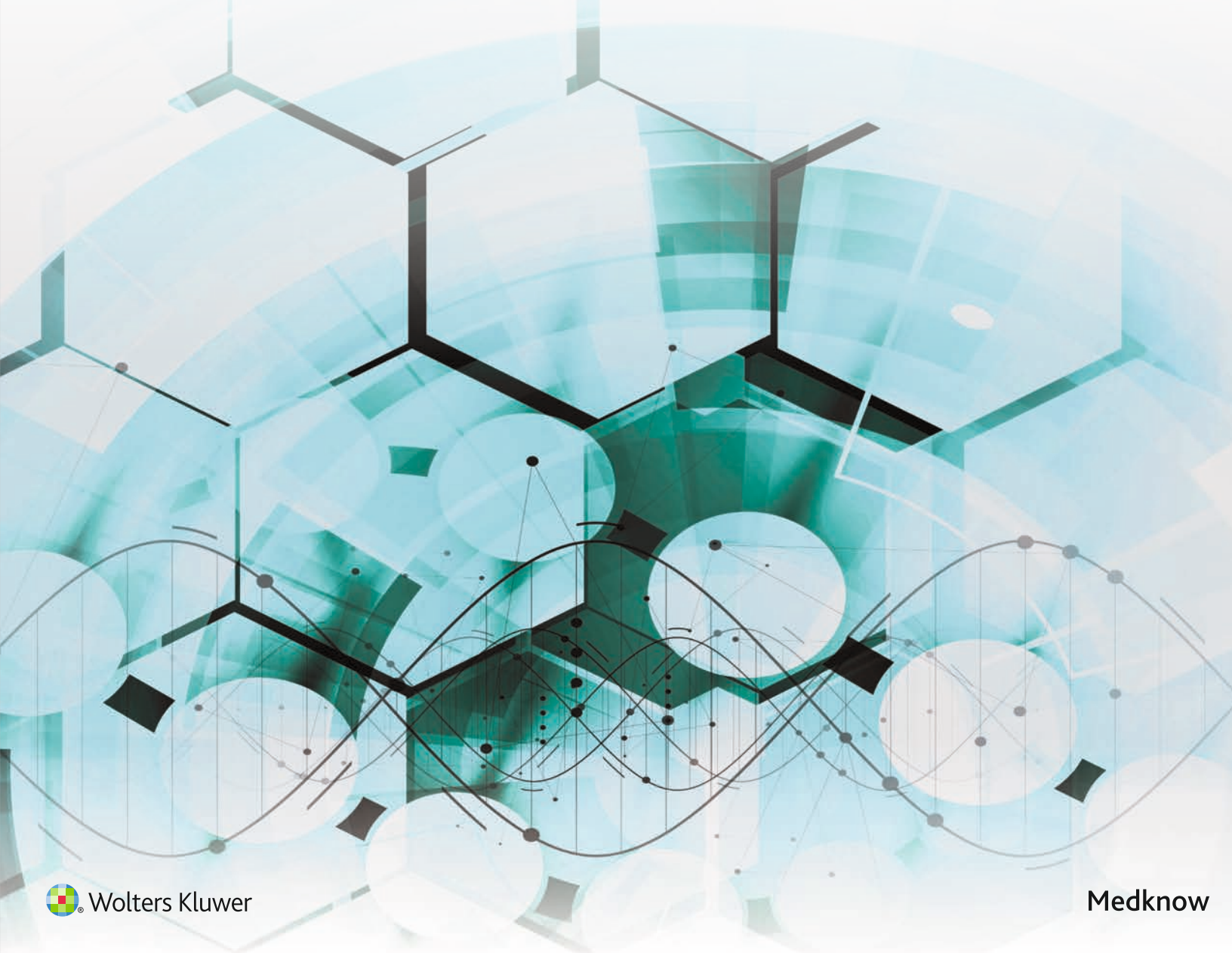
Volume 1 • Issue 3 • September-December 2022

Annals of Medical Science and Research

(An Official Publication of Institute of Post Graduate Medical Education and Research)

https://journals.lww.com/amsr



Wolters Kluwer

Medknow

Annals of Medical Science and Research

An official publication of the Institute of Post Graduate Medical Education and Research

Volume No. 1 Issue No. 3 September-December 2022

Editorial Office

Annals of Medical Science & Research
Address: 6th Floor, Academic Building, Institute of Post Graduate Medical Education and Research, 244, AJC Bose Road, Kolkata-700020
Phone No.: 033 22041 832, E-mail ID: amsreditor@gmail.com

Annals of Medical Science and Research

General Information

The journal

Annals of Medical Science and Research (AMSR) is a peer-reviewed journal published on behalf of Institute of Post Graduate Medical Education and Research. The journal devoted to basic, clinical, epidemiological and experimental studies of the various disciplines of medical and health sciences. It seeks to contribute significantly to the pathogenesis, diagnosis, prognosis, and the effective treatment or prevention of disease. The Journal is published in April, August and December.

Information for Authors

There are no page charges for AMSR submissions. Please check https://journals.lww.com/amsr/Pages/informationforauthors.aspx for details.

All manuscripts must be submitted online at https://review.jow.medknow.com/amsr

Subscription Information

Copies of the journal are provided free of cost to the members of Institute of Post Graduate Medical Education and Research. A subscription to Annals of Medical Science and Research comprises 3 issues.

For mode of payment and other details, please visit www.medknow.com/subscribe.asp.

Claims for missing issues will be serviced at no charge if received within 60 days of the cover date for domestic subscribers, and 3 months for subscribers outside India. Duplicate copies cannot be sent to replace issues not delivered because of failure to notify publisher of change of address.

The journal is published and distributed by Wolters Kluwer India Private Limited. Copies are sent to subscribers directly from the publisher's address. It is illegal to acquire copies from any other source. If a copy is received for personal use as a member of the association/society, one cannot resale or give-away the copy for commercial or library use.

The copies of the journal to the members of the association are sent by ordinary post. The editorial board, association or publisher will not be responsible for non receipt of copies. If any member/subscriber wishes to receive the copies by registered post or courier, kindly contact the publisher's office. If a copy returns due to incomplete, incorrect or changed address of a member/subscriber on two consecutive occasions, the names of such members will be deleted from the mailing list of the journal. Providing complete, correct and up-to-date address is the responsibility of the member/subscriber.

Information regarding change of address should be sent to wkhlrpmedknow_subscriptions@wolterskluwer.com

Advertising policies

The journal accepts display and classified advertising. Frequency discounts and special positions are available. Inquiries about advertising should be sent to Wolters Kluwer India Private Limited, advertise@medknow.com

The journal reserves the right to reject any advertisement considered unsuitable according to the set policies of the journal.

The appearance of advertising or product information in the various sections in the journal does not constitute an endorsement or approval by the journal and/or its publisher of the quality or value of the said product or of claims made for it by its manufacturer.

Copyright

Permissions

For information on how to request permissions to reproduce articles/information from this journal, please visit https://journals.lww.com/amsr/pages/default.aspx

Disclaimer

The information and opinions presented in the Journal reflect the views of the authors and not of the Journal or its Editorial Board or the Publisher. Publication does not constitute endorsement by the journal. Neither the Annals of Medical Science and Research nor its publishers nor anyone else involved in creating, producing or delivering the Annals of Medical Science and Research or the materials contained therein, assumes any liability or responsibility for the accuracy, completeness, or usefulness of any information provided in the Annals of Medical Science and Research, nor shall they be liable for any direct, indirect, incidental, special, consequential or punitive damages arising out of the use of the Annals of Medical Science and Research. The Annals of Medical Science and Research, nor its publishers, nor any other party involved in the preparation of material contained in the Annals of Medical Science and Research represents or warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such material. Readers are encouraged to confirm the information contained herein with other sources.

Addresses

Editorial Office

Prof. (Dr.) Dilip Kumar Pal,
Editor in Chief,
Annals of Medical Science and Research
Address: 6th Floor, Academic Building, Institute of Post Graduate Medical Education and Research, 244, AJC Bose Road, Kolkata-700020, India
E-mail: amsreditor@gmail.com
Website:https://journals.lww.com/amsr/pages/default.aspx

Published by

Wolters Kluwer India Private Limited.
A-202, 2nd Floor, The Qube, C.T.S. No.1498A/2 Village Marol
Andheri (East), Mumbai - 400 059, India.
Phone: 91-22-66491818
Website: www.medknow.com

We are glad to share about our planting partnership with "Grow Trees" to reduce carbon emission and help the world to be a greener environment!

Printed at

Nikeda Art Printers Pvt. Ltd.,
Building No. C/3 - 14,15,16, Shree Balaji Complex, Vehele Road,
Village Bhatale, Taluka Bhiwandi, District Thane - 421302, India.

Annals of Medical Science and Research

Volume No. 1 Issue No. 3 September-December 2022

CONTENTS

Neglected bone health needs more care

Good bone health means maintaining a sound and healthy skeletal system for proper body functioning. Like other systems, a good skeletal health is essentially required for a favorable disease outcome. Osteoporosis is the most common metabolic bone disease, characterized by low bone mass with microarchitectural deterioration and increased fragility. Osteoporosis is an inevitable consequence of the aging process due to increased life span and also the effects of major lifestyle changes occurring following urbanization and seen increasingly contributing serious health issues as well as economic stress worldwide.[1] Unfortunately, very often the treating physician fails to address the problem properly either ignoring completely or treating inadequately.

Osteoporosis is very much prevalent and currently about 200 million or more people have osteoporosis globally but the problem is just like the tip of an iceberg, because mostly this disorder remains silent, unless a fracture occurs. In India, osteoporosis is one of the major public health problems, and the prevalence of osteopenia and osteoporosis was reported as high as 49.9% and 18.3% cases, respectively, and osteoporosis was seen slightly higher in females than in males (19.4 vs. 17.3%).[2] It had been reported from the USA that annually about 2 million bone fractures were due to osteoporosis and that demanded 82% of total Medicare payments. It had been also projected that more than half of the world's hip fractures due to osteoporosis will be seen in Asian countries by 2050.[3,4] Patients with a history of hip fracture are seen to have a 2.5-fold increased risk of repeat fracture in the near future and increased mortality rate (up to 38%) within a year.[5] Moreover, following recovery from a fracture, there might be a permanent disability, due to resultant residual bony deformity and persistent chronic pain at the fracture site, that might lead to excess anxiety, depression, long absence from work, inability to attend social gatherings, poor quality of life, and sometimes death.[6]

Bone growth and development gradually gear up since infancy and peak bone mass is usually attained at the third decade of life. Bone growth is mainly influenced by heredity but other factors such as dietary habits, adequacy of sun exposure, hormonal influences, and physical activities. In normal physiologic condition, bone formation and resorption occur as a dynamic process and remains in a balancing state but in cases of any disturbances, there will be more bone resorption and that will lead to osteoporosis. In most of the cases, osteoporosis does not produce much of symptoms, but patients may complain of limb pain, body ache, weakness, acute or chronic low back pain, progressive stooped posture, bowing of legs, etc.

Evaluation of each patient should be done in respect to age, sex, occupation, family history of osteoporosis, dietary habits, adequacy of sun exposure, menstrual status, presence of diabetes, thyroid disorders, chronic kidney disease, HIV/AIDS, chronic obstructive pulmonary diseases, use of certain drugs such as corticosteroids (5 mg or more taken >3 months), furosemide, proton pump inhibitors, heparin, and phenytoin history of smoking, alcohol drink (>3 drinks/day), and any kind of physical activities. Clinical examination includes measurement of body weight (body mass index), height (any reduction from the previous one), any kind of spinal deformities including dowager hump (spinal hyperkyphosis) and tenderness, any chronic diseases (rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus (SLE), chronic lung diseases, diabetes, thyroid disorders, malabsorption syndrome, etc.), and details of the musculoskeletal examination. Blood biochemistry and specific laboratory tests where indicated has to be done to exclude secondary causes of osteoporosis such as serum calcium, thyroid functions, 25 hydroxy Vitamin D, phosphorus, serum cortisol, parathyroid hormone, testosterone and gonadotrophin level, serum protein electrophoresis, and 24-hour urinary calcium excretion. Bone mineral density (BMD) test is required in cases of postmenopausal women, women of 65 years or male of 70 years, the patient having malabsorption syndrome, or ankylosing spondylitis, rheumatoid arthritis, and SLE who are on prolonged corticosteroid therapy. In some special situations, vertebral imaging (computed tomography scan/magnetic resonance imaging) and biochemical markers for bone turnover (both formation and resorption markers) may be advised. Another fracture risk assessment tool (FRAX) can be used in predicting fracture risks in the future by using clinical risk factors without the help of BMD. Thus, combining BMD and FRAX tool, and in some special situation, bone turnover markers are all might be helpful in the prediction of fracture risk and also helps to evaluate the patients having low bone mass or osteoporosis.

The goals of treatment and its beneficial effects have to be explained to both the patients and their family members

for a comprehensive and successful management. Lifestyle modification is most important step and should be strictly imposed to all participants starting from childhood, adolescence, adulthood, and older age groups. These include daily physical activities of any form, avoidance of excess alcohol, quitting smoking, adequate intake of calcium (dairy products), Vitamin D (400 IU for an adult and 800 IU for older patients with associated severe osteoporosis), adequate exposure to sunlight (at least thrice a week for 30 min duration), and maintaining ideal body weight.

Secondary causes of osteoporosis aggravating bone loss need thorough evaluation and appropriate treatment to minimize bone loss.

Antiresorptive therapy with alendronate, risedronate, or zoledronic acid reduces bone loss by decreasing osteoclastic bone resorption in early postmenopausal women and in turn increase bone density.[7] Selective estrogen receptor modulators such as raloxifene and bazedoxifene act as an estrogenic agonist on bones and prevent excess bone turnover by downmodulating the activity of osteoclasts in a transforming growth factor-β3-dependent manner. These drugs have shown significant increase BMD and reduce the risk of vertebral fractures by 30% in postmenopausal women.[8]

Parathyroid hormone analogs (teriparatide and abaloparatide), when administer intermittently, produce new bone formation and improve the microarchitecture of the skeleton. These drugs significantly reduce both the incidence of vertebral and nonvertebral fractures and should be considered in postmenopausal women, men with severe osteoporosis, and patients on prolonged glucocorticoid therapy with a higher risk of fractures. Testosterone is recommended for men with serum testosterone levels <200 ng/dL, with higher risk of fracture (FRAX score), and where other antiosteoporotic therapies are contraindicated.[8] Calcitonin is approved for treatment in postmenopausal osteoporosis (intranasal spray or injectable preparation). However, its efficacy has not been established and thus not recommended as a primary drug therapy for osteoporosis and is presently used as an adjunct therapy with others. The Anti-receptor activator of nuclear factor kappa beta ligant (RNKL) drugs (denosumab and romosozumab) are humanized monoclonal antibodies and very much effective in the treatment of postmenopausal osteoporosis and malignancy associated osteoporosis.[9] Soya isoflavone preparations are natural products, structurally and functionally related to 17-beta-estradiol and these are seen suppressing the rate of bone resorption and also concomitantly enhance the rate of bone formation, although few studies found either a limited effect or no effect on bone loss.[10]

Monitoring of drug treatment and response to therapy is best done by repeating dual energy X-ray absorptiometry (DEXA) scan serially at the spine or hip annually, if possible, to note the changes in bone density and rarely measurement of the level of bone turnover markers, which is costly, might be helpful as an early response to the therapy.

