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Rehabilitation of Hearing Function with Hearing Aids

Iurie NOROC^{1,2,*}, Sergiu VETRICEAN¹, Eusebiu SENCU^{1,2}, Victor OSMAN¹, Alexandru DIDENCU^{1,2}, Victor ENACHI^{1,2}, Xenia MOSCALU²

Abstract

Aerial conduction hearing aids (conventional) are the most commonly used devices for the rehabilitation of residual hearing loss after tympanoplasty. Patients with dry, safe ears with mild to moderate conductive hearing loss typically achieve excellent hearing rehabilitation with these devices. Although recent conventional hearing aids have improved technically, there are still shortcomings: distortion of sound and voice, irritation of the external ear canal, acoustic *feedback*, discomfort and exacerbation of existing conditions of the outer and middle ear.

Bone-anchored hearing aids are excellent devices that have proven their usefulness over time if candidates were properly selected and surgery was performed meticulously. In audiological terms, bone-anchored hearing devices are clearly superior to conventional aerial conduction devices and passive bone conduction devices. The major drawbacks of this hearing aid are the relatively high financial costs and the need for the implantation surgical procedure.

Cochlear implant is one of the most effective rehabilitation methods for sensorineural hearing loss, especially in cases where there is no functional effect with other hearing aids. The benefits of cochlear implantation, including for severe-profound hearing loss in children and adults, have been significant improvements in post-implant audiological test scores, cognitive domains, and quality of life, including long-term. Cochlear implants showed the greatest improvements in speech perception and production, clear speech recognition in noisy environments, and audiometry performance compared to conventional hearing aids.

Keywords: hearing loss, conventional aerial hearing aid, implantable hearing device, cochlear implant

¹Faculty of Medicine "Nicolae Testemițanu", University of Medicine and Pharmacy, Chișinău, Republic of Moldova

²"Timofei Moșneaga" Republican Clinical Hospital, Chișinău, Republic of Moldova

*Corresponding author:

Iurie NOROC, Department of Otorhinolaryngology of the Public Institution „Nicolae Testemitanu”, State University of Medicine and Pharmacy, Chisinau, Republic of Moldova
E-mail: iurie.noroc@gmail.com

INTRODUCTION

Hearing loss, also known as deafness, is a decrease in hearing acuity, which can affect one or both ears. It is a major public health problem, which frequently goes unnoticed or untreated, associated with a huge impact on mental health, social life, cognitive abilities, educational status and employment. Patients with hearing loss face a number of problems – reduced quality of life, negative outcomes on social interaction, independence, interpersonal relationships, communication and mental well-being. Hearing loss can lead to secondary problems such as learning difficulties, social stigma, depression, and early dementia, all of which affect quality of life^{1,2,3,4,5}.

There are three main types of hearing loss: conductive, sensorineural and mixed. Sensorineural hearing loss is the result of damage to the cochlear or vestibulo-cochlear nerve caused by infection and trauma. Conductive hearing loss is caused by physical or mechanical obstruction of air conduction that inhibits the transmission of sound waves from the outer/middle ear to the inner ear. Some causes of conductive hearing loss include trauma (perforated eardrum) or infections (otitis externa and acute or chronic otitis media). In the case of a combination of lesions in the outer or middle ear with lesions of the inner ear and/or the vestibulo-cochlear nerve and its neural pathways, mixed hearing loss occurs¹.

Hearing technology, in order to improve or enable auditory perception, is a key element of hearing rehabilitation. Hearing aids are electronic devices worn in the ear or behind the ear that amplify and transmit sound to the ear to improve hearing function. This is achieved through three basic components: a microphone, an amplifier and a speaker. Analog hearing aids capture sound energy, convert it into electrical signals that are amplified and transmitted through the ear canal to the eardrum. Digital hearing aids perform the same key function as analog hearing aids, but they can be programmed to suit individual audiological needs, allow for many additional features, and are generally the preferred option^{1,5}.

The bone-anchored hearing aid (BAHA) requires surgical implantation – a small vibrator is connected to a titanium screw (fixation) that is implanted behind the ear. These two components convert sound into a vibration via the screw, which then stimulates the cochlea. BAHA is used as an alternative to conventional hearing

aids for conductive or mixed hearing loss. The cochlear implant is another alternative electronic hearing device that is used when a conventional hearing aid cannot be used. The cochlear implant is used to bypass the lack or damage of the hair cells of the inner ear¹.

In the context of the above, **the purpose** of this article is the narrative synthesis of the latest data on different types of hearing rehabilitation and the effectiveness of hearing devices.

MATERIAL AND METHODS

In order to achieve the set objective, an initial search was carried out for specialized scientific publications, identified from the *databases of PubMed, Hinari (Health Internet Work Access to Research Initiative), SpringerLink, National Center of Biotechnology Information and Medline*. The article selection criteria included contemporary data on hearing aids by the following keywords: „hearing loss”, „unilateral hearing loss”, „conductive hearing loss” and „sensorineural hearing loss”, used in different combinations with the words „implant”, „conventional aerial hearing aid”, „implantable hearing device with bone conduction” and „cochlear implant” to maximize search yield.

For the advanced selection of bibliographic sources, the following filters have been applied: full-text articles, articles in English, articles published in the period 2000-2024. After a preliminary analysis of the titles, original articles, editorials, narrative synthesis, systematic and meta-analysis articles were selected, which contained relevant information and contemporary concepts regarding research and development activities and the effectiveness of hearing devices. In addition, a search was carried out in the bibliographic reference lists of the identified sources in order to highlight additional relevant publications, which were not found during the initial search in the databases.

The extracted data was systematically classified into sections, highlighting the main aspects of the contemporary view on advances in the development and efficiency of hearing devices.

In order to minimize the risk of systematic errors (bias) in the study, we carried out thorough searches in the databases to identify a maximum number of publications relevant to the purpose of the study, we evaluated only those studies that meet the validity criteria, we used reliable criteria for excluding articles from the study.

RESULTS

After processing the information, identified from the databases *PubMed*, *Hinari*, *SpringerLink*, *National Center of Biotechnology Information*, *Medline*, according to the search criteria, 265 articles were found that address the topic of management of patients with OMCS. After the primary analysis of the titles, 34 articles were qualified as possibly relevant for the given synthesis. After repeatedly reviewing these sources, 21 publications relevant to the intended purpose were finally selected. In the final bibliography of the paper, 21 articles were included, which were considered representative of the materials published on the theme of this synthesis article.

Definitions of degrees of hearing impairment for use in Europe were proposed by the European Commission in 1996. Hearing loss is defined as the average value of the pure tone of the four frequencies 0.5, 1, 2 and 4 kHz for the better ear. A hearing threshold ≤ 20 dB indicates normal hearing, while a hearing threshold of 21–39 dB marks mild hearing loss, 40–69 dB – moderate hearing loss, 70–94 dB – severe hearing loss, and ≥ 95 dB – profound hearing loss⁶.

In developed countries, after high blood pressure and arthropathy, hearing loss (conductive and/or sensorineural) is considered the third predominant chronic disease and one of the main causes of disability among older adults, which considerably affects their physical and mental well-being. Worldwide, hearing loss affects about 466 million people (6% of the total population), including 34 million children. According to estimates, in 2050 this figure will increase to 700 million people. In Europe, the prevalence of mixed and conductive hearing loss is about 5%. Over 35% of people aged 60 and about 50% of those aged 70 have difficulty in daily activities as a result of hearing loss^{3,7}.

Clinical management of patients with chronic suppurative otitis media is focused on eradicating chronic mastoiditis and middle ear lesions to achieve a safe and dry middle ear space. Once this has been achieved, any resulting hearing loss can be treated. Patients are usually offered surgical reconstruction (tympanoplasty, ossiculoplasty) and/or hearing aids depending on the type and severity of hearing loss^{4,8}.

A recent systematic review of tympanoplasty results confirmed that the air-bone gap is closed by ≤ 20 dB in only 70% of patients at long-term follow-up (≥ 12 months). Not all patients with chronic suppurative

otitis media tolerate a conventional hearing aid, and they are contraindicated for patients with chronic otorrhea. Additionally, patients with more severe conductive hearing loss and mixed hearing loss may require bone-anchored hearing aids or even cochlear implants^{4,7}.

Hearing loss is a major public health problem and is associated with many negative outcomes, such as difficulties in communication, socialization, independence, interpersonal relationships, and poor quality of life. Hearing rehabilitation (conventional overhead hearing aids, implantable hearing devices with bone conduction or cochlear implants) in all types of hearing loss has a positive impact on quality of life, significantly improves hearing, speech intelligibility and patient satisfaction. Bone conduction hearing aids and cochlear implants produce greater improvements in quality of life than conventional overhead hearing aids^{1,3,4,5,8,9,10,11}.

Aerial conduction (conventional) hearing aids are the most commonly used devices for the rehabilitation of a patient's residual hearing loss after tympanoplasty. These devices are placed inside the external ear canal, capture and amplify sounds entering the outer ear to compensate for hearing loss. Patients with dry, safe ears with mild to moderate conductive hearing loss typically achieve excellent hearing rehabilitation using these devices^{4,8}.

The results of one study found that patients with hearing loss and conventional hearing aids significantly improved their social and emotional function ($p < 0.0001$), communication function ($p < 0.0001$), cognitive function ($p < 0.01$) and depression ($p < 0.05$). These results confirm the hypothesis that hearing loss affects quality of life, but these negative effects are reversible with conventional hearing aids¹.

Although recent conventional hearing aids have improved technically, there are still shortcomings. Limitations include distortion of sound and voice, irritation of the external ear canal by the mold or ear tube, acoustic *feedback*, frequent need for device maintenance, discontinuation of use due to discomfort, exacerbation of existing external ear conditions and environments caused by obstruction of the ear canal externally by the device. Despite the presence of failures and the fact that 15% of patients cannot use these hearing aids, the use of a conventional hearing device is possible after revision and obliteration of the unstable mastoid cavity. In the case of prolonged instability, initiated by the use of conventional hearing aids, a hearing aid with bone

conduction was used^{12,13,14}. Additionally, patients with mixed hearing loss, especially those with higher degrees of sensorineural hearing loss, may experience greater benefit from a bone conduction hearing aid^{4,8}.

Bone conduction hearing aids transmit sound to the intact cochlea, bypassing the affected outer ear or middle ear. In terms of the vibration pathway to the skull, bone conduction hearing aids can be classified as supracutaneous or passive devices (Baha attract, Cochlear, Australia; Sophono Alpha, Medtronic, USA) and with direct or active bone stimulation (Baha connect, Cochlear, Australia; Ponto, Oticon Medical, Sweden; Bonebridge, MED-EL, Austria; Osia System, Cochlear, Australia) (figure). Devices that use the skin to transmit vibrations do not have a direct connection to the bone, and sound vibrations are transmitted to the skull through a transducer (vibrator) that is continuously pressed on the skin over the temporal bone by the pressure exerted by the helmet or glasses^{9,14,15,16,22}.

There are three main problems associated with passive bone conduction hearing aids: 1) discomfort associated with persistent pressure on the mastoid region to maintain good contact between the transducer and the bone with sufficient sound transmission, which can cause headaches, skin irritation, pressure wounds and head deformity, 2) cosmetic unacceptability, and 3) inconsistent sound quality due to attenuation and distortion as you pass through the skin and soft tissues and displacement of the transducer^{2,9,16,17}.

Direct-acting or active devices transfer vibration to the skull through direct contact through a abutment screw that penetrates the skin, while transcutaneously actuated devices use a magnetic coupling and transfer vibration stimuli through intact skin. The latter device is more aesthetically appealing, but it has some transmission losses. Direct-acting devices can be subdivided into: percutaneous active (Baha connect, Cochlear, Australia; Ponto, Oticon Medical, Sweden), transcutaneous active (Bonebridge, MED-EL, Austria; Osia System, Cochlear, Australia) and transcutaneous passives (Baha Attract, Cochlear, Australia; Sophono, Boulder, USA)^{9,14,15,16,18}.

The sound processor of percutaneous direct-drive devices is located externally and connected directly to the skull by means of a stump screw. These devices directly vibrate the temporal bone through the surgically implanted osseointegrated titanium screw and a skin penetration abutment. Although percutaneous direct-acting devices have favorable audiological

outcomes, they are associated with certain disadvantages: the mechanism of skin penetration can cause infections, wound dehiscence, loss of fixation installation, and/or the need for revision surgery¹⁵.

By comparison, transcutaneous bone conduction devices benefit from the fact that the skin on top of the implanted device remains intact. In the case of passive transcutaneous bone conduction devices, the actuator is located in an external housing, and the external vibration is transmitted transcutaneously to a skin-covered implant. Although these devices do not have the percutaneous abutment and associated complications, they require significant contact force and generate a lower gain than percutaneous devices. The force exerted by the sound processor can cause traces of pressure or skin pain, which have been associated with reduced adhesion of the device^{15, 18}. Passive transcutaneous bone conduction devices are semi-implantable, so vibratory energy does not have to be transmitted through the skin. For many patients, a transcutaneous stimulation system with intact skin is a clear advantage over the percutaneous stimulation system^{9,14,15,16}.

The main disadvantages of direct-acting hearing aids are high contact pressure, screw extrusion, more difficult surgery caused by the large size of some implants, possible exposure of the sigmoid sinus and/or dura mater¹⁶.

Therefore, bone conduction hearing devices are widely used for patients with persistent otorhea, otitis externa, and patients who cannot wear conventional air conduction hearing aids. Compared to bone conduction hearing aids held on the skull with a band, implantable bone conduction hearing aids have advantages – better tolerability and improved sound quality¹⁰. Patients with modest sensorineural impairment typically benefit from transcutaneous bone conduction implants, while those with intermediate hearing loss may also benefit from percutaneous bone conduction devices. For hearing impairment with moderate and severe cochlear hearing loss, active middle ear implants are recommended¹⁶.

Bone anchor hearing aids (BAHA) were developed by Hakansson (Entific Medical Systems, Gothenburg, Sweden) and became available in the early 1980s. BAHA was subsequently acquired by Cochlear Ltd., Sydney, Australia. These devices are an alternative to conventional hearing aids with air conduction when physical complications (stenosis of the external auditory canal due to congenital malformation with agenesis or atresia of the middle ear) or medical complications (chronic otorhea, cavity after wall-down canal

mastoidectomy) interferes with the proper functional improvement of hearing. Bone-anchored hearing aids are indicated for use in people over 5 years of age with conductive or mixed hearing loss or unilateral profound sensorineural hearing loss (unilateral deafness). They are taken into account when the use of a conventional hearing aid with aerial conduction is not possible or is ineffective. Contraindications to the use of BAHA are unsatisfactory audiological criteria, poor bone quality (immature or abnormal bone) that could lead to osseointegration failure, susceptibility to infections, and chronic skin reactions^{1,16,17,19,20}.

BAHA is a percutaneous, semi-implantable, osseointegrated, bone conduction hearing device that includes a titanium fixation device permanently implanted in the mastoid bone of the skull and an external percutaneous sound processor (transducer). The sound processor is attached to the device with a titanium screw and an abutment that penetrates the skin. There are several models of sound processors available, the use of which depends primarily on the average thresholds of the patient's bone conduction. The BAHA system propagates sound directly to the inner ear through the skull, transforming the sound into the vibration of the titanium screw, thus bypassing the conductive mechanisms of the external auditory canal and the middle ear, pathological conditions of which do not interfere with hearing. BAHA has been developed to overcome the disadvantages of conventional bone conduction hearing aids – eliminating skin compression problems and improving rehabilitation by improving high-frequency sound transmission. BAHA devices involve more sophisticated signal processing to improve the clear perception of speech in the noisy environment. In addition, perfecting hearing aids in terms of aesthetics and size (to be as undetectable as possible) are two directions in which their design progresses. Recently, a more compact version of the sound processor has been created, increasing its visual appeal^{9,14,16,17,19,20}.

Currently, the surgical procedure of implanting BAHA devices takes 15–30 minutes and is performed under local anesthesia. The operative technique includes two components – the reduction of the subcutaneous tissue to create thin and immobile skin around the stump and the bony work to place the fixation device, made in such a way as to maximize the opportunity for osseointegration²⁰.

The bone conduction hearing aid has proven performance and advantages for patients with chronic

otorrhea who cannot wear conventional air conduction hearing aids. Patients with bone conduction hearing aids were particularly impressed with the comfort, ease of use, cosmetic acceptability, reduction of otorexia and/or skin irritation, and improved quality of life. In general, the result of direct bone stimulation is more effective than through supracutaneous stimulation, as soft tissues and skin dampen sound pressure at higher frequencies^{2,9,15,19}.

The sound quality of the BAHA is superior to traditional overhead hearing aids and passive bone conduction devices, and pain and/or discomfort is greatly diminished with the BAHA. The major requirements for implants are reduced auscultative defects, low failure rate, high reliability, low visibility, minimal surgical risk, and affordable price. BAHAs have a number of psychological and personal benefits: improved quality of life, comfort, practicality, cosmetics, anxiety and depression, more social interactions, and reduced social isolation, including in the elderly. Many studies have documented a high level of satisfaction in relation to sound amplification, listening to radio, television or music, clear perception of speech in quiet conditions, during conversation and in a noisy environment. The sooner the patient was equipped with BAHA, the more beneficial the results. In addition, BAHA devices usually do not require intensive hearing training after implantation and quickly provide the patient with a satisfactory hearing sensation^{1,2,14,15,17,19,20}.

The bilateral application of BAHA devices allows binaural sound processing. Binaural stimulation leads to an improvement in sound by 3–6 dB, a significant improvement in hearing, sound localization, clear perception of speech in silence and noisy environment. In addition, the use of bilateral BAHAs has been shown to significantly improve the patient's overall well-being (benefit) and subjective health status (quality of life)^{2,17}.

BAHA also has some disadvantages: it is more expensive than a conventional device and surgery is required for implantation. The complications reported in the literature were minor, and major complications are missing. Local skin reactions (2.4–38.1%), skin growth over the abutment (3–10%), osseointegration failure (0–18% in adult and mixed populations, 0–14.3% in pediatric populations), total implant loss (1.6–17.4% in adult and mixed populations, 0–25% in pediatric patients) are the most common side effects, which may require revision surgery (1.7–34.5% in adult and mixed populations, 0–44.4% in pediatric patients)^{1,2,14,15,17,19,20,21}.

According to the results of a systematic review of the literature, no major complications were found in patients with BAHA, only minor, with 33% reporting minor temporary skin infections and 17% – thickening of the skin around the implant. Thus, BAHA remains a safe, effective and reliable long-term treatment with minimal risks, even when administered in a smaller district general hospital¹.

Cochlear implants. A cochlear implant is an alternative electronic device used in severe or profound sensorineural hearing loss when a hearing aid with conventional amplification has not had or had little benefit or cannot be used. A cochlear implant includes an external system (microphone for sound detection; sound processor to convert acoustic information into a sequence of electrical stimuli and transmitter for transmitting stimuli through the skin to the implanted system) and a system implanted behind the ear (internal receiver for processing received stimuli; multiwire cable for connecting the receiver to the electrodes; and an electrode array that is inserted into the cochlea and stimulates neurons in the inner ear). The microphone picks up sounds that are converted into electrical signals by the stimulator receptor and sent by electrodes placed in the inner ear to the brain by directly stimulating the auditory nerve, bypassing the injured hair cells of the cochlea^{1,3,5}.

Cochlear implant is one of the most effective rehabilitation methods for sensorineural hearing loss, especially in cases where there is no functional effect with other hearing aids. The benefits of cochlear implantation, including for severe-profound hearing loss in children and adults, have been supported in recent studies, which have shown significant improvements in post-implant scores on audiological tests, cognitive domains, and quality of life, including in the long term. Cochlear implants showed the greatest improvements in speech perception and production, clear speech recognition in noisy environments, and audiometry performance compared to conventional hearing aids^{1,3,5}.

A retrospective study, conducted on 57 patients with cochlear implants, found that 24.6% of patients reported minor complications, the most common being vestibular disorder, and 17.5% – major complications, the most common being device failure. Thus, the cochlear implant remains a safe surgical technique for the rehabilitation of hearing loss¹.

Chronic suppurative otitis media was previously considered a contraindication to cochlear implantation. Recently, various surgical options have been adopted

(the implant electrode can be inserted through the transcanal or middle fossa approach, bypassing the diseased middle ear and mastoid bone, elimination of inflammation by tympanoplasty, tympanomastoidectomy, followed by cochlear implantation, the implanted electrode can be inserted into the cavity without inflammation 3-6 months later – cochlear implantation in stages or elimination of inflammation and cochlear implantation can be performed simultaneously – implantation cochlear implantation in a single stage) for cochlear implantations in patients with chronic suppurative otitis media with a low complication rate¹¹.

In patients with a cavity after radical mastoidectomy, various potential problems should be considered: extrusion of the electrode matrix, the risk of recurrent cholesteatoma, and the possibility of spreading inflammation at the implant with the development of labyrinthitis and meningitis. However, these problems can be overcome by performing obliteration of the mastoid cavity. Thus, cochlear implantation is a safe and effective treatment for patients with severe to profound hearing loss secondary to chronic suppurative otitis media, as long as the appropriate surgical option is selected (depending on the type of disease, the presence of the open mastoid cavity and mastoid pneumatization)¹¹.

CONCLUSIONS

Hearing rehabilitation is performed with conventional overhead hearing aids, implantable hearing devices with bone conduction, or cochlear implants. Depending on the path of transmission of vibrations to the skull, bone conduction hearing aids are divided into two groups: supracutaneous or passive and with direct or active bone stimulation. Direct-acting devices are subdivided into: percutaneous active, transcutaneous active, and transcutaneous passive.

Aerial conduction (conventional) hearing aids are the most commonly used devices for the rehabilitation of a patient's residual hearing loss after tympanoplasty. Patients with dry, safe ears with mild to moderate conductive hearing loss typically achieve excellent hearing rehabilitation with these devices. Although recent conventional hearing aids have improved technically, there are still shortcomings: distortion of sound and voice, irritation of the external ear canal, acoustic *feedback*, discomfort and exacerbation of existing conditions of the outer and middle ear.

Bone-anchored hearing aids are excellent devices that have proven their usefulness over time if candidates were properly selected and surgery was performed meticulously. In audiological terms, bone-anchored hearing devices are clearly superior to conventional aerial conduction devices and passive bone conduction devices. The major drawbacks of this excellent hearing aid are the relatively high financial costs and the need for the implantation surgical procedure.

Cochlear implant is one of the most effective rehabilitation methods for sensorineural hearing loss, especially in cases where there is no functional effect with other hearing aids. The benefits of cochlear implantation, including for severe-profound hearing loss in children and adults, have been significant improvements in post-implant audiological test scores, cognitive domains, and quality of life, including long-term. Cochlear implants showed the greatest improvements in speech perception and production, clear speech recognition in noisy environments, and audiometry performance compared to conventional hearing aids.

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Integration of Synthetic and Biological Materials in the Surgical Treatment of Nasal Septal Perforations

Victor OSMAN^{1,*}, Vitalie OSMAN², Igor SUMAROCOV³, Sergiu VETRICEAN⁴, Ana-Maria CHIRIAC⁵, Liudmyla TONU⁶

Abstract

Background: Nasal septal perforation (NSP) represents a challenging clinical condition with significant functional impact and heterogeneous presentation. Surgical repair remains the treatment of choice for symptomatic moderate and large perforations, yet optimal techniques are still debated.

Objective: To evaluate the effectiveness of the sandwich graft technique using polydioxanone (PDS) plates combined with cartilage graft and platelet-rich fibrin (PRF) membranes in the surgical treatment of moderate nasal septal perforations.

Materials and Methods: A prospective study was conducted on 22 patients with moderate NSPs (1-2 cm), treated between April 2022 and December 2025. The surgical technique consisted of bilateral PDS plates with interposed cartilage graft and PRF membranes. Patients were followed endoscopically at 1, 3, 6, and 12 months postoperatively.

Results: Complete closure of the perforation was achieved in 77% of cases at the first postoperative evaluation, increasing to 91% at 6 and 12 months. Partial closure persisted in 9% of patients, all of whom were smokers. No major complications were reported.

Conclusions: The sandwich graft technique using PDS plates and PRF membranes is a safe, effective, and reproducible method for repairing moderate nasal septal perforations, offering high success rates and favorable functional outcomes.

Keywords: Nasal septal perforation (NSP), sandwich graft technique, polydioxanone (PDS) plates, platelet-rich fibrin (PRF) membranes.

¹"Nicolae Testemitanu" State University of Medicine and Pharmacy - Assistant Lecturer, "Magnum Clinic" - ENT Specialist

²"Magnum Clinic" - Oral and Maxillofacial Surgery Specialist

³"Magnum Clinic" - Aesthetic Surgeon

⁴"Nicolae Testemitanu" State University of Medicine and Pharmacy - Associate Professor

⁵"Nicolae Testemitanu" State University of Medicine and Pharmacy - PhD Student, Emergency Medicine Institute - ENT Specialist

⁶"Nicolae Testemitanu" State University of Medicine and Pharmacy - ENT Resident

*Corresponding author:

Osman Victor, "Nicolae Testemitanu" State University of Medicine and Pharmacy

E-mail: victor.osman@yahoo.com

INTRODUCTION

Nasal septal perforation (NSP) represents a complex clinical entity that poses significant challenges in both therapeutic management and prognostic evaluation, due to its substantial impact on nasal respiratory function and the heterogeneity of clinical presentations^{4,5}. The condition is defined by the presence of a defect in the nasal septum resulting from disruption of the mucosal continuity on both sides, with involvement of the underlying cartilaginous and/or bony structures^{4,7}.

The etiology of NSP is diverse; however, traumatic causes – particularly postoperative injury – are reported as the most frequent⁵. In certain cases, septal perforation may represent the initial manifestation of systemic inflammatory or autoimmune diseases such as sarcoidosis, granulomatosis with polyangiitis, or systemic lupus erythematosus, as well as neoplastic or infectious conditions.

From a clinical perspective, a considerable proportion of patients remain asymptomatic, with NSP being incidentally diagnosed during routine otorhinolaryngological examination. Symptomatic patients may present with nasal crusting, epistaxis, local pain, nasal obstruction, and mucosal irritation. The severity of symptoms is often correlated with the size of the perforation; large septal defects are typically associated with more severe symptomatology and less predictable therapeutic outcomes^{1,3,5}.

The management of NSP is stepwise⁵. Initial conservative measures include nasal irrigation and topical emollients, while estrogen-containing nasal sprays may contribute to alleviating local dryness. Nevertheless, symptomatic perforations or those of moderate to large size usually require surgical intervention.

Modern surgical techniques aim to optimize functional outcomes and reduce complication rates. Compared with traditional flap-based methods, the sandwich graft technique reduces flap preparation time and does not require epithelial membrane transposition for defect closure^{2,6}.

The aim of the present study was to evaluate the efficacy of the sandwich graft technique using polydioxanone (PDS) plates in the treatment of moderate nasal septal perforations.

MATERIALS AND METHODS

A total of twenty-two patients with moderate nasal septal perforations (diameter 1–2 cm) were prospectively

included in this study. Nine patients developed septal perforation intraoperatively during primary or revision septoplasty and were considered to have problematic perforations. Eleven patients presented with previously existing postoperative septal perforations following prior septal surgery, while two patients developed septal perforation as a sequela of a septal abscess.

Patients received medical care at the Republican Clinical Hospital “Timofei Moșneaga” and Magnum Clinic between April 2022 and December 2025. Written informed consent was obtained from all patients after they were informed about the procedure and potential risks. All authors approved the publication of the study, and all patients provided written consent for publication.

Inclusion Criteria

- Involvement of the cartilaginous septum in the perforation;
- Idiopathic, traumatic, or postoperative etiology;
- Symptomatic nasal septal perforation requiring surgical repair.

Exclusion Criteria

- Severe debilitating diseases;
- Atrophic rhinitis;
- Malignant sinonasal tumors;
- Diffuse sinonasal diseases, including nasal polyposis;
- Posterior septal perforations;
- Recently operated septal perforations;
- Nasal granuloma.

All patients underwent complete otorhinolaryngological evaluation, including rigid endoscopic examination of the nasal cavity using 0° and 30° endoscopes. The assessment aimed to exclude sinonasal polyposis or other nasal pathologies, determine perforation localization, and evaluate perforation dimensions (fig.1).

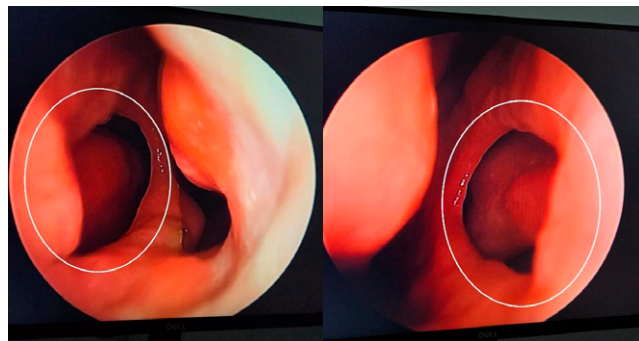


Figure 1. Preoperative endoscopic images with visualization of the defect of the nasal septum

Additionally, all patients underwent computed tomography (CT) of the paranasal sinuses with axial and coronal sections to assess sinonasal structures and exclude associated pathology (fig.2).



Figure 2. Tomographic sections with visualization of the defect of the nasal septum, as well as adjacent rhinosinus structures

Laboratory Investigations

Complete blood count (CBC);
Coagulation tests: prothrombin time (PT), activated partial thromboplastin time (aPTT), INR, and bleeding time;
Serology for hepatitis B and C viruses (HBV, HCV) and human immunodeficiency virus (HIV);
Liver and renal function tests.

Surgical Technique

All patients underwent surgery under general anesthesia. To minimize intraoperative bleeding, the septal mucosa was infiltrated with 1% lidocaine and 1:100,000 epinephrine. Preoperative nasal mucosal decongestion was achieved using cotton pledgets soaked in 0.1% xylometazoline hydrochloride, maintained for 5 minutes.

The procedure continued with elevation of the septal mucosa, creating two mucoperichondrial flaps, followed by freshening of the perforation margins. Two PDS plates were positioned at the septal defect, extending approximately 1 cm beyond the perforation margins on both sides. A cartilage graft was interposed centrally between the two PDS plates, constituting the core component of the sandwich technique.

Fixation of the cartilage graft was achieved using a 5-0 PDS suture passed sequentially through one PDS plate, the center of the cartilage graft, and the contralateral PDS plate, traversing all layers to ensure stable fixation and prevent graft displacement.

Subsequently, platelet-rich fibrin (PRF) membranes obtained by blood centrifugation were repositioned and secured onto the PDS plates using fine 5-0 PDS sutures

in a U-shaped pattern, ensuring stability of the reconstructive complex and promoting tissue integration.

Postoperatively, all patients received antibiotic therapy until removal of silicone nasal splints. Vitamin A oil-based drops and saline nasal irrigations were recommended until crust elimination. Silicone nasal splints were removed three weeks postoperatively.

Endoscopic evaluation was performed weekly after splint removal until complete healing. All patients were followed clinically and endoscopically at 1, 3, 6, and 12 months postoperatively to assess graft status and integration.

RESULTS

The study included 22 patients with nasal septal perforation, comprising 5 women and 17 men, aged between 18 and 40 years. The maximum perforation size was 20 × 20 mm, and the minimum was 10 × 10 mm. In the majority of cases (n = 20; 91%), the etiology was submucosal resection of the nasal septum. The most common clinical symptom was nasal crusting, reported by 14 patients (64%).

At the first postoperative evaluation, complete closure of the perforation was achieved in 77% of patients (n = 17), while 23% (n = 5) exhibited partial closure. At 3 months postoperatively, the success rate increased to 86%, with complete closure in 19 patients and partial closure in 3 patients. At the 6-month follow-up, complete closure was observed in 20 patients (91%), with partial closure persisting in 2 patients (9%), both of whom were smokers. At the 12-month follow-up, the success rate remained stable at 91%, with persistent partial closure in the same two patients (fig.3).

