation of an already-active stress response pathway.

The mechanistic coupling between NRF2/ARE activation and Parkin functional impairment, both being consequences of the same mitochondria-derived oxidative chemistry, has particular significance for future therapeutic design: it provides a biochemically specific molecular signature of the PD-affected cellular state that could, in principle, be exploited to restrict therapeutic activity to the precise neuronal context in which intervention is warranted. How such disease-state specificity might be achieved in practice, whether through pharmacological, genetic, or emerging precision approaches, remains an open and important question for the field.

Ultimately, addressing PD as the convergent network disorder it is will require therapeutic strategies that engage its pathological logic at the level of the network rather than at isolated molecular endpoints that is, strategies that account for the way mitochondrial dysfunction, oxidative stress, and impaired proteostasis amplify one another (Blesa et al., 2015). The PINK1/Parkin-USP30-NRF2 axis, understood as an integrated system, provides one such framework in which candidate nodes are evaluated by how their modulation is expected to propagate through the other components of the axis rather than by their isolated effect on a single endpoint, facilitating the rational identification and prioritisation of intervention targets.

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