Recommendations and preventions of osteoporosis are vital approaches which include lifestyle modification that should start as early as infancy and extend to elderly age groups with special attention to high-risk groups such as postmenopausal women, perimenopausal women with low bone mass, elderly male, adults suffering from another systemic inflammatory, or metabolic diseases. Modalities are performing regular exercise of any kind, quitting smoking, and restricting alcohol, and caffeine, with a goal to achieve and maintain optimum bone mass and to prevent bone loss. Physical activities are one of the most important measures that help to improve bone strength, muscle power, body balance and hence help to prevent fall and fracture.[11] The National Osteoporosis Foundation guidelines recommend daily weight-bearing exercises such as jogging, brisk walking, playing basketball, and dancing, and in elderly participants, low-impact exercises such as walking, cycling, and chair exercises are much helpful. Smoking should be quitted as this is associated with low bone and muscle mass.[12] Restricting alcohol drink is also important for preventing osteoporosis because alcoholics are seen having low bone mass and increased risk of fracture due to poor nutrition, impaired Vitamin D and calcium metabolism, and increased risks of fall. Adequate intake of dietary calcium is essentially required for all to prevent increased bone resorption and osteoporosis. Dairy products such as milk, yogurt, cheese, and foods fortified with calcium are the major sources of calcium and adequate intake of these will fulfill the requirement. Vitamin D is the principal hormone that plays an important role in maintaining bone health by enhancing intestinal absorption of calcium and it had been seen that intake of both calcium and Vitamin D simultaneously will rise BMD by 2%–10% and also lowered the fracture rates by up to 50%.[13]

Prevention of fall is an important step in elderly individuals in the presence of chronic diseases (inflammatory joint diseases and neurodegenerative diseases), sarcopenia, severe osteoporosis, and history of multiple fractures. Protective measures to prevent fall include wearing shoes with nonskid

rubber soles, using spectacles for clear vision, floors of all the rooms including washroom should have anti-skid arrangement instead of tiles having slippery surfaces, stairs should have handrails to provide support, arrangement of adequate lights in all rooms including washroom and uses of external hip protectors that will reduce hip fractures.

Hence, in conclusion, achieving optimal bone mass and maintaining a good bone health status are all necessary for a healthy lifestyle and these targets could be achieved only with health education (through school health programs and community education by health workers, debates, seminars, etc.), extensive lifestyle modification, proper dietary approaches, and adequate sun exposure. Finally, all the treating physicians must address the bone health status of all patients at every opportunity while treating other primary diseases and treat adequately if required for maintaining a good bone health along with treatment of the primary diseases.

Alakes Kumar Kole

Department of General Medicine, Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India

Address for correspondence: Prof. Alakes Kumar Kole, Department of General Medicine, Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India. Victoria Green, Flata3/204, 385, Garia Main Road, Kolkata - 700 084, West Bengal, India.
E-mail: dralakeskole72@gmail.com

Submitted: 06-Sep-2022, **Revised:** 19-Sep-2022, **Accepted:** 21-Sep-2022, **Published:** 23-Nov-2022.

REFERENCES

1. Johnston CB, Dagar M. Osteoporosis in older adults. Med Clin North Am 2020;104:873-84.
2. Cooper C, Campion G, Melton LJ 3rd. Hip fractures in the elderly: A world-wide projection. Osteoporos Int 1992;2:285-9.
3. Majumdar SR, Lier DA, Beaupre LA, Hanley DA, Maksymowych WP, Juby AG, *et al.* Osteoporosis case manager for patients with hip fractures: Results of a cost-effectiveness analysis conducted alongside a randomized trial. Arch Intern Med 2009;169:25-31.
4. Babhulkar S, Seth S. Prevalence of osteoporosis in India: An observation of 31238 adults. Int J Res Orthop 2021;7:362-8.
5. Colón-Emeric C, Kuchibhatla M, Pieper C, Hawkes W, Fredman L, Magaziner J, *et al.* The contribution of hip fracture to risk of subsequent fractures: Data from two longitudinal studies. Osteoporosis Int 2003;11:879-83.
6. Lewiecki EM, Laster AJ. Clinical review: Clinical of vertebral fracture applications assessment by dual-energy X-ray absorptiometry J Clin Endocrinol Metab 2006;91:4215-22.
7. van de Glind EM, Willems HC, Eslami S, Abu-Hanna A, Lems WF, Hooft L, *et al.* Estimating the time to benefit for preventive drugs with the statistical process control method: An example with alendronate. Drugs Aging 2016;33:347-53.
8. Boonen S, Ferrari S, Miller PD, Eriksen EF, Sambrook PN, Compston J, *et al.* Postmenopausal osteoporosis treatment with antiresorptives: Effects of discontinuation or long-term continuation on bone turnover and fracture risk – A perspective. J Bone Miner Res 2012;27:963-74.
9. Cummings SR, Martin JS, McClung MR, Siris ES, Eastell R, Reid IR, *et al.* Denosumab for prevention of fractures in postmenopausal women with osteoporosis. New Engl J Med 2009;361:756-65.
10. Adami S, Bufalino L, Cervetti R, Di Marco C, Di Munno O, Fantasia L, *et al.* Ipriflavone prevents radial bone loss in postmenopausal women with low bone mass over 2 years. Osteoporos Int 1997;7:119-25.
11. National Osteoporosis Foundation. Physician's Guide to Prevention and Treatment of Osteoporosis. Available from: http://www.nof.org. [Last accessed on 2002 Jun 21).
12. Hollenbach KA, Barrett-Connor E, Edelstein SL, Holbrook T. Cigarette smoking and bone mineral density in older men and women. Am J Public Health 1993;83:1265-70.
13. Reid IR. The role of calcium and vitamin din the prevention of osteoporosis. Endocrinol Metab Clin North Am 1998;27:389-98.

Access this article online

Quick Response Code:

Website: https://journals.lww.com/amsr

DOI: 10.4103/amsr.amsr_53_22

How to cite this article: Kole AK. Neglected bone health needs more care. Ann Med Sci Res 2022;1:101-3.

Review Article

Monkeypox: Current trends and therapeutic challenges

Pritama Paul, Ritika Singh, Nikhil Era, Sritama Paul[1], Uttam Kumar Paul[2]

Department of Pharmacology, [2]Department of Medicine, MGM Medical College, Kishanganj, Bihar, [1]Department of Biotechnology, Amity University, Kolkata, West Bengal, India

Abstract

Monkeypox is a viral zoonotic disease that mostly affects tropical rainforest regions of Central and West Africa, with infrequent exportations to other parts of the world. The cause of monkeypox is the monkeypox virus, a species of the Orthopoxvirus genus in the family Poxviridae. Monkeypox often has symptoms that last between 2 and 4 weeks and is a self-limiting condition. Severe cases could exist. Monkeypox can be spread to humans by close contact with infected animals or people, as well as through coming into contact with contaminated materials. By coming into intimate contact with lesions, bodily fluids, respiratory droplets, and contaminated things like bedding, the monkeypox virus can be transferred from one person to another. The smallpox immunizations used in the smallpox eradication campaign also provided immunity to that illness. One of the more recent vaccinations, which is now accessible, is approved for the protection of monkeypox. Hexadecyloxypropyl-cidofovir (CDV) has superior oral bioavailability, less toxicity, and improved cellular penetration compared to CDV, administered intravenously. The European Medicines Agency authorized tecovirimat, an antiviral medicine developed to treat smallpox, to treat monkeypox in 2022 on the basis of data from both animal and human testing. It is still not readily accessible.

Keywords: Contact, monkeypox, orthopoxvirus, rash, smallpox, vaccine

Address for correspondence: Dr. Uttam Kumar Paul, Department of Medicine, MGM Medical College, Kishanganj, Bihar, India.
E-mail: druttam131065@gmail.com
Submitted: 21-Aug-2022, **Revised:** 10-Sep-2022, **Accepted:** 12-Sep-2022, **Published:** 23-Nov-2022.

INTRODUCTION

Monkeypox is a viral zoonotic disease that has a large and complex genome. The disease has been detected outside Africa in the US (New York), the UK, Israel, India, and other countries. Transmission occurs from rodents to man by coming in contact, eating meat, or getting scratched by prairie dogs or rodents. It is a rapidly growing epidemic that was deemed "a public health emergency of worldwide concern" on July 23, 2022, by WHO Director-General Tedros Adhanom Ghebreyesus.

It has symptoms that are comparable to those of smallpox but clinically less severe than smallpox. Monkeypox has taken over as the most significant orthopoxvirus for public health following the elimination of smallpox in 1980 and the consequent end of smallpox immunization. Primarily found in Central and West Africa tropical rainforests, monkeypox has been increasing in cities. Numerous rodent species and nonhuman primates serve as animal hosts.[1]

The Orthopoxvirus genus of the Poxviridae family contains the enclosed double-stranded DNA virus known as the monkeypox virus. The Central African (Congo Basin) clade and the West African clade are two separate genetic clades of the monkeypox virus. A special

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/amsr
	DOI: 10.4103/amsr.amsr_46_22

How to cite this article: Paul P, Singh R, Era N, Paul S, Paul UK. Monkeypox: Current trends and therapeutic challenges. Ann Med Sci Res 2022;1:104-10.

immune evasion technique that the monkeypox virus MPV employs to evade both CD4 and CD8 T-cell immunological responses. Because supernatants from MPV-infected cells were not inhibitory and T-cell inhibition was seen in the presence of brefeldin A, a strong inhibitor of the secretory pathway, this appears to need direct cell-to-cell interaction. It was necessary to overcome this immunosuppressive impact by ultraviolet-inactivating the virus before incubation with peripheral blood mononuclear cells. This immunosuppressive effect required transcription of an early Viral gene product. This suggests that MPV encodes a novel class of immune modulators that either directly or indirectly inhibit host-induced antiviral T-cell responses. Cell-associated virus pathogenesis and systemic transmission are anticipated to be significantly affected by this inhibition.[2]

TRANSMISSION

- Through regular, close-proximity, skin-to-skin contact, monkeypox can spread to anybody, including direct contact with a rash, scabs, or body fluids from an infected person[3]
- Interacting with objects, textiles (such as clothing, beds, or towels), and surfaces that have been occupied by patients with monkeypox
- Coming into contact with respiratory droplets.

When health-care workers remove contaminated personal protective equipment, there is a risk of self-contamination and potential transmission of monkeypox to health-care professionals.

In the course of intimate touch, this direct touch may manifest as:

- Engaging in oral, anal, or vaginal sex with the infected person
- Hugs, body rubs, and kisses
- Prolonged face-to-face contact for at least 3 h and within 6 feet
- Touching objects previously contaminated by secretions of monkeypox patients but have not been sanitized, such as bedding, towels, fetish items, and sex toys
- A pregnant woman can transmit the virus to her fetus through the placenta
- Aside from being bitten or scratched by infected animals, humans can also contract monkeypox from infected animals through consuming meat, utilizing products made from infected animals, or obtaining the disease through the preparation of the meat.

SIGNS AND SYMPTOMS

Monkeypox typically takes 6–13 days to incubate before symptoms appear, although it can take anywhere from 5 to 21 days.[4] The infection has two distinct phases:

THE INVASION PERIOD (0–5 DAYS)

Fever, severe headache, lymphadenopathy (swelling of the lymph nodes), back discomfort, myalgia (muscle aches), and severe asthenia are the symptoms of the invasion period (lack of energy). Compared to other diseases that may initially seem similar, monkeypox has a specific characteristic called lymphadenopathy (chickenpox, measles, and smallpox).

THE SKIN ERUPTION PERIOD

Often starts 1–3 days after a fever appears. Instead of the trunk, the rash is more frequently found on the face and limbs. In 95% of cases, it affects the face, and in 75% of cases, it affects the palms of the hands and the soles of the feet. Additionally impacted are the cornea, conjunctivae, genitalia, and oral mucous membranes (in 70% of cases). The progression of the rash goes from macules (flat, firm lesions) to papules (slightly raised, firm lesions), vesicles (clear fluid-filled lesions), pustules (yellowish fluid-filled lesions), and crusts that dry up and break off [Figure 1]. Umbilication is a feature of evolving rash [Figure 1a]. Lesions can range in number from a few to several thousand. Lesions may combine in severe circumstances to form vast areas of skin slough off.