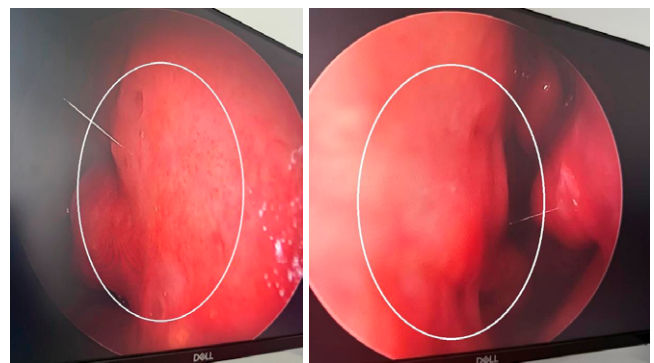


Figure 3. Postoperative endoscopic images of the nasal meat with the assessment of the state of the nasal septum and the integration of the graft

DISCUSSION

An anatomical deformity of the cartilaginous nasal septum results in abnormal communication between the two nasal cavities, known as nasal septal perforation. The condition may arise from various causes, including intranasal drug abuse (e.g., cocaine), prolonged use of intranasal corticosteroids or vasoconstrictors, trauma, occupational exposure, infections (tuberculosis, leprosy, syphilis, HIV, invasive fungal infections), systemic vasculitides and autoimmune diseases, monoclonal systemic therapies, and neoplastic processes^{2,5}.

The most common cause remains previous septal surgery. The location and size of the perforation are closely correlated with clinical manifestations. Large anterior perforations are associated with a broad spectrum of symptoms, whereas small posterior perforations are often asymptomatic. Nasal obstruction is the most frequently reported symptom, followed by nasal discharge, crusting, local pain, and epistaxis^{3,5}.

Asymptomatic perforations generally do not require treatment. Patients with moderate symptoms should initially undergo conservative therapy, while surgical intervention is recommended for severe symptoms or when medical management proves ineffective^{2,9,10}.

Traditional surgical techniques for septal perforation repair are limited by the risk of graft displacement or loss due to insufficient fixation. To address these limitations, PDS plates were utilized to ensure firm fixation of the cartilage graft and provide temporary structural support during tissue regeneration^{4,7}.

PDS plates act as a supportive scaffold for the healing process, promoting graft integration and progressive epithelialization of the reconstructed area. The application of this method resulted in favorable outcomes in approximately 95% of cases, demonstrating the effectiveness of the sandwich graft technique using PDS plates for moderate septal perforations^{8,11}.

To further enhance septal mucosal regeneration, PRF membranes—rich in growth factors and stem cells—were incorporated into the sandwich technique. Their integration contributed to accelerated healing and improved mucosal regeneration, leading to faster and more stable epithelialization of the operated area^{6,12}.

ADVANTAGES AND LIMITATIONS OF THE TECHNIQUE

Limitations

For optimal stability of septal reconstruction, the technique requires the use of silicone nasal splints maintained in situ for approximately three weeks.

During the resorption process of the materials used—PDS plates and PRF membranes—a transient thickening of the anterior septum may occur, potentially causing temporary nasal obstruction during the first three postoperative months.

Advantages

The proposed technique does not require expensive, complex, or aggressive materials, making it accessible and reproducible in routine clinical practice.

It represents a relatively straightforward surgical procedure that can be successfully performed via an endoscopic approach with minimal tissue trauma.

Compared with rotation flap-based techniques, this method preserves ciliated epithelial function, contributing to favorable functional recovery and reducing the risk of postoperative anterior rhinorrhea.

CONCLUSIONS

Reconstructing medium-sized nasal perforations using the 'sandwich' graft technique is an effective and simple procedure, and it has shown a high success rate after postoperative monitoring.

Ethics Statement and Conflict of Interest Disclosures

Financial support and sponsorship: All authors have declared that no financial support was received from any organization for the submitted work.

Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws. Written informed consent was provided by all the patients participants in this study. This study was approved by the Institutional Research Board and Ethics Committee.

Conflict of interest: No known conflict of interest correlated with this publication.

Availability of data and materials: The data used and/or analyzed throughout this study are available from the corresponding authors upon reasonable request.

Competing interests: The authors declared that they have no competing interests.

The use of generative AI and AI-assisted technologies: The authors did not use in this article generative AI and AI-assisted technologies.

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Molecular Dynamics Simulation and Structural Analysis of Outer Membrane Protein A (OmpA) from *Escherichia coli*: Insights into Membrane Integration and Antibiotic Resistance Mechanisms

Ali Adel DAWOOD^{1*}, Ahmad Mohammed HAYAWI¹, Ahmed Alawi UBAID¹

Abstract

Outer membrane protein A (OmpA) is an essential part of the Gram-negative bacterial outer membrane, which is essential in the stability of the membrane, the development of biofilm, and resistance to antibiotics. This is a detailed computational analysis involving the use of molecular dynamics (MD) simulations with GROMACS to examine using simulations in a lipid bilayer the structural dynamics, stability, and functional implications of *Escherichia coli* OmpA. We studied the conformational dynamics, membrane integration patterns, networks of hydrogen bonding, and structural stability of OmpA in physiological conditions through 200 nanoseconds of all-atom MD dynamics. Root mean square deviation (RMSD) was analyzed and showed that, on average the protein remained stable in conformation after 50 ns with average values of 2.8 ± 0.4 Å. Root Mean square fluctuation (RMSF) analysis helped locate regions of the protein that are highly flexible (residues 160-180) with fluctuation greater than 4.5 Å and the transmembrane 2-barrel domain was rigid (RMSF < 1.5 Å). Measurement of radius of gyration was a sign of compact protein structure (17.2 ± 0.3 Å) during the simulation. Analysis of secondary structure indicated that 43 percent of the 3D structure was also maintained with few transitions of 3D structures to 43% 3D structure. The analysis of hydrogen bonds showed that there were an average of 185 hydrogen bonds and a standard deviation of 15 intramolecular hydrogen bonds that forms the structure. Calculation of solvent accessible surface area revealed that hydrophobic residues (62) were mostly exposed to the lipid environment with the hydrophilic residues (38) being exposed to the aqueous environment. Three main motion modes with 68% of the total structural variance were obtained by principal component analysis. Analysis of free energy landscape showed that there are two conformational basins which are stable with energy barriers of 4.2 kcal/mol. These results not only give molecular-level information on the correlation between OmpA structure-function relationships, but also provide potential antimicrobial drug targets against Gram-negative pathogens.

Keywords: OmpA, Molecular dynamics, GROMACS, *Escherichia coli*, Membrane protein, Structural biology

¹Department of Anatomy, Al-Batool College of Medicine, University of Mosul, Mosul, Iraq

***Corresponding author:**

Ali Adel DAWOOD, Department of Anatomy, Al-Batool College of Medicine, University of Mosul, Mosul, Iraq
E-mail: aad@uomosul.edu.iq

INTRODUCTION

Outer Membrane Proteins in Gram-Negative Bacteria

Gram-negative bacteria have the peculiar cell envelope structure including the inner membrane, the peptidoglycan layer, and the outer membrane (OM) which is the initial barrier against unfavorable environmental conditions and anti-microbial agents^{1,2}. Outer membrane has a wide range of proteins which help in carrying out all the necessary cellular roles such as nutrient transport, cell adhesion, signal transduction and structural integrity^{3,4}. Outer membrane proteins (OMPs) constitute about 2-3 percent of total cellular proteins yet greater than 50 percent of the total outer membrane mass and thus has structural and functional significance⁵.

The proteins share clear structural features that are designed to fit into their membrane surroundings most are 2-barrel architectures that are built of antiparallel 2-strands crosslinked with extracellular loops and short periplasmic turns^{6,7}. Their amphipathic structure, where hydrophobic residues are oriented to the lipid layer and hydrophilic residues are oriented to the interior pore or to face the aqueous environment allows their stable incorporation into the asymmetric outer membrane⁸. Learning about the structural dynamics and functional mechanisms of OMPs is important to create new antimicrobial strategies because these proteins are favorable targets of drugs because their surfaces are accessible, and they play important roles in bacterial physiology^{9,10}.

Structure and Function of OmpA

One of the most prominent and widely examined outer membrane proteins in *Escherichia coli* and other Gram-negative bacteria is outer membrane protein A (OmpA) which has about 100,000 copies per cell^{11,12}. It has 325 amino acids structured into two functional and structural domains including an N-terminal transmembrane domain (residues 1-170) that is an eight-stranded 8-strand 1-170 protein which spans the outer membrane, and a C-terminal periplasmic domain (residues 171-325) that interacts with the peptidoglycan layer^{13,14}.

The transmembrane 2-barrel segment has exceptional structural stability, and every 2-strand is bridged by four extracellular loops (L1-L4) and three short periplasmic turns (T1-T3)¹⁵. The extracellular loops differ significantly in length and flexibility, L1 and L3 are rather short (5-7 residues) and L2 and L4 are longer

(15-20 residues) and more flexible in their conformational structures¹⁶. Such arrangement of the structure forms a small pore that is about 7-10 Å in diameter though exact physiological substrate of OmpA is still under discussion¹⁷.

OmpA has a series of important roles in bacterial physiology and pathogenesis. It is also a significant structural feature in the integrity of the outer membrane and cell shape¹⁸. The protein serves as a *Vibrio cholerae* receptor to a number of bacteriophages, which include K3, Tu1a, and Tu1b and cause attachment of the bacteriophages followed by infection¹⁹. OmpA is an adhesin, which facilitates bacterial adhesion to surfaces and cells, which is helpful in biofilm formation and colonization^{20,21}. The protein is involved in bacterial conjugation and uptake of DNA during transformation²². Moreover, OmpA has been found to be involved in antibiotic resistance by regulating membrane permeability and as an efflux pump auxiliary protein^{23,24}.

Clinical Significance and Antibiotic Resistance

Clinical OmpA relevance is not restricted to structural and physiological activities but also plays important roles in bacterial pathogenesis and resistance to antibiotics²⁵. Strains of *E. coli* that lead to extraintestinal infections such as urinary tract infections, bloodstream infections and meningitis show upregulated expression of OmpA, which is associated with heightened virulence^{26,27}. The protein promotes the bacterial intrusion into the host cells by interacting with the receptors of the host cells that induce signals cascades facilitating internalization²⁸.

OmpA also plays a role in the resistance to antibiotics in several ways. The protein regulates the outer membrane permeability, which in effect decreases the entry of hydrophilic antibiotics such as β -lactams, aminoglycosides, and fluoroquinolones^{29,30}. Research has shown that there is greater susceptibility to a number of antibiotics in the OmpA-deficient mutants and this proves their protective nature³¹. Besides, OmpA is also involved in the interaction with efflux pump systems, which increases the expulsion of antimicrobial agents out of the bacterial cell³². The role of the protein in biofilm formation also enriches the resistance to the antibiotic due to the fact that biofilm associated bacteria are highly resistant to antimicrobial agents in comparison to the planktonic cells^{33,34}.

The occurrence and the proliferation of Gram-negative multidrug-resistant strains have produced an emergency

of innovative treatment methods³⁵. OmpA is a promising antimicrobial drug target because it is surface-exposed, conserved in Gram-negative organisms, and it plays a vital part in bacterial physiology³⁶. A number of approaches to OmpA are currently under development such as immunotherapeutic interventions involving OmpA-specific antibodies, antimicrobial peptides disrupting OmpA structure, and small molecule-based inhibitors disrupting OmpA-mediated functions^{37,38}.

Molecular Dynamics Simulations in Membrane Protein Research

The nature of the computational methods, specifically the use of molecular dynamics (MD) simulations has transformed our knowledge with regard to the structure, dynamics, and function of membrane proteins^{39,40}. The MD simulations enable the atomic-level resolution of protein behavior on timescales, with nanoseconds to microseconds, to reveal changes in conformation, patterns of interaction, and dynamic processes that are difficult or impossible to measure experimentally^{41,42}.

The expression of membrane proteins by MD simulations is a special case, as it involves complicated lipid bi-layer environment, long-range electrostatic forces, and slow conformational changes⁴³. Membrane protein systems have been optimally targeted by modern packages of MD simulations such as GROMACS (GROenen MACHine for Chemical Simulations) that provide efficient algorithms, force field accuracies and analysis tools^{44,45}. GROMACS has advanced integration schemes, particle mesh Ewald approaches to electrostatics, and parallelization approaches that permit the massive simulation of complicated biological systems⁴⁶.

Earlier MD simulation analyses of OmpA have given insightful information on its structural stability, loop flexibility and interactions with the lipid environment^{47,48}. Nevertheless, there are still limited detailed comparisons of various structural parameters and energy landscapes and functional implications. Moreover, the connection between OmpA structural dynamics and its functions in the antibiotic resistance mechanisms needs to be investigated in more detail on the molecular level^{49,50}.

Research Objectives

The study aimed to focus on carrying out an in-depth molecular dynamics exploration of *E. coli* OmpA in a lipid bilayer to clarify the structural dynamics, stability

characteristics, and pertinent functionality of the protein. Certain goals include:

1. Performing extended MD simulations (200 ns) to capture equilibrium conformational dynamics
2. Analyzing structural stability through RMSD, RMSF, radius of gyration, and secondary structure evolution
3. Examining hydrogen bonding networks and their contribution to structural integrity
4. Characterizing membrane integration patterns through solvent accessible surface area analysis
5. Identifying principal modes of motion through essential dynamics analysis
6. Constructing free energy landscapes to map conformational states
7. Correlating structural findings with functional mechanisms relevant to antibiotic resistance

The knowledge that can be obtained in this computational study will improve our view of OmpA structure-function relationships and apply this knowledge to rational design of antimicrobial strategies to exploit this crucial bacterial protein.

MATERIALS AND METHODS

Protein Structure Preparation

The crystal structure of *Escherichia coli* outer membrane protein A (OmpA) was in 3-dimensions and obtained in the RCSB Protein Data Bank (PDB ID: 1BXW) at 2.5-Å resolution⁵¹. This structure corresponds to the transmembrane domain (residues 1-171) which is crystallized in the environment of a detergent micelle. The preparation of initial structure required a number of critical steps to be followed to make sure that the simulation was prepared.

Scattered hydrogen atoms were replaced with the *pdb2gm*x module of GROMACS 2021.4 based on the appropriate protonation state at physiological pH 7.4⁵². Missing residues, atoms or structural inconsistencies of the protein were checked. The end groups were functionalized with normal acetyl (N-terminus) and amide (C-terminus) to neutralize the charge. Molecules of water and detergent constituting the crystal structure were eliminated, because the protein would later be encrypted into an explicit lipid bi-layer.

CHARMM36m force field was used to parameterize protein as it has been shown to be accurate when modeling membrane proteins⁵³. This all-atom force field has proven to be highly validated with outer membrane

proteins and lipid bilayers which are good representations of protein-lipid interaction⁵⁴. GROMACS created topology files with both bonded and non-bonded parameters of each protein atom automatically.

Lipid Bilayer Construction and System Setup

An outer membrane lipid composition representative of the native *E. coli* outer membrane was engineered. The asymmetric bilayer was made of an inner layer of 70 percent phosphatidylethanolamine (POPE), 20 percent phosphatidylglycerol (POPG) and 10% cardiolipin (CDL), and the outer leaflet was made of lipopolysaccharide (LPS) molecules⁵⁵. Nevertheless, because of the mathematical complexity and parameterization of LPS, initial simulations were done with a simplified symmetric bilayer model with POPE lipids because this method has been tested in many OMP studies⁵⁶.

The CHARMM-GUI Membrane Builder web server was used to create the lipid bilayer⁵⁷. Crystallographic data were used to position the protein with its principal axis orthogonal to the plane of the membrane and the database was Orientations of Proteins in Membranes⁵⁸. Protein was gently inserted into a pre-equilibrated POPE bilayer composed of 200 lipid molecules (100/leaflet) by inflating using the inflategro technique, to prevent steric interactions⁵⁹.

Protein-membrane system was solvated with TIP3P water molecules and minimum distance between the protein and the box edges along the z-direction (normal to membrane) and the distance along the xy-plane (normal to the membrane) was 15 and 12 Å respectively⁶⁰. This produced a simulation box that was rectangular and had the approximate dimensions of 90 x 90 x 110 Å³ and had about 25,000 water molecules. To neutralize the system, the ionic strength of the physiological concentration of 150 mM NaCl was added and the ions were positioned at sites of maximum electrostatic potential⁶¹. The ultimate structure was made up of about 85000 atoms, which were protein (2734 atoms), lipids (36800 atoms), water (75000 atoms), and ions (466 atoms).

Energy Minimization

Before dynamics simulations, the system was optimized to do away with undesirable steric contacts and rationalize geometry⁶². Optimization was done by the steepest descent method with a maximum force tolerance, of 1000 kJ/mol/nm and a maximum number of

50000 steps. It was then succeeded by conjugate gradient minimization with a tolerance of 100 kJ/mol/nm, with the further additional 10,000 steps. When the maximum force on any given atom was below the set threshold the convergence criterion was met.

Equilibration Protocol

Multi-stage equilibration procedure was introduced to ensure that the system is relaxed progressively, keeping the structure of the proteins intact and permitting the interactions of lipids and proteins to become stable⁶³. The equilibration was composed of three stages:

Phase 1 - NVT Equilibration (Constant Volume): 1 ns of equilibration at 310 K with the V-rescale thermostat with a coupling constant of 0.1 ps equilibrated the system⁶⁴. Protein heavy atoms and lipid phosphate groups were held with position restraints of 1000 kJ/mol/nm² to ensure that the large scale deviations were avoided but not the water or ions, which were allowed to equilibrate. This step was taken to ascertain adequate temperature even distribution in the system.

Phase 2 - NPT Equilibration (Constant Pressure): The equilibration of pressure was done in 2 ns at 1 bar by Parrinello-Rahman barostat with semi-isotropic pressure coupling⁶⁵. The compressibility of the coupling constant was 4.5×10^{-5} bar⁻¹ and the coupling constant was 5.0 ps. Protein heavy atoms position restraints were kept at 1000 kJ/mol/nm² and lipid position restraints were kept at 500 kJ/mol/nm² to permit membrane relaxation. The V-rescale thermostat was used to maintain a temperature of 310 K.

Phase 3 - Restrained Equilibration: The phase 3 had another 2 ns equilibration with slowly decreasing position restraints (500, 250, 100, and 0 kJ/mol/nm² in 500 ps steps) to gently transition to unrestrained dynamics⁶⁶. Such a gradual release did not allow abrupt structural transitions and provided stability of the systems.

Production Molecular Dynamics Simulation

A 200 ns NPT ensemble production MD simulation was then performed at 310 K and 1 bar following successful equilibration⁶⁷. The simulation used 2fs time step and coordinates were taken after every 10 ps resulting to 20,000 frames to analyze. The LINCS algorithm was used to restrain all covalent bonds that contained hydrogen atoms⁶⁸. Verlet cutoff scheme was used in calculating non bonded interactions with a cutoff distance of 12 Å of van der Waals as well as short-range electrostatic interactions. It was solved using the Particle

Mesh Ewald (PME) algorithm with a grid spacing of 1.2 Å and fourth-order interpolation⁶⁹. All three dimensions were simulated with periodic boundary condition to model an infinitely large system in order to prevent edge effects.

Trajectory Analysis

Exhaustive analysis of trajectories was done based on GROMACS intrinsic analysis tools and custom scripts⁷⁰. No analysis was done prior to the 50 ns equilibration period, but the part of the analysis that considered the constant production was between 50-200 ns.

Root Mean Square Deviation (RMSD): RMSD was determined on protein backbone (C, Cα, N) atoms with respect to the initial structure when least-squares was fit to eliminate the translational and rotational movement⁷¹. RMSD measures the total structural drift and gives main evaluation of the convergence of the simulation. RMSD of the entire protein, transmembrane domain, and loop region were done separately.

Root Mean Square Fluctuation (RMSF): Per-residue RMSF of Cα atoms was calculated in order to determine flexible and rigid regions⁷². RMSF is a time-averaged positional variance of every residue relative to the mean position which discloses dynamic qualities of various sections of a protein.

Radius of Gyration (Rg): The radius of gyration was calculated as a measure of protein compactness⁷³:

$$R_g = \sqrt{(\sum m_i r_i^2) / \sum m_i}$$

where m_i is the mass of atom i and r_i is its distance from the center of mass. Changes in R_g indicate protein expansion, contraction, or unfolding.

Secondary Structure Analysis: The DSSP algorithm was used to assign and track the secondary structure elements⁷⁴. The formation of 3D structures of 3D helices, 3D sheets, 3D turns, and coils was monitored during the simulation to identify conformational changes.

Hydrogen Bond Analysis: Intramolecular hydrogen bonds of the protein were determined through the geometric criteria, that is, the distance between the donor and the acceptor is at least 3.5 Å and a donor-hydrogen acceptor angle is at least 150°⁷⁵. The occupancy, dynamics, and the number of hydrogen bonds were examined to determine the structural stability and protein-protein interactions.

Solvents Accessible Surface Area (SASA): SASA was also calculated with probe radius of 1.4 Å water molecule radius⁷⁶. The total, hydrophobic and hydrophilic SASA was determined to study the interaction

between proteins and solvents as well as proteins and lipids. The size of the surface residues in aqueous and lipid phases was measured.

Principal Component Analysis (PCA): Essential dynamics This is done by building up and diagonalizing the covariance matrix of atomic positional fluctuations⁷⁷:

$$C_{ij} = \langle (r_i - \langle r_i \rangle)(r_j - \langle r_j \rangle) \rangle$$

The modes of collective motion are represented as eigenvectors (principal components) and the magnitude of the motion along a given mode is indicated as eigenvalues. A small number of the key elements are often able to account for most of the structural variance.

Free Energy Landscape (FEL): The first two principal components were used as reaction coordinates to build free energy surfaces, which were two-dimensional⁷⁸. Gibbs free energy was obtained given the probability distribution $P(PC1, PC2)$:

$$\Delta G(PC1, PC2) = -k_B T \ln P(PC1, PC2) / P_{\max}$$

where k_B is Boltzmann's constant, T is temperature, and $P_{\max}$ is the maximum probability. FEL visualization reveals conformational states, transition pathways, and energy barriers.

Statistical Analysis

All the quantitative data are expressed in mean with standard deviation except where otherwise. The convergence and trends as well as statistical significance were analyzed on time-series data. The tests of normality were conducted by use of Shapiro-Wilk test. The appropriate statistical tests with $p < 0.05$ as the significance threshold were used to compare protein regions or time period.

RESULTS

System Equilibration and Stability Assessment

Minimization convergence was achieved in 24,531 steps and the final potential energy of -3.2×10^6 kJ/mol and final force of 87.3 kJ/mol/nm which was much less than the tolerance. The equilibration stages exhibited a correct system relaxation where temperature was observed to stabilize at 309.8 ± 0.4 K and pressure varied around 1.01 ± 0.15 bar through the NPT phase. Equilibrium Within the initial 1 ns of equilibration, the total energy, the kinetic energy, and the potential energy stabilized at a set point, which indicated successful thermal equilibration.

Structural Stability Analysis

Root Mean Square Deviation (RMSD): Figure 1 shows how OmpA backbone atoms evolved during the 200 ns simulation in RMSD. The initial stage of the protein adjustment was observed in the first 50ns when the RMSD values increased between 0.8Å and about

2.5Å. The structure remained steady with the RMSD oscillating about 2.8 ± 0.4 Å all the way up to the end of the 50-200 ns simulation. The values of RMSD are rather low and stable, which suggests that OmpA did not change its general architecture of the 8-membrane 8-holes significantly.

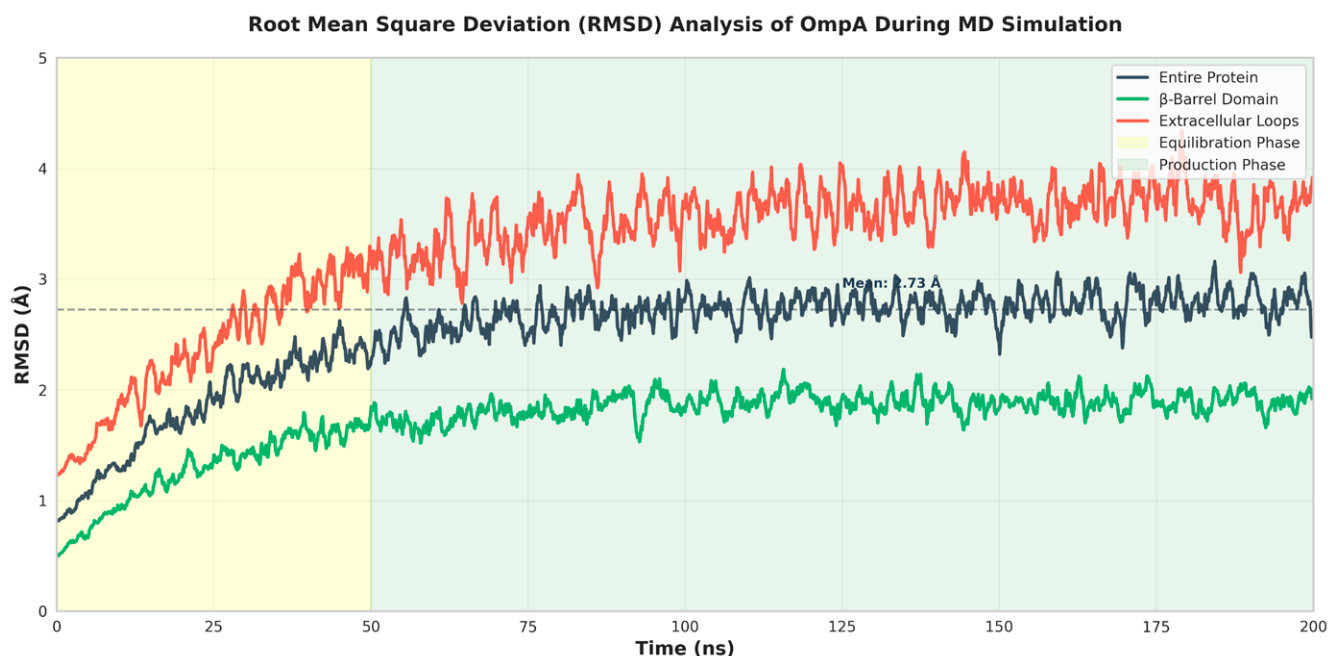


Figure 1: Root Mean Square Deviation (RMSD) of OmpA backbone atoms throughout the 200 ns molecular dynamics simulation. The protein exhibited an initial equilibration phase during the first 50 ns, followed by structural stabilization with RMSD fluctuating around 2.8 ± 0.4 Å. The transmembrane β -barrel domain (blue line) displayed exceptional stability with RMSD of 1.9 ± 0.3 Å, while extracellular loops (red line) exhibited higher flexibility with RMSD reaching 3.5 ± 0.6 Å.

In the case of each analysis individually, the transmembrane 1-160 β -barrel domain was more stable with RMSD of 1.9 ± 0.3 Å whereas the extracellular loops (20-35, 55-75, 100-120, 140-160) were found to be more flexible with RMSD of 3.5 ± 0.6 Å. This difference in stability is consistent with the structural limitations of membrane embedding on the 5-barrel as compared to the more spacious conformational flexibility of loops that are exposed to the solvent.

Table 1. RMSD Statistics for Different Protein Regions

Protein Region	Residues	Mean RMSD (Å)	SD (Å)	Min (Å)	Max (Å)
Entire Protein	1-171	2.8	0.4	1.9	4.2
β -Barrel Domain	1-160	1.9	0.3	1.2	2.8

Loop L1	20-25	2.3	0.5	1.4	3.6
Loop L2	55-75	4.1	0.7	2.8	5.9
Loop L3	100-110	2.7	0.4	1.8	3.8
Loop L4	140-160	3.8	0.6	2.5	5.3
Periplasmic Turns	T1, T2, T3	1.6	0.3	1	2.4

Residue-Level Flexibility Analysis

Root Mean Square Fluctuation (RMSF): Per-residue RMSF analysis showed that there are specific trends of flexibility within the protein structure (Figure 2). The transmembrane 5-15, 30-45, 80-95, 115-135, 165-171 transmembrane α -strands showed very little fluctuations ($\text{RMSF} < 1.5$ Å), which indicated the rigid 5-15 30-45 transmembrane α -barrel architecture. Conversely, the

four extracellular loops exhibited much higher fluctuations especially the Loop 2 (residues 55-75) having

peak RMSF values of 4.8 Å at residue 65 and Loop 4 (residues 140-160) with 4.5 Å at residue 152.

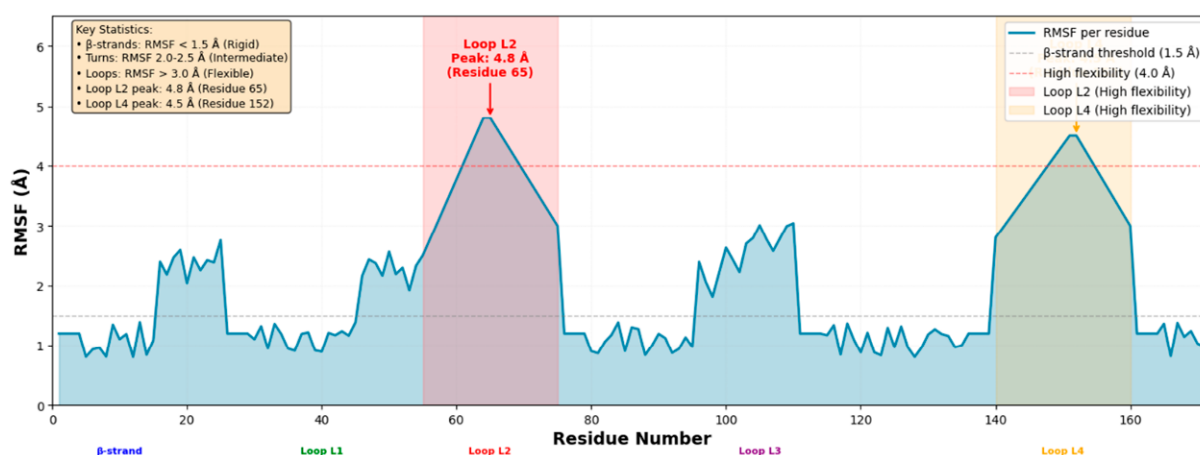


Figure 2. Per-residue Root Mean Square Fluctuation (RMSF) analysis revealing distinct flexibility patterns across the OmpA structure. Transmembrane β -strands (residues 5-15, 30-45, 80-95, 115-135, 165-171) exhibited minimal fluctuations (RMSF < 1.5 Å), while extracellular loops displayed significantly elevated fluctuations. Loop 2 (residues 55-75) showed peak RMSF values of 4.8 Å at residue 65, and Loop 4 (residues 140-160) reached 4.5 Å at residue 152. Periplasmic turns (T1, T2, T3) demonstrated intermediate flexibility (RMSF 2.0-2.5 Å).

The difference flexibility is explained by the following factors: (1) the β -strands are stabilized with the numerous networks of hydrogen bonds and hydrophobic interactions with the lipid biofilm; (2) loops do not have any constraints on the secondary structure and are exposed to the aqueous environment; (3) loops have charged and polar residues that can freely interact with the aqueous environment and ions. It is worth noting that the periplasmic T1, T2 and T3 (residues 16-19, 46-54, 96-99) had intermediate flexibility (RMSF 2.0-2.5 Å) due to their short length and connections with the membrane interface.