Children are more likely to experience severe cases, which are correlated with the level of viral exposure, the patient's condition, and the type of sequelae. The results could be worse if immunological deficits were present. Although smallpox immunization proved protective in the past, people under the age of 40–50 years (depending on the country) may now be more susceptible to monkeypox due to the worldwide discontinuation of smallpox vaccine campaigns after the disease's elimination. It is unknown how widespread an asymptomatic infection might be. The case fatality ratio of monkeypox has historically ranged from 0 to 11 per 100 people in the general population and has been higher among young children. In recent times, the case fatality ratio has been around 3–6 per 100 people.[5]

In addition to the typical symptoms, novel monkeypox symptoms can include:[4]

- A painful rash that may first appear on genitalia, pubic region, or in the vicinity of the anus with fewer bumps (one to two bumps)

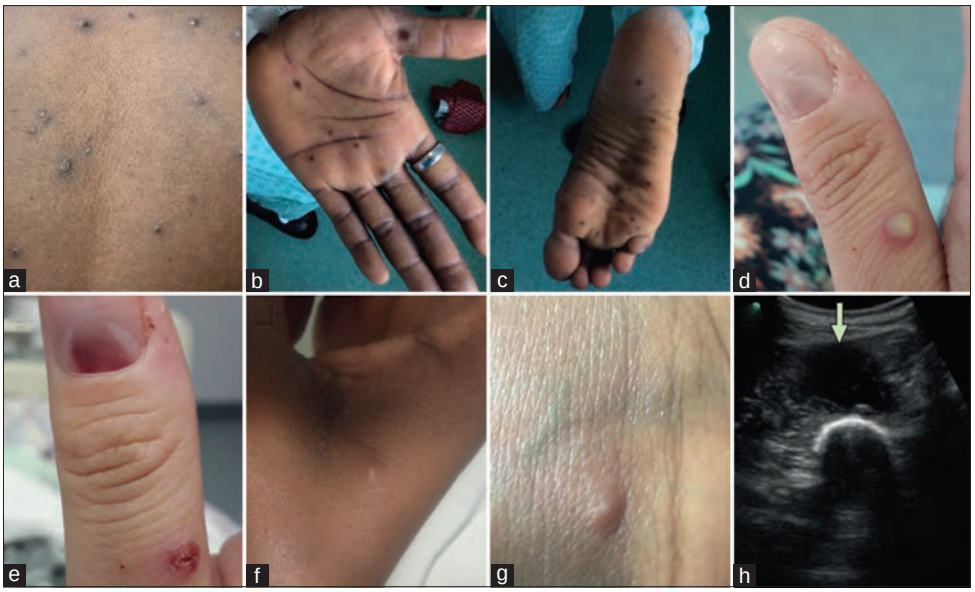


Figure 1: Skin and soft tissue manifestations of monkeypox skin and soft tissue feature included: (a and d) vesicular or pustular lesions; (b and c) macular lesions involving the palms and soles; (d and e) a subungual lesion; (f and g) more subtle papules and smaller vesicles; (h) and a deep abscess (arrow, image obtained during ultrasound-guided drainage)[4]

- Pimples that resemble blisters, pus-filled bumps, or open sores bumps that are in various phases, even when they are found in the same location
- Before the rash, some patients may not experience a fever or flu-like symptoms. Some others never even experience a fever
- Other symptoms, such as pain around the anus, the urgency to urinate, bleeding from the rectum, and painful inflammation of the anus and rectum lining (proctitis), have occasionally been described by patients.

COMPLICATIONS

Serious monkeypox complications could include subsequent infections such:[6]

- Bronchopneumonia
- Sepsis
- Corneal infection with potential visual loss
- Encephalitis.

INVESTIGATION

For monkeypox viral DNA, real-time polymerase chain reaction (RT-PCR) was positive in all seven patients' pleomorphic skin lesions, which included papules, vesicles, pustules, umbilicated pustules, ulcerating lesions, and scabs [Figure 1]. The upper respiratory tract swabs from all patients revealed viral DNA, with DNA being found in the blood of six patients and the urine of four others.[7]

A new strain of monkeypox linked to an individual diagnosed with the virus after recent travel to West Africa has been identified in the UK.

DIAGNOSIS

Other rash disorders, such as chickenpox, measles, bacterial skin infections, scabies, syphilis, and medication-associated allergies, must be taken into account when making a clinicaldifferential diagnosis. As a clinical characteristic, lymphadenopathy during the prodromal stage of the illness can help differentiate monkeypox from chickenpox or smallpox.[8]

Clinical samples must be taken from cases in accordance with the listed criteria [Table 1].[9]

The right sample and arrange for it to be delivered safely to a laboratory with the right equipment if monkeypox is detected. The kind of laboratory test used and the type and quality of the pecimen used determine whether monkeypox is confirmed. Therefore, samples should be sent and packaged in line with local, national, and international regulations. Given its precision and sensitivity, RT-PCR is the primary laboratory test. The best diagnostic samples for monkeypox come from skin lesions, such as dry crusts and the liquid that comes from vesicles and pustules. A biopsy is a possibility when it is practical.[10]

Lesion samples must be maintained cool and stored in a dry, sterile tube without a viral transport medium. Due to

Table 1: Traveller from outbreak/endemic region or Community Transmission

Asymptomatic	Observe for the development of any signs and symptoms for 21 days post exposure If signs and symptoms develop, collect specimens as per the duration of illness as mentioned below	
Symptomatic	Rash phase** *Lesion roof- with scalpel or plastic scrapper collected in plain tube *Lesion fluid with intradermal syringe *Lesion base scrapings with sterile polyester swab collected in plain tube *Lesion crust in plain tube NPS/OPS in dry plain tube [without any bacterial medium or VTM] Blood collected in SSGT (4-5 ml) Blood collected in EDTA (2-3ml) Urine in sterile urine container (3-5ml)	Recovery phase Blood collected in SSGT (4-5 ml) Urine in sterile urine container (3-5ml)

the short period of viremia about the date of specimen collection, after symptoms begin, RT-PCR blood tests are typically inconclusive and should not be regularly obtained from patients.

Antigen and antibody detection techniques do not offer proof of monkeypox-specific infection because orthopoxviruses are serologically cross-reactive. Therefore, in cases, where resources are scarce, serology and antigen detection procedures are not advised for diagnosis or case inquiry. Furthermore, recent or distant immunization with a vaccinia-based vaccine (e.g., anyone immunized before the eradication of smallpox, or more recently due to heightened risk, such as orthopoxvirus laboratory employees) may result in false-positive results.

The following patient data may be included with the specimens to interpret test results:

a. Age
b. Date of onset of fever
c. Date of the specimen collection
d. Date of the current condition of the patient (stage of rash)
e. Date of the beginning of rash.

PREVENTION

To avoid catching monkeypox:

- Avoid contact with animals that may be carrying the virus, especially deceased animals in places where monkeypox is prevalent
- Don't use bedding or other items that may have come into contact with an animal or human who has a monkeypox
- Keep infected people and animals apart from those who are at risk of acquiring the disease. If one comes near a person or animal who is infected, often wash hands with soap and water
- If one can't prevent contact, wear protective equipment including masks, safety goggles or glasses, and gloves.[10]

VACCINATION

Numerous observational studies have shown that the smallpox vaccine is around 85% effective at preventing monkeypox. There may be a milder sickness as a result of previous smallpox vaccination. A scar on the upper arm is typically present as proof of previous smallpox vaccination. The first-generation (original) smallpox vaccines are no longer accessible to the general population. Some laboratory or health-care employees may have had a more current smallpox vaccination to safeguard them from orthopoxvirus exposure at work.

In 2019, a brand newer vaccine based on the Ankara strain of the modified attenuated vaccinia virus was authorized for the prevention of monkeypox. This two-dose vaccine is still only partially available. Because the vaccinia virus provides cross-protection for the immune response to orthopoxviruses, formulations of smallpox and monkeypox vaccines are based on this virus.[11]

JYNNEOS is a vaccine that has received the Food and Drug Administration (FDA) approval in the United States (U. S.) and is permitted for use in those 18 years of age and older who are deemed to be at high risk for contracting smallpox or monkeypox. It is a live viral vaccine made from the attenuated, nonreplicating orthopoxvirus strain Modified Vaccinia Ankara – Bavarian Nordic, which was initially created for use in the event of a smallpox bioterrorist attack in specific populations (e.g., immunocompromised individuals). JYNNEOS is authorized for use as a 2-dose (0.5 mL each) subcutaneous injection regimen, with the doses delivered 4 weeks apart.[12]

The ACAM2000 vaccine has been made available to help prevent monkeypox and has been licensed to help protect against smallpox. ACAM2000 is an alternative to the JYNNEOS vaccine. It is advised to administer the ACAM2000 vaccine in a single dose through several skin pricks with a specialized needle.[12]

Table 2: Comparison between cidofovir, brincidofovir, tecovirimat, and vaccinia immune globulin

	Cidofovir[13]	Brincidofovir[14]	Tecovirimat[15]	VIG[18]
Name	GS-504	CMX 001	TPOXX	VIG
Mechanism of action	The active metabolite of cidofovir is cidofovir diphosphate, bearing structural similarity to nucleotides, competing with dCTP for viral DNA polymerase and gets incorporated into the growing viral DNA strands and prevents further DNA polymerization and disrupts DNA replication of viruses[13]	Brincidofovir (lipid conjugate of cidofovir) crosses the intestinal wall and penetrates target viral-infected cells before being cleaved to the free antiviral agent cidofovir. The active metabolite of cidofovir is cidofovir diphosphate, bearing structural similarity to nucleotides, competing with dCTP for viral DNA polymerase and gets incorporated into the growing viral DNA strands and prevents further DNA polymerization and disrupts DNA replication of viruses[14]	Tecovirimat is an inhibitor of viral p37 and blocks the ability of virus particles to be released from infected cells[15]	Antibodies obtained from pooled human plasma of individuals immunized with the smallpox vaccine provide passive immunity[18]
Route of administration	Intravenous route	Oral (tablets)	Intravenous route oral (capsules)	Intravenous route
Dose	5 mg/kg body weight (given as an intravenous infusion at a constant rate over 1 h) administered once weekly for 2 consecutive weeks	100 mg (suspension available). 200 mg once weekly for 2 doses (on days 1 and 8)	200 mg. The dose varies by body weight: 200–600 mg BID for 14 days	6000 U/kg as soon as symptoms appear; may repeat based on the severity of symptoms and response to treatment; 9000 U/kg may be considered if the patient does not respond to the initial dose
Approval status	Authorized at the EU level and US for the treatment of CMV retinitis in patients with AIDS and normal renal function Proven activity against poxviruses in vitro and animal studies	Approved by FDA in June 2021 under the agency's Animal Rule treatment of human smallpox disease caused by variola virus in adult and pediatric patients, including neonates	Authorized in the EU for the treatment of orthopoxvirus-associated infections (smallpox, monkeypox, cowpox, vaccinia virus) since January 2022 under exceptional circumstances	A preparation of VIG suitable for IV use (VIG-IGIV) is manufactured by Calgene, Canada; has been approved by the FDA, and is available through the CDC (under IND protocols) and the US Department of Defense[18]
Side effects	Decreased serum bicarbonate, proteinuria, neutropenia, infection, hypotony of the eye, iritis, uveitis, nephrotoxicity, fever	Diarrhea, nausea, vomiting. Elevation in hepatic transaminases. Increased risk for mortality when used for a longer duration (24 weeks). May cause embryo-fetal toxicity	Headache, nausea, abdominal pain	Headache, nausea, rigors, dizziness
Drug Interactions	Amikacin amphotericin B deoxycholate contrast media (iodinated) cyclosporine ioversol neomycin PO tacrolimus teicoplanin etc.	Ivermectin Ketoconazole	Repaglinide, midazolam	Contains maltose: May result in elevated glucose readings that can lead to untreated hypoglycemia or inappropriate insulin administration; may impair the efficacy of the life attenuated virus vaccines. May interfere with some serological tests

dCTP: Deoxycytosine-5-triphosphate, VIG: Vaccinia immune globulin, IGIV: Immune globulin intravenous, IND: Investigational new drug, PO: Per os (orally), CMV: Cytomegalovirus, FDA: Food and Drug Administration, CDC: Centers for Disease Control and Prevention, BID: two times a day

MANAGEMENT

General measures

As per the current various guidelines, this is the current mode of management for monkeypox. Typically, monkeypox is a self-limiting illness with symptoms that last for 2 to 4 weeks. Monkeypox typically resolves on its own without medical intervention. Following a diagnosis, the patient should be kept in isolation for 2 to 4 weeks or till the symptoms subsides with proper ventilation placing a surgical mask over the nose and mouth of the patient, while the medical team monitors their status, administers fluids to prevent dehydration, and maintains nutritional support. Treat subsequent bacterial infections with medication if necessary and keep an eye out for any further complications.

Specific treatment

Based on information from both animal and human research, the European Medicines Agency granted tecovirimat (TPOXX), an antiviral drug created to treat smallpox, a license to treat monkeypox in 2022. It is still not readily accessible.

Cidofovir

A broad-spectrum antiviral medication with activity against a variety of DNA viruses, including MPXV, is now another available therapeutic option [Table 2].[13]

Brincidofovir

Hexadecyloxypropyl-cidofovir (HDP-CDV), commonly known as CMX001 or brincidofovir (BCV), is a lipid compound of the nucleotide analog CDV. In comparison to CDV, BCV has better intracellular enzyme conversion to the active form and enhanced cellular absorption.[14] In contrast to CDV, BCV is orally accessible, enabling the use of tablet and solution drug delivery formulations. In contrast to TPOXX, BCV's antiviral activity results in inhibition of the viral DNA polymerase after incorporation into viral DNA [Table 2].

Tecovirimat

TPOXX is an orthopoxvirus VP37 envelope wrapping protein inhibitor. It stops the body from becoming infected with viruses. This medication prevents its molecular target, a protein by the name of p37, from engaging with intracellular transport elements required for the creation of enveloped virus and consequently viral dissemination.

TPOXX prevents the development of extracellular viral forms, which are in charge of the infection's systemic transmission and prevent the cytopathic consequences brought on by viruses. The smallpox infection is slowed to the point that the immune system can kill the virus attributable to TPOXX's inhibition of envelopment, which prevents viral particles from leaving an infected cell. TPOXX does not block the production of intracellular forms of the virus. TPOXX blocks the viral protein p37 from interacting with cellular proteins involved in membrane trafficking, interfering with the viral protein's ability to localize within cells. Orthopoxviruses have demonstrated a high level of selectivity and specificity for TPOXX.[15] With no significant side effects, TPOXX is well-tolerated. Headache, nausea, abdominal pain, and vomiting were common adverse responses in healthy adult individuals (2%) when repaglinide and TPOXX are administered together, mild-to-moderate hypoglycemia may result. At dose levels up to 2000 mg/kg/day, oral administration of TPOXX is often well-tolerated (mice). If administered with midazolam it can reduce the effectiveness [Table 2].

Vaccinia immune globulin

A hyperimmune globulin called vaccinia immune globulin (VIG) has been approved by the FDA to treat a few vaccine-related side effects. These include severe widespread vaccinia, progressive vaccinia, eczema vaccinatum, and vaccinia infections in persons with skin issues, abnormal infections brought on by the vaccinia virus, and (save in unusual situations) keratitis (for instance, eye infections).[16]

Moreover, there are few studies on the use of VIG for either monkeypox or smallpox in people. Since it is not advised to get the vaccinia virus vaccine, patients with exposure histories who have severe immunodeficiency in T-cell function may instead have a VIG be given.[17] VIG therapy should be administered according to an investigational new drug filing. [Table 2].

VIG may also be needed as prophylaxis in patients, for whom preexposure smallpox vaccine is contraindicated (such as those with eczema or pregnant women), although it is currently not licensed in these cases.[16]

Future prospective

The Indian Council of Medical Research, New Delhi, invites Expression of Interest for collaboration for the development of *in vitro* diagnostic kits and vaccine candidates against monkeypox virus (MPXV). This master plan holds the key prospect for the development of an indigenous vaccine against the monkeypox virus by India.