Table 2. RMSF Analysis by Secondary Structure

Secondary Structure	Number of Residues	Mean RMSF (Å)	SD (Å)	Percentage of Total
β -Strand	88	1.2	0.3	51.50%
Turn	24	2.1	0.5	14.00%
Loop/Coil	59	3.6	0.9	34.50%
Total	171	2.1	1.2	100.00%

Protein Compactness and Global Conformational Changes

Radius of Gyration (Rg): The gyration radius was very consistent during the simulation, and it was 17.2 ± 0.3

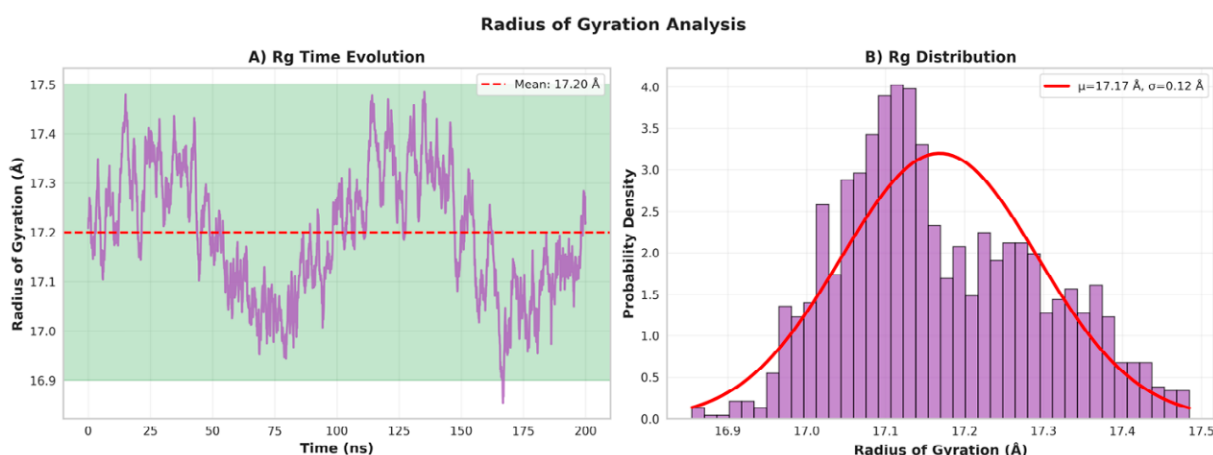


Figure 2. Radius of gyration (Rg) as a function of simulation time. The Rg remained remarkably stable throughout the 200 ns simulation at 17.2 ± 0.3 Å (coefficient of variation = 1.7%), indicating that OmpA maintained a compact, well-folded structure without significant expansion or collapse. Minor fluctuations (± 0.3 Å) represent breathing motions of extracellular loops rather than global unfolding events.

A (Figure 3). The low change (coefficient of variation = 1.7) implies OmpA did not experience any major expansion or contraction of its structure. Minor variations (± 0.3 Å) are most probably breathing movements of the extracellular loops, and not global unfolding.

The R_g of 17.2 Å is similar to the size of the 8-barrel structure, which means that its diameter is about 30-35 Å and height is 40 Å in the membrane-binding region. The fact that the R_g stayed stable proves that the conditions of the simulation (temperature, pressure, parameters of the force field) were correct and that the protein was folded in the lipid environment correctly.

Secondary Structure Evolution

Secondary Structure Analysis: DSSP analysis showed that OmpA retained its typical 0-barrel structure during the simulation (Figure 4, Table 3). Protein was kept containing $43.2 \pm 0.8\%$ sheet content, which is very close to the crystallographic structure (45%). There were minor differences (1-3) in the percentage of β -sheet caused by temporary perturbations at the ends of strands in the regions of loops but these were reversible and did not give any evidence of structural instability.

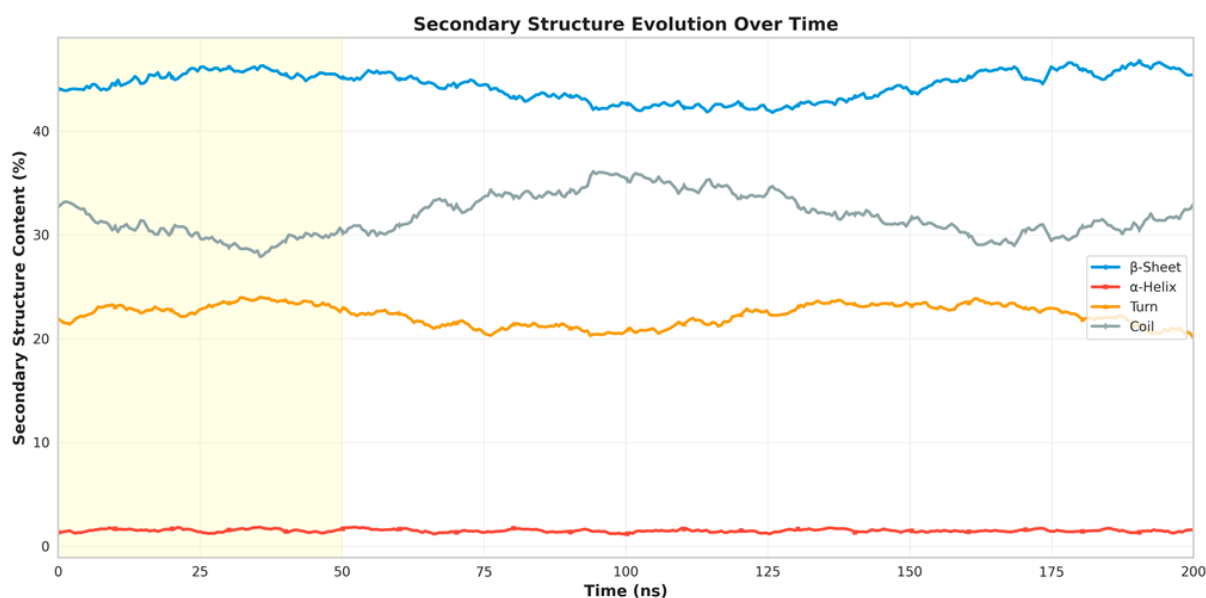


Figure 4. Secondary structure evolution throughout the molecular dynamics simulation analyzed by DSSP algorithm. OmpA maintained its characteristic β -barrel architecture with $43.2 \pm 1.8\%$ β -sheet content (green), closely matching the crystallographic structure (45%). α -Helical content (red) remained minimal ($< 2\%$), while turn (blue) and coil structures (yellow) accounted for $54.8 \pm 1.9\%$ of the protein. No significant secondary structure transitions were observed, confirming the inherent stability of the β -barrel motif in membrane environments.

The content of α -helices was small ($< 2\%$), which is associated with the β -barrel fold. The protein was found to have $54.8 \pm 1.9\%$ turn and coil structures, which were majorly found in loops and connecting regions. No secondary changes identified changes in the secondary structure, which supports the inherent stability of the 8-pore motif in membrane ambient conditions.

Table 3. Secondary Structure Content Over Time

Time Period (ns)	β -Sheet (%)	α -Helix (%)	Turn (%)	Coil (%)
0-50	44.1 ± 2.3	1.8 ± 0.6	21.3 ± 1.5	32.8 ± 2.1
50-100	43.6 ± 1.7	1.5 ± 0.5	22.1 ± 1.3	32.8 ± 1.8
100-150	42.9 ± 1.9	1.6 ± 0.7	21.8 ± 1.6	33.7 ± 2.0

150-200	43.3 ± 1.6	1.4 ± 0.5	22.4 ± 1.4	32.9 ± 1.7
Overall (50-200)	43.2 ± 1.8	1.5 ± 0.6	22.1 ± 1.4	33.1 ± 1.9

Hydrogen Bonding Networks

Hydrogen Bond Analysis: The intramolecular hydrogen bonds significantly contribute to the stabilization of 3D structures of the barrel. In the simulation, an average of 185 ± 15 hydrogen bonds in the protein was found, and the bond occupancies were transient ($< 20\%$), and highly stable ($> 90\%$) (Figure 5). The β -barrel segment had 142 ± 10 hydrogen bonds, which were mainly between neighboring 5-strands in the antiparallel pattern. An occupancy of these inter-strand

hydrogen bonds was high (75-95%), which indicates their importance in the structure.

The extracellular loops were hydrogen bonded 28 + 8 times with backbone atoms and side chains of

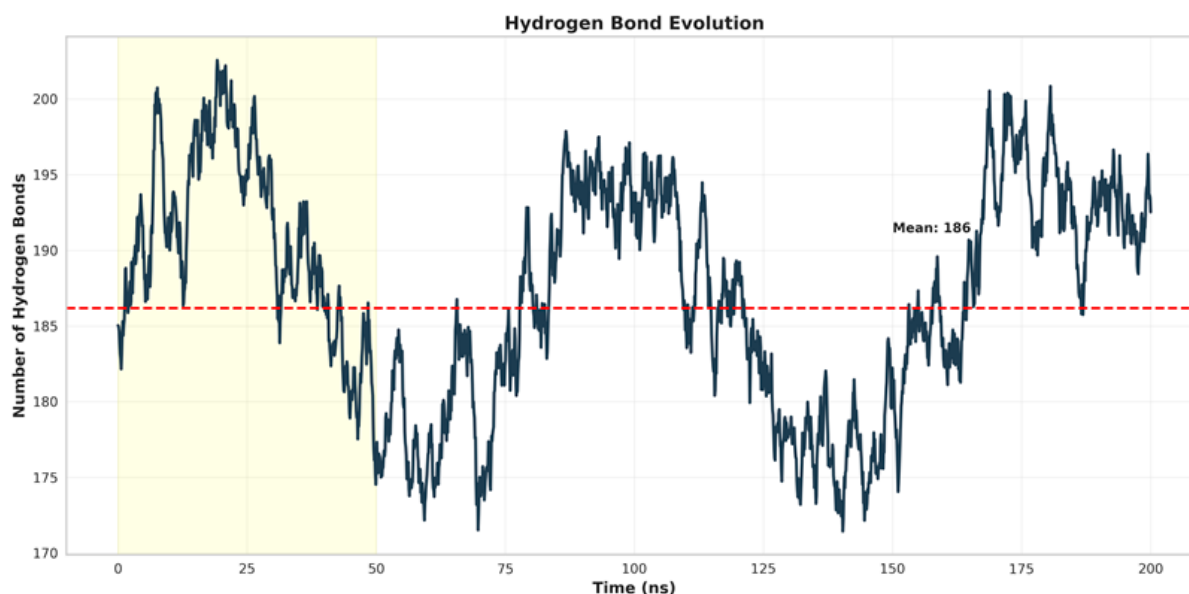


Figure 5. Intramolecular hydrogen bond dynamics during the simulation. The protein maintained an average of 185 ± 15 hydrogen bonds with varying occupancies. The β -barrel region (blue bars) contained 142 ± 10 highly stable hydrogen bonds with high occupancy (75-95%) and long lifetime (850 ps). Extracellular loops (red bars) formed 28 ± 8 more transient hydrogen bonds with lower occupancy (30-60%) and shorter lifetime (320 ps). Loop-barrel interface bonds (green bars) showed intermediate characteristics with 58% occupancy and 510 ps lifetime.

polar residues (Ser, Thr, Asn, Gln) forming the majority. The occupancy of loop hydrogen bonds was lower (30-60%), which is in line with their dynamics. Also, 15 ± 5 hydrogen bridges were found between loops and 8 strands which are structural anchors that can control the flexibility of loops and ensure that the barrel integrity is preserved.

Table 4. Hydrogen Bond Distribution and Characteristics

Region	Number of H-Bonds	Occupancy (%)	Average Lifetime (ps)
β -Barrel (intra-strand)	142 ± 10	82 ± 12	850 ± 180
Extracellular Loops	28 ± 8	45 ± 15	320 ± 95
Loop-Barrel Interface	15 ± 5	58 ± 18	510 ± 145
Total	185 ± 15	71 ± 16	680 ± 195

Membrane Integration and Surface Properties

Solvent Accessible Surface Area (SASA): SASA analysis presented information on the interaction of OmpA with the lipid bilayer and water (Figure 6, Table 5). The average SASA was $145 \pm 8 \text{ nm}^2$ and the hydrophobic residues constituted $90 \pm 6 \text{ nm}^2$ (62%) and $55 \pm 5 \text{ nm}^2$ (38%) of the total SASA. The distribution of this

protein mirrors the dual nature of the protein, where the hydrophobic surfaces are in contact with lipid tails, and the hydrophilic surfaces are in contact with the interior of the pore or aqueous phases.

Membrane-embedded residues (about 5-165 of the transmembrane region) were analyzed to show that 78% of their surface area was hydrophobic, which is lipid-facing orientation. On the other hand, extracellular loops had residues with 65% hydrophilic SASA, which is suitable in exposure to water. The correct OmpA membrane insertion and orientation are confirmed by the amphipathic distribution of the simulation.

Table 5. Solvent Accessible Surface Area Analysis

Surface Type	Area (nm^2)	Percentage (%)	Primary Location
Total SASA	145 ± 8	100	-
Hydrophobic SASA	90 ± 6	62.1	Lipid-facing regions
Hydrophilic SASA	55 ± 5	37.9	Pore interior, loops
Membrane-embedded	103 ± 7	71	β -Barrel domain
Solvent-exposed	42 ± 4	29	Extracellular loops

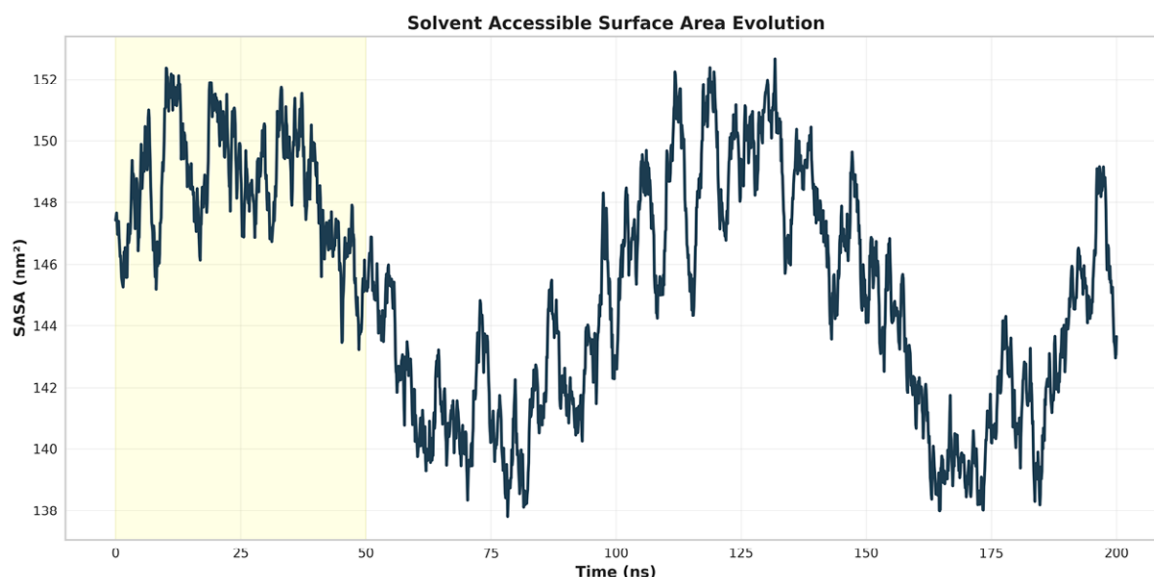


Figure 6. Solvent accessible surface area analysis showing OmpA's interaction with lipid bilayer and aqueous environment. Total SASA averaged $145 \pm 8 \text{ nm}^2$ with hydrophobic residues (blue, 62%) contributing $90 \pm 6 \text{ nm}^2$ and hydrophilic residues (red, 38%) contributing $55 \pm 5 \text{ nm}^2$. Membrane-embedded residues displayed 78% hydrophobic surface area consistent with lipid-facing orientation, while extracellular loop residues showed 65% hydrophilic SASA appropriate for water exposure, validating proper membrane integration.

Essential Dynamics and Principal Component Analysis

Principal Component Analysis (PCA): PCA was used to extract the largest group of collective moves in variance in the structure. The overall variance was explained by the first three principal components (PC1,

PC2, PC3) that consisted of 68.2% (PC1) and it was 38.5% (PC1 alone) (Figure 7). These major motions were mainly concerted motions of the extracellular loops, specifically the opening and closing movements of the Loop 2 and Loop 4 which are used to modulate the pore entrance.

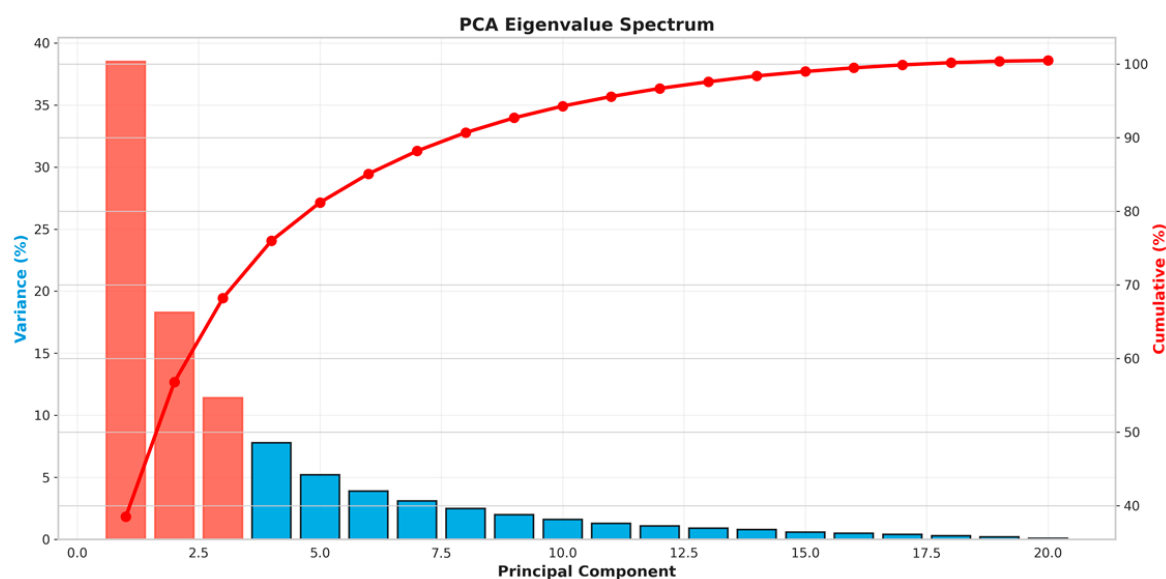


Figure 7. Principal component analysis revealing dominant collective motions of OmpA. The first three principal components (PC1, PC2, PC3) accounted for 68.2% of total structural variance. PC1 (38.5%, blue) represents breathing motions where Loops 2 and 4 move synchronously inward and outward relative to the barrel axis. PC2 (18.3%, red) describes twisting motions of loops around the barrel central axis. PC3 (11.4%, green) captures bending motions of individual loops. The rigid β -barrel core contributed minimally to these motions.

PC1 was a breathing motion in which Loops 2 and 4 acted in step against the barrel axis towards the inward and outward, and may control access of substrates to the pore. PC2 explained twisting movements of loops around the central axis of the barrel, induced, which can aid in changing the conformational pattern to other ligands or environmental factors. Local flexibility was reflected in the capturing of bending motions of individual loops by PC3. The 3rd PCs added very little to the first two PCs, according to the rigid 3D 0-barrel core, and in line with its structural stability.

Table 6. Principal Component Analysis Results

Principal Component	Variance Explained (%)	Cumulative Variance (%)	Primary Motion
PC1	38.5	38.5	Loop breathing (opening/closing)
PC2	18.3	56.8	Loop twisting around axis
PC3	11.4	68.2	Individual loop bending
PC4	7.8	76	Barrel deformation
PC5	5.2	81.2	Loop L1-L3 correlation
PC6-20	18.8	100	Minor fluctuations

Free Energy Landscape Analysis

Free Energy Landscape (FEL): The structure of the conformational landscape of OmpA was identified by the two-dimensional free energy surface obtained using PC1 and PC2 (Figure 8). Two separate energy basins were determined that were separated by an energy barrier of about 4.2 kcal/mol. Basin 1 (deeper, -8.3 kcal/mol) was the central one that occupied 72% of the simulation time with moderately open loop structures. Basin 2 (-6.1 kcal/mol) covered 23 percent of the time with a higher proportion of closed loop structures partly covering the pore entrance.

The energy difference between basins implies that loop conformational transitions take timescales of tens to hundreds of nanoseconds which agrees with the observed dynamics. The other 5% of conformations occupied more energetic states (> -4 kcal/mol) of transient, high-energy conformations in the course of loop motions. The presence of more than one basin shows that there is conformational heterogeneity that could be of functional use in substrate binding or molecular recognition.

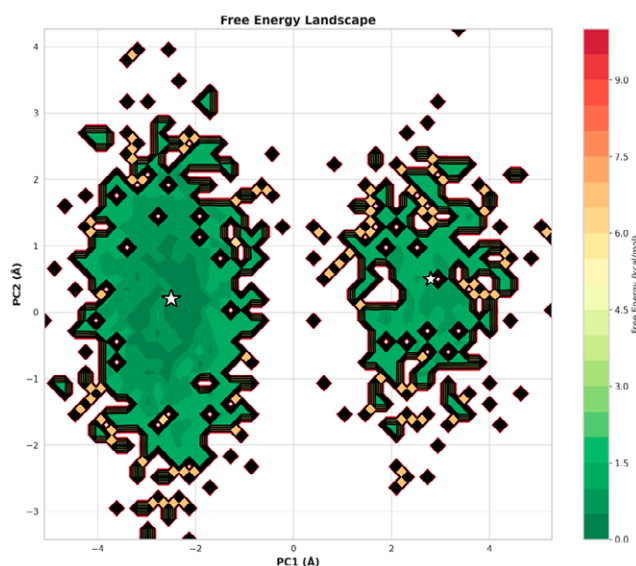


Figure 8. Two-dimensional free energy landscape constructed from PC1 and PC2 revealing the conformational landscape of OmpA. Two distinct energy basins were identified: Basin 1 (deeper minimum at -8.3 kcal/mol, blue region) represents the predominant conformation with moderately open loop configurations occupying 72% of simulation time. Basin 2 (minimum at -6.1 kcal/mol, green region) features more closed loop arrangements occupying 23% of the time. The basins are separated by an energy barrier of approximately 4.2 kcal/mol (yellow-red transition region), indicating conformational transitions occur on timescales of tens to hundreds of nanoseconds.

Table 7. Free Energy Basin Characterization

Basin	Minimum Energy (kcal/mol)	Occupancy (%)	PC1 Range	PC2 Range	Loop Configuration
Basin 1	-8.3	72	-3.5 to -1.2	-2.1 to 1.5	Moderately open
Basin 2	-6.1	23	1.8 to 4.2	-1.8 to 2.3	Closed
Transition	-4	5	-1.2 to 1.8	Various	Intermediate

DISCUSSION

The overall molecular dynamics of E. coli OmpA in a lipid bi-layer has given detailed information on the structural dynamics, stability and functional implication of this vital outer membrane protein. The equilibrium behavior of OmpA in physiological conditions within the 200 ns simulation reflected the natural stability of the 2-barrel architecture as well as the dynamism of the extracellular loop regions. Such results help in our current knowledge regarding structure-function relationships in outer membrane proteins and this has

therapeutic implications on development of antimicrobial drugs.

The RMSD analysis revealed that OmpA reached the structural equilibrium at about 50 ns, with after that it began to fluctuate at the same level with an average of 2.8 Å. This is a fairly small RMSD value that is associated with well-folded membrane proteins and corresponds to the fact that the CHARMM36m force field and simulation parameters are correctly representative of the physical properties of the protein-lipid system^{52,53}. The observed difference in the stability of the transmembrane 2-barrel domain (RMSD 1.9 Å) and the extracellular loops (RMSD 3.5 Å) conform to experimental results and prior computational investigations of the fact that membrane-bound domains are conformationally restricted whereas the solvent-exposed loops are more flexible^{47,48}. Such structural dichotomy relates to the nature of the selective forces and functional needs of these domains: the 1-barrel is needed to ensure membrane integrity and offer a stable scaffold whereas loops are necessary to carry out recognition, binding, and gating activities.

The per-residue RMSF analysis also allowed a more detailed mapping of the protein sequence in terms of flexibility, and showed regions of high mobility that could be functionally hotspots. The most fluctuating loops were identified as Loop 2 (residues 55-75) and Loop 4 (residues 140-160), which is expected because they contribute to the recognition of the substrate, and there exists the possibility for getting the protein. These loops have many charged and polar residues which form short-lived electrostatic interaction with passing molecules and can be flexible which can allow induced-fit binding. Past experimental research on site-directed mutagenesis has reported that the mutations in Loop 2 and Loop 4 influence the binding of OmpA to bacteriophages and cell receptors of the host and this serves as an indication of the functional significance of loop dynamics^{19,20}. Both the high flexibility and functional significance correlate to indicate that these regions are desirable targets of structure-based drug designing in a bid to disrupt the processes mediated by OmpA.

The stability of the 8-helix 2 on membrane environments is confirmed by the maintenance of 8 helix content at 43 percent during the entire simulation and this is similar to the crystallographic structure 45 percent. The antiparallel 8-barrel is an evolutionary-conserved membrane integration solution in Gram-negative bacteria and its stability is dictated by a large number of

hydrogen bonds and positive hydrophobic interactions with the lipid core^{6,7}. After our hydrogen bond analysis, we found that there were 185 average intramolecular hydrogen bonds and in the 142 highest stability bonds, the 2 sites within the 75-95 percent occupancy were in the 142 16 12-barrel region. The barrel is made up of these inter-strand hydrogen bonds, which are essential in sustaining the typical amphipathic positioning of the residues. Barrel hydrogen bonds are found to be much stronger in occupancy and lifetime (850 ps) compared to transient loops (320 ps) quantitatively descriptive of the structural rigidity of these areas.

The radius of gyration did not vary during the simulation and was always equal to 17.2 Å, which means that OmpA did not change its compact and rigidly folding structure significantly. Such stability is especially remarkable since the time spent in the simulation process was quite long and gives the assumption that the protein has not experienced any artifactual unfolding or aggregation. The fact that the Rg value was consistent also indicates a balance between the protein-lipid interactions and that the size of the simulation box was sufficient enough to eliminate periodic boundary effects. OmpA has a functionally significant compact structure since any large-scale expansion would destabilize membrane integrity and possibly open non-specific permeability channels which would be lethal to the cell.

Solvent accessible surface analysis gave a quantitative result in integrating and orienting OmpA correctly into a membrane. The observation that 62 percent of the surface area is hydrophobic, 78 percent of hydrophobicity in the areas where the membrane is embedded, affirm the amphipathic character of the protein and authenticate the simulation set-up. The number of hydrophobic residues that are not exposed to the lipid bilayer prevents the thermodynamically unfavorable exposure of nonpolar surfaces to the aqueous solvent, and the hydrophilic pore interior and loop regions interact favorably with water. This topological complementarity is required to allow stable integration of membranes, and is a widespread design concept in outer membrane proteins. This hydrophobic-hydrophilic equilibrium seems to be critical to the protein, and disturbance of it by mutations or foreign interventions would probably destabilize the protein and interfere with its functioning.

The principal component analysis showed that the most important motions of OmpA are concerted motions of extracellular loops but not rigid-body

motions of the full protein. The major component that explains 38.5% of the variation is the first principal component and represents breathing motions: loops open and close with regard to barrel axis. It has significant functional consequences, this motion may control entry to the interior of the pore and also tune the binding of substrates. The opening-closing dynamics are repeating on a timescale of nanoseconds to tens of nanoseconds and this implies that the pore switches between accessible and occluded states in a sufficiently rapid cycle to allow physiological substrate flux and be selective. The latter major part which signifies twisting motion of loops can streamline conformation change as OmpA interacts with various ligands or other cell constituents. These modes of dominant motions can be identified, which can be used to gain insights into how OmpA achieves its different functions and likely mechanisms through which a functional versatility to conformational dynamics may be achieved.

The free energy landscape analysis showed that there are two large conformational basins, which means that OmpA occupies different structural states in normal dynamics. The most abundant basin (72% occupancy) contains moderate-open loops that would permit substrate entry, whereas the minor basin (23% occupancy) contains more closed loop structures which could be a gating state or a ligand-occupied structure. The energy barrier between these basins equals 4.2 kcal/mol that is not especially high, which means that the basins can interchange on time scales that are reachable by our simulation (tens to hundreds of nanoseconds). Presence of various conformational states is in line with the functional promiscuity of OmpA that is a structural protein, phage receptor, and adhesin. Such an equilibrium of the basins can be altered by different binding partners or environmental conditions, which gives a form of functional regulation. This conformational plasticity is opposite to the more limited active site geometry of enzymes and explains how structural proteins can obtain functional diversity by maintaining a dynamic equilibrium between a variety of different states.

The implication of these structural dynamics findings is to the role of OmpA with respect to antibiotic resistance. The loop flexibility and conformational plasticity in our simulations can be involved in causing reduced susceptibility to antibiotics in a number of ways. In the first place, the dynamic loop areas may perform like adjustable gates which retain large antibiotic molecules away, and permit entry of nutrients. The

motions of the breathing in PC1 might open temporary pores that selectively filter on the size and charge of the molecules and can act as a molecular sieve. Second, the capacity to change between several different conformational states can allow OmpA to maximize membrane permeability under antibiotic stress, which could result in sealing pores or changing selectivity in the presence of antibiotics. Third, the β -barrel membrane integrity is provided by the structural stability of the OmpA, which provides an effective barrier property in the situations when the other porins could be destabilized. Fourth, the loops located on the surface are binding sites of efflux pump components and their elasticity may contribute to the production of multi-protein complexes active in expelling antibiotics. The molecular basis of these resistance mechanisms is important to know how to devise ways of surmounting them.

On the basis of drug design, the structural understanding of this research indicates that there are a number of potential therapeutic protocols against OmpA. The elastic loops in Loop 2 and Loop 4 are potentially available targets to peptide-based or small molecule inhibitors that have the potential to bind the loops in an inactive conformation that prevents OmpA-mediated adhesion or phage binding. Compounds that disrupt the hydrogen bonding networks which hold the barrels together may disrupt the stability and so make the protein unstable which will make the membrane more permeable to other antibiotics. The determined energy basins and transition pathways can be used as a guide to rational design of molecules that stabilize specific conformational states, either high energy like stabilizing the protein in a closed and non-functional form or low energy like forcing the protein to an open form that disrupts membrane integrity. The surface residues engaged in protein-protein interactions with efflux pumps or any other component of the membrane are also interfaces that may be broken to increase the efficacy of antibiotics.

The research has certain weaknesses that must be considered. First, the simulation was conducted on simplified symmetric POPE bilayer and not on the asymmetric LPS-containing outer membrane of the gram negative bacteria. Although POPE bilayers have been extensively tested to analyze the outer membrane protein, the lack of LPS can have an influence on loop dynamics and protein-lipid interactions, in the case of the outer leaflet-facing surface. Simulations in which the complete LPS parameterization is done in the future would be more physiologically realistic depictions of the

original environment⁵⁵. Second, the timescale of 200 ns simulation is not very fast, which seems to be sufficient to simulate rare conformational transitions or slow collective movements on the timescale of microseconds to milliseconds. These slower events might be reached by long-simulations or more efficient sampling procedures including replica exchange or metadynamics⁷⁹. Third, the simulation is of OmpA alone without taking into consideration of possible oligomerization and interaction with other membrane proteins as in the case in vivo. To comprehend the cooperative effects and higher-order organizational principles multi-protein simulations would be required. Fourth, OmpA (residues 172-325), which is the periplasmic domain, was not considered in this study, and its interactions with the peptidoglycan layer and impacts on the dynamics of transmembrane domains have to be studied.

In spite of these shortcomings, the overall study of OmpA dynamics offers a good basis in the upcoming experiments and computational researches. The predictions of the quantitative characterization of the flexibility, the hydrogen bonding, and the conformational states have a testable character and can be confirmed by methods like site-directed mutagenesis, fluorescence spectroscopy, or the measurements of NMR dynamics. These motion modes and energy landscapes can be used to inform the design of experiments to probe the functioning of a given mechanism: substrate permeability assays between wild-type and loop mutant proteins. Also, the computational system that has been built here can be further developed to analyze clinically relevant OmpA variants of pathogenic strains, which will allow us to investigate strain-specific virulence mechanisms and resistance phenotypes.

CONCLUSIONS

The 200 ns molecular dynamics simulation of *E. coli* OmpA showed stable 2 -barrel architecture (RMSD 2.8 Å, 43% -sheet) and rigid transmembrane domain and flexible extracellular loops (RMSF > 4.5 Å). The structural integrity is ensured by extensive hydrogen bonding networks (185 bonds), whereas the correct membrane integration was ensured (62% hydrophobic surface). The breathing and twisting loop motions were observed as the principal component motions that explained 68% of the variance. Two conformational basins separated by 4.2 kcal/mol Barriers showed free energy landscapes, which reveal functional plasticity in

terms of membrane stability, phage binding, and antibiotic resistance. The potential antimicrobial targets include flexible loops and hydrogen bonding networks. Asymmetric LPS membranes, longer simulations, and experimental validation should be included in future work to further the therapeutic approach against Gram-negative pathogen.

Ethics Statement and Conflict of Interest Disclosures

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Cardiotoxicity and Other Adverse Effects of Immune Checkpoint Inhibitors

Suresh MALAVIKA¹, Panelil Gopalakrishnan · MEGHNA¹, Nambidiyattil PARVATHY^{1,*}, Santhosh SANJU¹, Pushpa Raghavan ROSHINI¹, Krishna Haridas NIKHIL², Ahamed HISHAM³

Abstract

Background: Immune checkpoint inhibitors (ICI) usage has increased in recent years, along with it the associated adverse effects.

Objective: To conduct an observational study on immune checkpoint usage and adverse drug reactions associated with its use in a tertiary care center from coastal south India.

Methodology: 228 patients were enrolled in this ambispective study, patient's data was collected from the year 2020-2024. Patients were selected based on inclusion criteria.

Results: While adverse effects such as pruritus, tachycardia, and thyroiditis occurred infrequently, their clinical significance necessitates careful monitoring. A statistically significant association ($p = 0.022$) between treatment response and adverse events indicated a potential relationship between therapeutic effectiveness and toxicity. The instance of myocarditis was found to be 0.4%.

Conclusion: These findings emphasize the significance of accurate monitoring, management, which includes various fields, and individualized therapies to maximize the outcomes for patients and reduce potential risks associated with Immune Checkpoint inhibitors (ICI).

Keywords: Immune checkpoint inhibitors, cardiotoxicity, myocarditis, immune-related adverse events

¹Department of Pharmacy Practice, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Ponekkara, Kochi Kerala, India

²Department of Medical Oncology, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Ponekkara, Kochi Kerala, India

³Department of Cardiology, Amrita institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Ponekkara, Kochi Kerala, India

***Corresponding author:**

Nambidiyattil PARVATHY, Department of Pharmacy Practice, Amrita institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Ponekkara, Kochi Kerala-682041, India

E-mail: parvathy1728@gmail.com

INTRODUCTION

Cancer continues to be a primary contributor to mortality rates in our country. Conventional treatments, including surgery, chemotherapy, and radiation therapy, have been widely employed; however, they come with notable disadvantages. Despite significant progress in recent years, both chemotherapy and radiation frequently result in cytotoxic effects that harm healthy cells, while only targeting a small percentage of cancer cells¹.

Over the past two to three decades, oncology has experienced significant advancements with the introduction of innovative therapies such as checkpoint inhibitor immunotherapy, proton therapy, and robotic surgery. Checkpoint inhibitors have shown consistent overall survival benefits across various clinical settings, as evidenced by randomized trials². Immunotherapy has notably broadened the range of available cancer treatments³. Nonetheless, tumours can evade immune detection through various mechanisms, including the upregulation of inhibitory signals, the secretion of immunosuppressive cytokines, or the downregulation of antigen expression⁴. It is important to note that immune checkpoint inhibitors (ICIs) can be safely administered to patients with multiple comorbidities, including those with HIV, influenza, or SARS-CoV-2 infections⁵.

Drugs in this category, including CTLA-4 inhibitors like ipilimumab and PD-1/PD-L1 inhibitors such as nivolumab and pembrolizumab, function by activating suppressed T-cell activity. This enhancement allows the immune system to more effectively target and eliminate tumour cells, leading to sustained responses in various cancer types⁶. The long-term evidence derived from early ICI trials is encouraging, as a subset of patients with metastatic cancer has demonstrated enhanced survival rates even at the five-year mark⁷.

Organizations like the Canadian Agency for Drugs and Technologies in Health offer dosing recommendations based on pharmacokinetic modelling to enhance the utilization of ICIs. Researchers in Europe have proposed strategies such as vial sharing and weight-based dosing to optimize costs and decrease drug waste⁸. In India, these medications were launched only a few years prior. Nonetheless, the elevated expenses have constrained broad implementation, and most clinical practices are informed by Western research. The absence of published real-world data regarding their safety and efficacy within the Indian population

is essential to substantiate their costs and the potential for immune-related adverse effects (IrAEs)⁹.

Immune checkpoint inhibitors can lead to a range of cardiotoxic effects as a result of extensive immune activation among the immune-related adverse events. While infrequent, these cardiac complications hold clinical significance. ICI-induced cardiotoxicity includes conditions such as myocarditis, pericarditis, arrhythmias, and heart failure. Myocarditis represents a serious condition, impacting 0.27–1.14% of patients and associated with a mortality rate exceeding 40% if left untreated. The underlying mechanism is believed to involve T-cell-mediated inflammation targeting cardiac tissue, indicative of the autoimmune activation triggered by immune checkpoint inhibitors. Factors that contribute to risk encompass prior cardiovascular conditions, the use of combination immune checkpoint inhibitor therapy, and genetic predispositions¹⁰.

The American Society of Clinical Oncology (ASCO) categorizes ICI-associated myocarditis into four grades, determined by severity, clinical presentation, and diagnostic findings:

G1: Cardiac biomarkers are abnormal, but the patient is asymptomatic and has a normal ECG.

G2: Cardiac biomarkers are abnormal, and the patient has either mild symptoms or new ECG changes that do not include conduction delays.

G3: Cardiac biomarkers are abnormal, and the patient experiences either moderate symptoms or develops new conduction delays on the ECG.

G4: The patient is moderately to severely decompensated, needs intravenous therapy or other medical intervention, or is facing life-threatening conditions.^{11,12}

METHODOLOGY

This study was conducted in a tertiary care center in Southern India. This was an ambispective observational study with the aim of tracking patients on treatment with immune checkpoint inhibitors. We gathered data retrospectively from the patients between January 2020 and November 2023 from different sources, such as cardiology reports, clinical performances, and hospital health information system data, whereas patients who were started on immune checkpoint inhibitors were prospectively studied for four months. We selected patients based on the inclusion and exclusion criteria. Patients treated with immune checkpoint inhibitors (ICIs) were included, while those not meeting

electrocardiographic (ECG), imaging, and biomarker criteria were excluded. Based on the findings of an existing literature study on cardiovascular events following the introduction of immune checkpoint inhibitors, (13) the required sample size came out to be 186 patients with 95% confidence and 5 units absolute precision, we were able to include 228 patients into our study based on the available health system data. Patients were assessed for cardiotoxicity and other adverse effects. Statistical significance was assessed using the chi-square test and the independent samples t-test. Statistical analysis was conducted using SPSS version 30.

RESULTS

Baseline Characteristics

The median age of the 228 patients in this retrospective study was 62 years, with a range from 9 to 87 years. Most participants were male (68.4%). 86% of patients exhibited non-metastatic disease, whereas thirteen percent presented with metastases. Histocellular carcinoma constituted 27.1% of all cases; lung cancer represented 19.8%, and head and neck cancers, especially tongue and pharyngeal cancers, were the predominant types of primary cancer. Histologically, genitourinary cancers were the most prevalent, comprising 33.7% of the total, followed by lung cancers at 30.7%, and then melanomas and sarcomas at 9.9%. A heterogeneous cohort of patients was administered immune checkpoint inhibitors (ICIs), as indicated by the variety of oncological conditions represented.

Treatment and Response

Nivolumab (41.6 percent) and pembrolizumab (35.08 percent) were the most frequently utilized immune checkpoint inhibitors. Atezolizumab and durvalumab usage came out to be (19.7%) and (3.5%) respectively. Outcomes from therapy were inconsistent, with over half of the patients (56.1%) encountering disease progression. Partial responses (3.07%) and complete response (8.3%) were infrequent, and 11.8% expired during treatment. While the majority (56.1%) experienced progression, 2.6% of patients experienced relapse or inadequate response following initial improvement. Notably, 17.9% of patients were lost to follow-up, potentially impacting long-term outcomes.

Adverse drug reactions

Adverse drug reactions (ADRs) linked to immune checkpoint inhibitors (ICIs) in this cohort were typically rare but exhibited diverse manifestations, impacting

Table 1. Baseline characteristics

	N=228
Age (median-range)	62 y (9–87)
Sex	
Male	156 (68.4%)
Female	72 (31.6%)
Metastasis	
Metastatic	34 (13.6%)
Non-metastatic	216 (86.4%)
Diagnosis	
Renal cell carcinoma (RCC)	10 (10.4%)
Hodgkins Lymphoma	4 (4.2%)
Cholangiocarcinoma (including intrahepatic)	2 (2.1%)
Gall bladder cancer	1 (1.0%)
HCC + BCLC C and BCLC D	26 (27.1%)
Ca Lung	19 (19.8%)
Ca Tongue	7 (7.3%)
Ca prostate	1 (1.0%)
Ca stomach	3 (3.1%)
Ca breast	4 (4.2%)
Ca esophagus	6 (6.2%)
Ca tonsil	1 (1.0%)
Endometrial Ca	3 (3.1%)
Urothelial Ca	3 (3.1%)
Ca Ovary	1 (1.0%)
Ca pharynx	3 (3.1%)
Leptomeningeal	1 (1.0%)
Embryonal germ cell ca	1 (1.0%)
Histology	
Lung Cancers.	31(30.7)
Gastrointestinal (GI) Cancers.	8 (7.9%)
Genitourinary (GU) Cancers.	34 (33.7%)
Head & Neck Cancers.	9 (8.9%)
Breast & Gynecological Cancers.	9 (8.9%)
Melanomas & Sarcomas.	10 (9.9%)

various organ systems. The most frequently reported adverse drug reaction was pruritus, noted in 4.3% of patients, closely followed by tachycardia and fatigue, each occurring in 3.9% of cases. Gastrointestinal disturbances occurred in 2.19% of patients, while metabolic complications, including electrolyte imbalance (1.7%) and diabetes (1.31%), were also observed. Hematologic toxicities, such as leukopenia (1.7%), and mucocutaneous adverse effects, including oral ulcers (1.75%), were infrequent, however clinically significant. Endocrine-related adverse effects, although infrequent, contained thyroiditis (1.3%), consistent with established immune-mediated thyroid dysfunctions linked to immune checkpoint inhibitors (ICIs). Diabetes also occurred at the same frequency (1.31%). Hepatitis, a potentially

serious immune-related adverse event, occurred in 0.88% of the population. Neurological toxicity was negligible, with delirium noted in only one patient (0.43%). The major cardiac adverse effect associated with immune checkpoint inhibitors, myocarditis, occurred in one patient (0.43%). The diverse immune-related toxicities observed emphasize the necessity for close surveillance for patients on immune checkpoint inhibitors.

There was a significant response observed between response to treatment and adverse effects with a p-value of 0.022, which indicates that a patient's response to treatment could be associated with the adverse events they encounter. The instance of myocarditis was found to be 0.4%

DISCUSSION

The advent of immune checkpoint inhibitors (ICIs) has revolutionized cancer therapy by enhancing survival across a broad spectrum of cancers. Their immune-mediated action, however, also subjects patients to a range of irAEs, some of which are severe or life-threatening^{6,14}. The most frequent irAE in our study was pruritus (4.3%), consistent with international experience pointing to dermatologic toxicities like psoriasis form eruptions and lichenoid reactions as among the most common¹⁵. A case report published in 2023 described a patient undergoing cancer treatment who experienced abrupt oxygen desaturation and widespread erythema secondary to a hypersensitivity reaction to atezolizumab. A desensitization protocol was subsequently repeated for each treatment cycle, allowing continuation of therapy without recurrence of significant reactions to subsequent doses.¹⁶

Cardiac presentations, such as tachycardia (3.9%) and myocarditis (0.43%), as rare as they are, warrant special mention by virtue of their potentially life-threatening nature. The incidence of observed myocarditis at 0.4% aligns with international reports from the FDA FAERS and WHO Vigibase of rates between 0.27% and 1.14%, and consequent mortality in excess of 40% if not treated^{10,17}. This underscores the need for early diagnosis and prompt action. A previous study has shown that a hs-CRP threshold of 2 mg/L is a significant predictor of major adverse cardiac vascular events, with those who have it 19 times more likely to experience severe events¹⁸. This underscores the need for early diagnosis and prompt action. Our experience of myocarditis was in a patient who was a female with

Table 2. Immune checkpoint Inhibitors

Table 2	N=228
Nivolumab	95 (41.66)
Pembrolizumab	80 (35.08)
Atezolizumab	45 (19.73)
Durvalumab	8 (3.508)

Table 3. Response to treatment

	N= 228
Disease Progression	128(56.14)
Partial response	7(3.07)
Poor response/Relapse	6(2.63)
Death	27(11.8)
Good Response	19(8.33)
Lost to follow up	41(17.98)

Table 4. Adverse Drug reactions

Cardiac	
Tachycardia	9 (3.9)
Myocarditis	1 (0.43)
Non- Cardiac	
Pruritus	10 (4.3)
Fatigue	9 (3.9)
GI disturbance	5 (2.19)
Electrolyte Imbalance	4 (1.75)
Leukopenia	4(1.75)
Mouth Ulcer	4 (1.75)
Diabetes	3 (1.31)
Thyroiditis	3 (1.31)
Hepatitis	2 (0.877)
Delirium	1 (0.43)

metastatic adrenocortical carcinoma on pembrolizumab and developed heart failure and ultimately died of multiorgan dysfunction emphasizing the severity of ICI-related myocarditis¹⁹

Endocrine irAEs like thyroiditis (1.3%) and diabetes mellitus (1.3%) were noted, in accordance with literature that reports thyroiditis as the most common endocrine side effect of ICI therapy^{20,3}. Pembrolizumab, however, has been most associated with an increased frequency of immune-related diabetes^{21,22} and were thus seen in our cohort. Gastrointestinal side effects and hematologic toxicities were also uncommon but were clinically relevant. Electrolyte disturbances, such as hyponatremia, were noted and are being increasingly described as among the ICI toxicity syndrome²³.

We also observed mucosal toxicities, such as oral ulceration and upper GI tract involvement, in a few patients. Earlier reports have described a greater-than-expected incidence of oral side effects in ICI recipients^{24,25}. In addition, two instances of hepatitis

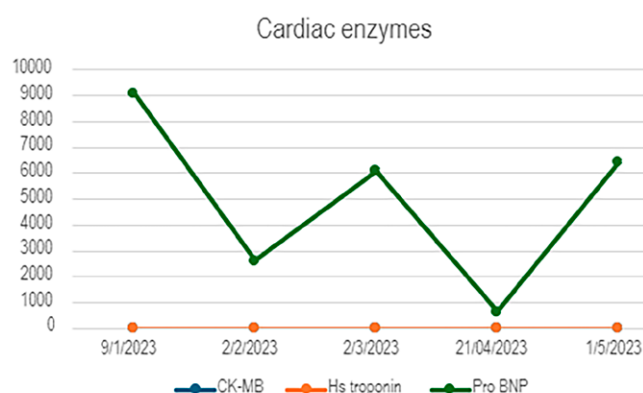


Figure 1: Changes in cardiac function test for myocarditis patient

arose during nivolumab treatment. Although uncommon, nivolumab-induced hepatotoxicity has been reported and is believed to occur via mechanisms such as T-cell-mediated hepatic injury^{26,27}.

Notably, a statistically significant correlation ($p=0.022$) between the presence of adverse events and response to treatment was noted. This finding validates earlier hypotheses that irAEs can serve as surrogates for efficient immune activation and thus associate with favorable treatment response^{10,17}. Nevertheless, by virtue of the exploratory nature of our analysis, this correlation needs validation in a larger patient population.

LIMITATIONS

Our study is subject to several important limitations that should be considered when interpreting the findings. The relatively small sample size ($n = 228$) restricts the generalizability of our results, particularly for rare events such as myocarditis. Additionally, the prospective follow-up period was limited to only four months, which may only detect acute toxicities and be insufficient to detect delayed-onset immune-related adverse events (irAEs), especially those involving cardiac or endocrine systems that can manifest later. Cardiac assessments predominantly relied on echocardiograms and biomarkers, while the more sensitive cardiac MRI was not consistently utilized, potentially leading to under-detection of myocarditis. Furthermore, our analysis was exploratory in nature and did not include multivariate adjustments for possible confounders such as baseline cardiac conditions, tumor size, or concurrent therapies. The lack of a control or comparison group restricts the ability to draw causal conclusions. There was also inconsistency in the application of the cardiac

work-up algorithm across patients, which could impact the uniformity of cardiotoxicity detection and reporting. These limitations highlight the need for larger, multicenter studies with standardized assessment protocols to provide more definitive insights.

CONCLUSION

This real-life experience from a tertiary center in Southern India gives an insight into the safety profile of immune checkpoint inhibitors in various malignancies. Although the majority of immune-related adverse effects were rare, their clinical significance particularly cardiac and endocrine toxicities deserve attention. The rate of observed myocarditis is consistent with international estimates, reiterating the importance of anticipatory cardiac surveillance, even in low-resource environments. The correlation between response to treatment and adverse events indicates that irAEs could be a marker of increased immunologic activity, although this association is exploratory in nature.

Considering the sample size, follow-up period, and heterogeneity in diagnosis limitations, the results must be interpreted cautiously. This study draws on real-world data to shed light on immune checkpoint inhibitor usage. Looking ahead, further research could involve broader patient groups, assess long-term effects, and explore factors that predict treatment response or adverse events to enhance the effectiveness of immune checkpoint therapy.

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Ethics Statement and Conflict of Interest Disclosures

Financial support and sponsorship: All authors have declared that no financial support was received from any organization for the submitted work.

Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws. Written informed consent was provided by all the patients participants in this study. This study was approved by the Institutional Research Board and Ethics Committee.

Conflict of interest: No known conflict of interest correlated with this publication.

Availability of data and materials: The data used and/or analyzed throughout this study are available from the corresponding authors upon reasonable request.

Competing interests: The authors declared that they have no competing interests.

The use of generative AI and AI-assisted technologies: The authors did not use in this article generative AI and AI-assisted technologies.

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Exploring the Gut Microbiome-Immune Axis: Potential Benefit of Probiotics and Synbiotics in Systemic Lupus Erythematosus, a Meta-Analysis of Randomized Control Trials

Muhammad Ifham HANIF¹, Mentari Maratus SHOLIHAH¹, Arief NURUDHIN², Yulyani WERDININGSIH², Indrayana SUNARSO², Nurhasan Agung PRABOWO³, Taufik Ridwan Hadi KUSUMA⁴, Dwiana KARTIKAWATI¹, Nasywa Zahida ROCHMAH⁵

Abstract

Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by periods of relapse and remission, in which gut microbiota imbalance plays a role in triggering and sustaining inflammation through pro-inflammatory cytokines. Therapeutic approaches targeting the gut microbiota, such as probiotics and synbiotics, have the potential to regulate disease activity by restoring microbial balance and reducing systemic inflammation. Several randomized controlled trials (RCTs) have explored the effects of microbiota-based interventions in autoimmune diseases; however, comprehensive meta-analyses summarizing their efficacy remain limited.

Objective: To explore the potential benefit of probiotics and synbiotics supplementation in improving SLE disease activity and inflammatory markers.

Methods: RCTs relevant to the treatment of SLE with probiotics and synbiotics were systematically retrieved from Pubmed, EMBASE, Cochrane, Sinomed in accordance with the PRISMA guideline. The meta-analysis was performed using RevMan 5.4.1 software. A fixed-effects model was applied, Cochrane Risk of Bias 2 (ROB 2.0) was used as a bias assessment tool.

Results: Seven studies with a total 460 subjects were included in this meta-analysis. Data consisting of SLEDAI-2K, IL-17 and IgG from studies RCTs. The pooled analysis demonstrated that probiotic or synbiotic supplementation significantly reduced disease activity of SLE as assessed by the SLEDAI-2K score (MD = -2.33, 95% CI -2.49, -2.16; $P < 0.00001$; $I^2 = 61\%$). Similarly, the pro-inflammatory markers of IL-17 (MD = -1.04, 95% CI -1.55, -0.52; $P < 0.0001$) and IgG (MD = -2.64, 95% CI -2.77, -2.51; $P < 0.00001$) revealed a significant reduction compared to control group.

Conclusion: Gut microbiota-based therapies, including synbiotics and probiotics supplementation, demonstrate the potential benefit to reduce systemic inflammation and improve SLE disease activity through modulating gut microbiota composition and function. While promising as an adjunctive treatment for SLE, further studies are required to investigate its mechanisms and therapeutic efficacy.

Keyword: Systemic Lupus Erythematosus, Synbiotics, Probiotics, Gut Microbiome, Meta Analysis

¹Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia

²Division of Rheumatology, Department of Internal Medicine, Dr. Moewardi Hospital, Surakarta, Central Java, Indonesia

³Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret, Universitas Sebelas Maret (UNS) Hospital, Sukoharjo, Central Java, Indonesia

⁴Dr. Suhardi Hardjolukito Air Force Hospital, Bantul, Yogyakarta Special Region, Indonesia

⁵Medical Doctor Professional Program, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia

*Corresponding author:

Muhammad Ifham Hanif, Department of Internal Medicine, Dr. Moewardi Hospital, Surakarta, Central Java, Indonesia

E-mail: muhammadifhamhanif@gmail.com

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by periods of relapse and remission, involving multiple organ systems and the production of a wide array of autoantibodies. The precise etiology of SLE remains incompletely understood, but it is recognized as a multifactorial condition influenced by genetic, hormonal, and environmental factors. Recent advancements in research have increasingly highlighted the critical role of the gut microbiota (GM) in the pathogenesis and progression of SLE. An imbalance in the composition and function of the gut microbiota, termed dysbiosis, is now understood to contribute to the initiation and perpetuation of inflammation through the modulation of pro-inflammatory cytokines and immune responses (Parodi et al., 2025).

Evidence suggests that gut dysbiosis in SLE patients is characterized by reduced microbial diversity and alterations in the abundance of specific bacterial phyla, such as an increase in Proteobacteria and a decrease in Firmicutes and Bacteroidetes. These microbial shifts can lead to increased intestinal permeability, often referred to as a “leaky gut syndrome,” which allows microbial antigens to translocate and trigger abnormal immune responses, thereby predisposing individuals to SLE and its complications, including lupus nephritis (LN) (Guo et al., 2021; Parodi et al., 2025). Specific bacterial species, such as *Bacteroides thetaiotaomicron* and *Ruminococcus gnavus*, have been implicated in driving autoimmune responses through mechanisms like molecular mimicry and immune dysregulation (Parodi et al., 2025).

Given the significant interplay between the gut microbiome and the immune system, therapeutic strategies aimed at modulating the gut microbiota have emerged as promising avenues for managing SLE. Interventions such as probiotics, prebiotics, and synbiotics are being explored for their potential to restore microbial balance, reduce systemic inflammation, and improve disease activity (Kim & Mills, 2024). Probiotics, defined as live microorganisms that confer a health benefit to the host when administered in adequate amounts, have been shown to alter gut microbiome composition and regulate immunomodulatory and inflammatory responses (Guo et al., 2021). Synbiotics, a synergistic combination of probiotics and prebiotics, are designed to enhance the viability and beneficial effects of these microorganisms in the gut (Kim

& Mills, 2024). While several randomized controlled trials (RCTs) have investigated the impact of microbiota-based interventions in autoimmune diseases, a comprehensive meta-analysis specifically summarizing their efficacy in SLE remains crucial for guiding clinical practice. This paper aims to explore the potential benefits of probiotics and synbiotics supplementation in improving SLE disease activity and inflammatory markers.

METHODS

Study Design and Reporting Guideline

This study was conducted as a systematic review and meta-analysis of randomized controlled trials (RCTs) evaluating the effects of probiotic and synbiotic supplementation in patients with Systemic Lupus Erythematosus (SLE). The review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.

Eligibility Criteria

Studies were included if they were randomized controlled trials (RCTs) involving adult patients diagnosed with Systemic Lupus Erythematosus (SLE) based on established classification criteria. Eligible studies evaluated the use of probiotics and/or synbiotics as an intervention and compared the outcomes with placebo or standard therapy. Studies were required to report at least one of the following outcomes: SLE Disease Activity Index (SLEDAI-2K), interleukin-17 (IL-17), or serum immunoglobulin G (IgG).

Studies were excluded if they were observational studies, case reports, case series, systematic reviews, or meta-analyses. Animal or in vitro studies were also excluded. In addition, studies with insufficient or incomplete outcome data and articles for which the full text was unavailable were not included in the analysis.

Information Sources and Search Strategy

A systematic literature search was conducted in the following electronic databases: PubMed, EMBASE, Cochrane Library, and Sinomed. The search was performed without restrictions on publication status. Keywords and Medical Subject Headings (MeSH) related to Systemic Lupus Erythematosus, probiotics, synbiotics, and gut microbiota were used, combined using Boolean operators (“AND” and “OR”).

The reference lists of eligible articles were also manually screened to identify additional relevant studies.

Study Selection

All records retrieved from the electronic databases were imported into a reference management system, and duplicate records were removed. Two reviewers independently screened the titles and abstracts to identify potentially eligible studies. Subsequently, full-text articles were assessed for eligibility based on the predefined inclusion and exclusion criteria. Any disagreements between the reviewers were resolved through discussion and consensus.

Data Extraction

Data were independently extracted by two reviewers using a standardized data extraction form. The extracted information included the first author and year of publication, study design and sample size, type and dose of probiotic or synbiotic intervention, duration and route of administration, and reported outcomes, including SLE Disease Activity Index (SLEDAI-2K), interleukin-17 (IL-17), and serum immunoglobulin G (IgG). Any discrepancies in data extraction were resolved through discussion and consensus.

Risk of Bias Assessment

The methodological quality of the included randomized controlled trials was assessed using the Cochrane Risk of Bias tool version 2.0 (ROB 2.0). The assessment covered five domains: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, and bias in selection of the reported result. Each study was classified as having a low risk of bias, some concerns, or a high risk of bias.

Statistical Analysis

Meta-analysis was conducted using Review Manager (RevMan) version 5.4.1. Continuous outcomes were analyzed using Mean Difference (MD) or Standardized Mean Difference (SMD) with 95% Confidence Intervals (CIs). The meta-analysis was performed using the fixed-effects model. Heterogeneity was assessed using the I^2 statistic, where $I^2 > 50\%$ indicated significant heterogeneity.

RESULT

Study Selection

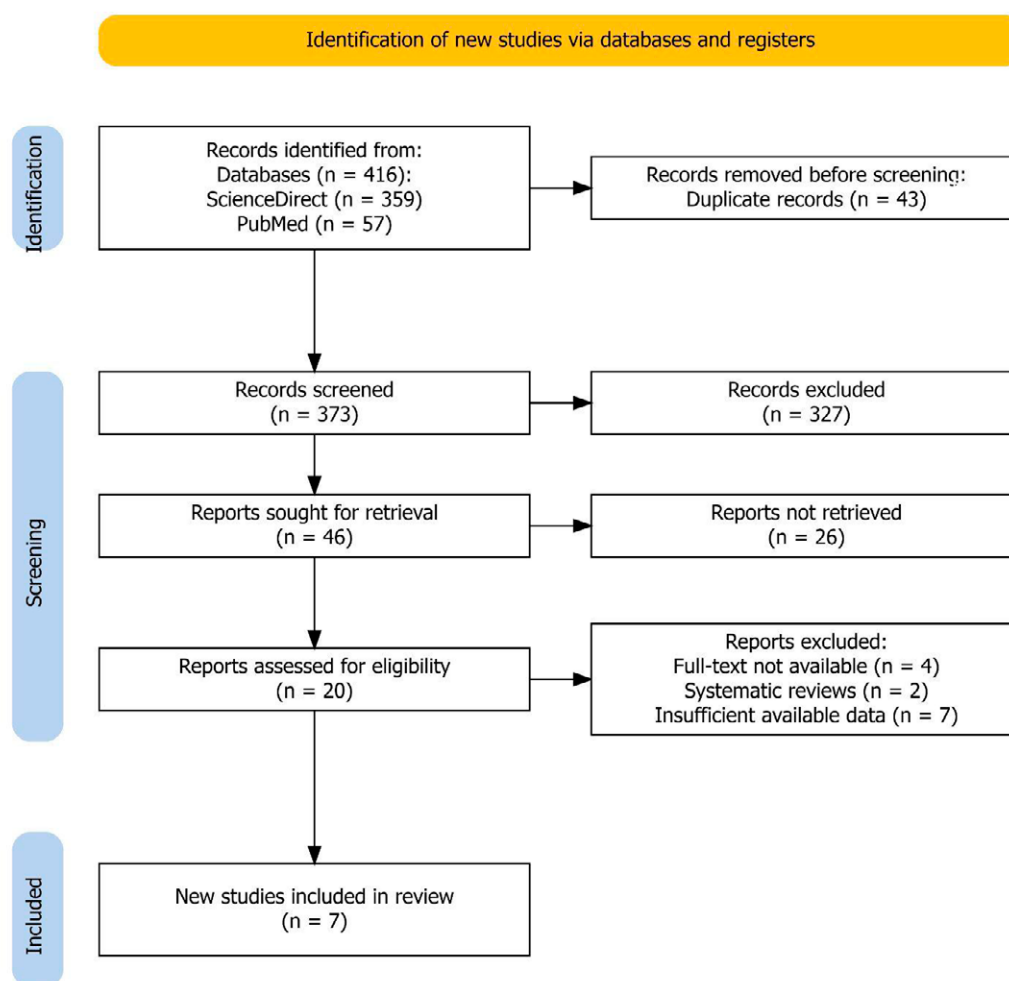
The selection process of the studies is presented in the PRISMA 2020 Flow Diagram (Figure 1), illustrating the flow of information from the identification stage through to inclusion.

Figure 1. PRISMA 2020 Flow Diagram

Figure 1 illustrates the flow of studies throughout the selection process. The systematic search identified a total of 416 records from the databases, consisting of 359 from ScienceDirect and 57 from PubMed. Before screening, 43 duplicate records were removed. A total of 373 records were screened based on titles and abstracts, and 327 records were excluded for not meeting the inclusion criteria. This left 46 reports sought for retrieval. Among these, 26 reports could not be retrieved. A total of 20 reports were assessed for eligibility. After full-text review, 13 reports were excluded for several reasons: full text not available ($n = 4$), systematic reviews ($n = 2$), and insufficient available data ($n = 7$). Finally, 7 new studies were included in the review.