Key points

- The monkeypox virus, a species of the Orthopoxvirus genus in the family Poxviridae, is the culprit behind monkeypox
- The virus that causes monkeypox can be spread from one person to another by coming into close contact with lesions, bodily fluids, respiratory droplets, and contaminated objects like bedding
- Smallpox is more contagious and results in a more serious sickness than monkeypox
- Monkeypox often manifests clinically as fever, rash, and swollen lymph nodes
- The monkeypox vaccines employed in the smallpox eradication operation also offered protection from that disease. There are more recent vaccines (JYNNEOS and ACAM2000) available that are authorized for the prevention of monkeypox
- Compared to CDV, which is given intravenously, HDP-CDV shows better oral bioavailability, less toxicity, and enhanced cellular penetration.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Fine PE, Jezek Z, Grab B, Dixon H. The transmission potential of the MPXV virus in human populations. Int J Epidemiol 1988;17:643-50.
2. Hammarlund E, Dasgupta A, Pinilla C, Norori P, Früh K, Slifka MK. Monkeypox virus evades antiviral CD4+ and CD8+ T cell responses by suppressing cognate T cell activation. Proc Natl Acad Sci U S A 2008;105:14567-72.
3. World Health Organization: "Multi-Country Monkeypox Outbreak in Non-Endemic Countries," "Monkeypox," "Emergencies: International Health Regulations and Emergency Committees," "WHO Director-General's Statement at the Press Conference Following IHR Emergency Committee Regarding the Multi-Country Outbreak of Monkeypox – 23 July 2022."Available from: https://www.who.int/news-room/fact-sheets/detail/monkeypox. [Last retrieved on 2022 Aug 12].
4. Adler H, Gould S, Hine P, Snell LB, Wong W, Houlihan CF, *et al*. Clinical features and management of human monkeypox: A retrospective observational study in the UK. Lancet Infect Dis 2022;22:1153-62.
5. WHO. Monkeypox, 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/monkeypox. [Last accessed on 2022 Sept 9].
6. Learned LA, Reynolds MG, Wassa DW, Li Y, Olson VA, Karem K, *et al*. Extended interhuman transmission of monkeypox in a hospital community in the Republic of the Congo, 2003. Am J Trop Med Hyg 2005;73:428-34.
7. McCollum AM, Damon IK. Human monkeypox. Clin Infect Dis 2014;58:260-7.
8. Damon IK. Status of human MPXV: Clinical disease, epidemiology, and research. Vaccine 2011;29 Suppl 4:D54-059.
9. MoHFW. National Guidelines for Management of Monkeypox Diseases by MoHFW, Government of India 2022. Available from: https://main.mohfw.gov.in/diseasealerts-0. [Last accessed on 2022 Sept 9].
10. Moore MJ, Rathish B, Zahra F. Monkeypox. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK574519/. [Last updated on 2022 Jul 16].
11. Petersen BW, Kabamba J, McCollum AM, Lushima RS, Wemakoy EO, Muyembe Tamfum JJ, *et al*. Vaccinating against monkeypox in the Democratic Republic of the Congo. Antiviral Res 2019;162:171-7.
12. CDC. Monkeypox Vaccination Basics 2022. Available from: https://www.cdc.gov/poxvirus/monkeypox/vaccines/vaccine-basics.html. [Last accessed on 2022 Sept 9].
13. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012. Cidofovir. Available from: https://www.ncbi.nlm.nih.gov/books/NBK548417. [Last updated 2017 Oct 10].
14. National Center for Biotechnology Information 2022. PubChem Compound Summary for CID 483477, Brincidofovir. Available from https://pubchem.ncbi.nlm.nih.gov/compound/Brincidofovir#source=PubChem. [Last retrieved on 2022 Aug 12].
15. National Center for Biotechnology Information 2022. PubChem Compound Summary for CID 16124688, Tecovirimat. Available from https://pubchem.ncbi.nlm.nih.gov/compound/Tecovirimat. [Last retrieved on 2022 Aug 12].
16. Wittek R. Vaccinia immune globulin: Current policies, preparedness, and product safety and efficacy. Int J Infect Dis 2006;10:193-201.
17. Nalca A, Rimoin AW, Bavari S, Whitehouse CA. Reemergence of monkeypox: Prevalence, diagnostics, and countermeasures. Clin Infect Dis 2005;41:1765-71.
18. Rizk JG, Lippi G, Henry BM, Forthal DN, Rizk Y. Prevention and Treatment of Monkeypox. Drugs 2022:82;957963.

Review Article

Diagnostic dilemmas in young onset diabetes mellitus

Avijit Saha, Parna Roy

Department of General Medicine, Malda Medical College and Hospital, Malda, West Bengal, India

Abstract

The prevalence of diabetes among teenagers is increasing worldwide. Diabetes in young has profound implications on long term health of individuals and for the society as well. A wide range of specific type of diabetes can occur in this age group. The diagnosis of diabetes actually involves two steps. The first step is to document the elevated blood glucose as per the autoimmune diabetes in adults cutoffs and the second step is to characterize the type of diabetes the particular person has. The diagnosis of specific type of diabetes in young individuals poses several unique challenges. The things get further complicated by the fact that Asian-Indians represent diverse ethnicity where Type 2 diabetes tends to occur two decades earlier and relatively at lower body mass index. Treatment outcome depends on the correct diagnosis of diabetes type and subsequent targeted therapy. Correct diagnosis also enables clinicians to provide information to the patient about disease course and nature of therapy that the particular patient needs. A detailed patient history and physical examination provide clues to the diagnosis. However, to make appropriate diagnosis, sometimes, we need to take the help of special tests such as islet cell autoantibodies, fasting and stimulated c-peptide, lipid profile, USG of the abdomen, and homeostatic model assessment of insulin resistance. The results of these tests need to be interpreted cautiously as many of these tests' results cannot reliably discriminate between types of diabetes and moreover results are keep changing as the disease evolves. In this review, we discuss the characteristics of each type of diabetes that can occur in young individuals.

Keywords: C-peptide, diabetes in young, homeostatic model assessment of insulin resistance, islet cell autoantibodies

Address for correspondence: Dr. Avijit Saha, Malda Medical College Doctor's Quarter, Type 1, Flat No. 4B, Malda Town, Malda - 732 101, West Bengal, India.
E-mail: sahaavijit46@gmail.com
Submitted: 09-Aug-2022, **Revised:** 07-Sep-2022, **Accepted:** 12-Sep-2022, **Published:** 23-Nov-2022.

INTRODUCTION

Diabetes mellitus is a chronic and progressive complex metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Its prevalence is rising worldwide. Global estimates suggest that 463 million individuals have diabetes as of 2019.[1] India is home to around 77 million diabetic people and 2nd highest in the world in diabetic population only after China and supposed to surpass China very soon.[1] Actually, India is facing twin epidemics of diabetes and obesity. The driving forces behind these epidemics are urbanization and economic development with resultant increase in GDP, sedentary lifestyle, and Western diet on a background of genetic susceptibility. The rising number of young people (children and adolescent) with diabetes is of particular concern, and they have a different clinicodemographic profile. Earlier onset of diabetes leads to

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/amsr
	DOI: 10.4103/amsr.amsr_43_22

How to cite this article: Saha A, Roy P. Diagnostic dilemmas in young onset diabetes mellitus. Ann Med Sci Res 2022;1:111-5.

longer lifetime exposure to hyperglycemia and consequently greater propensity for long-term complications. We know that Indian diabetic patients are different from rest of the world. Type 2 diabetes mellitus (T2DM) onset in Indians is nearly 2 decades earlier than Western counterparts. This partly explains high prevalence of type 2 diabetes in the young. Diabetes in the young has been classically defined as onset of diabetes <35 years of age, but there is heterogeneity in the literature about the age cutoff, and some considered age below 25 years is young-onset diabetes.[2] A study was done in young Indian diabetic patients by Sahoo *et al*.[3] and they reported equal prevalence of type 1 diabetes mellitus (T1DM) and T2DM (40% each) in their study cohort. India has one of the highest global rates of prediabetes progression to T2DM (14%–18%), 6% (Finland), and 2.5% (USA) annually, highlighting a more aggressive diabetes phenotype in Indians.[4] This young group of people is very crucial for nation. Hence, we should give them correct diagnosis regarding diabetes type and treat them accordingly. There is a 31% increase in type 2 diabetes in the age group between 10 and 19 years in the USA between 2001 and 2009.[5] Data from the SEARCH study[6] also show an annual increase of about 7% in the incidence of type 2 diabetes among people aged 10–19 years in the USA. The registry of youth-onset diabetes in India (YDR) reported that over 25% of youth-onset diabetes <25 years were T2DM.[7] In some countries such as Taiwan, Japan, and Hong Kong, type 2 diabetes is now more common than type 1 diabetes in adolescent age group.[8] There has been a unique challenge in the diagnosis, management, and monitoring of these individuals. We will explore different types of diabetes in young age group and their phenotypic features.

SPECTRUM OF DIABETES MELLITUS IN YOUNG

The diagnosis of types of diabetes in middle and older adults does not require much consideration as the majority of patients have T2DM in this age group. However, it poses lots of challenges in children, adolescents, and young adults where a spectrum of hyperglycemic disorders need to be considered in the differential diagnosis such as type 1 DM, T2DM, latent autoimmune diabetes in adults, maturity-onset diabetes of young (MODY), fibrocalcific pancreatic diabetes (FCPD), flatbush diabetes, malnutrition-modulated diabetes mellitus, endocrinal causes, and drug-induced diabetes.[9] It is important to note some children may develop two different types of diabetes concomitantly or subsequently like double diabetes.[10] As a result of widespread epidemic of obesity, our young population get obese, making body mass index (BMI) and age at diagnosis are unreliable parameters in differentiating types of diabetes. The following section discusses the salient features of different types of young onset diabetes.

CHARACTERISTICS OF YOUNG-ONSET DIABETES

It has been observed from previous studies that in adult persons at the time of diagnosis of T2DM, 50% reduction in pancreatic beta-cell secretary function has taken place. In contrast, recent studies suggest that in adolescents at the time of diagnosis of T2DM, insulin secretion is impaired by ~85%.[11] Moreover, recent data from the Treatment Options for T2DM in Adolescents and Youth (TODAY) study suggest that deterioration in beta-cell function is even more rapid in adolescents than what has been reported in adults.[12] The TODAY study showed a 20%–35% annual decline in β-cell function in adolescents aged 10–19 years with type 2 as compared to a 7% decline in older individuals with type 2 diabetes.[12] Furthermore, data from the TODAY and SEARCH studies[13] indicate that cardiometabolic comorbidities are prevalent with youth-onset T2DM and are associated with worse morbidity and mortality than the same age group T1DM patients.

WHEN TO SUSPECT TYPE 1 DIABETES MELLITUS?

The incidence of T1DM is maximum up to adolescence but can occur at any ages. Mostly, lean and thin patients with lack of family history (85% cases are sporadic) present with marked osmotic symptoms that evolved rapidly over days to weeks. The classical symptoms are polyuria, polydipsia, nocturia, weight loss, reduced school performance, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. In its most severe form, ketoacidosis may develop and lead to stupor, coma, and in the absence of effective treatment, death can occur. As per the Research Society for the Study of Diabetes in India guideline, every sick child should be tested for blood glucose value and blood glucose should be considered as fifth vital sign. As a result of chronic exposure to obesogenic environment some of the type 1 diabetic patients are becoming obese and thus making body weight at the time of diagnosis an unreliable parameter for differentiation from other types of diabetes. Nearly 90% of individuals with type 1 diabetes have presence of one or more islet autoantibodies such as insulin autoantibody, glutamic acid decarboxylase, insulinoma-associated autoantigen 2, and zinc transporter 8.[14] These autoantibodies may present months to years before onset of hyperglycemic symptoms, thus enabling identification of at-risk individuals. Although 10% of type 1 diabetic patients may be tested negative for islet

cell autoanbodies and this type of T1DM is termed as idiopathic (type 1B).[15,16] Islet cell autoantibody titers tend to diminish as the disease advances and measurement of c-peptide is going to help in this situation. Persistently, low/undetectable c-peptide (below 0.6 ng/ml) confirms the diagnosis. It is important to note that honeymoon phase may occur in the initial course of disease when endogenous insulin production improves, so higher c-peptide response may be present. A recent consensus statement from the Juvenile Diabetes Research Foundation, American Diabetes Association and the Endocrine Society,[17] has identified three stages of T1DM, Stage 1, where the individual has evidence of autoimmunity but is normoglycemic, Stage 2, where there is evidence of glucose intolerance, and Stage 3, characterized by symptomatic hyperglycemia where the patient has typical osmotic and catabolic symptoms. Hence, we need to identify these stages for appropriate interventions.

WHEN TO SUSPECT LATENT AUTOIMMUNE DIABETES IN ADULT?

This is an adult form of type 1 DM where individuals present with subacute clinical course in their third decades of life with a normal range BMI with detectable C-peptide and islet cell autoantibodies.[18] This type of patients are initially well controlled with oral anti-diabetic agents and then exhibit rapid downhill course.Later they behave like T1DM patients and ultimately need exogenous insulin within few years of diagnosis.[19]

WHEN TO SUSPECT TYPE 2 DIABETES MELLITUS?

Young-onset T2DM occurs most often during the second decades of life with a median age of diagnosis of 13.5 years.[20] This coincides with the peak of physiologic pubertal insulin resistance.[21] These subjects are usually either overweight or obese with positive family history of diabetes in the first or second-degree relatives. Young-onset T2DM rarely occurs before puberty.[20] This type of patients initially may be asymptomatic and detected during screening. Presentations of this type of patients are not aggressive like that of T1DM patients. They occasionally have clinical signs of insulin resistance such as abdominal obesity, acanthosis nigricans, skin tag, hypertension, polycystic ovarian syndrome in adolescent females, nonalcoholic fatty liver disease, and characteristic dyslipidemia. T2DM patients do not develop ketoacidosis is a myth, can occur in up to 25% of patients.[21] By definition, islet cell autoimmunity is absent in this type, but a tiny percentage of patients may have positive islet cell autoantibodies. Measurement of C-peptide is not routinely required in establishing T2DM diagnosis, but for predicting response to therapy, sometimes, we can recommend this test as it is a useful index of endogenous insulin reserve. C-peptide is usually measured in the fasting state and after stimulation either by means of glucagon administration or ingestion of a standard meal. It should be noted that patients with profound hyperglycemia and hyperlipidemia of any etiology may have suppressed c-peptide levels; this phenomenon is termed "glucolipotoxicity."[22] C-peptide estimation is therefore not recommended in the acute phase of hyperglycemia. After successful correction of hyperglycemia, c-peptide level tends to improve in T2DM in initial course of the disease.[23]

It is also now clearly known that there are at least four distinct phenotypes of ketosis-prone diabetes mellitus: A− B−, autoantibody negative and with absent β-cells; A+ B−, autoantibody positive and with absent β-cells (autoimmuneT1DM); A− B+, autoantibody negative and with present β-cells; and A+ B+, autoantibody positive and with present β-cells.[24]

WHEN TO SUSPECT FLATBUSH DIABETES?

Flatbush diabetes has been widely recognized as a clinical entity since 1984 where patients have both features of T1DM and T2DM and described as type 1.5 DM. It is now being recognized as an important clinical entity in Sub-Saharan Africans, Asian and Indian populations, and Hispanic populations.[25] Flatbush diabetes is defined as a syndrome in which diabetes starts with ketoacidosis like presentation in individuals who are GAD and anti-islet cell antibody negative.[26] These patients are young adults/middle aged individuals who admitted in hospital setting with unprovoked ketoacidosis and phenotypically resemble as T2DM patients.[26] After intensive initial insulin therapy, many patients become insulin independent and can be well controlled with oral antidiabetic medications.

WHEN TO SUSPECT MATURITY-ONSET DIABETES OF THE YOUNG?

In contrast to T1DM and T2DM which are polygenic in nature, monogenic diabetes is caused by defect in a single gene and depending upon these fourteen different types of monogenic diabetes have been identified.[27] Monogenic diabetes accounts for 1%–2% of diabetes in the young in the U.K and the USA.[27] In a clinic based study from India, genetic defects were found in approximately 12% of T2DM patients.[28] MODY 1 and MODY 3 are relatively common among the different types and these patients show mild fasting hyperglycemia only and mostly remain asymptomatic except during pregnancy.[27] Certain forms

of monogenic diabetes are associated with extra pancreatic features also like renal cyst, genitourinary abnormalities etc.[28] Early age at onset (<25 years), normal weight individuals with absence of signs of insulin resistance, exhibiting istlet cell autoantibody negativity, extreme sensitivity to sulfonylureas in certain types and well controlled with oral agents for at least initial 5 years from diagnosis, autosomal dominant inheritance pattern, family history of diabetes in three successive generations, and absence of ketosis when insulin is stopped are considered to be the clinical diagnostic criteria for MODY.[29] Those patients who have initially been diagnosed as having T1DM due to younger age at onset but who are requiring low dose of insulin(<0.5 U/Kg/day) and having measurable c-peptide years after diagnosis and who have not developed ketosis even after stoppage of insulin should have undergone genetic testing for MODY genes.[29] This type of diabetic patients are less prone to develop diabetes related long term complications.

WHEN TO SUSPECT FCPD (FIBROCYSTIC PANCREATIC DIABETES)?

This is an uncommon form of diabetes secondary to chronic calcific nonalcoholic pancreatitis.[30] It is found most commonly in southern Asia and parts of Africa where cassava consumption seems to be the underlying cause.[31] When an young malnourished underweight subject with recurrent history of severe type abdominal pain and without any positive family history of diabetes presents with hyperglycemia and in whom islet cell autoantibodies are absent, we should suspect FCPD and go for straight X ray of the abdomen or ultrasonogarphy of abdomen for documentation of pancreatic calculi. Interestingly, despite phenotypically similar to T1DM patients and requiring life-long insulin therapy, they rarely develop diabetic ketoacidosis.[30] They are prone to develop microvascular complications of diabetes, but macrovascular complications are rare.[32] The most dreaded long-term complication is the development of pancreatic carcinoma. Another, increasingly frequent, form of pancreatic diabetes occurs in children with cystic fibrosis.[33]

WHEN TO SUSPECT MALNUTRITION-MODULATED DIABETES MELLITUS?

Malnutrition-modulated diabetes was previously known as protein deficient DM. Its presentation is similar to that of T1DM, but it occurs in the background of chronic malnutrition from childhood. In spite of severe hyperglycemia ketosis does not occur.[34] These patients are extremely lean and require high doses of insulin to the tune of 2.0 U/kg/day.[34] These patients are regularly suffering from infections of skin, soft tissue and pulmonary tuberculosis. Exceptionally micro- and macrovascular complications related to diabetes are rare in them.[35] The only difference of this from FCPD is absence of pancreatic morphological abnormalities.

MANAGEMENT ISSUES IN YOUNG-ONSET DIABETES

Most patients with T1DM require multiple daily doses of insulin or a continuous subcutaneous insulin infusion pump for control of diabetes. During ketoacidosis they need fluid therapy along with insulin infusion after getting admitted in hospital setting. Insulin and metformin are the only agents approved for the management of T2DM in children and adolescents.[36] These children also tend to have other co morbidities such as hypertension and dyslipidemia which need to be treated according to age-specific guidelines.[37] Patients with some types of monogenic diabetes exhibit hyperresponsiveness to low doses of sulfonylureas and some form can be managed by lifestyle measures only. A few patients may need insulin as the beta-cell defect progress.[29] Most patients with FCPD require insulin injections. Diabetes is usually brittle and difficult to control in those patients.[30]

CONCLUSION

Diabetes is now becoming the most common endocrine disorder among teenagers across the world. Diabetes in young poses a diagnostic challenge to the clinician as a wide spectrum of diabetes types may occur in this age group. It is important to correctly categorize the index patient into particular type of diabetes as it will decide the type of therapy that the particular patient needs. Correct diagnosis of type of diabetes is also enables clinician to provide information about the future course and prognosis of the disease and to decide the need for family screening and to decide screening for other autoimmune diseases such as vitiligo, celiac disease, and hypothyroidism. After proper diagnosis and subsequent judicious use of antidiabetic agents, these young people can lead to fruitful and complication-free life.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, *et al.* Global and regional diabetes prevalence estimates for 2019

and projections for 2030 and 2045: Results from the international diabetes federation diabetes Atlas, 9th edition. Diabetes Res Clin Pract 2019;157:107843.
2. Thanabalasingham G, Pal A, Selwood MP, Dudley C, Fisher K, Bingley PJ, *et al*. Systematic assessment of etiology in adults with a clinical diagnosis of young-onset type 2 diabetes is a successful strategy for identifying maturity-onset diabetes of the young. Diabetes Care 2012;35:1206-12.
3. Sahoo SK, Zaidi G, Vipin VP, Chapla A, Thomas N, Yu L, *et al*. Heterogeneity in the aetiology of diabetes mellitus in young adults: A prospective study from North India. Indian J Med Res 2019;149:479-88.
4. Anjana RM, Shanthi Rani CS, Deepa M, Pradeepa R, Sudha V, Divya Nair H, *et al*. Incidence of diabetes and prediabetes and predictors of progression among Asian Indians: 10-year follow-up of the Chennai Urban rural epidemiology study (CURES). Diabetes Care 2015;38:1441-8.
5. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, *et al*. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. JAMA 2014;311:1778-86.
6. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, *et al*. Incidence trends of type 1 and type 2 diabetes among youths, 2002-2012. N Engl J Med 2017;376:1419-29.
7. Praveen PA, Madhu SV, Mohan V, Das S, Kakati S, Shah N, *et al*. Registry of youth onset diabetes in India (YDR): Rationale, recruitment, and current status. J Diabetes Sci Technol 2016;10:1034-41.
8. Ma RC, Chan JC. Type 2 diabetes in East Asians: Similarities and differences with populations in Europe and the United States. Ann N Y Acad Sci 2013;1281:64-91.
9. Shah AS, Nadeau KJ, Dabelea D, Redondo MJ. Spectrum of phenotypes and causes of type 2 diabetes in children. Annu Rev Med 2022;73:501-15.
10. Kietsiriroje N, Pearson S, Campbell M, Ariëns RA, Ajjan RA. Double diabetes: A distinct high-risk group? Diabetes Obes Metab 2019;21:2609-18.
11. Gungor N, Bacha F, Saad R, Janosky J, Arslanian S. Youth type 2 diabetes: Insulin resistance, beta-cell failure, or both? Diabetes Care 2005;28:638-44.
12. TODAY Study Group, Zeitler P, Hirst K, Pyle L, Linder B, Copeland K, *et al*. A clinical trial to maintain glycemic control in youth with type 2 diabetes. N Engl J Med 2012;366:2247-56.
13. Dabelea D, Stafford JM, Mayer-Davis EJ, D'Agostino R Jr., Dolan L, Imperatore G, *et al*. Association of type 1 diabetes versus type 2 diabetes diagnosed during childhood and adolescence with complications during teenage years and young adulthood. JAMA 2017;317:825-35.
14. Ziegler AG, Nepom GT. Prediction and pathogenesis in type 1 diabetes. Immunity 2010;32:468-78.
15. Unnikrishnan AG, Singh SK, Sanjeevi CB. Prevalence of GAD65 antibodies in lean subjects with type 2 diabetes. Ann N Y Acad Sci 2004;1037:118-21.
16. Kanungo A, Sanjeevi CB. IA-2 autoantibodies are predominant in latent autoimmune diabetes in adults patients from Eastern India. Ann N Y Acad Sci 2003;1005:390-4.
17. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, *et al*. Staging presymptomatic type 1 diabetes: A scientific statement of JDRF, the endocrine society, and the American diabetes association. Diabetes Care 2015;38:1964-74.
18. Pozzilli P, Pieralice S. Latent autoimmune diabetes in adults: Current status and new horizons. Endocrinol Metab (Seoul) 2018;33:147-59.
19. Buzzetti R, Tuomi T, Mauricio D, Pietropaolo M, Zhou Z, Pozzilli P, *et al*. Management of latent autoimmune diabetes in adults: A consensus statement from an international expert panel. Diabetes 2020;69:2037-47.
20. Copeland KC, Zeitler P, Geffner M, Guandalini C, Higgins J, Hirst K, *et al*. Characteristics of adolescents and youth with recent-onset type 2 diabetes: The today cohort at baseline. J Clin Endocrinol Metab 2011;96:159-67.
21. Rosenbloom AL. Obesity, insulin resistance, beta-cell autoimmunity, and the changing clinical epidemiology of childhood diabetes. Diabetes Care 2003;26:2954-6.
22. Poitout V, Amyot J, Semache M, Zarrouki B, Hagman D, Fontés G. Glucolipotoxicity of the pancreatic beta cell. Biochim Biophys Acta 2010;1801:289-98.
23. Weir GC. Glucolipotoxicity, β-cells, and diabetes: The emperor has no clothes. Diabetes 2020;69:273-8.
24. Maldonado M, Hampe CS, Gaur LK, D'Amico S, Iyer D, Hammerle LP, *et al*. Ketosis-prone diabetes: Dissection of a heterogeneous syndrome using an immunogenetic and beta-cell functional classification, prospective analysis, and clinical outcomes. J Clin Endocrinol Metab 2003;88:5090-8.
25. Lebovitz HE, Banerji MA. Ketosis-prone diabetes (Flatbush Diabetes): An emerging worldwide clinically important entity. Curr Diab Rep 2018;18:120.
26. Shankar M, Chowdhury T, Gousy N, Parthasarathi A. An insight into flatbush diabetes: A rare form of diabetes. Cureus 2022;14:e21567.
27. Hattersley A, Bruining J, Shield J, Njolstad P, Donaghue KC. The diagnosis and management of monogenic diabetes in children and adolescents. Pediatr Diabetes 2009;10 Suppl 12:33-42.
28. Radha V, Ek J, Anuradha S, Hansen T, Pedersen O, Mohan V. Identification of novel variants in the hepatocyte nuclear factor-1alpha gene in South Indian patients with maturity onset diabetes of young. J Clin Endocrinol Metab 2009;94:1959-65.
29. Tattersall RB, Fajans SS. A difference between the inheritance of classical juvenile-onset and maturity-onset type diabetes of young people. Diabetes 1975;24:44-53.
30. Barman KK, Premalatha G, Mohan V. Tropical chronic pancreatitis. Postgrad Med J 2003;79:606-15.
31. Girish BN, Rajesh G, Vaidyanathan K, Balakrishnan V. Assessment of cassava toxicity in patients with tropical chronic pancreatitis. Trop Gastroenterol 2011;32:112-6.
32. Praveen G, Mohan V. Fibrocalculous pancreatic diabetes-current scenario in developing countries. Int J Diabetes Dev Ctries 2018;38:131-32.
33. Litvin M, Nwachukwu S. Cystic fibrosis related diabetes: A unique challenge in diabetes care. Mo Med 2016;113:384-9.
34. Samal KC, Kanungo A, Sanjeevi CB. Clinicoepidemiological and biochemical profile of malnutrition-modulated diabetes mellitus. Ann N Y Acad Sci 2002;958:131-7.
35. Yessoufou A, Ategbo JM, Moutairou K, Dramane KL, Khan N. Malnutrition-Modulated Diabetes Mellitus (MMDM): A state of review. Int J Biol Chem Sci 2008;2:44-58.
36. Tamborlane WV, Klingensmith G. Crisis in care: Limited treatment options for type 2 diabetes in adolescents and youth. Diabetes Care 2013;36:1777-8.
37. Reinehr T. Metabolic syndrome in children and adolescents: A critical approach considering the interaction between pubertal stage and insulin resistance. Curr Diab Rep 2016;16:8.

Original Article

Analysis of expression pattern of proteins associated with AKT/mTOR signaling pathway in kidney cancer development

Ritesh Satardey, Ravi Yadav[1], Madhusudan Das[1], Dilip Kumar Pal

Department of Urology, Institute of Post Graduate Medical Education and Research, [1]Department of Zoology, University of Calcutta, Kolkata, West Bengal, India

Abstract

Introduction: The purpose of this study was to analyze the expression status, reciprocal interplay, and prognostic significance of AKT1 and hypoxia-inducible factor 1 alpha (HIF-1α) in AKT/mechanistic target of the rapamycin pathway and to enable them to be studied as possible therapeutic targets.

Materials and Methods: This prospective study included 25 patients with clear cell renal cell carcinoma (ccRCC) operated between December 2019 and January 2022. Tumor and adjacent normal tissue samples were subjected to immunohistochemical analysis, RNA extraction, cDNA synthesis, and quantitative real-time polymerase chain reaction for AKT and HIF-1α. The fold changes were then calculated by ΔΔCt method.

Results: The included 25 ccRCC patients had 1.5-fold greater HIF-1 mRNA expression and 0.9-fold higher AKT1 gene expression in the ccRCC tissues compared to the corresponding healthy control. High, moderate, and low expression of HIF-1α was seen in 15, 6, and 1 of 25 samples, respectively. High, moderate, and low expression of p-AKT1 was seen in 18, 2, and 3 of 25 samples, respectively.