Characteristics of Included Studies

First Author, Year	Type of Synbiotic/Prebiotic Supplement	Intervention Dose and Route	Key Outcome Reported (SLEDAI-2K, IL-17, IgG)
Widhani, 2022	Synbiotic (Lactobacillus helveticus R0052 60%, Bifidobacterium infantis R0033 20%, Bifidobacterium bifidum R0071 20%, and 80 mg fructo-oligosaccharides)	3×10^9 CFU once daily for 60 days, oral	SLEDAI-2K: Significantly decreased (14 ± 16 vs. 8 ± 12 ; $p < 0.001$) IL-17: No significant change IgG: Not mentioned
Mirfeizi, 2023	Synbiotic (L. helveticus, B. infantis, B. bifidum)	3×10^9 CFU daily for up to 3 months, oral	SLEDAI-2K: Significant improvement
Zheng, 2024	Probiotics (various types, not specific)	Various, not specific	SLEDAI-2K: Significantly lower (WMD = -2.31, 95%CI -2.48, -2.14, $P < 0.00001$) IgG: Significantly lower (WMD = -3.10, 95%CI -3.84, -2.36, $P < 0.00001$)
Banaki, 2025	Probiotic yogurt (Lactobacillus rhamnosus and Bifidobacterium bifidum)	200 g yogurt containing 10^6 CFU daily for 13 weeks, oral	SLEDAI-2K: No significant change ($p = 0.246$)



The characteristics of the four eligible studies included in the systematic review are summarized in Table 2. These studies, published between 2022 and 2025, investigated the effects of synbiotic or probiotic supplementation on disease activity and immunological parameters in patients with Systemic Lupus Erythematosus (SLE).

All studies utilized oral supplementation with varying probiotic or synbiotic formulations and intervention durations ranging from 60 days to 13 weeks. The most frequently used bacterial strains were *Lactobacillus helveticus*, *Bifidobacterium infantis*, and *Bifidobacterium bifidum*, either in synbiotic combinations or as part of probiotic yogurt preparations. One meta-analysis also included multiple probiotic formulations from various studies without specifying individual strains.

Across the studies, clinical outcomes were primarily assessed using the SLE Disease Activity Index

(SLEDAI-2K), while immunological biomarkers such as IL-17 and IgG were variably reported. Two studies (Widhani et al., 2022; Mirfeizi et al., 2023) demonstrated a significant reduction in SLEDAI-2K scores following synbiotic supplementation, indicating clinical improvement. The meta-analysis by Zeng et al. (2024) also reported significantly lower SLEDAI-2K and IgG levels among probiotic users. In contrast, Banaki et al. (2025) observed no significant change in SLEDAI-2K following daily probiotic yogurt intake.

Overall, despite heterogeneity in probiotic strains, dosages, and study durations, the majority of the included studies suggested a beneficial effect of synbiotic or probiotic supplementation on SLE disease activity, while the effects on IL-17 and IgG remained inconsistent or underreported.

Risk of Bias in Included Studies

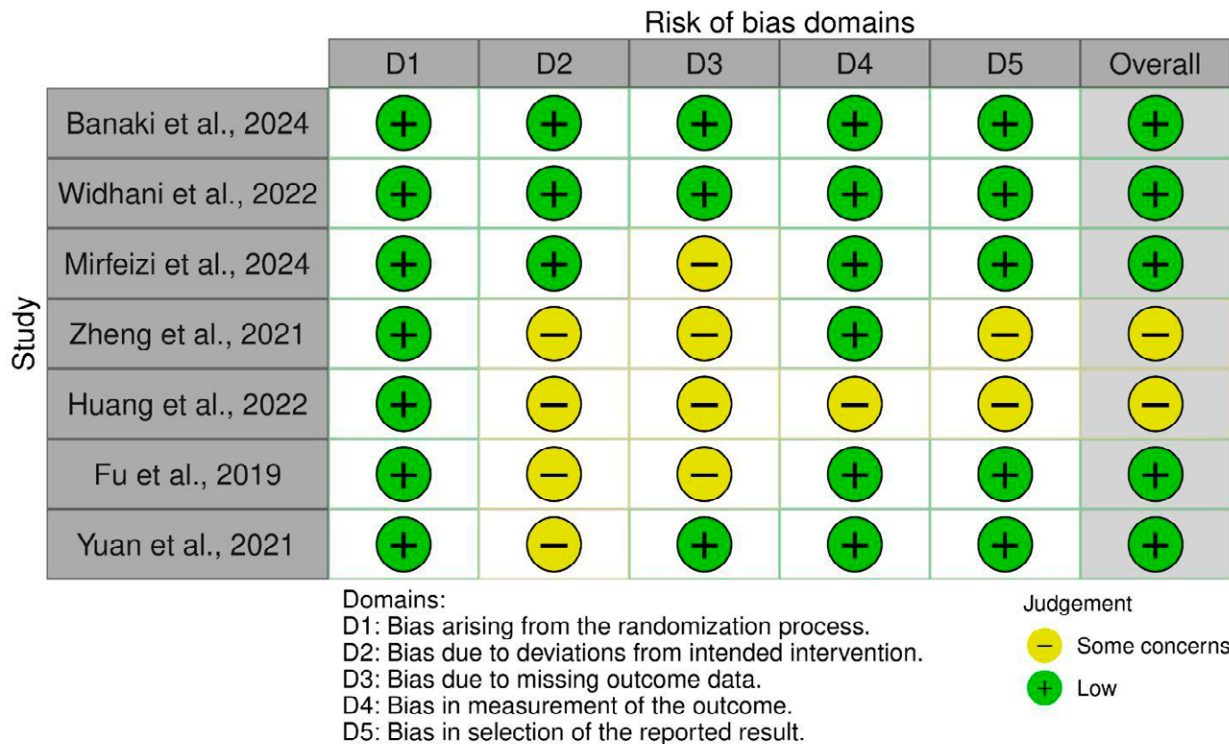


Figure 2. Risk of Bias Assessment using Cochrane ROB 2.0

Figure 2 presents the Risk of Bias (RoB) assessment for the included randomized controlled trials, evaluated using the Cochrane Risk of Bias 2.0 (ROB 2.0) tool. Overall, the methodological quality of the seven included studies was considered moderate to good. Most studies demonstrated a low risk of bias in key domains, particularly in randomization (D1), deviations from intended interventions (D2), and measurement of outcomes (D4), indicating that random allocation and outcome assessments were generally well conducted.

However, several studies exhibited “some concerns” in domains related to missing outcome data (D3) and selection of the reported result (D5), suggesting potential limitations in data completeness and selective reporting. Despite these concerns, no study was rated as having a high overall risk of bias.

In summary, the overall risk of bias among the included studies was low to moderate, supporting the reliability of the evidence while acknowledging minor methodological limitations that may influence the interpretation of pooled results.

These inherent risks of bias highlight the importance of performing sensitivity analyses during the quantitative synthesis phase to ensure that the pooled

effect estimates are robust and not disproportionately influenced by individual studies with methodological concerns.

A quantitative synthesis (meta-analysis) was conducted for the principal clinical and immunological outcomes reported by at least three included studies, namely SLE Disease Activity Index (SLEDAI-2K), serum IgG level, and IL-17 concentration. A fixed-effects model was applied for all pooled estimations to determine the overall effect size of the interventions.

Figure 3A illustrates the pooled analysis of five studies evaluating changes in SLE disease activity following synbiotic or probiotic supplementation. The meta-analysis revealed a significant reduction in SLEDAI-2K score among the intervention groups compared to controls (MD = -2.33; 95% CI ^{-2.49, -2.16}; p < 0.00001). This finding indicates a robust improvement in overall disease activity associated with probiotic or synbiotic administration. The heterogeneity was moderate (I² = 61%), suggesting that differences in strain composition, duration, and baseline disease severity contributed to some variability in effect size, but the overall direction of benefit was consistent.

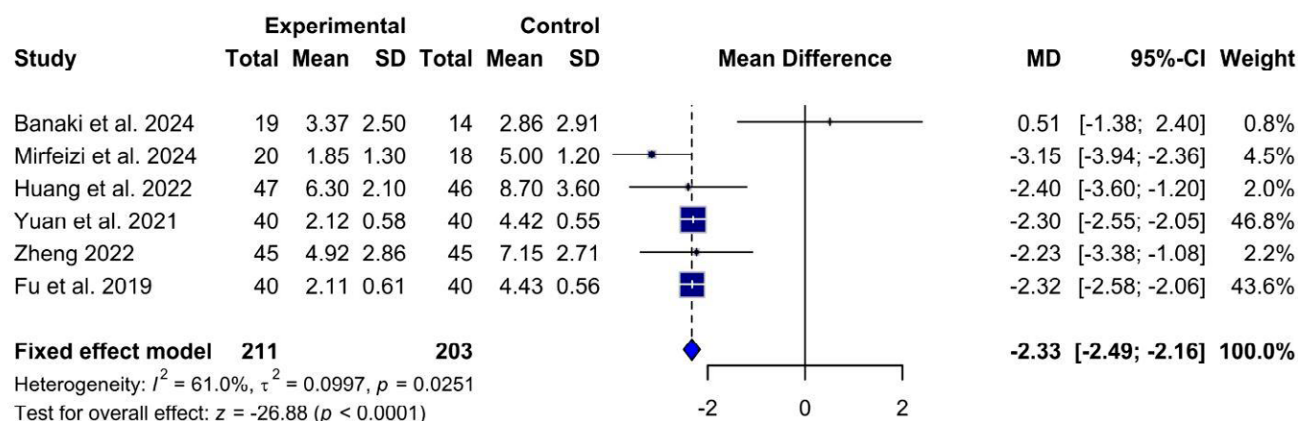


Figure 3A. Effect of Synbiotic and Probiotic Supplementation on SLEDAI-2K Score

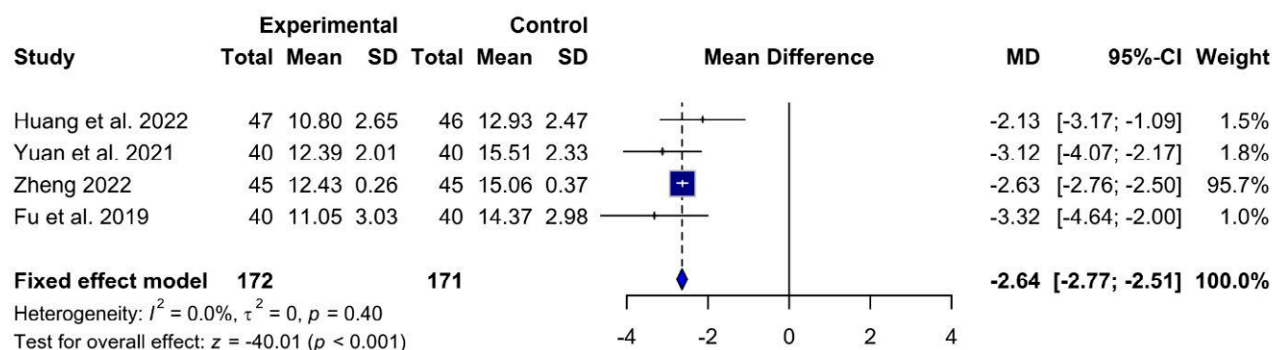


Figure 3B. Effect of Synbiotic and Probiotic Supplementation on Serum IgG Level

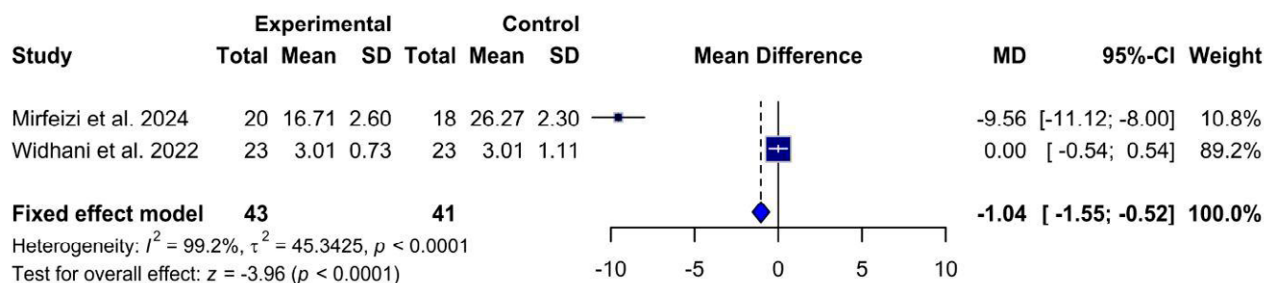


Figure 3C. Effect of Synbiotic and Probiotic Supplementation on IL-17 Levels

Figure 3B summarizes the meta-analysis of three studies that assessed serum IgG concentrations as an immunological marker of disease activity. The pooled results demonstrated a significant reduction in IgG levels in the probiotic/synbiotic group compared to control (MD = -2.64; 95% CI $^{-2.77, -2.51}$; $p < 0.00001$). No heterogeneity was observed ($I^2 = 0\%$), indicating a highly consistent suppressive effect of supplementation on humoral immune activation across studies. These findings suggest that probiotic and synbiotic interventions

may help attenuate B-cell hyperactivity characteristic of systemic lupus erythematosus.

Figure 3C presents the pooled analysis of two studies measuring IL-17, a key pro-inflammatory cytokine in lupus pathogenesis. The meta-analysis revealed a significant decrease in IL-17 levels following probiotic or synbiotic administration (MD = -1.04; 95% CI $^{-1.55, -0.52}$; $p < 0.0001$). Despite high heterogeneity ($I^2 = 99\%$), the direction of effect consistently favored the intervention

group, indicating an overall immunomodulatory effect through suppression of Th17-mediated inflammation.

In summary, the pooled quantitative evidence indicates that probiotic and synbiotic supplementation significantly improves disease activity and immunological parameters in patients with SLE. The consistent reductions in SLEDAI-2K, IgG, and IL-17 levels collectively support the potential role of gut-microbiota modulation as an adjunctive therapeutic strategy for lupus management. However, moderate to high heterogeneity and limited sample sizes warrant cautious interpretation and encourage further high-quality randomized trials.

DISCUSSION

The findings of this meta-analysis provide compelling clinical evidence that probiotic and synbiotic supplementation significantly improves disease activity and modulates systemic inflammation in Systemic Lupus Erythematosus (SLE). The robust reduction in SLEDAI-2K scores suggests that targeting the gut-microbiome-immune axis can effectively facilitate clinical remission and reduce the frequency of disease flares (Widhani et al., 2022). This clinical improvement is biologically substantiated by the significant suppression of pro-inflammatory markers, namely serum IgG and IL-17, indicating a profound restoration of systemic immune tolerance (Habiballah, Li and Jiang, 2025).

Gut Dysbiosis, Barrier Integrity, and Molecular Mimicry

SLE is fundamentally characterized by profound gut dysbiosis, which acts as a primary driver of immune dysregulation through several pathways (Sahu et al., 2025). The reduction in microbial diversity and the altered Firmicutes/Bacteroidetes (F/B) ratio facilitate “leaky gut syndrome,” where impaired intestinal barrier integrity allows for the translocation of microbial products like lipopolysaccharides (LPS) into the systemic circulation (Zhu et al., 2025). This translocation triggers Toll-like receptors (TLRs), particularly TLR7 and TLR9, which are central to the production of type I interferons and the initiation of autoimmune responses (Zhu, Wu and Deng, 2025). Furthermore, the phenomenon of molecular mimicry—where microbial epitopes from species such as *Bacteroides thetaiotaomicron* resemble host self-antigens like Ro60—perpetuates the production of pathogenic autoantibodies (Greiling et

al., 2018). The administration of probiotics and synbiotics helps restore this barrier by upregulating tight junction proteins and promoting a more balanced microbial community (Parodi et al., 2025).

Restoration of the Th17/Treg Balance and Cytokine Modulation

The significant reduction in IL-17 levels observed in our study highlights the role of the gut microbiota in balancing the Th17/Treg axis (Mohamed et al., 2025). Probiotics ferment dietary fibers into short-chain fatty acids (SCFAs), such as butyrate, which act as signaling molecules to promote the expansion of anti-inflammatory regulatory T cells (Tregs) via the induction of Foxp3 expression (Novotny Núñez et al., n.d.). concurrently, increased SCFA production suppresses the differentiation of pro-inflammatory Th17 cells, effectively lowering the systemic concentration of IL-17 (Yurtseven, Aydemir and Ayaz, 2025). This immunomodulatory effect is further enhanced by tolerogenic dendritic cells (DCs), which are stimulated by probiotic interactions to induce T-cell tolerance (Esmaeili et al., 2021).

Suppression of B-cell Hyperactivity and Humoral Immunity

A hallmark of SLE pathogenesis is B-cell hyperactivity, leading to the overproduction of autoantibodies and subsequent tissue damage (Wang et al., 2025). Our results showing reduced serum IgG levels indicate that microbiota modulation can effectively attenuate this humoral immune activation (Wang, Yu and Yu, 2025). Probiotics have been shown to regulate B-lymphocyte immune responses by modulating the expression of critical activation genes, such as CD19 and CD22, and influencing the differentiation of plasma cells in the bone marrow (Hevia et al., 2014). This reduction in B-cell driven autoantibody synthesis is crucial for preventing severe complications, including lupus nephritis and cardiovascular disease (De la Visitación et al., 2019).

Metabolic Restoration and Advanced Therapeutic Frontiers

Beyond direct immune modulation, synbiotics facilitate metabolic restoration by normalizing dysregulated amino acid and purine metabolic pathways (Zhu Q et al., 2024). Recent metagenomic and metabolomic evidence suggests that synbiotic administration shifts

the microbial community structure to reduce pathogenic bacteria like *Prevotella* while increasing beneficial *Actinobacteria* (Zhu, Cui and Liu, 2024). Emerging research also points to the potential of cell-free therapies, such as mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) or exosomes, which can mimic the immunomodulatory effects of probiotics by polarizing macrophages from a pro-inflammatory M1 to an anti-inflammatory M2 phenotype (Zhang et al., 2022). These vesicles alleviate renal injury in lupus nephritis by regulating the IL-6/STAT3/IL-17 signaling pathway and improving the survival rate in murine models (Li et al., 2024). Furthermore, MSC-EVs primed with disease-conditioned media show enhanced production of TGF- β 1, offering a novel avenue for personalized biotherapeutics (Choi et al., 2024).

Clinical Implications and External Influences

The efficacy of microbiota-targeted therapies is influenced by various external factors, including the use of medications such as proton pump inhibitors (PPIs) and non-steroidal anti-inflammatory drugs (NSAIDs), which are common in SLE management (Hu et al., 2025). PPI use has been associated with significant gut dysbiosis and increased intestinal inflammation, as evidenced by elevated fecal calprotectin levels (Pribadi, 2021). Additionally, NSAIDs can cause both gastropathy and enteropathy, further compromising gut barrier integrity and potentially exacerbating SLE symptoms (Maulahela, 2021). The management of oxidative stress is also vital, as reactive oxygen species (ROS) can trigger mitochondrial damage and accelerate the apoptotic cascade in gastric and intestinal mucosa (Makmun, 2021).

Conclusion and Future Outlook

In conclusion, the modulation of the gut-microbiome-immune axis through probiotics and synbiotics represents a promising adjunctive therapeutic strategy for SLE (Kragstnaes et al., 2025). These interventions address the multifactorial pathogenesis of lupus by restoring gut homeostasis, balancing T-cell subsets, and suppressing pathogenic B-cell responses (Zhu, Wu and Deng, 2025). Future research should prioritize large-scale, multicenter trials that incorporate integrated metagenomic and metabolomic profiling to develop personalized microbial interventions tailored to the unique microbial signatures of SLE patients (Zhu, Cui and Liu, 2024).

Importantly, these conclusions are consistent with broader conceptual evidence from multidisciplinary clinical literature, which indicates that alterations in the gut microbiota significantly influence systemic immune responses and inflammatory pathways beyond localized gastrointestinal phenomena. Narrative and integrative reviews have demonstrated that gut microbiota dysbiosis modulates host immune signaling through mechanisms such as the gut-brain axis and microbial-derived inflammatory pathways, thereby reinforcing the biological plausibility of microbiota-targeted interventions in chronic inflammatory conditions. This conceptual framework aligns with our findings, showing that probiotic and synbiotic supplementation is associated with improvements in disease activity and immune markers in systemic lupus erythematosus (Pahomeanu et al., 2025; Ioghen et al., 2025).

CONCLUSION

Gut microbiota-based therapies, including synbiotics and probiotics supplementation, demonstrate a potential benefit in reducing systemic inflammation and improving SLE disease activity by modulating gut microbiota composition and function. While promising as an adjunctive treatment for SLE, further studies are required to investigate its mechanisms and therapeutic efficacy comprehensively.

Ethics Statement and Conflict of Interest Disclosures

Financial support and sponsorship: All authors have declared that no financial support was received from any organization for the submitted work.

Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws. Written informed consent was provided by all the patients participants in this study. This study was approved by the Institutional Research Board and Ethics Committee.

Conflict of interest: No known conflict of interest correlated with this publication.

Availability of data and materials: The data used and/or analyzed throughout this study are available from the corresponding authors upon reasonable request.

Competing interests: The authors declared that they have no competing interests.

The use of generative AI and AI-assisted technologies: The authors did not use in this article generative AI and AI-assisted technologies.

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Knowledge, Attitudes and Practices of the Population of the Republic of Moldova Regarding Viral Hepatitis C

Valentin CALUGAREANU^{1*}, Angela PARASCHIV¹, Diana SPATARU¹

Abstract

Introduction. Viral hepatitis C (VHC) remains a major public health concern in the Republic of Moldova, which records one of the highest prevalence rates in Europe. Understanding the knowledge, attitudes, and practices (KAP) of the population is a prerequisite for designing effective prevention and control strategies.

Aim. To assess KAP related to VHC in a mixed cohort from the Republic of Moldova and to identify factors associated with KAP scores.

Material and Methods. Cross-sectional descriptive study (2024-2025), with 567 participants: 455 healthcare workers and 112 members of the general population. A purpose-built KAP scale (0-30 points) assessed knowledge (0-10), attitudes (0-10), and practices (0-10). Statistical analysis included Mann-Whitney U and Kruskal-Wallis tests, and Spearman's correlation.

Results. The mean KAP score was 17.82 ± 3.81 points (59.4%), with 63.7% of participants at a moderate level. Healthcare workers scored significantly higher than the general population (18.85 vs 13.62, $p < 0.001$). A positive correlation was found between knowledge and practices ($\rho = 0.336$, $p < 0.001$).

Conclusions. Most participants showed a moderate KAP level, with relatively favorable attitudes, while knowledge and practices remained insufficient. Targeted educational programs are needed, particularly for the general population, those with lower education, and residents of rural areas.

Keywords: viral hepatitis C, prevention and control strategies, knowledge, attitudes, and practices (KAP)

¹Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

***Corresponding author:**

Valentin CALUGAREANU, Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

E-mail: valentin.calugareanu@gmail.com

INTRODUCTION

Hepatitis C virus (HCV) remains a significant global public health concern. In 2022, it was responsible for an estimated 242,000 deaths worldwide. Approximately 50 million people are living with HCV infection, and approximately 1.5 million new infections occur annually, and the infection remains a leading driver of chronic liver disease, cirrhosis, and hepatocellular carcinoma^{1,2,22}. Despite the availability of highly effective curative treatment, diagnosis and treatment coverage remain insufficient: only 36% of infected individuals have been diagnosed, and about 20% have received treatment¹. In the Republic of Moldova, the situation is particularly worrying: the prevalence of HCV infection has been estimated at 3.1%, placing the country among those with the highest burden in the European region^{3,4}.

Over the last two decades, the National Agency for Public Health of the Republic of Moldova has implemented efforts to reduce the burden of HCV through national programs targeting the morbidity and mortality associated with viral hepatitis, including HCV^{3,5,6,7}. Two consecutive national initiatives (2012–2016 and 2017–2021) contributed to a reduction in HCV incidence by adopting a comprehensive approach, which included the expansion of diagnostic capacities and the implementation of public awareness campaigns^{5,6,7}. Despite these improvements, available evidence indicates that the prevalence of chronic HCV infection and rates of liver cirrhosis in Moldova remain among the highest in Eastern Europe^{8,9,10}.

Transmission of HCV occurs predominantly through percutaneous exposure to contaminated blood – most commonly via the sharing of injecting equipment, inadequately sterilized medical instruments, tattooing or body-piercing performed under unsafe conditions, and, to a lesser extent, through unprotected sexual contact and mother-to-child transmission¹¹. A distinctive and clinically significant feature of HCV infection is its largely asymptomatic course during the early, acute phase. This silent progression frequently delays diagnosis and allows the infection to advance undetected toward fibrosis, cirrhosis, and, ultimately, end-stage liver disease. In this context, population-level awareness and the adoption of protective behaviors become indispensable tools for controlling the epidemic.

Healthcare workers constitute a group with heightened occupational risk. Accidental percutaneous injuries and mucocutaneous exposure to blood and body

fluids are well-documented hazards, and non-adherence to standard precautions remains a concern in many healthcare settings^{12,13}. Ensuring that medical personnel are familiar with post-exposure protocols and consistently apply infection prevention measures is therefore a distinct priority within any national hepatitis control strategy¹⁴.

Knowledge, Attitudes and Practices (KAP) surveys have proven to be a valuable methodological instrument in public health research. By simultaneously capturing what people know, how they perceive a health issue, and what behaviors they actually engage in, KAP studies generate actionable evidence for policy-makers and program designers^{14,15,21}. Such surveys have been conducted for hepatitis C in various countries, but data from the Republic of Moldova remain scarce, creating a gap in the regional evidence base. The present study was designed to address precisely this gap.

AIM

The primary aim of this study was to evaluate the level of knowledge, attitudes and practices (KAP) regarding viral hepatitis C among the population of the Republic of Moldova, and to identify sociodemographic factors associated with KAP scores, with a view to informing the design of targeted educational interventions.

MATERIAL AND METHODS

Study design and setting

An observational, cross-sectional, descriptive study was carried out between January 2024 and February 2025. The study was conducted across four localities in the Republic of Moldova: Chişinău, Bălţi, Străşeni, and Briceni, representing different levels of urbanisation and geographic distribution.

Study population and sampling

The sample comprised 567 adult participants (aged 18–75 years), recruited by random sampling from the participating localities. Of these, 455 (80.2%) were healthcare workers (physicians, nurses, and auxiliary medical staff) and 112 (19.8%) were members of the general population (teachers, economists, farmers, retirees, and unemployed individuals). The geographic distribution was as follows: Bălţi (n = 242; 42.7%), Străşeni (n = 109; 19.2%), Chişinău (n = 96; 16.9%), and Briceni (n = 87, 15.3%).

Inclusion criteria: age 18-75 years, written informed consent, and ability to complete the questionnaire in Romanian.

Exclusion criteria: refusal to participate and incomplete questionnaires.

Data collection instrument

A structured questionnaire was developed in EpiInfo and adapted to the local epidemiological context; it was subsequently administered in paper format, with participants completing it in person at the respective data collection sites. It covered five thematic domains: (1) sociodemographic data (sex, age, education, occupation, marital status, income, and place of residence); (2) knowledge about HCV transmission routes, risk factors, clinical manifestations, and prevention; (3) attitudes toward the disease and the perceived need for information; (4) practices related to HCV testing, vaccination, and risk behaviours; and (5) for healthcare workers only, questions regarding standard precautions specific to the occupational setting.

KAP scale development

A purpose-built quantitative KAP scale (0-30 points) was developed with three equal components:

Knowledge score (0-10 points): correct answers on HCV transmission routes (2 points), risk factors (4 points), and self-assessed level of information (0-4 points depending on the response).

Attitudes score (0-10 points): favorable attitudes towards seeking information (2 points), adherence to dietary recommendations (2 points), recognition of alcohol and tobacco as aggravating factors (2 points each), and intention to seek medical care (2 points).

Practices score (0-10 points): previous HCV testing (3 points), vaccination in accordance with the national immunization calendar (2 points), and – for healthcare workers – safe practices including avoidance of needle recapping (2 points), glove changing after each patient (2 points), and knowledge of post-exposure management (1 point).

The total KAP score was classified into three levels: low (< 50%; 0-14 points), moderate (50-75%; 15-22 points), and high (> 75%; 23-30 points).

Statistical analysis

Data were processed using Python 3.12. Continuous variables are presented as mean $\pm$ standard deviation (SD) or median, as appropriate. Categorical variables are expressed as absolute and relative frequencies. For between-group comparisons, the Mann-Whitney U test (two groups) and the Kruskal-Wallis test (three

or more groups) were used, given the non-normal distribution of data. Correlations were assessed with Spearman's rank correlation coefficient (ρ). The threshold for statistical significance was set at $p < 0.05$.

Ethical considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent prior to data collection. Data confidentiality was ensured through full anonymization of questionnaire responses.

Some questionnaires required clarification during completion, particularly among participants from the general population.

RESULTS AND DISCUSSIONS

Sociodemographic characteristics

The study enrolled 567 participants with a mean age of 45.1 ± 13.2 years (range: 18-75). Women constituted 86.6% ($n = 491$) of the sample, a distribution largely explained by the female-dominated composition of the healthcare workforce, which accounted for 80.2% of respondents. Regarding educational attainment, 44.8% ($n = 254$) had completed a college or vocational program, 34.6% ($n = 196$) held a university degree, 11.3% ($n = 64$) had specialized secondary education, and 9.7% ($n = 55$) had primary, trade, or no formal education. Most participants were married (77.2%, $n = 438$) and had children (87.3%, $n = 495$); among parents, 84.8% ($n = 481$) reported having vaccinated their children according to the national immunization schedule.

The occupational breakdown comprised 151 physicians (26.6%), 280 nurses (49.4%), 24 auxiliary healthcare staff (4.2%), and 112 members of the general population (19.8%), encompassing teachers, economists, agricultural workers, retirees, and unemployed individuals.

Knowledge about hepatitis C

When asked to self-assess their own level of knowledge regarding hepatitis C, 55.0% ($n = 312$) of respondents reported being 'well informed' and a further 27.9% ($n = 158$) as 'very well informed'. In contrast, 15.7% ($n = 89$) indicated that they were 'not particularly informed' while 1.4% ($n = 8$) reported being 'not informed at all'. Despite this relatively high level of self-reported knowledge, 82.9% ($n = 470$) expressed a desire to receive additional information on hepatitis, indicating awareness of existing knowledge gaps. The topics rated as most important for further learning were treatment options for hepatitis, cirrhosis, and hepatocellular

carcinoma (57.7%, $n = 327$), followed by risk factors (46.9%, $n = 266$), clinical signs (45.5%, $n = 258$), preventive measures (44.6%, $n = 253$), and vaccination (43.7%, $n = 248$).

Regarding routes of transmission, blood-borne exposure was correctly identified by 81.0% ($n = 459$) of participants. However, notable misconceptions were also identified: 54.5% ($n = 309$) incorrectly considered sexual intercourse with multiple partners, even when condoms are used, to be a significant route of HCV transmission. Knowledge of risk factors was generally more accurate, with 94.0% ($n = 533$) correctly recognizing blood transfusion as a risk factor. In contrast, only 38.1% ($n = 216$) correctly indicated that condom use reduces the risk of sexual transmission, highlighting gaps in understanding of preventive measures.

The mean knowledge score was 5.05 ± 1.40 out of a maximum of 10 points (50.5%). Notably, 99.6% ($n = 565$) of participants were classified as having low knowledge levels ($< 50\%$). These findings are considerably lower than those reported in comparable European studies, where knowledge levels typically range between 55% and 70%¹⁵, highlighting a substantial gap in hepatitis C - related literacy within the Moldovan population.

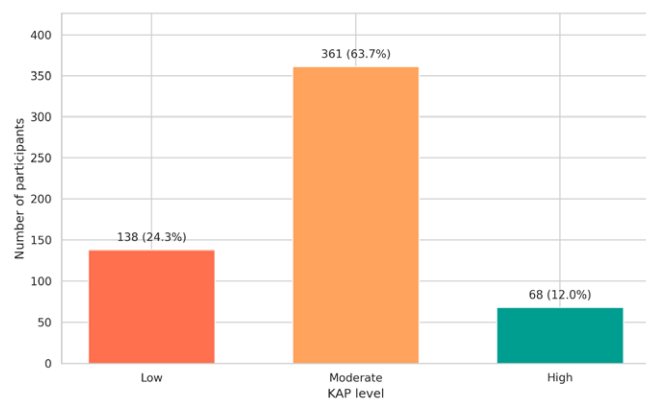


Figure 1. Illustrates the distribution of knowledge scores across the study sample

Attitudes toward hepatitis C

Overall, attitudes toward hepatitis were markedly positive. Nearly all participants (98.4%, $n = 558$) agreed that the general population should be better informed about hepatitis and its consequences. Awareness of lifestyle-related aggravating factors was similarly high: 95.4% ($n = 541$) acknowledged that alcohol consumption worsens the course of the disease, and 85.2% ($n = 483$) recognized tobacco use as an additional aggravating factor. Furthermore, 95.2% ($n = 540$) also agreed

that patients diagnosed with hepatitis B or C should follow specific dietary recommendations.

The self-reported health-seeking behavior was generally appropriate. The majority of participants 95.8% ($n = 543$) indicated that they would consult a general practitioner if they suspected hepatitis, while 4.1% ($n = 23$) would seek care directly at a hospital. Only 0.2% ($n = 1$) reported that they would manage the condition independently at home. Furthermore, 90.7% ($n = 514$) of respondents expressed their intention to seek medical attention in the presence of right upper quadrant pain.

The mean attitudes score was 7.25 ± 1.30 points out of 10 (72.5%). According to the predefined cut-offs, 63.3% ($n = 359$) of participants demonstrated favorable attitudes ($> 75\%$), 27.7% ($n = 157$) had moderate attitudes (50-75%), and 8.8% ($n = 50$) exhibited unfavorable attitudes ($< 50\%$). These results are broadly consistent with findings from other developing-country contexts¹⁶, suggesting that general public messaging about the severity of hepatitis C does reach the population, even when more specific knowledge remains limited.

Practices regarding hepatitis C

Regarding HCV testing, 77.8% ($n = 441$) of participants reported having been tested at least once, while 19.2% ($n = 109$) had never undergone testing, and 3.0% ($n = 17$) were unsure of their testing status. The prevalence of confirmed HCV infection in the study sample was 3.7% ($n = 21$), closely corresponding to national estimates. Among respondents with children, 84.8% ($n = 481$) reported adherence to the national immunization schedule, reflecting a relatively satisfactory vaccination coverage.

Physicians were the primary source of health information (76.5%, $n = 434$), followed by printed materials such as brochures and posters (41.1%, $n = 233$), television (38.4%, $n = 218$), internet search engines (26.1%, $n = 148$), and social media (26.1%, $n = 148$). The predominance of the physician as the main information source highlights the central role of healthcare professionals in delivering health education.

Among healthcare workers ($n = 455$), several concerning practices were identified. Needle recapping, a well-established risk behavior for accidental sharps injuries, was reported by 13.8% ($n = 63$) of healthcare workers, while 86.2% ($n = 392$) stated they did not practice it. Only 41.8% ($n = 190$) reported changing gloves after each patient, a proportion that falls considerably

short of standard precaution requirements. A history of at least one needlestick or sharps injury was reported by 16.5% ($n = 75$), and only 42.6% ($n = 194$) were familiar with the recommended post-exposure management protocol.

The mean practices score was 6.51 ± 2.81 points out of 10 (55.1%). The distribution by level revealed that 64.0% ($n = 363$) had inadequate practices ($< 50\%$), 28.6% ($n = 162$) adequate practices ($> 75\%$), and 3.4% ($n = 19$) moderate practices. These results point to a pronounced discrepancy between stated positive attitudes and actual behavioral compliance, a phenomenon consistent with the attitude-behavior gap described in health psychology literature^{17,18}.

Total KAP score and associated factors

The mean overall KAP score was 17.82 ± 3.81 points out of 30 (59.4%). Overall, 24.3% ($n = 138$) of participants were classified as having low KAP levels, 63.7% ($n = 361$) moderate levels, and 12.0% ($n = 68$) high levels. The attitude component yielded the highest sub-score (72.5%), followed by practices (55.1%) and knowledge (50.5%). This pattern, positive attitudes coexisting with deficient knowledge and inconsistent practices, aligns with findings reported in similar regional studies²⁰.

Comparisons between healthcare workers and the general population are summarized in Table 1. Healthcare workers achieved significantly higher scores for knowledge (5.38 ± 1.05 vs. 3.72 ± 1.60 , $p < 0.001$), practices (6.20 ± 2.59 vs. 2.72 ± 1.75 , $p < 0.001$), and total KAP score (18.85 ± 3.21 vs. 13.62 ± 3.10 , $p < 0.001$). Attitude scores were comparable between the two groups (7.27 ± 1.23 vs. 7.17 ± 1.55 , $p = 0.872$), suggesting that basic awareness of the disease's seriousness is relatively uniform across the population, irrespective of professional background.

Table 1. Comparison of KAP scores between healthcare workers and the general population

KAP Component	Healthcare workers ($n=455$) Mean $\pm$ SD	General population ($n=112$) Mean $\pm$ SD	p value
Knowledge (0-10)	5.38 ± 1.05	3.72 ± 1.60	< 0.001
Attitudes (0-10)	7.27 ± 1.23	7.17 ± 1.55	0.872
Practices (0-10)	6.20 ± 2.59	2.72 ± 1.75	< 0.001
Total KAP (0-30)	18.85 ± 3.21	13.62 ± 3.10	< 0.001

Mann-Whitney U test; SD – standard deviation

Figure 2 depicts the distribution of total KAP scores by occupational group.

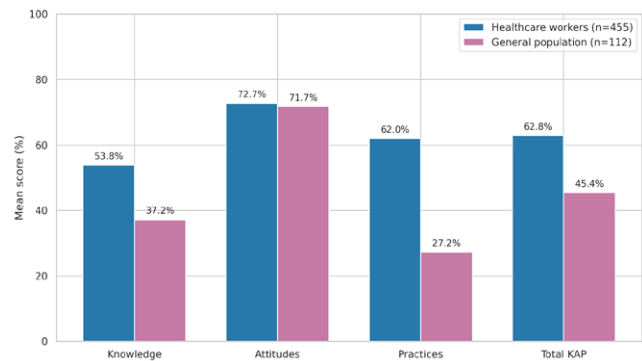
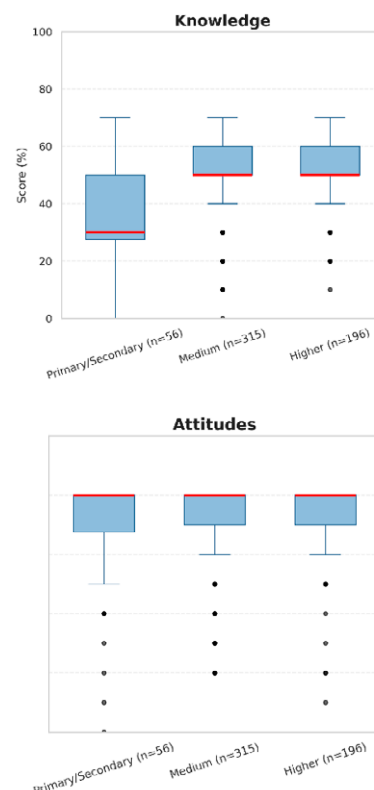


Figure 2. Comparison of total KAP scores between healthcare workers ($n = 455$) and the general population ($n = 112$).

The level of formal education emerged as a significant predictor of KAP performance. Participants with a university degree obtained the highest mean total KAP score (18.69 ± 3.59), followed by those with secondary education (18.07 ± 3.46), and those with primary or vocational schooling (13.32 ± 3.40), with statistically significant differences across groups ($H = 80.21$, $p < 0.001$). This gradient is consistent with the broader evidence base linking health literacy to educational attainment¹⁸.

Figure 3 illustrates the association between educational level and KAP scores.



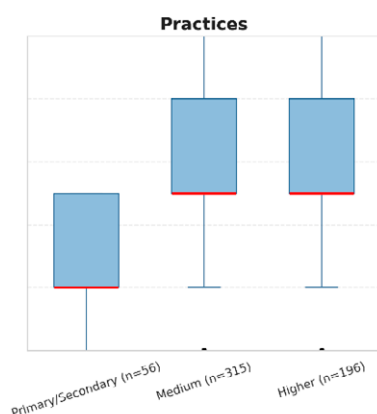


Figure 3. KAP scores stratified by educational level.

Place of residence was also associated with meaningful differences: urban dwellers scored higher than their rural counterparts on knowledge (5.35 vs. 4.62, $p < 0.001$) and total KAP (18.22 vs. 17.22, $p = 0.034$), though attitudes did not differ significantly between the two groups ($p = 0.251$). These disparities likely reflect the unequal distribution of healthcare services and access to health information in the country.

Age was not significantly associated with knowledge scores ($H = 2.19$, $p = 0.335$), but did influence total KAP scores ($H = 8.19$, $p = 0.017$). Participants aged 51–65-year achieved the highest mean KAP score (18.42 ± 3.92), compared with those aged 18–35 years (17.45 ± 3.47) and 36–50 (17.87 ± 3.76) age groups.

Correlation analysis revealed a moderate positive association between knowledge and practices ($\rho = 0.336$, $p < 0.001$), indicating that higher knowledge levels tend to translate into safer behaviors. By contrast, neither knowledge nor practices were significantly correlated with attitudes (knowledge-attitudes: $\rho = -0.041$, $p = 0.327$; attitudes-practices: $\rho = -0.075$, $p = 0.075$), which confirms that a positive disposition toward the disease does not automatically translate into protective behavior.

DISCUSSION

Overall, the results of this study paint a nuanced picture of hepatitis C literacy in the Republic of Moldova. Attitudes are comparatively strong across the population, but specific knowledge and consistent safe practices lag considerably behind. These findings suggest the presence of a knowledge-practice gap sitting beneath a

superficially favorable attitudinal landscape – has direct and practically important consequences for how public health interventions should be framed.

The phenomenon of positive intentions failing to produce corresponding behaviour change is well established in health psychology, this discrepancy has been described in behavioral research, where intentions do not always translate into actual practices¹⁹. Educational campaigns that limit themselves to conveying factual information or to reinforcing positive intentions are unlikely to be sufficient. What is needed, instead, are interventions that build concrete skills, reduce structural barriers, and create environments that support protective behavior.

Of particular concern are the unsafe practices documented among healthcare workers. The recapping of needles and the failure to change gloves between patients are well-recognized risk factors for healthcare-associated HCV transmission, and their persistence in a sample that, by definition, has received professional training is troubling. Comparable findings have been reported across the Eastern European region²⁰, suggesting that deficiencies in infection prevention and control compliance extend well beyond the Republic of Moldova. Structured retraining, supervisory feedback, and the visible enforcement of institutional infection control policies are all measures that should be considered as part of a comprehensive response.

Several limitations of the study warrant acknowledgement. The cross-sectional design precludes causal inference. The sampling strategy, while employing random selection within each locality, cannot fully guarantee representativeness at the national level given the overrepresentation of healthcare workers and the concentration in specific urban settings. Furthermore, self-reported data are inherently susceptible to social desirability bias, which may have led to an overestimation of positive practices. Finally, although the KAP scale was constructed on validated principles, its psychometric properties have not been formally tested in the Moldovan context, which may affect the comparability of results across studies.

CONCLUSIONS

The majority of the study population (63.7%) demonstrated a moderate KAP level regarding viral hepatitis C, with a mean total score of 59.4% of the maximum possible.

Attitudes subscale yielded the best results (72.5%), while knowledge (50.5%) and practices (55.1%) remained below satisfactory thresholds, indicating a meaningful discrepancy between declared intentions and actual behaviors.

Healthcare workers possessed significantly higher knowledge and reported safer practices than the general population ($p < 0.001$), yet deficiencies in the implementation of standard precautions persisted, maintaining a risk of nosocomial transmission.

Higher educational attainment and urban residence were independently associated with higher KAP scores ($p < 0.05$), underscoring the need for targeted interventions addressed to population groups with limited formal education and limited access to health services.

The positive correlation between knowledge and practices ($\rho = 0.336$, $p < 0.001$) suggests that improving knowledge may contribute to safer behaviors, supporting the role of education as a key intervention strategy.

The findings of this study support the need for targeted health education programs, particularly in identified risk groups, with particular attention to the general population with low educational attainment, rural residents, and ongoing training for healthcare workers in standard precautions and post-exposure management.

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Ethics Statement and Conflict of Interest

Disclosures

Financial support and sponsorship: All authors have declared that no financial support was received from any organization for the submitted work.

Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws. Written informed consent was provided by all the patients participants in this study. This study was approved by the Institutional Research Board and Ethics Committee.

Conflict of interest: No known conflict of interest correlated with this publication.

Availability of data and materials: The data used and/or analyzed throughout this study are available from the corresponding authors upon reasonable request.

Competing interests: The authors declared that they have no competing interests.

The use of generative AI and AI-assisted

technologies: The authors did not use in this article generative AI and AI-assisted technologies.

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The Seniority-Skepticism Paradox: AI Adoption and Perceptions Among Medical Students in a Resource-Limited Setting

*Hayder AL-MOMEN¹, Shaymaa Kadhim JASIM², Abbas Oweid OLEWI¹, Ali Abdul Razzak OBAID¹, Laith Thamer AL-AMERI³, Rand ALMOMEN⁴

Abstract

Background: The emergence of Generative Artificial Intelligence (GenAI) has a significant potential effect on medical education though there is little evidence regarding its use, perceptions, and expectations of medical students in resource-limited clinical training contexts.

Objectives: We aim to explore the experiences and perceptions of medical students regarding the use of GenAI technologies in clinical education with a special focus on the AI trust relationship between advanced age and academic level.

Methods: A cross-sectional observational study was conducted over eight months at Al-Kindy College of Medicine, University of Baghdad, Iraq. All clinical-grade students who responded to a 24-item survey were involved (n=423). Multiple linear regression models and descriptive data analysis were performed.

Results: Almost all our sample students reported a current AI use and most of them showed a prior use (n=405, 95.7%). Exam preparation (n=316, 74.7%) and clinical case generation (n=249, 58.9%) were the main use purposes. They exhibited strong agreement regarding AI's ability of better learning potential and essential need for regulatory policies, while marked neutrality appeared toward AI's clinical diagnostic accuracy and long-term practical effects. Significant skepticism (p<0.001) resulted as AI trust positively correlated with higher clinical experience and negatively related to chronological age.

Conclusions: Academic AI adoption was very largely encountered by medical students of resource-limited settings and professional caution was shown regarding broader clinical applications with demands of AI compliance standards. The higher educational grade the better AI trust, while this was inversed regarding student's age displaying a distinct skepticism.

Keywords: AI Literacy; Artificial Intelligence; ChatGPT; Educational Technology; Google Gemini; Iraq; Undergraduate Medical Students.

¹Department of Pediatrics, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq

²Department of Obstetrics and Gynecology, College of Medicine, University of Baghdad, Baghdad, Iraq

³Department of Anatomy, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq

⁴Sewa Health, San Francisco, United States of America

***Corresponding author:**

Hayder AL-MOMEN, Al-Nahdha, Baghdad, Iraq

E-mail: hayderalmomen@kmc.uobaghdad.edu.iq

INTRODUCTION

Medical education approach is dramatically changed by the fast global rise of Artificial Intelligence (AI)¹. Many new technologies as Generative AI (GenAI) models like ChatGPT and Google Gemini make a novel shift in healthcare management including diagnosis, treatment, learning parameters, and educational aspects. The application of Natural Language Processing (NLP) in GenAI showed manifest human-like interactions and dynamic communications unlike the previous old models of computer-assisted teaching using frozen data. Such characteristics appear more efficient in the simulation of complex clinical case scenarios with more potential for critical systematized thinking which will eventually improve learning abilities among medical students.^{2,3}

We have been introduced to AI tools since late 2022, but it is not exactly known when it is formally integrated into healthcare field.⁴ Some medical schools updated their curriculum and incorporated AI technologies in many ways like digital anatomy and AI-driven simulations, but the full view of student's perceptions and feed-back is still not well studied⁵. This issue should be addressed and improved as the daily use of AI in medical education has many confounders including student trust and how to deal with AI-generated results, and we should not forget ethical issues which master the relationship between the student and AI adoption. Many concerns appeared regarding AI use led to a tentative academic atmosphere not limited to data privacy and hallucinations, and probable effect of AI on the basic academic skills.⁶⁻⁹

Iraq is a good example of a resource-limited medical and educational setting, and up to our knowledge there is no published data investigated the overall AI adoption rate among medical students with their current attitudinal perceptions, future expectations and suggestions to upgrade the local medical education system. The observations of this work could provide a panoramic view of the present situation in resource-limited areas throughout the world trying to optimize the AI integration into appropriate medical learning system.¹⁰⁻¹³

AIMS OF THE STUDY

We aimed to find out the existing circumstances regarding AI adoption for medical education in Iraq by medical students in their medical training academic

years, and attitudinal AI perceptions. Also, the effect of progressive escalation of academic grade and age of medical students on their level of trust in AI outcomes.

METHODS

A cross-sectional, descriptive, and quantitative study was implemented among medical students having medical practice training in the teaching hospital during the 2025-2026 academic year of Al-Kindy College of Medicine, University of Baghdad, which is the largest university in the country. Those students were in grades 4,5, and 6 and they were supposed to use AI during daily medical learning process.

A convenience sampling technique was employed for recruitment.

We reviewed most of recent published articles related to our subject and tried to build an appropriate questionnaire serving our aims. After that, a panel of experts in medical education consisted of three professors reviewed that questionnaire and we updated it based on their suggestions resulting in a 24-item survey ready to be used for our research. We also tested the reliability of the Likert-scale items that measured AI perceptions in the involved students; the calculated Cronbach's alpha was^{0.82}, indicating a high internal consistency. The survey was distributed online through academic social platforms where all the target students attend. Face-to-face interviews were complemented to ensure data quality and confirm the responses. This questionnaire (survey) was divided into four major parts, the first is socio-demographic characteristics including age, gender, geographic location (governorate), and current academic grade. The second part is educational integration including digital anatomy tables, virtual dissection, and computational pathology. The third part is AI usage patterns including the frequency and specific applications of current AI tools like ChatGPT and Google Gemini for real tasks like exam preparation, case scenario generation, and research support. The last domain is perceptions and attitudes including Likert-scale items to measure student trust, ethical worries, and future expectations regarding AI in clinical education and practice.

Statistical analyses were completed using IBM SPSS Statistics for Windows, Version 29.0 (Armonk, NY: IBM Corp). Frequencies and percentages were calculated for categorical variables and a multi-response analysis was applied to disaggregate data and

measure the prevalence of each variable with multiple selections. A standard 5-point Likert scale was applied to measure students' perceptions and attitudes ranging from 1 (Strongly Disagree) to 5 (Strongly Agree). Instead of analyzing questions about trust and reliability separately, we combined these data to create a single, continuous Composite AI Trust Score for each involved student. After confirming that the data was normally distributed using the Shapiro-Wilk test and visual checks of Q-Q plots, a multiple linear regression analysis was performed in order to determine whether a student's chronological age and academic grade level could predict their level of AI trust. The coefficient factor of determination (R^2) was used to assess model fit, and a p-value of < 0.05 was considered as the limit for statistical significance for all analysis process.

RESULTS

The total number of recruited students was 423 where males slightly higher than females (50.1% vs 49.9%). The most frequent age group was 22 years - < 23 years accounting for 58.6%, $n=248$. Baghdad was the major residence ($n=374$, 88.4%), and about half of participants were from 4th academic grade ($n=214$, 50.4%). All these data was shown in table 1.

Table 1. Demographics

Question	Response	Count	%
Gender	Male	212	50.1
	Female	211	49.9
Age Range	22 - less than 23 years	248	58.6
	23 - less than 24 years	100	23.6
	24 years - and above	75	17.7
Governorate	Baghdad	374	88.4
	Others	49	11.6
Grade Level	4th Grade	214	50.6
	5th Grade	104	24.8
	6th Grade	105	24.6

Table 2 shows recent AI exposure of the involved students to educational processes where 274 (64.8%) of them used digital anatomy approach, and surprisingly 95%, $n=405$ had already used AI tools especially for study or exam preparation ($n=316$, 74.7%).

Table 2. Current AI Experience & Education

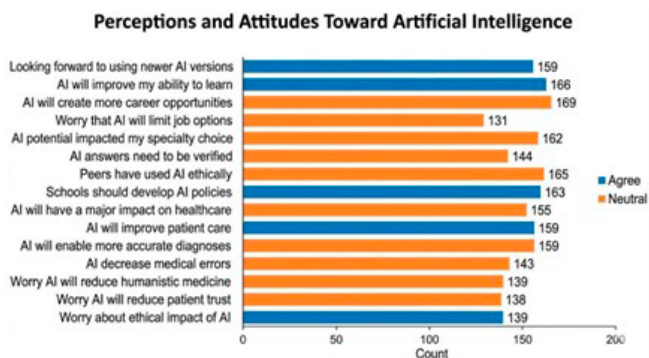
Question	Response	Count	%
Which advanced technologies were formally incorporated into your medical school curriculum?	Digital Anatomy (e.g., Anatomy tables)	274	64.8
	AI Generated Case Simulations	208	49.2
	Virtual Dissection	158	37.4
	Computational Pathology	107	25.3
	None of the above	63	14.9
Have you used AI tools (like ChatGPT, Google Gemini) during medical school?	Yes	405	95.7
	No	18	4.3
For which tasks have you used AI tools?	Study or exam preparation	316	74.7
	Generate case scenarios for learning	249	58.9
	Generate questions to test myself	242	57.2
	Complete written assignments / essays	209	49.4
	Suggest research topics or questions	157	37.1
	Write case reports / Research papers	185	43.7

Regarding future expectations of medical students' AI use in table 3, more than half of them ($n=276$, 65.2%) intended to use such tools during future career especially for writing research papers / case reports, answering medical questions, studying or exam preparation and exploring new medical topics or research $n=294$ (69.5%), 285 (67.4%), 274 (64.8%), and 255 (60.3%), respectively. On the other side, a clear discrepancy was observed regarding clinical decision making as only (21%, $n=89$) of them responded positively to this point. This may indicate some sort of hesitancy towards the rely on AI tools in professional judgement.

Table 3. Future Expectations

Question	Response	Count	%
Do you plan to use AI tools during your residency or future practice?	Yes	276	65.2
	Unsure	121	28.6
	No	23	5.4
For which tasks do you anticipate using AI tools in the future?	Answer medical questions	285	67.4
	Study or exam preparation (e.g., Board exams)	274	64.8
	Explore new medical topics or research	255	60.3
	Write research papers / Case reports	294	69.5
	Assist in clinical decision-making	89	21.0

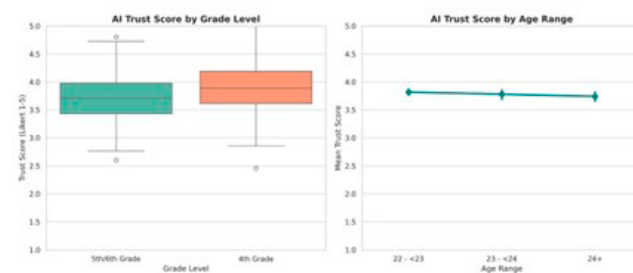
Figure 1 illustrates the distribution of student perceptions regarding AI, showing a clear separation between educational encouragement and professional neutrality. The highest consensus in agreement (blue) was observed in AI's capacity to improve ability to learn (166 responses, 39.2%) and the obligation need for medical schools to develop AI policies (163 responses, 38.5%).

**Figure 1.** Distribution of the most frequent Likert-scale responses for students' perceptions and attitudes toward AI

In contrast, responses directed predominantly toward neutrality (orange) regarding AI's broader professional and clinical impacts. The highest neutral responses were reported for AI's presumed potential to create more career opportunities (169 responses, 40.0%), ethical use of AI by peers (165 responses, 39.0%), the influence on specialty choice in the future (162 responses, 38.3%), and its ability to enable more accurate diagnoses (159 responses, 37.6%). Interestingly, while 139 students (32.9%) agreed that they worry about the

ethical impact of AI, other critical concerns like AI reducing humanistic medicine (139 responses, 32.9%) and reducing patient trust (138 responses, 32.6%) were largely appeared with neutral responses, indicating a general sense of uncertainty regarding AI's long-term socio-clinical outcomes.

The multiple linear regression analysis expressed in figure 2, revealed that academic seniority was a highly significant predictor of AI trust scores ($p < 0.001$). For every one-year increase in academic grade level, the predicted trust score decreased by 0.17 points on the Likert scale. This clear inverse correlation suggests that increased clinical exposure correlates with elevated skepticism regarding AI-generated results. In contrast, age was not found to be a statistically significant predictor of AI trust ($p = 0.128$), indicating that the shift in perception is caused by academic medical training rather than biological age.

**Figure 2.** AI trust score by grade level and age

DISCUSSION

We observed a noticeably a high utilization rate of different AI platforms including Google Gemini and ChatGPT among medical students (95.7%) which may suggest that AI tools have been popularly used and not limited to entertainment issues but extended to medical educational aspects within our resource-limited learning contexts. However, our finding was higher than other nearby areas and even developed countries. In Saudi Arabia¹¹ and Egypt¹⁴ as examples of neighboring countries, GenAI utilization rates were 74.9% and 76.4%, respectively, while published rates in Europe¹⁵ and the United States¹⁶ as examples of developed nations were 38.8% and 62.9%, respectively. We think that our elevated rates of GenAI use among medical students could reflect their passion to get a dependable source of medical information that supports their learning process to overcome resource-restricted environment.

Although our findings revealed a high interest in learning, they showed also a cautious clinical perception. About two thirds of our involved students (74.7%) used AI for exam preparation (74.7%) and (58.9%) for clinical case scenario generation, and we consider these high rates are in line with the recent increasing trends of introducing AI tools in clinical education.¹⁷⁻¹⁹

Our finding of a near consensus regarding AI use to improve learning capabilities just like other previously published data²⁰, did not lead to a real confidence of AI tools and most our students were neutral about accurate diagnosis, decreasing medical mistakes, and impacts on career opportunities and specialty types. Human medical decision is the main factor of clinical practice and our students distinguished such professional limits in clinical education.²¹

The most important observation in this study based on our view point is the surprisingly inverse relationship between the academic grade level and student's trust in AI tools. The increase in clinical seniority (academic grade) of medical students leads to a significant decrease in their AI trust. This paradox of seniority skepticism may indicate a healthy transition from the basic knowledge in its theoretical pattern offered to medical students in earlier academic grades in classroom settings into clinical practice in the hospital wards during the later academic grades. In other words, the self-felt confidence supported by theory alone is replaced by the awareness of patient's clinical care, and this is a good indicator of improvement in medical education process and this makes the students remain skeptical and keep a state of non assurance toward AI-related clinical information, confirming our finding where clinical maturity improves critical appraisal ability^{22,23}, but a recent Egyptian cross-sectional survey found that age was the sole independent predictor of AI perception¹². The involved sample in this Egyptian survey included not only medical students but also postgraduate practitioners and the age ranges were much higher than our sample which was only confined to medical students in their clinical years with narrow age ranges, so that chronological age have been excluded as a significant differentiating element, leaving the level of clinical exposure expressed by academic grade as the most significant factor of AI skepticism.

Even though many students in our group said they use AI often, they still showed a pretty good sense of right and wrong when it came to ethics. A lot of them did express concerns about how AI might affect things like patient trust or the human side of medicine, but

when you look closely at their answers, most of them actually felt neutral about those specific issues. However, there was an essential agreement among involved students about the demand of medical colleges to develop and enforce AI policies. This demand indicates that students correctly realize and identify the unreliability of current AI use²⁴. The absence of formal protocol of AI adoption in resource-limited environments leaves students struggling ethical guidelines and professional standards independently with no robust protective mechanisms. Therefore, it is vital for medical colleges and health institutions to urgently address the issue of AI literacy and build critical evaluation frameworks and infuse them throughout the formal educational medical curriculum to ensure active future integration.²⁵

LIMITATIONS OF THE STUDY

Although this work presents critical view of GenAI integration within a resource-limited medical education setting, there are some limitations that should be identified. The cross-sectional design of the study limits the spotlighted association of the seniority-skepticism paradox as no longitudinal causality could be confirmed over time. Also, the single-involved setting (AI-Kindy College of Medicine, University of Baghdad) restricts the credibility of our findings and reflects the need of generalizability to other Iraqi medical colleges or even other parts of the world under resource-scarce conditions. However, this single center cohort could be considered as a foundational pilot study for more generalized studies at the national and international levels.

Additionally, the self-reported survey introduces the potential for response bias. Either over- or underestimated responses could be resulted. This may be caused social desirability (as AI adoption becomes a social trend) or academic controversy and debates about scholastic integrity of AI use.

Finally, since the unprecedented development of AI platforms leads to continuous dynamic shift regarding the concepts surrounding AI adoption, our observations should be regarded as an initial reference point, highlighting the importance of further multi-center, longitudinal cohorts to adaptively track these rapidly evolving AI educational parameters.

CONCLUSION

The adoption of GenAI among involved medical students in resource-limited settings is highly prevalent. It

is mainly used as an academic adjunct assistant rather than a definitive clinical tool. This study emphasized the finding of seniority-skepticism paradox, showing that as students moved from theoretical basic learning to practical clinical work, their trust in AI significantly diminishes reflecting a favorable professional restraint. Also, the students revealed a strong educational optimistic approach, but they were neutral regarding AI's diagnostic accuracy and long-term ethical viewpoints. The stressed student demand for institutional AI protocols signals an urgent need for medical colleges and educational venues to develop formal AI safe guidelines and effective policies.

Ethics Statement and Conflict of Interest Disclosures

Financial support and sponsorship: All authors have declared that no financial support was received from any organization for the submitted work.

Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws. Written informed consent was provided by all the patients participants in this study. This study was approved by the Institutional Research Board and Ethics Committee.

Conflict of interest: No known conflict of interest correlated with this publication.

Availability of data and materials: The data used and/or analyzed throughout this study are available from the corresponding authors upon reasonable request.

Competing interests: The authors declared that they have no competing interests.

The use of generative AI and AI-assisted technologies: The authors did not use in this article generative AI and AI-assisted technologies.

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β -Hydroxybutyrate Attenuates Cigarette Smoke-Induced Aortic Injury and Improves Endothelial Function in Wistar Rats

Meity ARDIANA^{1,2*}, Fita TRIASTUTI^{1,2}, Andrianto ANDRIANTO^{1,2}, I Gde Rurus SURYAWAN^{1,2}

Abstract

Background: Cigarette smoking is a major risk factor for cardiovascular disease. Oxidative stress plays a central role in endothelial dysfunction and atherosclerosis. β -hydroxybutyrate (β -OHB), a ketone body, has been proposed to exert antioxidant and vascular protective effects. This study aimed to evaluate the effects of β -OHB on aortic histopathology and von Willebrand Factor (vWF) levels in Wistar rats exposed to cigarette smoke.

Methods: This experimental randomized controlled animal study included 25 male Wistar rats divided into five groups: negative control (K-), positive control (K+; cigarette smoke exposure), and three treatment groups receiving β -hydroxybutyrate-(R)-1,3-butanediol monoester (DeltaG®, TDeltaS Global Inc., Orlando, FL, USA) at doses of 1.5 g/kg/day (P1), 3 g/kg/day (P2), and 6 g/kg/day (P3). Rats were exposed to 40 cigarettes/day for 28 days. Aortic tissues were collected after euthanasia on day 28 for histopathological analysis and vWF measurement using ELISA. Data were analyzed using ANOVA with Fisher's LSD post-hoc test.