Conclusion: Our study data predicted higher gene expression as well as protein expression of HIF-1α and AKT. The proteins HIF-1α and AKT are localized in the nucleus of the RCC tumor samples compared to normal. Overexpression of these proteins might play significant roles in tumor development and differentiation as reported by others previously. This study can help clarify the biological role of HIF-1α and AKT in RCC to develop new strategies for this malignancy.

Keywords: AKT, hypoxia-inducible factor 1 alpha, mechanistic target of the rapamycin, renal cell carcinoma

Address for correspondence: Prof. Dilip Kumar Pal, Department of Urology, Institute of Post Graduate Medical Education and Research, 244, Acharya Jagdish Chandra Bose Road, Bhowanipore, Kolkata - 700 020, West Bengal, India.
E-mail: urologyipgmer@gmail.com
Submitted: 10-Jul-2022, **Revised:** 21-Aug-2022, **Accepted:** 29-Aug-2022, **Published:** 23-Nov-2022.

INTRODUCTION

Renal cell carcinoma (RCC) contributes to 2%–3% of all adult malignant neoplasms and is one of the most fatal urologic malignancies.[1] Men are at greater risk (1.9:1) than females.[1] Tobacco smoking, obesity, hereditary factors, hypertension and related medication, and chronic renal failure are some of the common risk factors associated with RCC.[2]

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/amsr
	DOI: 10.4103/amsr.amsr_38_22

RCC originates from the tubular structures of the kidney and is classified into four major histological cell types. Clear cell RCC (ccRCC) is the most common type, accounting for about 75%–80% of all cases of RCC. Around 4% of RCCs are hereditary.[3] Nearly, all familial cases of ccRCC are due to inherited mutation in the Von Hippel–Lindau (VHL) tumor suppressor gene. In at least two-thirds of sporadic

How to cite this article: Satardey R, Yadav R, Das M, Pal DK. Analysis of expression pattern of proteins associated with AKT/mTOR signaling pathway in kidney cancer development. Ann Med Sci Res 2022;1:116-20.

cases of ccRCC, the VHL gene is inactivated either by point mutation, deletion, or promoter hypermethylation.[4-6]

Cancer is unvaryingly accompanied by changes in signal transduction. Alterations in proto-oncogenes and tumor suppressor genes lead to dysregulated signal transduction that underlies the abnormal growth and proliferation of cancer cells. In some cases, tumor-associated mutations specifically target genes coding for critical signaling proteins. Signaling proteins that play an important role in cancer-associated signaling networks can serve as therapeutic targets even though their function is not specifically altered as part of the disease etiology.[7,8]

AKT/protein kinase B is a part of the serine/threonine protein kinase family. It has been implicated in the pathogenesis and progression of many human malignant tumors, such as prostate, breast, lung, ovary, and thyroid cancers, by regulating some key steps that control the balance of cell survival and apoptosis.[9,10] Akt activates and phosphorylates the mechanistic target of rapamycin (mTOR); consequently, activated mTOR regulates S6K1 activation and phosphorylation.[11]

MATERIALS AND METHODS

The study was conducted in a tertiary care hospital from December 2019 to January 2022. The present study protocol was reviewed and approved by the institutional ethical committee. Informed consent was obtained by all subjects when they were enrolled in the study.

This prospective, observational study recruited 25 patients who underwent surgery for suspected renal malignancy. Tumor biopsy and adjacent normal tissue were collected. The tissue samples were divided into three parts: one part was stored at −80°C until protein isolation, another part was stored in 10% formalin for paraffin-embedded tissue blocks, and the remaining part was kept in TRIzol reagent for RNA isolation

Immunohistochemical expression

The protein expression of AKT and VHL product HIF-1α was done by immunohistochemical analysis of 25 primary tumor samples according to the standard protocol.[12] In brief, 10% formalin-fixed tissues were used to make paraffin-embedded blocks. Deparaffinized tissue sections (3 μm) were incubated for 15 min in a methanol solution containing 3% H_2O_2 to block endogenous peroxidase activity. Following antigen retrieval with a 10 mM citrate buffer, the tissue sections were incubated overnight at 4°C with a polyclonal rabbit antibody (1:100) specific for AKT1 and HIF-1α (Abcam, Cambridge, UK). After rinsing in citrate buffer, a biotinylated goat anti-mouse antibody (1:500) labeled with streptavidin and chromogen as substrate was added (UltraVision Detection System Anti-Polyvalent, HRP/AEC, LabVision Corporation, Fremont, CA, USA). The evaluation of expression involved site and degree of reactivity. Degree of reactivity included evaluation for maximal staining intensity using a 0–3 scale (0, negative; 1, weak; 2, moderate; 3, strong). To obtain representative images, slides were scanned by the Leica 1000 DM ergonomic system.

mRNA expression study

Total RNA was isolated from paired tissue samples (renal tumor and adjacent nonmalignant renal tissue from patients) by using the TRIzol reagent according to Maiti *et al*., 2015.[12] After that, real-time quantitative polymerase chain reaction (qRT-PCR) analysis was performed to analyze the expression label of VHL and AKT with an ABI Prism 7500 using Power SYBR Green PCR Master Mix (Applied Biosystems, USA). Beta 2 microglobulin was used as a control.

Statistical analysis

For multivariate analysis, the Cox regression analysis was used. $P < 0.05$ was considered statistically significant. Statistical analysis employed the statistics software package SPSS 24.0.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1 shows the demographic and clinicopathologic characteristics of the study participants.

Quantitative polymerase chain reaction analysis

We examined the difference in gene expression levels of HIF-1α and AKT1 between tumor and adjacent normal tissues. We found a significant 1.5-fold higher mRNA expression of HIF-1α and 0.9-fold higher expression of AKT1 gene in the ccRCC tissues compared to the respective healthy control, respectively ($P < 0.05$ compared to normal).

Expression of hypoxia-inducible factor 1 alpha

The RCC tumor samples showed strong immunoreactivity to HIF-1α at the level of the nucleus, whereas control samples showed either none or weak, diffuse immunoreactivity. The integrity of morphology, the degree of vascularization, and the differentiation of cellular components are deformed in the case of RCC in comparison to normal cells. HIF-1α staining intensities were generally increased in RCC compared with normal tissues. Among the samples, high expression of HIF-1α was seen in 60% (15/25) of the

samples. Moderate expression was observed in 24% (6/25) of the samples and 4% (1/25) has low expression. Compared to normal tissues, significant increase in the expression of HIF-1α protein was seen in stage I + II tumor cells (27.85%, $P = 0.004$) and stage III + IV tumor cells (57.14%, $P = 0.009$). It is previously reported that overexpression of HIF-1α had significantly worse overall survival (177) [Figure 1]. Table 2 explains the percentage of patients and the scoring of expression.

Expression of AKT

Tumor-positive tissue had a higher frequency of activated Akt (p-Akt) than nearby normal tissues [Figure 1]. Furthermore, increased p-Akt was more often located in the nucleus than in the cytoplasm. Among the samples, high expression of p-AKT was seen in 72% (18/25) of the samples. Moderate expression was observed in 8% (2/25) of the samples and 12% (3/25) has low expression. Compared to normal tissues, a significant increase in the expression of AKT protein was seen in stage I + II tumor cells (18.85%, $P = 0.005$) and stage III + IV tumor cells (43.14%, $P < 0.0003$). Table 3 explains the percentage of patients and scoring of expression.

DISCUSSION

Multiple signaling pathways are coupled to the development of RCC. Among them, PI3K/AKT/mTOR is one of the universal signaling pathway characteristics of most cells, the central elements of which are the enzymes PI3K, AKT, and mTOR kinase. All of these occurrences set off unchecked mechanisms that aid in the development of cancer. Analysis of expression pattern of proteins associated with AKT/mTOR signaling pathway showed that (a) components of the mTOR pathway are constitutively activated in all cases (100%) of RCC; (b) higher expression of p-Akt1, an upstream effector, and the mTOR pathway component, HIF-1α are significantly upregulated and overexpressed in both gene and protein level expression

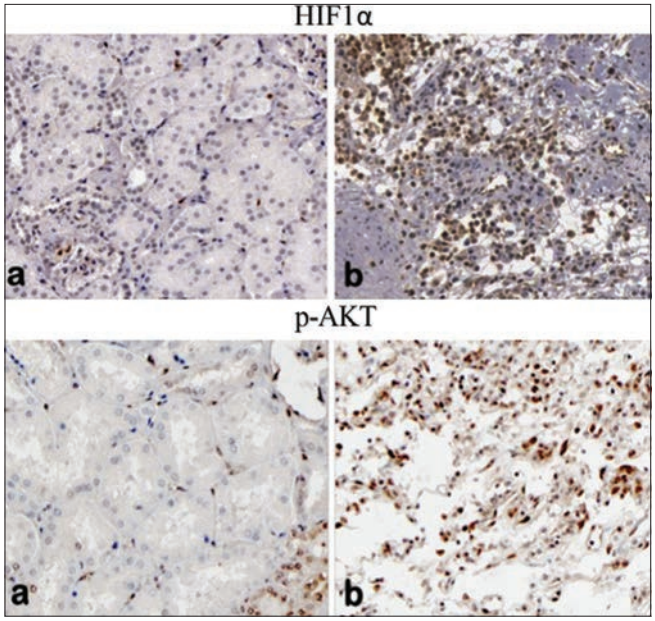


Figure 1: Representative expression image of HIF-1α and p-AKT by immunohistochemistry in renal tumor tissues (40). (a) Normal tissue (b) Renal tumor tissue, HIF-1α: Hypoxia-inducible factor 1 alpha

Table 1: Demographic and clinicopathologic characteristics of study participants

Characteristics	Number of cases
Age at diagnosis (years), mean	
Male	53
Female	54
Gender	
Male	15
Female	10
Stage at diagnosis	
I	6
II	12
III	4
IV	3
Location	
Right	9
Left	16
Smoking status	
Smoking	20
Nonsmoking	5
Drinking status	
Alcoholic	3
Nonalcoholic	22
Hypertension	
Yes	17
No	8
Obesity	
Yes	18
No	7

Table 2: Comparative analysis between Stage I+II and III+IV tumors for hypoxia-inducible factor 1 alpha

IHC scoring	Stage	
	I+II	III+IV
n=25	18	7
0	1	2
1	0	1
2	5	1
3	12	3

IHC scoring: 0: Negative, 1: Weak, 2: Moderate, 3: Strong.
IHC: Immunohistochemistry

Table 3: Comparative analysis between Stage I+II and III+IV tumors for AKT1

IHC scoring	Stage	
	I+II	III+IV
n=25	18	7
0	1	1
1	1	2
2	1	1
3	10	3

IHC scoring: 0: Negative, 1: Weak, 2: Moderate, 3: Strong.
IHC: Immunohistochemistry

in RCC tumor tissues compared to the normal kidney; and (c) correlative expression is evident of activated phosphorylation sites and signal transduction from p-Akt to HIF-1α. In support of our observations regarding a constitutively activated mTOR pathway in human RCC are various studies. These include (a) the Chuang *et al*.'s study[13] of the overexpression of glutathione s-transferase (GST)-α in the majority of primary and metastatic ccRCC, given that GST-α can be increased through PI3K/Akt/mTOR/p70S6K signaling;[14] (b) the efficacy of rapamycin as an inhibitor of human renal cancer pulmonary metastasis in a xenograft model;[15] and (c) the study by Thomas *et al*.[16] demonstrating that kidney cancer cells become more sensitive to the mTOR inhibitor CCI-779 when the VHL tumor suppressor gene VHL is lost and that VHL-deficient tumors exhibit increased uptake of the PET tracer fluorodeoxyglucose in an mTOR-dependent manner. HIF-1a plays a central role in RCC tumorigenesis by acting as a transcription factor for several proteins that are important in tumoral adaptation to a tissue microenvironment that is low in oxygen. Higher HIF-1α expression in the cytoplasm might indicate that HIF-1α has been translocated to the cytoplasm, does not transcribe DNA, and therefore leads to less aggressive tumors and better prognosis. Because high HIF-1α expression was associated with poor survival, targeting HIF-1α may be a promising therapeutic approach.[17] HIF-1α regulates angiogenesis, tumor growth, progression, metastatic spread, and glucose metabolism by acting as a transcription factor for crucial proteins, such as vascular endothelial growth factor, platelet-derived growth factor, epidermal growth factor receptor, insulin-like growth factor, glucose transporter-1), chemokine receptors, and carbonic anhydrase IX and XII. In addition, HIF-1α plays an important role in regulating the cell cycle and apoptosis.[18] We identified a significant higher expression of HIF-1α among tumor samples compared to normal. We observed a correlation between the gene and protein expression of HIF-1α and AKT genes and that both were found to be upregulated and overexpressed, respectively.

Activated Akt (p-Akt) was found more frequently in tumor positive than in uninvolved tissues. The activated serine/threonine kinase Akt (p-Akt) controls proteins involved in apoptosis and cell proliferation.[19] Overexpression of p-Akt, however, may contribute to the development and progression of various malignancies and consequently have a negative impact on prognosis.[19] It was previously shown that Akt, once activated, translocates from the cytoplasm to the nucleus. The role of this nuclear translocation, the specific nuclear target structures, and, above all, the consecutively initiated regulatory processes, which may increase tumor proliferation rate or dedifferentiation or a combination of both, have not been fully clarified so far. In conclusion, the present study found significantly increased nuclear p-AKT expression in RCC. Enhanced AKT kinase activity and subsequent downstream signal transduction are now accepted to play an important role in human malignancy.