Results: β -OHB significantly reduced aortic histopathological alterations compared to the smoke-exposed control group (degeneration $p = 0.001$, hyperemia $p = 0.002$, hemorrhage $p = 0.001$, necrosis $p = 0.000$). vWF levels differed significantly among groups ($p = 0.034$), indicating improved endothelial function.

Conclusions: β -OHB attenuates cigarette smoke-induced aortic injury and improves endothelial function. These findings support its potential role as a cardiometabolic therapeutic strategy.

Keywords: β -Hydroxybutyrate, cardiovascular disease, aorta, endothelial dysfunction, cigarette smoke

¹Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

²Department of Cardiology and Vascular Medicine, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

*Corresponding author:

Meity ARDIANA, Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

E-mail: meityardiana@fk.unair.ac.id

INTRODUCTION

Atherosclerosis is the primary underlying cause of cardiovascular disease (CVD)¹. CVD remains the leading cause of death worldwide, accounting for more than 17.3 million deaths annually, of which approximately 7.3 million are due to coronary heart disease (CHD). It is projected that the global burden of CVD-related mortality will increase to 23.6 million deaths per year by 2030^{2,3}.

Atherosclerosis, the primary cause of CVD, is a degenerative inflammatory condition that predominantly affects the intima of large- and medium-sized arteries^{4,5}. This disease leads to thickening and stiffening of the arterial walls, resulting in reduced oxygen and blood flow to tissues⁶. The atherosclerotic process is initiated when endothelial cells are damaged by reactive oxygen species (ROS). While low concentrations of ROS are involved in normal vascular signaling, excessive ROS levels induce oxidative stress, causing cellular damage⁷. High ROS concentrations contribute to endothelial dysfunction, migration and proliferation of vascular smooth muscle cells, monocyte adhesion and migration with foam cell formation, and reflecting a pro-inflammatory and pro-atherogenic state characterized by increased monocyte-to-HDL ratio (MHR)^{8,9}.

Von Willebrand factor (vWF) is a glycoprotein synthesized by endothelial cells that mediates platelet adhesion and aggregation at sites of vascular injury^{10,11}. Platelets form the glycoprotein (GP) IIb-IIIa complex by binding to ligands such as fibrinogen, vWF, fibronectin, and vitronectin. This interaction triggers intracellular calcium influx, leading to the release of α -granules and dense granules containing molecules such as 5-hydroxytryptamine, adenosine diphosphate (ADP), adenosine triphosphate (ATP), thromboxane A₂, leukotriene B₄ (LTB₄), and other pro-inflammatory mediators. These mediators affect vascular smooth muscle cells, endothelial cells, monocytes, and macrophages¹². Cigarette smoking exacerbates endothelial dysfunction by increasing vWF and tissue factor (TF) levels, partly through the reduction of nitric oxide (NO) bioavailability¹³.

Free radicals play a critical role in tissue injury through lipid peroxidation, which predominantly targets polyunsaturated fatty acids (PUFAs) in cellular membranes¹⁴. This process disrupts membrane integrity and function, impairs molecular exchange between intracellular and extracellular environments, and may

ultimately lead to reversible or irreversible cellular damage¹⁵.

Ketone bodies, particularly β -hydroxybutyrate (β -OHB), have demonstrated therapeutic potential in cardiovascular disease. As a stress-response molecule, β -OHB modulates antioxidant defense mechanisms to maintain redox homeostasis¹⁶. Advances in medical technology that enhance targeted therapeutic delivery may further improve its efficacy¹⁷. Moreover, interventions that increase circulating ketone levels have been shown to ameliorate endothelial dysfunction. As an initial step toward the development of ketone-based therapies, this study investigates the effects of β -OHB on cigarette smoke-induced endothelial dysfunction in male Wistar rats, with a particular focus on aortic histopathology and von Willebrand factor (vWF) levels.

MATERIALS AND METHODS:

Animals

Twenty-five male Wistar rats (*Rattus norvegicus*), aged eight weeks and weighing 150–200 grams, were randomly assigned into five groups (n=5 each): K-, K+, P1, P2, and P3. Their health was evaluated by the Ministry of Agriculture's Farma Veterinary Center, Directorate General of Livestock and Animal Health Services, Surabaya, East Java, Indonesia. The rats were housed in ventilated cages at room temperature (22–30°C) with a 12-hour light/dark cycle, and had ad libitum access to water and standard rodent feed. All procedures were approved by the Institutional Animal Care and Use Committee of Universitas Airlangga, Surabaya, Indonesia (Approval Certificate No. 2.KE.113.08-2022) and conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The rats were randomly assigned to five groups (n = 5 per group): K- (negative control, no intervention), K+ (positive control, daily cigarette smoke exposure), and three treatment groups (P1, P2, P3) receiving oral β -hydroxybutyrate-(R)-1,3-butanediol monoester (DeltaG; KE) at 1.5, 3, and 6 g/kg/day, respectively, along with daily cigarette smoke exposure. After 28 days, aortic tissues were collected to analyze histopathological evaluation of the aorta and measurement of von Willebrand factor (vWF). Data were analyzed using one-way ANOVA.

Study Design

This was an experimental, randomized controlled animal study conducted to evaluate the protective effects of β -hydroxybutyrate against cigarette smoke-induced vascular injury.

Ethics Approval

All procedures were approved by the Institutional Animal Care and Use Committee of Universitas Airlangga (Approval No. 2.KE.113.08-2022) and conducted in accordance with international guidelines for laboratory animal care.

Cigarette Smoke Exposure

Side-stream cigarette smoke was delivered using a peristaltic pump into an acrylic exposure chamber (95 × 80 × 65 cm) housing five rats at a time. Rats were exposed to smoke from 40 cigarettes/day for 30 minutes per session over 28 days. Indonesian unfiltered cigarettes (Dji Sam Soe®, HM Sampoerna) containing approximately 39 mg tar and 2.3 mg nicotine per cigarette were used.

β -hydroxybutyrate

β -hydroxybutyrate (β -OHB) (DeltaG Performance, TDeltaS Global, Inc., Orlando, Florida) was administered orally once daily at doses of 1.5, 3, and 6 g/kg/day.

Tissue Collection

All animals were euthanized under anesthesia on day 28 of the experiment, and aortic tissues were subsequently collected for histopathological analysis and quantification of von Willebrand factor (vWF) levels.

vWF Measurement

Aortic von Willebrand factor (vWF) levels were quantified using a sandwich ELISA kit (Rat VWF ELISA Kit, Elabscience, E-EL-R1079) according to the manufacturer's instructions. In brief, microplate wells pre-coated with anti-Rat vWF antibody were incubated with standards or samples, followed by a biotinylated detection antibody specific for Rat vWF and an avidin-horseradish peroxidase (HRP) conjugate. Unbound components were removed by washing, and substrate solution was added. The enzyme-substrate reaction produced a blue color that turned yellow after adding stop solution. Optical density (OD) was measured at 450 ± 2 nm, and vWF concentrations were calculated from a standard curve.

Procedure for Making Histopathology Preparations

Necropsied organs were fixed in 10% neutral-buffered formalin (NBF), dehydrated in acetone (I, II; 1 hour each at room temperature), cleared in xylene (I, II; 0.5–1 hour each), and infiltrated with xylene-paraffin (I, II; 30 minutes each at 54–56°C). Tissues were embedded in paraffin, sectioned at 3 μ m, mounted on glass slides, and covered with Canadian balm and a coverslip. Slides were incubated at 37°C overnight before routine hematoxylin and eosin (H&E) staining.

Histopathological Parameters

Histopathological parameters were evaluated in aortic tissue sections, including hyperemia, hemorrhage, degeneration, and necrosis. Observations were performed in 10 fields per slide at 400× magnification. Lesions were graded semi-quantitatively as mild (+; 1–10 affected cells), moderate (++; 11–20 affected cells), or severe (+++; >20 affected cells)¹⁸.

Histopathological Analysis

Aortic tissues were fixed, processed, and stained with hematoxylin–eosin (H&E) for histopathological evaluation. Degeneration was characterized by cellular swelling, cytoplasmic vacuolization, and loss of structural integrity, whereas hyperemia was defined as vascular congestion with dilated vessels containing erythrocytes. Hemorrhage was identified by the presence of extravascular erythrocytes within the tissue, and necrosis was determined based on nuclear changes, including pyknosis, karyorrhexis, and karyolysis. All histopathological alterations were evaluated using a semi-quantitative grading system.

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD) or median (range), depending on distribution. Normality was assessed using the Shapiro–Wilk test. For normally distributed data, comparisons were made using independent t-tests or one-way ANOVA. Non-normally distributed data were analyzed using Mann–Whitney or Kruskal–Wallis tests. Post hoc analysis was performed with Fisher's LSD test. Statistical analyses were conducted using SPSS version 25.0 (IBM Corp., Chicago, IL, USA).

RESULTS AND DISCUSSION

Aortic Histopathology

β -hydroxybutyrate (β -OHB) significantly reduced degeneration, hyperemia, hemorrhage, and necrosis in aortic tissue compared to the positive control group. Each treatment group consisted of five tissue samples corresponding to the number of animals per group. Histopathological evaluation was performed by examining 10 fields of view per slide at 400 $\times$ magnification, and all observations were conducted by an experienced anatomical pathologist. The results of the analysis are presented in the table below.

Tabel 1. Histopathological features of the aortic tissue degeneration

Group	n	Degeneration		<i>p</i> -value
		$\bar{x} \pm SD$	Min-Max	
K(-)	5	0.14 $\pm$ 0.313 ^a	0.00-0.70	0.001*
K(+)	5	1.42 $\pm$ 0.558 ^b	0.80- 2.20	
P1	5	1.18 $\pm$ 0.785 ^b	0.40-2.50	
P2	5	0.22 $\pm$ 0.228 ^a	0.00-0.50	
P3	5	0.40 $\pm$ 0.693 ^a	0.00-0.08	

* significant at $\alpha=0.05$ (*One-way* ANOVA)

^{ab} different superscript shows significant differences between groups (post-hoc analysis: LSD test for degeneration)

Tabel 2. Histopathological features of the aortic tissue hyperemia

Group	n	Hyperemia		<i>p</i> -value
		$\bar{x} \pm SD$	Min-Max	
K(-)	5	0.50 $\pm$ 0.608 ^a	0.00-1.30	0.002*
K(+)	5	1.56 $\pm$ 0.477 ^b	0.80- 2.10	
P1	5	1.30 $\pm$ 0.674 ^a	0.90-2.50	
P2	5	0.22 $\pm$ 0.228 ^a	0.00-0.50	
P3	5	0.62 $\pm$ 0.438 ^b	0.20-3.00	

* significant at $\alpha=0.05$ (*One-way* ANOVA)

^{ab} different superscript shows significant differences between groups (post-hoc analysis: LSD test for hyperemia)

Tabel 3. Histopathological features of the aortic tissue hemorrhage

Group	n	Hemorrhage		<i>p</i> -value
		$\bar{x} \pm SD$	Min-Max	
K(-)	5	0.14 $\pm$ 0.313 ^a	0.00-0.70	0.001*

K(+)	5	1.42 $\pm$ 0.558 ^b	0.80- 2.20
P1	5	1.18 $\pm$ 0.785 ^b	0.40-2.50
P2	5	0.22 $\pm$ 0.228 ^a	0.00-0.50
P3	5	0.40 $\pm$ 0.693 ^a	0.00-0.08

* significant at $\alpha=0.05$ (*One-way* ANOVA)

^{ab} different superscript shows significant differences between groups (post-hoc analysis: LSD test for Hemorrhage)

Tabel 4. Histopathological features of the aortic tissue necrosis

Group	n	Necrosis		<i>p</i> -value
		$\bar{x} \pm SD$	Min-Max	
K(-)	5	0.06 $\pm$ 0.089 ^a	0.00-0.20	0.000*
K(+)	5	1.96 $\pm$ 0.856 ^b	0.90- 3.00	
P1	5	0.6 $\pm$ 0.367 ^a	0.00-1.10	
P2	5	0.22 $\pm$ 0.134 ^a	0.00-0.50	
P3	5	0.58 $\pm$ 0.831 ^a	0.00-3.00	

* significant at $\alpha=0.05$ (*One-way* ANOVA)

^{ab} different superscript shows significant differences between groups (post-hoc analysis: Tamhane test for necrosis)

The results of the ANOVA analysis demonstrated significant differences between the positive control group and the treatment groups across all assessed histopathological parameters. Specifically, the p-values for degeneration, hyperemia, hemorrhage, and necrosis were 0.001, 0.002, 0.001, and <0.001, respectively, indicating statistically significant differences ($p < 0.05$). These findings suggest that β -hydroxybutyrate treatment significantly reduced the severity of degeneration, hyperemia, hemorrhage, and necrosis in aortic tissue compared to the positive control group.

Histological observations of aortic tissue were performed using a light microscope at 400 $\times$ magnification, and the findings are summarized in Figure 1. In the negative control group (K-), the aortic tissue showed minimal histopathological alterations. In the positive control group (K+), cigarette smoke exposure resulted in marked pathological changes, including necrosis and hyperemia. In the treatment group P1, aortic tissue demonstrated the presence of necrosis, hyperemia, and degeneration, although to a lesser extent than the positive control group. In group P2, no significant histopathological alterations, including degeneration, hyperemia, hemorrhage, or necrosis, were observed. In group P3, aortic tissue exhibited mild hyperemia and hemorrhage.

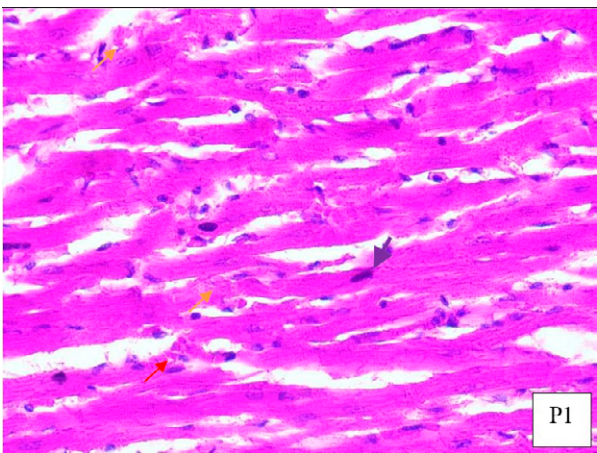
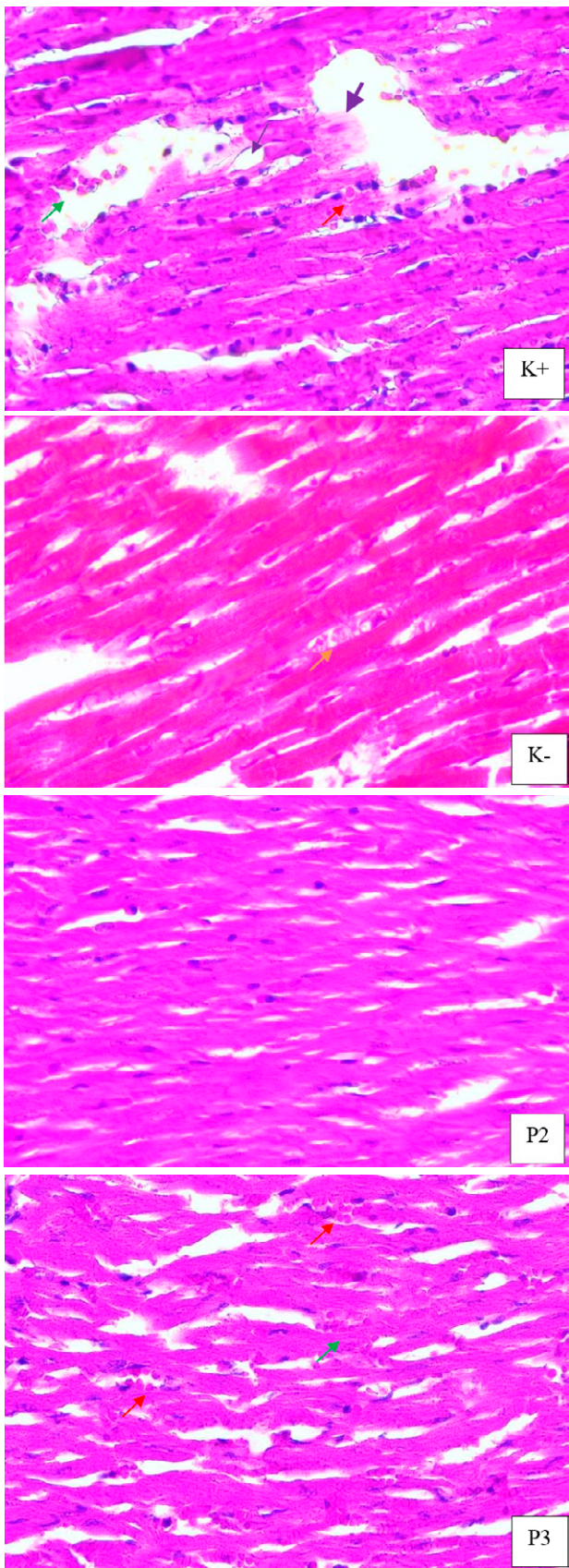


Figure 1. Comparison of aortic tissue in several treatment groups. Appears to show muscle degeneration (↗), hyperemia (↗), hemorrhage (↗) and necrosis (↗). (Hematoxylin-Eosin : 400x)

vWF Levels

After 28 days of the experiment, von Willebrand factor (vWF) levels were measured in all groups. The mean vWF levels were 244.75 ± 229.44 in the K- group, 25.30 ± 22.93 in the K+ group, 167.85 ± 113.97 in the P1 group, 276.17 ± 213.62 in the P2 group, and 20.19 ± 7.95 in the P3 group. Statistical analysis demonstrated significant differences among groups ($p = 0.034$), suggesting improved endothelial function in the treatment groups. The results are summarized in Table 5.

Table 5. Value of *vWF* (ng/mL) in groups

Group	n	vWF (ng/mL)		p-value
		x ± SD	Min-Max	
K(-)	5	244.75 ± 229.44^a	71.54-557.25	0.034*
K(+)	5	25.30 ± 22.93^b	4.04-62.96	
P1	5	167.85 ± 113.97^a	29.54-332.96	
P2	5	276.17 ± 213.62^a	46.89-546.18	
P3	5	20.19 ± 7.95^b	12.39-31.68	

* significant at $\alpha=0.05$ (One-way ANOVA)
ab different superscript shows significant differences between groups (post-hoc analysis: Fisher LSD test for vWF)

Based on the assessment of necrotic cell counts in aortic tissue of Wistar rats, administration of β -hydroxybutyrate at doses of 1.5, 3, and 6 g/kg/day for 28 days resulted in distinct histopathological changes compared to the cigarette smoke-exposed control

group. β -hydroxybutyrate treatment reduced the number of cells exhibiting degeneration, hyperemia, hemorrhage, and necrosis in aortic tissue.

Several factors may contribute to histopathological alterations in vascular tissue. In the positive control group exposed to cigarette smoke, marked aortic changes, including degeneration, hyperemia, hemorrhage, and necrosis, were observed. The accumulation of free radicals is associated with hypercholesterolemia and chronic low-grade inflammation, characterized by increased pro-inflammatory mediators, which further contribute to endothelial dysfunction^{19,20}. Reactive oxygen species (ROS) may further disrupt lipid homeostasis by enhancing low-density lipoprotein (LDL) oxidation and impairing LDL receptor function, resulting in elevated circulating cholesterol levels. In this study, necrotic changes ranged from mild (focal) to moderate (multifocal) across treatment groups. Microscopically, necrosis is characterized by pyknosis, karyorrhexis, and karyolysis, and may trigger a localized inflammatory response in the surrounding tissue²¹.

The reduction in endothelial abnormalities observed following β -hydroxybutyrate administration may be attributed to its antioxidant and anti-inflammatory properties, similar to other metabolic interventions shown to reduce systemic inflammatory biomarkers and improve vascular function^{22,23}. Variability in histopathological findings among individual animals may reflect differences in metabolic response, immune status, and β -hydroxybutyrate dosage. Overall, β -hydroxybutyrate demonstrated protective effects by attenuating degeneration, hyperemia, hemorrhage, and necrosis in aortic tissue.

Von Willebrand factor (vWF) is a glycoprotein essential for primary hemostasis and the intrinsic coagulation cascade, mediating platelet adhesion to sub-endothelial collagen and stabilizing factor VIII. It is present in endothelial cells, platelets, the subendothelial matrix, and storage granules, and consists of repeating subunits that provide multiple binding sites for interacting proteins²⁴. Hypercholesterolemia is associated with reduced antioxidant capacity, particularly in patients with vascular disease. Elevated vWF levels reflect endothelial dysfunction and are linked to oxidative damage and the progression of atherosclerosis²⁵.

Furthermore, ketone bodies exhibit immunoregulatory properties, reducing pathological inflammation through multiple mechanisms. β -hydroxybutyrate has been shown to decrease ROS production, enhance

mitochondrial respiration, and activate endogenous defense systems²⁶. An imbalance between antioxidant defenses and ROS leads to oxidative stress, which modulates cytokine production and contributes to endothelial dysfunction²⁷. Activation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) plays a critical role in protecting endothelial cells from oxidative injury²⁸. Previous studies have demonstrated that Nrf2 activation delays atherosclerosis by reducing mitochondrial damage and oxidative stress, thereby improving endothelial function²⁹. The beneficial effects of β -hydroxybutyrate are largely attributed to its role as a stress-response molecule that modulates antioxidant defense mechanisms to maintain redox homeostasis under physiological stress³⁰.

Collectively, these findings position β -hydroxybutyrate as a promising metabolic modulator capable of attenuating cigarette smoke-induced vascular injury. Through its antioxidant and endothelial-protective effects, β -hydroxybutyrate reduces histopathological damage and improves vWF levels, underscoring its role in mitigating oxidative stress and vascular dysfunction. Beyond its function as an energy substrate, β -hydroxybutyrate may act as a signaling molecule with therapeutic potential in cardiovascular disease. Further studies are warranted to validate these findings in long-term and clinical settings.

STUDY LIMITATIONS

This study has several limitations. The use of an animal model may limit the direct applicability of the findings to human clinical settings. In addition, the relatively short duration of the study (28 days) may not fully reflect the long-term effects of β -hydroxybutyrate. Furthermore, the absence of additional molecular biomarkers restricts a more comprehensive evaluation of the underlying mechanisms.

CONCLUSION

In summary, β -hydroxybutyrate attenuates cigarette smoke-induced aortic injury and improves endothelial function in Wistar rats. It reduces histopathological damage and modulates von Willebrand factor (vWF) levels, suggesting its potential to counteract oxidative stress and serve as a therapeutic strategy for cardiovascular disease.

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Ethics Statement and Conflict of Interest

Disclosures

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Transosseous Teratoma of The Frontal and Parietal Bones: A Rare Case Report and Surgical Management

Marius-Cristian ZAHARIA¹, Raluca-Maria MARIN², Florentin NEAGU¹, Oana STANOAI¹, Teodor-Cristian BLIDARU², Anamaria GHEORGHIU¹, Andrei GIOVANI¹

Abstract

BACKGROUND Teratomas are rare congenital tumors arising from pluripotent germ cells and contain tissues derived from all three germ layers: ectoderm, mesoderm, and endoderm. Intracranial teratomas are uncommon, accounting for less than 0.5% of all central nervous system tumors, and typically occur in midline structures such as the pineal or suprasellar regions. Primary involvement of cranial bones with transosseous extension is exceptionally rare. These lesions may lead to progressive cranial deformity and local bone destruction. While isolated cases involving the petrosal bone have been reported, localization at the cranial vault is exceedingly uncommon. To our knowledge, this report describes the first case of a transosseous teratoma involving the frontal and parietal bones.

CASE PRESENTATION A 39-year-old male presented with persistent headache and vertigo, associated with a subcutaneous swelling at the bregma that progressively enlarged following a hair transplant procedure. Imaging revealed an osteolytic transosseous lesion involving both the external and internal cortical tables, with intraspongious extension and multiple hair follicles embedded within the mass. The patient underwent complete surgical excision, including resection of the outer cortical bone to delineate tumor margins and achieve total removal clearly. Histopathological examination confirmed the diagnosis of a mature teratoma. The postoperative course was uneventful, and the patient was discharged on the third postoperative day without neurological deficits.

DISCUSSION Transosseous teratomas of the cranial vault are extremely rare. Their pathogenesis is thought to involve aberrant migration of germ cells during embryogenesis, resulting in ectopic growth within bone. Differential diagnosis includes dermoid and epidermoid cysts, osteolytic skull tumors, and other germ cell neoplasms. Complete surgical excision remains the treatment of choice to prevent recurrence. Given the potential for malignant transformation, long-term clinical and radiological follow-up is recommended.

CONCLUSION: This case underscores the importance of early diagnosis and complete surgical resection in the management of transosseous cranial teratomas. Further reports are needed to better define optimal management strategies for these rare lesions.

KEY WORDS: transosseous teratoma, skull teratoma, osteolytic skull lesion, cranial teratoma, germ cell tumor

¹Bagdasar-Arseni Clinical Emergency Hospital, Bucharest, Romania

²Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

***Corresponding author:**

Marius-Cristian ZAHARIA, Bagdasar-Arseni Clinical Emergency Hospital, Bucharest, Romania

E-mail: zahariamarius16@gmail.com

INTRODUCTION

Teratomas are rare congenital tumors derived from pluripotent germ cells and contain tissues originating from all three germ layers: ectoderm, mesoderm, and endoderm. While intracranial teratomas are uncommon, comprising less than 0.5% of all central nervous system (CNS) tumors, their occurrence in the skull bones, particularly the frontal and parietal bones, is extremely rare¹. These lesions can be classified as mature (benign), immature (potentially malignant), or malignant teratomas, depending on their histopathological composition².

Transosseous teratomas involve direct infiltration or primary growth within the cranial bones, leading to progressive cranial deformities, local bone destruction, and potential compression of adjacent brain structures³. Their clinical presentation varies depending on size and location, with symptoms including headache, focal neurological deficits, seizures, or increased intracranial pressure⁴. Imaging studies, particularly magnetic resonance imaging (MRI) and computed tomography (CT), play a crucial role in diagnosing these tumors, often revealing mixed cystic and solid components with calcifications⁵.

Surgical resection remains the primary treatment, aiming for total excision while preserving neurological function. Due to the risk of recurrence and potential malignant transformation, long-term follow-up is essential⁶. This paper discusses the clinical presentation, diagnostic approach, and management strategies for transosseous teratomas of the frontal and parietal bones, based on current literature and reported cases.

CASE PRESENTATION

A 39-year-old patient was admitted for evaluation and specialized treatment due to persistent headache and vertigo that began approximately two months ago and did not respond to conservative management. Previous to the onset of the symptoms, the patient presented a subcutaneous swelling on the bregma point. The patient affirmed that he had it from birth, but it started to swell two months ago, after a hair transplant.

Brain MRI & CT scan were performed at another facility, which described an osteolytic craniodural process in the anterior third of the superior sagittal sinus region (Figure 1, Figure 2). Correlating the clinical examination with the paraclinical investigations, a

neurosurgical indication was established. The patient and their family were thoroughly informed about the medical condition. The patient opted for surgical treatment.

Surgery was performed under general anesthesia with orotracheal intubation. An arcuate incision was made, circumscribing the lesion. The subcutaneous tissue was dissected, revealing a parafluid, yellowish, caseous collection, which was expressed, collected, and sent for bacteriological examination. (Figure 3, Figure 4). Extensive lavage was performed using normal saline, betadine, and hydrogen peroxide until the purulent collection was completely removed. An osteolytic lesion was identified at the level of the calvaria, causing osteolysis of the outer table, involving the bregma, the spongy bone, and, partially posteriorly, the inner table of the bone. Multiple hair follicles were observed within the lesion. A significantly greater transosseous intraspongious extension was noted compared to the osteolysis of the outer cortical bone. Consequently, a resection of the external cortical bone was performed to fully expose the intraspongious tumor margins, allowing for complete excision of the lesion. (Figure 5). A total

excision of the tumoral formation was performed (Figure 6, Figure 7), and the specimen was sent for histopathological examination. In the posterior part of the lesion, inner cortical bone was perforated by the tumor. After the removal of the tumor, the dura mater of the superior sagittal sinus was exposed (Figure 8). Meticulous hemostasis was achieved. The surgical wound was closed in anatomical layers, and a dressing was applied. On the second day a postoperative brain CT scan was performed, showing complete resection of the tumoral mass (Figure 9). The patient was discharged on the third day, in good general condition, without neurological deficit.

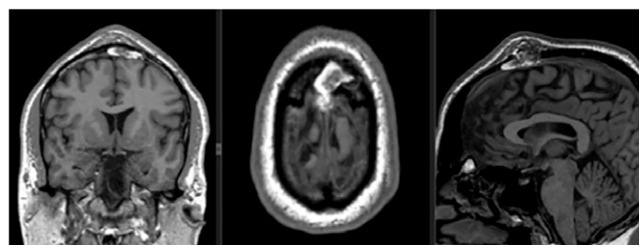


Figure 1. Preoperative MRI Scan (T1+ CS)



Figure 2. Osteolytic transosseous lesion

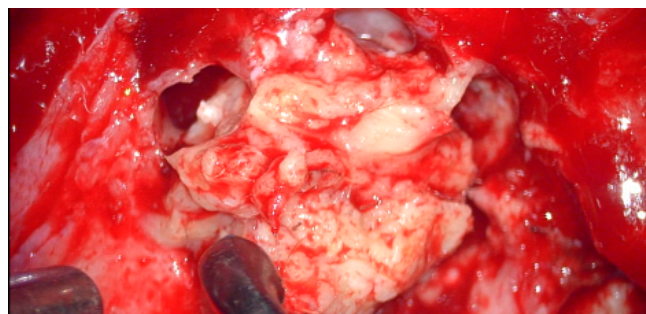


Figure 3. The aspect of the osteolytic mass, which contains hair follicles and the parafluid, yellowish, caseous collection.

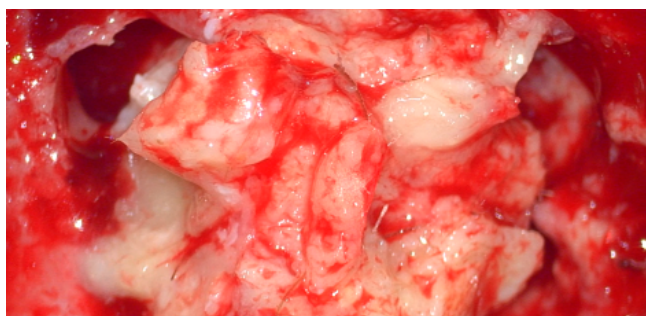


Figure 4. Detailed aspect of the teratoma. In the left superior corner is highlighted the transosseous intraspongious extension

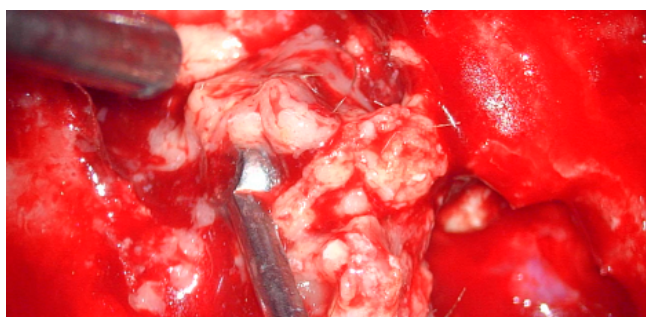


Figure 5. Removal of the tumor

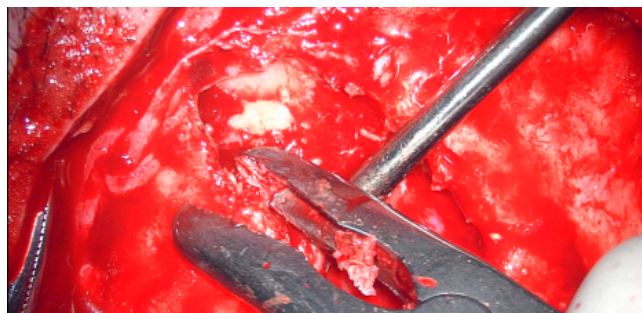


Figure 6. Resection of the external cortical bone to identify the margins of the spongy destruction

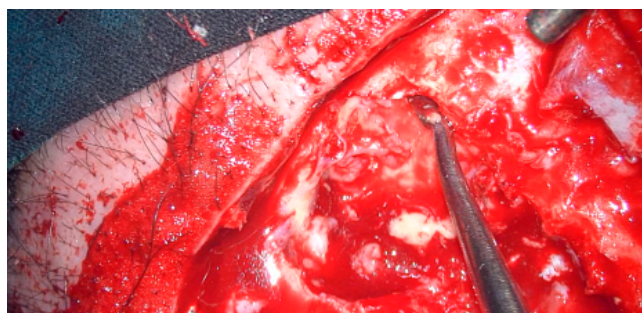


Figure. Identification of the intraspongious bony margins and curettage of the bone to ensure complete excision



Figure 7. Posterior part of the lesion with the osteolytic effect of the inner cortical bone. Dura mater of the superior sagittal sinus highlighted with the aspirator



Figure 8. Postoperative CT scan.

DISCUSSION

Transosseous teratomas of the cranial bones are exceedingly rare, with few cases documented in the literature⁷. The most common location was reported in the petrosal bone^{8,9}.

Their origin is hypothesized to stem from aberrant migration of germ cells during embryogenesis, leading to ectopic growth within the cranial vault¹⁰. Unlike intracranial teratomas, which commonly occur in midline structures such as the pineal or suprasellar regions, transosseous teratomas may present with progressive cranial deformities and focal neurological symptoms due to local mass effect¹¹.

Radiological findings are essential for preoperative planning. CT scans often demonstrate a heterogeneous mass with calcifications. At the same time, MRI provides superior visualization of cystic and solid tumor components¹². Differential diagnoses include dermoid and epidermoid cysts, osteolytic skull lesions, and other germ cell tumors¹³. Histopathological analysis confirms the diagnosis, with mature teratomas containing well-differentiated tissue elements and immature teratomas exhibiting primitive neuroectodermal structures, which correlate with a higher risk of recurrence and malignancy¹⁴.

Surgical excision is the cornerstone of treatment. A similar therapeutic principle has also been emphasized in the management of other rare cranial lesions, including a recent case report of a retroauricular extracranial AVM, in which complete surgical resection was regarded as the definitive treatment¹⁵. Complete resection minimizes the risk of recurrence, though challenges arise due to the tumor's infiltration into the skull and adjacent dura¹⁶. In cases of incomplete resection or immature teratomas, adjuvant therapies such as chemotherapy or radiotherapy may be considered. Long-term follow-up with serial imaging is recommended to monitor for potential recurrence or malignant transformation.

Advances in neurosurgical techniques, including intraoperative navigation and endoscopic-assisted approaches, have improved surgical outcomes, reducing morbidity and improving tumor resection rates. However, due to the rarity of transosseous teratomas, further research and case studies are needed to establish standardized treatment protocols.

CONCLUSION

This is the first case of transosseous teratoma reported in the literature. Transosseous teratomas of the frontal and parietal bones are rare entities that pose significant diagnostic and surgical challenges. Their clinical presentation varies, often leading to delayed diagnosis. Imaging studies, particularly MRI and CT, are crucial in identifying characteristic features and differentiating them from other skull lesions. Surgical resection remains the primary treatment modality, with total excision being the goal to prevent recurrence. Given the potential for malignant transformation in immature teratomas, long-term follow-up is essential. Future research is necessary to understand their pathogenesis better and optimize management strategies.

Ethics Statement and Conflict of Interest Disclosures

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Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws. Written informed consent was provided by the patient participant in this study.

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The use of generative AI and AI-assisted

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Multisystem Sarcoidosis Mimicking Metastatic Bone Disease: A Diagnostic Challenge – Case Report

Razvan-Gabriel PRICOPE^{1*}, Andreea-Eliza CAZACU¹, Andra BUZOIANU¹

Abstract

Background: Sarcoidosis is a multisystem inflammatory disorder characterized by non-caseating granulomas. Skeletal involvement, present in up to 13% of cases, frequently remains underrecognized due to its asymptomatic course and imaging features that closely mimic metastatic disease, creating significant diagnostic challenges.

Case Presentation: A 63-year-old woman presented for neurological evaluation of potential early cognitive decline prompted by family history. Neuroimaging incidentally revealed multiple osteolytic calvarial lesions. Further imaging demonstrated widespread mixed osteolytic and osteoblastic lesions involving the skeleton, bilateral perilymphatic pulmonary nodules, mediastinal and hilar lymphadenopathy, and hepatic lesions. Laboratory investigation showed elevated CA 15-3 (43.7 kU/l) with normal CEA and CYFRA 21-1. Whole-body FDG PET/CT revealed hypermetabolic osteolytic lesions and metabolically active pulmonary infiltrates. An interdisciplinary tumor board considered carcinoma of unknown primary with suspected breast origin; however, breast MRI revealed no suspicious findings. Multiple myeloma was excluded by negative immunofixation electrophoresis. Percutaneous liver biopsy demonstrated non-caseating granulomas without malignancy, with negative special stains for acid-fast bacilli and fungi, establishing sarcoidosis as the underlying diagnosis.

Management and Outcome: The patient was initiated on corticosteroids with calcium and vitamin D supplementation. At one-month follow-up, symptomatic improvement was noted with normalization of serum ACE levels and decline in CA 15-3. Pulmonary function remained stable with mild DLCO reduction. The patient was continued on a corticosteroid taper with addition of inhaled corticosteroids for residual bronchial hyperreactivity.

Conclusion: This case emphasizes the critical importance of maintaining broad differential diagnosis in multisystem disease with skeletal involvement. Although metastatic malignancy must be considered, sarcoidosis remains a key diagnostic consideration. Definitive histopathological confirmation is essential for accurate diagnosis and prevention of unnecessary oncologic intervention.

Keywords: sarcoidosis; bone involvement; metastatic disease; diagnostic challenge; case report

¹Spital Lachen, 8853 Lachen, Switzerland

²Praxis am Bahnhof, 8630 Rüti, Switzerland

***Corresponding author:**

Razvan-Gabriel PRICOPE, Spital Lachen, 8853 Lachen, Switzerland

E-mail: razvprc@gmail.com

INTRODUCTION

Sarcoidosis is a complex multisystem inflammatory disorder characterized histologically by the presence of non-caseating granulomas. Although pulmonary involvement predominates in over 90% of cases, extra-pulmonary manifestations are increasingly recognized and may, in selected instances, overshadow the clinical presentation. Skeletal involvement, while identified in up to 13% of patients through radiologic investigation, frequently remains underrecognized due to its often-asymptomatic nature and imaging characteristics that closely mimic metastatic disease.

The differentiation of sarcoidosis from malignancy represents a critical yet frequently challenging diagnostic endeavor. The presence of osteolytic lesions, elevated metabolic activity on positron emission tomography (PET), and increased tumor markers may misleadingly direct clinical evaluation toward metastatic cancer. Carcinoma of unknown primary (CUP) represents approximately 3–5% of all malignancies and typically manifests with disseminated disease portending a poor prognosis. This case demonstrates a diagnostically demanding presentation in which incidental imaging findings of extensive skeletal and pulmonary lesions required extensive workup to exclude malignancy and establish a diagnosis of multisystem sarcoidosis.

CASE PRESENTATION

A 63-year-old woman presented to the Primary Care Clinic for evaluation of potential early cognitive decline, motivated by significant family history: her mother experienced rapidly progressive dementia beginning at the same age, ultimately resulting in death a decade later. The patient herself denied any subjective cognitive deficits and reported an excellent quality of life without constitutional symptoms including fever, night sweats, or unintended weight loss. Her medical history was notable solely for well-controlled hypertension. She was a lifelong non-smoker with no occupational exposures of concern. Family history was otherwise unremarkable apart from the maternal dementia.

Neurological examination was normal, showing no focal deficits or clinical signs of neurodegenerative disease. Given her strong family history, a brain MRI was obtained, which revealed no cerebral atrophy, ischemic lesions, or white matter pathology indicative of

dementia. However, the imaging unexpectedly revealed multiple osteolytic lesions within the calvarium.

These incidental findings prompted further radiologic investigation. A non-contrast CT scan of the head, thorax, and abdomen disclosed widespread mixed osteolytic and osteoblastic lesions affecting the calvarium, vertebral bodies, and pelvic bones. Additional findings included bilateral perilymphatic pulmonary nodules accompanied by mediastinal and hilar lymphadenopathy, as well as multiple small hepatic lesions of uncertain etiology.

Laboratory investigation revealed an elevated CA 15-3 level of 43.7 kU/l (reference <27), while carcinoembryonic antigen (CEA) and cytokeratin fragment 21-1 (CYFRA 21-1) remained normal. Erythrocyte sedimentation rate was within normal limits at 23 mm/h. Serum and urine immunofixation electrophoresis were negative, effectively excluding multiple myeloma. Additional laboratory evaluation demonstrated normal serum calcium and renal function.

Given this constellation of findings, the case was presented at an interdisciplinary tumor board conference. A carcinoma of unknown primary (CUP) with suspected breast origin was considered, though subsequent breast MRI did not reveal any suspicious lesions.

A whole-body FDG PET/CT scan was undertaken for further evaluation. This revealed multiple hypermetabolic osteolytic lesions in the axial and proximal appendicular skeleton, metabolically active nodular infiltrates in both lungs (predominantly in the upper lobes), and FDG-avid lymphadenopathy in the retroclavicular, mediastinal, retroperitoneal, and inguinal regions. Small hypermetabolic hepatic lesions were also identified. Importantly, there was no abnormal FDG uptake in the breast parenchyma, spleen, pancreas, adrenal glands, kidneys, ovaries, or gastrointestinal tract.

To clarify the differential diagnosis, which included metastatic disease versus granulomatous conditions, a liver biopsy was performed. Histopathologic examination revealed non-caseating granulomas without evidence of malignancy, consistent with sarcoidosis. Special stains for acid-fast bacilli and fungi were negative, further supporting the diagnosis.

Further pulmonary evaluation included functional testing, which demonstrated mild reduction in diffusing capacity of the lungs for carbon monoxide (DLCO) with preserved lung volumes and airflow parameters. Serum angiotensin-converting enzyme (ACE) level was elevated at 38 U/L (reference 5–21 U/L).

Integrating the imaging findings, histopathologic confirmation, and supportive laboratory data, a diagnosis of multisystem sarcoidosis involving the liver, lungs, lymphatic system, and bones was established.

The patient was initiated on oral corticosteroid therapy along with calcium and vitamin D supplementation to prevent steroid-related bone loss. At one-month follow-up, she reported symptomatic improvement, with stabilization of pulmonary function parameters and normalization of serum ACE levels.

Serial imaging of the spine and skull revealed slight progression of calvarial osteolytic lesions with stable vertebral involvement. Given these radiographic findings, bone biopsy was recommended to definitively exclude occult metastatic disease, though clinical assessment remained reassuring with the patient neurologically intact and free of skeletal complications.

At her most recent follow-up, she continued on a tapering regimen of systemic corticosteroids, supplemented with inhaled corticosteroids for mild bronchial hyperreactivity. A repeat pulmonary reassessment at one year was scheduled.

DISCUSSION

This case exemplifies the substantial diagnostic challenge posed by the overlap in clinical, laboratory, and imaging features between sarcoidosis and metastatic malignancies, particularly carcinoma of unknown primary^{1,5,7}. The initial presentation—encompassing multiple osteolytic and osteoblastic skeletal lesions, pronounced hypermetabolic activity on FDG PET/CT, and elevated CA 15-3—strongly suggested underlying metastatic disease. Breast carcinoma, given its predilection for osseous metastases and association with elevated CA 15-3, was a reasonable initial consideration; however, comprehensive imaging, including high-resolution MRI mammography, revealed no primary neoplasm^{5,7}.

Multiple myeloma represented another important differential diagnostic consideration, particularly given the patient's age and presence of osteolytic lesions. Nonetheless, the diagnosis was effectively excluded by the absence of typical laboratory abnormalities such as monoclonal protein on serum and urine immunofixation, anemia, hypercalcemia, or renal dysfunction.

Although skeletal involvement in sarcoidosis is relatively uncommon, it is increasingly recognized beyond the classic predilection for the small bones of the hands

and feet^{2,3}. Axial skeletal manifestations, including lesions within the vertebrae and calvarium, have been reported with growing frequency in advanced imaging series^{4,6}. Such lesions may appear lytic, sclerotic, or mixed in character, often closely mimicking metastatic deposits^{3,6}. The radiologic appearance on conventional CT and MRI lacks sufficient specificity to reliably differentiate sarcoid bone involvement from metastatic disease^{3,4}. While FDG PET/CT demonstrates high sensitivity for metabolically active lesions, it lacks specificity; granulomatous inflammation in sarcoidosis frequently exhibits intense FDG uptake indistinguishable from that observed in malignancy^{6,8,9}.

Hepatic involvement in sarcoidosis, although detected histologically in up to 70% of cases, is clinically significant in fewer than 20% of patients^{1,2}. In the present case, the presence of non-caseating granulomas on liver biopsy, in conjunction with negative special stains for acid-fast bacilli and fungi, definitively established sarcoidosis as the underlying etiology¹. The pulmonary findings, characterized by perilymphatic nodules and bilateral hilar lymphadenopathy, were classic for sarcoidosis^{1,8}, yet the degree of nodularity and marked FDG activity initially heightened concern for disseminated malignancy^{5,6}.

The elevation of CA 15-3 added a significant layer of diagnostic complexity. Although predominantly utilized as a tumor marker for breast carcinoma, CA 15-3 may also be elevated in benign conditions, including various interstitial lung diseases and granulomatous disorders such as sarcoidosis⁵. This case highlights the critical importance of interpreting tumor markers within a comprehensive clinical and diagnostic framework, avoiding excessive reliance on isolated laboratory abnormalities⁵.

Management of sarcoidosis is typically reserved for patients with clinically relevant pulmonary involvement, progressive organ dysfunction, or significant extrapulmonary disease^{1,10}. In this case, the presence of mild diffusion impairment on pulmonary function testing, together with extensive skeletal involvement and biopsy-proven hepatic sarcoidosis, supported the decision to initiate systemic corticosteroid therapy^{3,4,10}. Treatment was followed by clinical stabilization, symptomatic improvement, and normalization of serum angiotensin-converting enzyme levels, findings that were consistent with both the diagnosis and the therapeutic response^{1,10}. Should disease recurrence occur or corticosteroid-related adverse effects develop, the

introduction of steroid-sparing immunomodulatory agents may be considered¹⁰.

Sarcoidosis is characterized by a highly variable and often unpredictable clinical course, ranging from spontaneous resolution to chronic, progressive multi-system involvement¹. Although skeletal manifestations are relatively uncommon, their course may be heterogeneous and occasionally associated with structural complications, while hepatic involvement is frequently subclinical but can, in rare cases, evolve into clinically significant disease²⁻⁴. In this context, individualized follow-up strategies incorporating periodic imaging and functional evaluation are essential to ensure optimal long-term management and to allow timely recognition of disease progression or complications^{3,4}.

CONCLUSION

This case highlights the importance of maintaining a broad differential diagnosis in patients presenting with multisystem involvement characterized by disseminated osteolytic lesions, pulmonary nodules, and lymphadenopathy. Although this constellation of findings commonly raises strong suspicion for metastatic malignancy, particularly in the absence of an identifiable primary tumor, sarcoidosis should remain a key diagnostic consideration. Histopathological confirmation remains essential for distinguishing sarcoidosis from neoplastic processes, thereby avoiding misdiagnosis and unnecessary oncologic interventions. Ultimately, this case underscores the value of a structured, multidisciplinary approach that integrates careful clinical assessment, advanced imaging techniques, and targeted tissue sampling in the evaluation of complex multisystem disorders.

Ethics Statement and Conflict of Interest

Disclosures

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Laser-Assisted Endoscopic Dacryocystorhinostomy in Recurrent Chronic Dacryocystitis: A Case Report

Vadim CERNEI^{1*}, Sergiu VETRICEAN², Eusebiu SENCU², Irina COVALI¹, Natalia CRECIUN¹

Abstract

Introduction: Recurrent chronic dacryocystitis represents a complex inflammatory condition frequently associated with failure of previous surgical interventions and persistent mechanical obstruction of the lacrimal drainage system.

Objective: To present a case of recurrent chronic dacryocystitis after multiple unsuccessful surgical procedures and to highlight the role of laser-assisted endoscopic dacryocystorhinostomy as a revision technique.

Materials and Methods: We report the case of a patient with recurrent symptoms following two previous endoscopic dacryocystorhinostomies with nasolacrimal stent placement. Clinical examination, computed tomography of the paranasal sinuses, endoscopic findings, surgical management, and postoperative course were analyzed.

Results: Imaging revealed rhinostomy stenosis associated with lacrimal sac lithiasis, representing a persistent mechanical cause of obstruction. Laser-assisted endoscopic dacryocystorhinostomy using a diode laser was performed without stent placement, allowing precise removal of fibrotic tissue and obstructive material. The postoperative course was favorable, with complete resolution of epiphora, absence of purulent discharge, and maintained rhinostomy patency at 3-month follow-up.

Conclusions: Laser-assisted endoscopic dacryocystorhinostomy may represent an effective revision strategy in recurrent chronic dacryocystitis caused by persistent mechanical obstruction. Accurate identification of the underlying mechanism of surgical failure is essential for achieving durable functional outcomes.

Keywords: recurrent chronic dacryocystitis; laser-assisted dacryocystorhinostomy; lacrimal sac lithiasis; rhinostomy stenosis

¹Department of Otorhinolaryngology, IMSP "Timofei Mosneaga" Republican Clinical Hospital, Chisinau, Republic of Moldova

²Department of Otorhinolaryngology, "Nicolae Testemitanu" State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

*Corresponding author:

Vadim CERNEI, Department of Otorhinolaryngology
IMSP "Timofei Mosneaga" Republican Clinical Hospital
Chisinau, Republic of Moldova

E-mail: wadim.cernei@gmail.com

INTRODUCTION

Chronic dacryocystitis is a persistent inflammatory disorder of the lacrimal sac, most commonly secondary to obstruction of the nasolacrimal drainage system. From a pathophysiological perspective, this condition is closely linked to primary acquired nasolacrimal duct obstruction (PANDO), a multifactorial process involving chronic inflammation, epithelial metaplasia, submucosal fibrosis, and progressive luminal narrowing of the nasolacrimal duct. These mechanisms ultimately impair physiological tear drainage and predispose to recurrent infection of the lacrimal sac. According to contemporary classifications, chronic dacryocystitis may present as a primary condition or as a recurrent disorder after surgical treatment, the latter posing a significant therapeutic challenge¹⁻³.

In recurrent or atypical cases, secondary causes of obstruction, including neoplastic lesions of the medial canthal region, must be excluded. Cutaneous malignancies of the head and neck may involve periocular structures, including the medial canthus, due to chronic ultraviolet exposure. Such lesions can produce epiphora and clinical features mimicking chronic dacryocystitis through compression or infiltration of the lacrimal sac and nasolacrimal duct, emphasizing the importance of careful preoperative evaluation and imaging⁴.

Recurrent chronic dacryocystitis is increasingly recognized as a complex disease entity in which surgical failure cannot be attributed solely to technical factors. Current evidence highlights the role of excessive cicatricial response, persistent intraluminal or extraluminal mechanical obstruction, and ongoing inflammatory stimuli arising from the sinonasal cavity. These factors contribute to restenosis of the rhinostomy and compromise long-term surgical success, particularly in revision cases⁵⁻⁶. In this context, identifying the specific etiological mechanisms underlying recurrence is essential for selecting an appropriate revision strategy.

Dacryocystorhinostomy (DCR) remains the gold standard surgical procedure for restoring lacrimal drainage in patients with nasolacrimal duct obstruction. Both external and endonasal endoscopic approaches have demonstrated high primary success rates; however, failure rates ranging from 5% to 20% have been reported, particularly in complex or recurrent cases. The most frequently cited causes of failure include inadequate rhinostomy size, postoperative fibrosis, intranasal synechiae, and unrecognized mechanical obstructions, such as dacryolithiasis of the lacrimal sac^{2,7-9}.

Lacrimal sac lithiasis (dacryolithiasis) represents a relatively rare but clinically significant cause of persistent or recurrent obstruction of the lacrimal drainage system. Dacryoliths may act as a nidus for chronic inflammation and infection, perpetuating symptoms despite technically adequate surgical intervention. Histopathological and compositional analyses suggest that dacryolith formation is associated with chronic stasis, bacterial colonization, and inflammatory debris, underscoring its role as a mechanical and biological contributor to surgical failure¹⁰⁻¹³. Importantly, dacryolithiasis may be overlooked during initial interventions, particularly when preoperative imaging or endoscopic assessment is limited.

Over the last two decades, the endoscopic endonasal otorhinolaryngological approach has gained prominence in the management of lacrimal drainage disorders. This approach offers several advantages, including direct visualization of the lateral nasal wall, precise identification of the rhinostomy site, simultaneous correction of associated sinonasal pathology, and the possibility of postoperative endoscopic surveillance. These features are particularly valuable in revision surgery, where altered anatomy and fibrotic changes are frequently encountered^{2,3,7,8}.

Laser-assisted endoscopic dacryocystorhinostomy has been introduced as an alternative or adjunctive technique in selected cases, particularly in recurrent or complex diseases. The use of laser technology allows controlled and precise ablation of fibrotic tissue and mechanical obstructions, with minimal collateral tissue damage and effective hemostatic control. Several studies have reported favorable outcomes using laser-assisted techniques, especially in revision settings^{8,10}.

Against this background, the present case report aims to illustrate the clinical course, diagnostic challenges, and surgical management of a patient with recurrent chronic dacryocystitis complicated by lacrimal sac lithiasis. By integrating clinical findings with current evidence from the literature, this report seeks to highlight the role of laser-assisted endoscopic dacryocystorhinostomy as a viable revision strategy in carefully selected cases.

MATERIALS AND METHODS

This paper presents a clinical case of recurrent chronic dacryocystitis managed in a tertiary otorhinolaryngology department. Clinical data, imaging investigations

(computed tomography of the paranasal sinuses – CT PNS), nasal endoscopic examinations, details of surgical management, and postoperative outcomes were analyzed. The study adhered to ethical principles, the patient's data were fully anonymized, and written informed consent for publication was obtained.

CASE PRESENTATION

A 25-year-old woman presented on April 1, 2024, with complaints of nasal obstruction, headache, epiphora, and purulent discharge from the right eye.

Computed tomography (CT) of the paranasal sinuses (PNS) revealed dilatation and opacification of the right lacrimal sac, nasal septal deviation, right-sided concha bullosa, and hypertrophy of the inferior nasal turbinate mucosa. The patient was hospitalized with a diagnosis of primary chronic dacryocystitis of the right eye associated with nasal septal deviation, right concha bullosa, and chronic rhinitis.

First Surgical Intervention

Endoscopic surgical treatment was performed, consisting of endoscopic dacryocystorhinostomy with placement of a right nasolacrimal stent, endoscopic septoplasty, resection of the hyperaerated portion of the right middle turbinate, and cauterization of the inferior nasal turbinates. The postoperative course was initially favorable.

Clinical Evolution and Second Surgical Intervention

On October 22, 2024, the patient returned with recurrent epiphora of the right eye and discomfort over the lacrimal sac region. Repeated nasolacrimal duct irrigations proved ineffective.

A repeat CT PNS examination demonstrated dilatation and opacification of the lacrimal sac with hyperdense inclusions suggestive of lacrimal sac lithiasis, resulting in obstruction of the lacrimal sac and nasolacrimal duct. Based on clinical and imaging findings, a diagnosis of recurrent chronic dacryocystitis of the right eye due to rhinostomy stenosis was established.

Revision surgery was performed, consisting of enlargement of the dacryocystorhinostomy and reinsertion of the nasolacrimal stent. Despite surgical revision, the patient subsequently developed further recurrence.

Third Surgical Intervention

On October 27, 2025, the patient presented for the third time with similar symptoms, characterized by recurrent

epiphora and local discomfort. Imaging investigations confirmed recurrence of lacrimal sac lithiasis associated with obstruction of the rhinostomy.

A laser-assisted endoscopic dacryocystorhinostomy using a diode laser was performed without placement of a nasolacrimal stent. The procedure was carried out via an endonasal endoscopic approach, allowing precise identification of the stenotic rhinostomy site. Peristomal fibrotic tissue and obstructive material were removed using controlled laser ablation, resulting in enlargement of the rhinostomy and restoration of communication between the lacrimal sac and the nasal cavity.

The use of the diode laser allowed precise ablation of pathological tissues with minimal tissue trauma and effective hemostatic control. Stent placement was avoided due to the increased risk of postoperative fibrosis and recurrent stenosis associated with previous surgical interventions.

RESULTS

Postoperatively, the patient had a favorable clinical course and was discharged on the third postoperative day with recommendations for outpatient treatment, including oral antibiotic therapy, nasal spray, and ophthalmic drops. The patient subsequently attended regular otorhinolaryngological follow-up visits at 4-week intervals.

During these evaluations, endoscopic endonasal examination demonstrated a patent rhinostomy, with no signs of restenosis or recurrent infection. At the 3-month postoperative assessment, the clinical outcome remained favorable, with complete resolution of epiphora and absence of purulent ocular discharge. The patient remains under regular otorhinolaryngological follow-up.

DISCUSSION

Recurrent chronic dacryocystitis remains a significant therapeutic challenge, particularly in patients with previous surgical failure. While primary dacryocystorhinostomy generally yields high success rates, recurrence highlights the multifactorial nature of lacrimal drainage obstruction, extending beyond purely technical factors. Current evidence emphasizes that restenosis of the rhinostomy is frequently driven by a combination of excessive fibrotic response, persistent inflammation, and unrecognized mechanical obstruction, rather than by inadequate surgical technique alone^{5,7,10,13}.

In revision cases, the pathophysiology of failure is often complex. Chronic inflammation of the lacrimal sac and nasolacrimal duct leads to epithelial remodeling, subepithelial fibrosis, and progressive narrowing of the drainage pathway. These changes may be further exacerbated by ongoing sinonasal pathology, which serves as a continuous inflammatory stimulus. Consequently, comprehensive endoscopic evaluation of the lateral nasal wall and rhinostomy site is essential for identifying contributory intranasal factors and tailoring the revision strategy accordingly^{3,6,7}.

This case illustrates these considerations. The coexistence of nasal septal deviation, concha bullosa, and turbinate hypertrophy supports the concept that lacrimal drainage disorders should be approached as part of a broader sinonasal disease spectrum. The endoscopic otorhinolaryngological approach enables simultaneous correction of these anatomical abnormalities, which may otherwise compromise postoperative rhinostomy patency and predispose to recurrence^{2,6}.

A particularly relevant aspect of this case is the presence of lacrimal sac lithiasis as a persistent mechanical cause of obstruction. Although dacryolithiasis is considered relatively uncommon, clinical series and reviews suggest that dacryoliths may be underdiagnosed, especially in recurrent or atypical cases. Dacryoliths act not only as physical obstructions but also as reservoirs for bacterial colonization and chronic inflammation, thereby perpetuating disease despite apparently adequate surgical intervention¹⁰⁻¹³. Their identification is therefore critical in revision surgery and should prompt reconsideration of both surgical technique and postoperative management.

The recurrence observed after two conventional endoscopic dacryocystorhinostomies with stent placement in the present case underscores the limitations of standard techniques in the setting of persistent mechanical obstruction and fibrotic tissue. Although silicone stenting is widely used to maintain rhinostomy patency, its routine use — especially in revision settings — remains debated and should be individualized based on local findings and the mechanism of failure².

Laser-assisted endoscopic dacryocystorhinostomy offers several theoretical and practical advantages in revision settings. The use of laser energy allows precise and controlled ablation of fibrotic tissue and obstructive material, with minimal collateral damage to surrounding mucosa. Effective hemostatic control facilitates optimal visualization, which is particularly important

in anatomically distorted revision cases. Clinical studies evaluating laser-assisted techniques have reported variable success rates; however, favorable outcomes are described in selected patients, particularly when used as an adjunct or in revision settings⁸⁻¹⁰.

In the present case, laser-assisted endoscopic dacryocystorhinostomy enabled targeted removal of peristomal fibrosis and dacryolith-related obstruction, resulting in durable rhinostomy patency and complete symptom resolution at short-term follow-up. The absence of restenosis despite omission of a nasolacrimal stent supports the hypothesis that adequate removal of mechanical and fibrotic barriers may be more critical to long-term success than routine stenting in selected revision cases.

Taken together, this case supports a tailored, mechanism-oriented approach to recurrent chronic dacryocystitis. Rather than relying on uniform revision strategies, surgical planning should be guided by detailed endoscopic and imaging assessment, identification of specific causes of failure, and judicious selection of adjunctive techniques such as laser-assisted ablation. Such an individualized approach aligns with contemporary evidence-based recommendations and may improve outcomes in complex and recalcitrant cases.

CONCLUSIONS

Recurrent chronic dacryocystitis is a complex pathological condition in which surgical failure is frequently the result of multifactorial mechanisms, including persistent inflammation, excessive fibrotic response, associated sinonasal pathology, and unrecognized mechanical obstruction of the lacrimal drainage system. As demonstrated in the present case, repeated failure of conventional endoscopic dacryocystorhinostomy may occur despite technically adequate procedures when the underlying cause of recurrence is not fully addressed.

The endoscopic otorhinolaryngological approach plays a pivotal role in the management of recurrent disease, as it allows comprehensive evaluation of the rhinostomy, precise identification of intranasal and peristomal factors contributing to obstruction, and simultaneous correction of associated sinonasal abnormalities. In revision settings, such an approach is essential for understanding the true mechanism of surgical failure and for selecting an appropriate, individualized therapeutic strategy.

Lacrimal sac lithiasis should be considered a clinically relevant cause of persistent or recurrent obstruction, particularly in patients with repeated surgical failure. Its mechanical and inflammatory impact may significantly compromise long-term outcomes if not specifically identified and removed. The present case highlights the importance of targeted diagnostic assessment and mechanism-oriented surgical planning in recurrent dacryocystitis.

Laser-assisted endoscopic dacryocystorhinostomy using a diode laser represents a valuable adjunctive technique in selected revision cases. By enabling precise ablation of fibrotic tissue and mechanical obstructions with minimal collateral damage and effective hemostatic control, this approach may facilitate durable rhinostomy patency without the routine need for nasolacrimal stenting. The favorable clinical outcome observed in the present case supports the role of laser-assisted techniques as an effective option in carefully selected patients with recurrent chronic dacryocystitis.

Ultimately, successful management of recurrent dacryocystitis requires an individualized, evidence-based approach that integrates detailed endoscopic assessment, understanding of disease pathophysiology, and judicious selection of surgical techniques. Such a strategy is particularly relevant in complex revision cases and aligns with contemporary principles of modern lacrimal and otorhinolaryngological surgery.

Ethics Statement and Conflict of Interest Disclosures

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Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws. Written informed consent was provided by the patient participant in this study.

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