CONCLUSION

Our study data predicted a higher gene expression as well as protein expression of HIF-1α and AKT. The proteins HIF-1α and AKT are localized in the nucleus in RCC tumor samples compared to normal. Overexpression of these proteins might play significant roles in tumor development and differentiation as reported by others previously. This study can help clarify the biological role of HIF-1α and AKT in RCC to develop new strategies for this malignancy.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017;67:7-30.
2. Grossman E, Messerli F. Diuretics and renal cell carcinoma – What is the risk/benefit ratio? Kidney Int 1999;56:1603-4.
3. Pavlovich CP, Schmidt LS. Searching for the hereditary causes of renal-cell carcinoma. Nat Rev Cancer 2004;4:381-93.
4. McRonald FE, Morris MR, Gentle D, Winchester L, Baban D, Ragoussis J, *et al*. CpG methylation profiling in VHL related and VHL unrelated renal cell carcinoma. Mol Cancer 2009;8:31.
5. Ratcliffe PJ. New insights into an enigmatic tumour suppressor. Nat Cell Biol 2003;5:7-8.
6. Kaelin WG Jr. Molecular basis of the VHL hereditary cancer syndrome. Nat Rev Cancer 2002;2:673-82.
7. Shaw RJ, Cantley LC. Ras, PI(3) K and mTOR signalling controls tumour cell growth. Nature 2006;441:424-30.
8. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science 2009;324:1029-33.
9. McCarty MF. Targeting multiple signaling pathways as a strategy for managing prostate cancer: Multifocal signal modulation therapy. Integr Cancer Ther 2004;3:349-80.
10. David O, Jett J, LeBeau H, Dy G, Hughes J, Friedman M, *et al*. Phospho-Akt overexpression in non-small cell lung cancer confers significant stage-independent survival disadvantage. Clin Cancer Res 2004;10:6865-71.
11. Gingras AC, Raught B, Sonenberg N. Regulation of translation initiation by FRAP/mTOR. Genes Dev 2001;15:807-26.
12. Maiti GP, Ghosh A, Mondal P, Ghosh S, Chakraborty J, Roy A, *et al*. Frequent inactivation of SLIT2 and ROBO1 signaling in head and neck lesions: Clinical and prognostic implications. Oral Surg Oral Med Oral Pathol Oral Radiol 2015;119:202-12.
13. Chuang ST, Chu P, Sugimura J, Tretiakova MS, Papavero V, Wang K, *et al*. Overexpression of glutathione s-transferase alpha in clear cell renal cell carcinoma. Am J Clin Pathol 2005;123:421-9.

14. Kim SK, Abdelmegeed MA, Novak RF. Identification of the insulin signaling cascade in the regulation of alpha-class glutathione S-transferase expression in primary cultured rat hepatocytes. J Pharmacol Exp Ther 2006;316:1255-61.
15. Luan FL, Ding R, Sharma VK, Chon WJ, Lagman M, Suthanthiran M. Rapamycin is an effective inhibitor of human renal cancer metastasis. Kidney Int 2003;63:917-26.
16. Thomas GV, Tran C, Mellinghoff IK, Welsbie DS, Chan E, Fueger B, *et al*. Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. Nat Med 2006;12:122-7.
17. Hoefflin R, Harlander S, Schäfer S, Metzger P, Kuo F, Schönenberger D, *et al*. HIF-1α and HIF-2α differently regulate tumour development and inflammation of clear cell renal cell carcinoma in mice. Nat Commun 2020;11:4111.
18. Gkagkalidis K, Kampantais S, Dimitriadis G, Gourvas V, Kapoukranidou D, Mironidou-Tzouveleki M. Expression of HIF-2a in clear-cell renal cell carcinoma independently predicts overall survival. Med Mol Morphol 2020;53:229-37.
19. Hager M, Haufe H, Kemmerling R, Hitzl W, Mikuz G, Moser PL, *et al*. Increased activated Akt expression in renal cell carcinomas and prognosis. J Cell Mol Med 2009;13:2181-8.

Original Article

Tubulointerstitial nephritis in cases of lupus nephritis: An experience from a tertiary care referral center of Eastern India

Keya Basu, Subhrajyoti Karmakar, Abhirup Bhunia[1], Soma Addya, Moumita Sengupta, Arpita Roy Chowdhury[1]

Departments of Pathology and [1]Nephrology, Institute of Postgraduate Medical Education and Research, Kolkata, West Bengal, India

Abstract

Context: Up to 60% of patients with systemic lupus erythematosus develop lupus nephritis (LN). Tubulointerstitial nephritis (TIN) includes interstitial inflammation, tubulitis, tubular atrophy (TA), and interstitial fibrosis. These are considered independent risk factors for renal outcome.

Aims: To evaluate the occurrence of TIN in LN and to correlate it with clinical and histopathological variables and renal outcome.

Settings and Design: It was a prospective, single-center study.

Subjects and Methods: One hundred and thirty-two LN cases were evaluated. Light microscopic scoring of interstitial inflammation, fibrosis, and TA was done as follows: 0 (nil); 1+ (mild - <25% of the area of observed cortex); 2+ (moderate - >25% to 50% of the area of observed cortex); 3+ (severe - >50% of the area of observed cortex). For direct immunofluorescence study, fluorescein isothiocyanate-conjugated polyclonal rabbit antisera against human IgG, IgA, IgM, C3c, C1q, kappa, and lambda antibodies (DAKO, Germany) were used.

Statistical Analysis Used: Statistical software GraphPad Prism version 6.1.

Results: Significant TIN was present in 6% of cases associated with high National Institutes of Health activity and chronicity indices irrespective of the modified International Society of Nephrology and Renal Pathology Society class of LN. In the cases where inflammation and fibrosis are marked, significantly raised serum creatinine, low estimated glomerular filtration rate, high 24-h urinary protein excretion, and reduced survival without any complete remission were seen. Severe interstitial and tubular inflammations without chronicity were also associated with low survival rate due to frequent relapse and significant hypertension.

Conclusions: Activity and chronicity indices describing TIN components become essential to predict the survival, therapeutic response, and disease prognosis in LN.

Keywords: Interstitial fibrosis, interstitial inflammation, lupus nephritis, tubular atrophy, tubular basement membrane, tubulitis, tubulointerstitial nephritis

Address for correspondence: Dr. Keya Basu, Flat No. 301, Priyodarshan Apartment, 233A/1 N S C Bose Road, Kolkata - 700 092, West Bengal, India.
E-mail: kbasughosh@gmail.com

Submitted: 30-Jun-2022, **Revised:** 22-Jul-2022, **Accepted:** 05-Aug-2022, **Published:** 23-Nov-2022.

Access this article online	
Quick Response Code:	**Website:** https://journals.lww.com/amsr
	DOI: 10.4103/amsr.amsr_35_22

How to cite this article: Basu K, Karmakar S, Bhunia A, Addya S, Sengupta M, Chowdhury A. Tubulointerstitial nephritis in cases of lupus nephritis: An experience from a tertiary care referral center of Eastern India. Ann Med Sci Res 2022;1:121-30.

INTRODUCTION

Lupus nephritis (LN) is a major cause of morbidity and mortality of systemic lupus erythematosus (SLE) patients. Up to 60% of SLE patients develop LN and require treatment with immunosuppressive agents.[1-3] Despite aggressive treatments, some patients do not respond to therapy and progress to end-stage renal disease (ESRD).[4] The clinical and pathological manifestations are also diverse.[5,6] A precise description of renal histopathology and an appropriate classification of LN are both essential for nephrologists to guide treatment and predict prognosis. Tubulointerstitial nephritis (TIN) is frequent in LN with immune deposits being present in up to one-third of patients along the tubular basement membrane (TBM).[7] The independent risk factors of LN are interstitial inflammation, tubular atrophy (TA), and interstitial fibrosis (IF).[8] At present, LN is classified by Modified International Society of Nephrology and Renal Pathology Society (ISN/RPS) into six Classes (I, II, III, IV, V, and VI) along with modified National Institutes of Health (NIH) activity and chronicity indices.[9] This classification was primarily based on glomerular lesions, despite the fact that LN could involve all renal components, including the glomeruli, tubules, interstitium, and blood vessels. The aim of this prospective study was to analyze TIN on a series of native renal biopsies of SLE patients and to delineate correlations between clinical variables and therapeutic outcome.

SUBJECTS AND METHODS

Study design

This prospective study was performed in the department of pathology in collaboration with the department of nephrology of a tertiary care center of Eastern India from February 2018 to March 2020.

Renal biopsies from 132 LN cases were classified with activity and chronicity indices according to the modified ISN/RPS classification by two experienced nephropathologists. Patients fulfilling the 1997 American College of Rheumatology revised criteria for SLE were included.[10] The disease activity was assessed by the SLE Disease Activity Index (SLEDAI).[11]

Exclusion criteria

1. Patients with inadequate glomeruli (<2 glomeruli)
2. Known superimposed tubulointerstitial diseases
3. Known superimposed diseases like anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis, antiglomerular basement membrane disease, and monoclonal gammopathy of renal significance.
4. Patients with other comorbidities
5. Patients unwilling to participate in the study.

Renal histopathology

Renal biopsy specimens were examined by light microscopy (LM) and direct immunofluorescence (DIF) techniques. For LM, 4.5% formalin-fixed renal biopsy specimens were cut in 2-mm serial sections and stained with hematoxylin and eosin, periodic acid Schiff, Jones Methenamine Silver, and Masson's trichrome. All cases were classified according to the ISN/RPS classification and modified NIH score was used for activity and chronicity.[9] The scoring of interstitial inflammatory cell infiltration was as follows:[12]

- Score 0: No inflammation
- Score 1 (mild): <25% of nonscarred interstitium affected
- Score 2 (moderate): 25%–50% of nonscarred interstitium affected
- Score 3 (severe): >50% of nonscarred interstitium affected.

The scoring of IF and TA was assessed similarly. Grading of inflammatory infiltrate was limited to the nonscarred areas of renal cortex. The percentage of tubules with tubulitis was evaluated only in nonatrophic cortical tubules. Interobserver variation was resolved by re-reviewing the biopsies and thus reaching a consensus.

Direct immunofluorescence examination

Frozen sections were incubated with fluorescein isothiocyanate-conjugated polyclonal rabbit antisera against human IgG, IgA, IgM, C3c, C1q, kappa, and lambda (DAKO, Germany) and viewed using an immunofluorescence microscope (Olympus KX 21 with Magvision software). The extent and intensity of staining were scored semiquantitatively in the glomeruli and along the TBM:

- Score 1+ (0%–5%)
- Score 2+ (5%–25%)
- Score 3+ (25%–75%)
- Score 4+ (>75%).

Control slides were also examined simultaneously.

Clinical evaluation

Clinical parameters such as age, sex, duration of disease, arthralgia or arthritis, hypertension, and history of medications were recorded. Laboratory parameters such as complete blood count, erythrocyte sedimentation rate, urine routine and microscopic examination, albumin creatinine ratio (ACR), 24-h urinary protein estimation,

serum albumin, creatinine, complement level (C3 and C4), serology (hepatitis B and C and HIV), and autoantibodies such as antinuclear antibodies (ANA), ANCA, and anti-double-stranded DNA antibodies (anti-dsDNA) were analyzed and recorded at the time of the biopsy. Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease Study equation.[13]

Response to therapy

In general, Class II LN patients do not receive immunosuppressive treatment.[14] Class III–IV patients are treated with immunosuppressive agents, such as corticosteroids in combination with cyclophosphamide and mycophenolate mofetil (MMF), during the initial treatment phase. Azathioprine and MMF were administered in the maintenance phase of the therapy.[14] Patients were followed up in outpatient lupus clinics.

Outcome parameters

The following outcome and prognostic parameters were recorded and used for statistical analysis: development of ESRD, all-cause mortality, complete remission (CR), partial remission (PR), acute tubular necrosis (ATN), chronic kidney disease (CKD), and relapse detailed in previous studies.[15-18] CR is defined as decline in urine protein-to-creatinine ratio (uPCR) to 0.5 g/g (≤50 mg/mmol) accompanied by return of serum creatinine to previous baseline. PR is defined as the reduction in proteinuria by at least 50% and to <3 g/g measured as uPCR from a 24 – hour urine collection, along with stabilization or improvement in the kidney function (± 10-15% of baseline), within 6-12 months of commencement of therapy.[19] CKD is defined as a glomerular filtration rate <60 ml/min/1.73 m^2 for 3 months.

Relapse was defined as an increase in SLEDAI score of at least four points. ATN is a kidney disorder involving damage to the tubular epithelial cells which can lead to acute kidney failure. Poor prognosis was indicated by ESRD, ATN, and relapse. Good prognosis was indicated by PR or CR.

Ethical issues

This study was approved by the Institutional Ethics Committee and Research Advisory Committee of Institute. All the patients had given informed consent before participating in this study. The research was in compliance with the Declaration of Helsinki.

Statistical analysis

Quantitative data were expressed as mean ± standard deviation. Categorical variables were presented as frequencies and percentages. One-way analysis of variance was carried out during comparative analysis between the two groups. Statistical software GraphPad Prism version 6.1 (2365 Northside Dr., Suite 560, San Diego, CA 92108, USA) was used for statistical analysis. The Pearson's correlation test was performed between various lesions ($r = 0.2–0.4$ as mild correlation; 0.4–0.8 as moderate correlation; 40.8 as high correlation). Percentage of survival between different groups was compared by log-rank test. Statistical significance was considered $P < 0.05$.

RESULTS

We assessed the components of TIN in a total 132 cases of LN. Total 91 (68.9%) patients showed evidence of TIN. TIN was assessed by four components, such as interstitial inflammation, tubulitis, TA, and IF. Patients were divided into 5 groups depending on the degree of involvement of the components of TIN. In Group A, there were 106 (80.3%) patients with absent to mild involvement of all four compartments [Figure 1]. In Group B, there were 6 (4.5%) patients with absent to mild inflammation of interstitium and tubules and moderatetosevere TA and IF [Figure 2]. In Group C, there were 7 (5.3%) patients with moderatetosevere inflammation of interstitium and tubules and absent to mild TA and IF [Figure 3]. In Group D, 8 (6%) patients in total showed moderatetosevere degree of involvement of all four compartments [Figure 4]. In Group E, which had 5 (3.8%) patients, any one component was moderatetosevere in intensity and rest three components are absent to mild in intensity. In three patients, interstitial inflammation was moderate and the rest three (tubulitis, TA, and IF) were absent to mild [Figure 5]. In two cases, TA

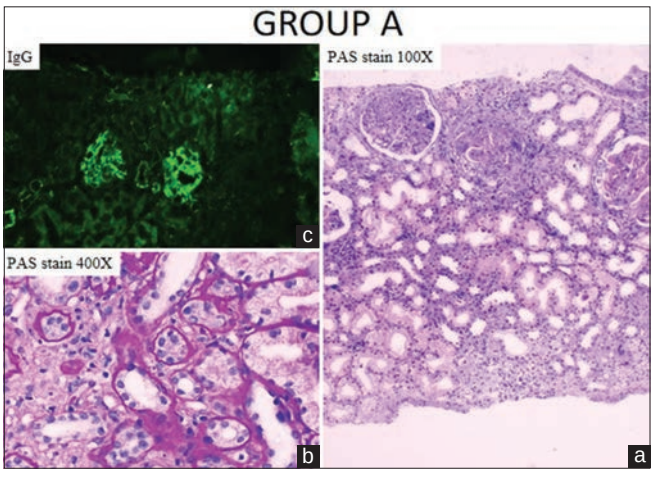


Figure 1: Group A: (a) Mild interstitial inflammation without any tubulitis, tubular atrophy or interstitial fibrosis (PAS X100) (b) Mild focal tubular atrophy with minimal interstitial inflammation (PAS X400) (c) No DIF deposits around tubules or interstitium in spite of remarkable intraglomerular positivity (IgG DIF X 100)

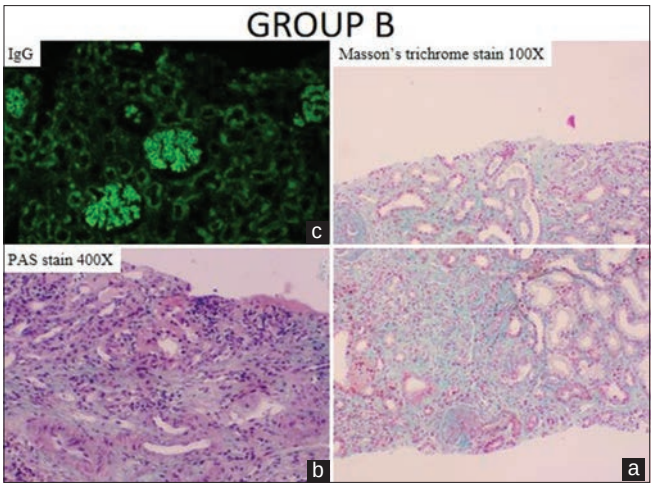


Figure 2: Group B: (a) Moderate interstitial fibrosis and tubular atrophy (IFTA 40%) mild interstitial inflammation and absent tubulitis (MT X100) (b) Tubular atrophy with tubular dropout and interstitial fibrosis with minimal interstitial inflammation (PAS X400) (c) No DIF deposits around tubules or interstitium in spite of remarkable intraglomerular positivity (IgG DIF X 100)

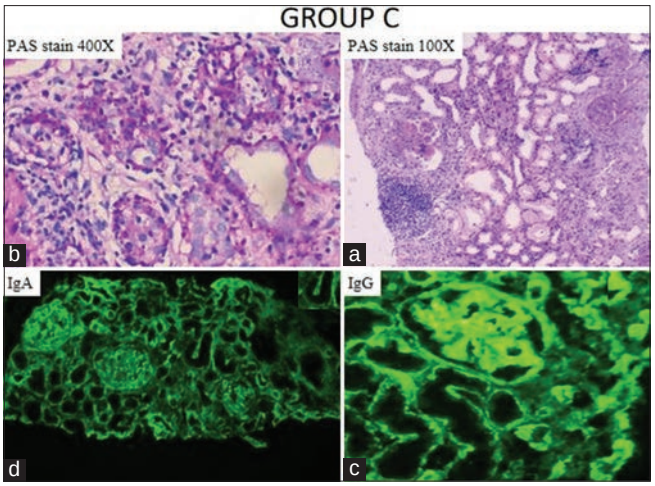


Figure 3: Group C: (a) Moderate interstitial inflammation with prominent lymphoid follicle formations and tubulitis without any tubular atrophy and interstitial fibrosis (IFTA 5%) (PAS X100) (b) Marked tubulitis with polymophonuclear inflammatory cells in the tubulointerstitial compartment (PAS X 400). (c) Significant DIF positivity along the tubulointerstitial compartment in addition to the remarkable intraglomerular positivity (IgG DIF X 200). (d) Significant DIF deposits around tubules and interstitium in addition to remarkable intraglomerular positivity (IgA DIF X 100)

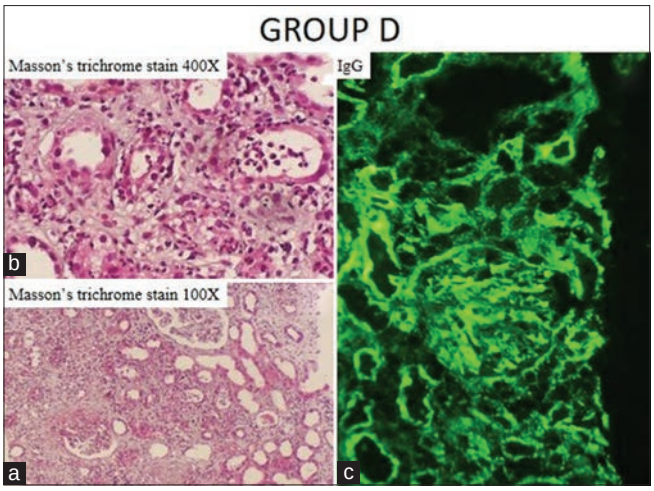


Figure 4: Group D: (a) Marked interstitial inflammation and fibrosis with marked tubulitis and tubular atrophy (IFTA 70%) (MT X100) (b) Marked tubulitis with polymophonuclear inflammatory cells in the tubulointerstitial compartment along with thick TBM indicating tubular atrophy (MT X 400). (c) Significant DIF positivity along the tubulointerstitial compartment in addition to the remarkable intraglomerular positivity (IgG DIF X 200)

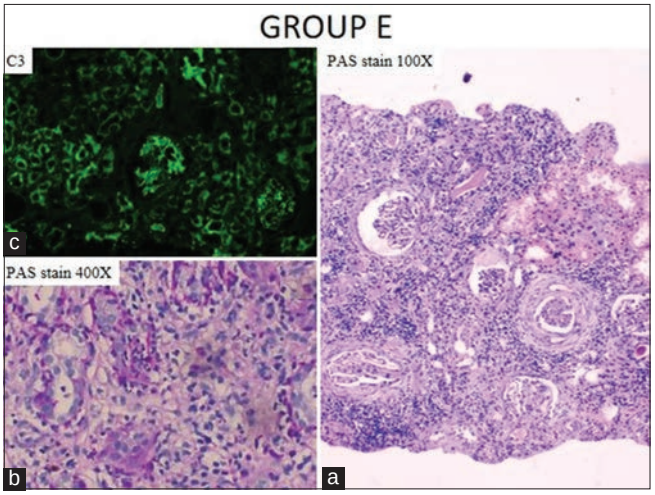


Figure 5: Group E: (a) Severe interstitial inflammation without any tubulitis, tubular atrophy or interstitial fibrosis (PAS X100). (b) Sheets of polymorphs present in the interstitium without any evidence of tubulitis, interstitial fibrosis or tubular atrophy (PAS X400). (c) Antinuclear antibody (ANA) effect in the tubules without any DIF deposits around tubules or interstitium in spite of remarkable intraglomerular positivity (C3c DIF X 100)

was moderate and the rest three (interstitial inflammation, tubulitis, and IF) were absent to mild [Table 1].

All the LN cases belonged to third to fourth decade with marked female preponderance in each group. Serum ANA and anti-dsDNA antibody positivity and reduced C3 and C4 levels were comparable in all the groups. Serum albumin was also comparable in all the groups with minimum level of 1.0 g/dL to maximum level of 4.4 g/dL. SLEDAI score was also comparable in all the groups with maximum value of 27 and minimum value of 0 [Table 2].

When comparing the clinical characteristics and biochemical parameters among the 5 groups, Group D was associated with severe clinical manifestations, hypertension, and raised serum creatinine level. Twenty-four hour urinary protein excretion was significantly high and eGFR was significantly low in Group D cases. All the patients of Group D showed hematuria and the presence of active urinary sediments including pus cells. Patients of Group C and D also showed hypertension which was statistically significant [Table 2].

Table 1: Combined distribution of the four tubulointerstitial nephritis featuresin the 132 lupus nephritis patients

Inflammatory infiltrare	Tubulitis	Tubular atrophy	Interstitial fibrosis	Number of cases, *n* (%)	Group
Absent-mild	Absent-mild	Absent-mild	Absent-mild	106 (80.3)	A
Absent-mild	Absent-mild	Moderate-severe	Moderate-severe	6 (4.5)	B
Moderate-severe	Moderate-severe	Absent-mild	Absent-mild	7 (5.3)	C
Moderate-severe	Moderate-severe	Moderate-severe	Moderate-severe	8 (6)	D
Other combinations (with at least one moderate to severe tin features)				5 (3.8)	E

Table 2: Clinical characteristics and biochemical parameters of different tubulointerstitial nephritis groups in lupus nephritis

Clinlcal parameters	Group A (*n*=106)	Group B (*n*=6)	Group C (*n*=7)	Group D (*n*=8)	Group E (*n*=5)	*P*
Age (years), mean±SD	25.23±8.78	30.17±12.29	25.57±5.78	27.25±9.36	22.80±4.38	0.6351
Sex (male: female)	1:6	0:6	0:7	0:8	0:5	0.3940
Hypertension, *n* (%)	25 (23.6)	1 (16.6)	5 (71.4)	5 (62.5)	3 (60)	0.0047
Duration (months), mean±SD	14.44±16.92	9.33±7.66	10.00±10	8.18±11.78	11.60±13.07	0.7263
ANA positivity, *n* (%)	103 (97.2)	5 (83.3)	7 (100)	8 (100)	5 (100)	0.3576
Anti-dsDNA positivity, *n* (%)	59 (55.6)	4 (66.6)	6 (85.7)	5 (62.5)	3 (60)	0.6180
Decreasing C3, *n* (%)	66 (62.2)	4 (66.6)	6 (85.7)	6 (75)	4 (80)	0.6494
Decreasing C4, *n* (%)	59 (55.6)	4 (66.6)	6 (85.7)	6 (75)	3 (60)	0.4794
Serum creatinine (mg/dL), mean±SD	0.94±0.39	1.13±0.34	1.13±0.56	1.79±0.92	1.19±0.75	<0.0001
Serum albumin (g/dL), mean±SD	3.02±0.78	3.00±0.66	3.06±0.43	2.32±0.65	2.88±0.60	0.1697
eGFR, mean±SD	88.57±32.27	69.58±32.81	70.34±28.76	47.04±26.33	81.48±44.78	0.0063
24-h urinary protein (g), mean±SD	1.98±1.68	1.74±0.90	2.67±1.36	4.70±3.72	3.07±2.87	0.0022
Microscopic hematuria, *n* (%)	48 (45.2)	4 (66.6)	5 (71.4)	8 (100)	3 (60)	0.0252
Active sediment, *n* (%)	15 (14.1)	0	3 (42.8)	4 (50)	2 (40)	0.0200
SLEDAI, mean±SD	9.98±6.47	10.17±6.94	14.86±5.87	15.25±7.08	14.60±9.94	0.0551

ANA: Antinuclear antibodies, SD: Standard deviation, dsDNA: Anti-double-stranded DNA antibodies, eGFR: Estimated glomerular filtration rate, SLEDAI: Systemic lupus erythematosus disease activity index

The total number of glomeruli was comparable between the abovementioned groups. Globally sclerosed glomeruli were present in all the groups. Modified ISN/RPS class for LN showed comparable class distribution with maximum number of Group A patients belonging to ISN/RPS Class II. Most frequent class of Group B patients were ISN/RPS Class IV and V and that of Group C patients were ISN/RPS Class III, III + V, and IV + V. Maximum number of Group D patients belonged to ISN/RPS Class IV and IV + V. Group E patients were equally distributed in ISN/RPS Class III, IV, V, III + V, and IV + V [Table 3].

NIH activity indices were significantly high in Group C and Group D due to the presence of higher rate of cellular and fibrocellular crescents in Group D and increased interstitial inflammation in Group C. The rest of the parameters contributing to the activity indices such as endocapillary hypercellularity, neutrophilic infiltration and karyorrhexis, fibrinoid necrosis, and subendothelial hyaline deposit were comparable among all the groups [Table 3].

NIH Chronicity indices were significantly high in Group B and Group D due to the presence of higher rate of fibrous crescents, tubulitis, TA, and IF in Group D. Group B patients showed significantly increased TA and IF. Maximum tubulitis was present in Group C. Global and segmental glomerulosclerosis were comparable in all the groups. Vascular lesions such as perivascular deposits, thrombi, vasculitis, leukocytoclastic vasculitis, and arteriosclerosis were comparable in all the groups [Table 3].

DIF microscopy showed full house intraglomerular positivity of immune markers in 90 patients (68.2%). DIF staining intensity for IgG, IgA, IgM, C3c, C1q, kappa, and lambda was comparable in all the groups within the glomeruli. Total 24 patients (18.2%) showed positivity for IgG along the TBM in DIF. Group D showed notably intense IgG positivity along the TBM. The rest of the markers (IgA, IgM, C3c, and C1q) showed comparable intensity along the TBM [Table 4].

All the patients were treated and followed up from minimum 15 days to maximum 72 months. Serial measurement of clinical and biochemical parameters along with urinary findings was noted. None of the patients died in our follow-up period. The highest rate of CR was seen in group A. CKD Stage 3 was notably seen in Group D followed by Group B. ESRD was remarkably present in Group E. PR, CKD 4, AKI, and relapse were comparable in all the groups. None of the patients had CKD Stage 1 or 2 [Table 5].

Survival data analysis showed lowest percentage of survival in Group C and Group D and >50% survival in Group E and Group B and <50% in Group A. The longest follow-up data were available in Group A. The survival data considering ESRD, AKI, CKD 4,

Table 3: Histopathological findings in different groups of tubulointerstitial nephritis in lupus nephritis

Histopathological parameters	Group A (*n*=106)	Group B (*n*=6)	Group C (*n*=7)	Group D (*n*=8)	Group E (*n*=5)	*P*
Total glomeruli, mean±SD	13.20±9.29	9.00±3.95	14.57±8.34	17.25±6.92	12.40±5.27	0.5275
Global sclerosis, *n* (%)	0.47±1.42	0.83±2.04	1.28±1.89	1.87±1.72	1.0±1.0	0.0776
ISN/RPS Class II, *n* (%)	26 (19.6)	0	0	0	0	
ISN/RPS Class III, *n* (%)	27 (20.4)	1 (16.6)	2 (28.5)	1 (12.5)	1 (20)	
ISN/RPS Class IV, *n* (%)	23 (17.4)	2 (33.3)	1 (14.2)	3 (37.5)	1 (20)	
ISN/RPS Class V, *n* (%)	20 (15.1)	2 (33.3)	0	0	1 (20)	
ISN/RPS Class III+V, *n* (%)	24 (18.1)	0	2 (28.5)	1 (12.5)	1 (20)	
ISN/RPS Class IV+V, *n* (%)	12 (9)	1 (16.6)	2 (28.5)	3 (37.5)	1 (20)	
ISN/RPS Class, mean±SD	2.26±1.83	3.83±1.17	2.28±1.11	2.57±1.13	3.00±1.58	0.2569
Activity index, mean±SD	3.00±3.28	5.50±4.23	7.28±4.99	7.87±2.10	6.00±4.63	<0.0001
Endocapillary hypercellularity, mean±SD	0.55±1.01	0.83±1.17	0.57±1.13	0.87±1.13	1.20±1.64	0.6283
Cellular±fibrocellular crescents, mean±SD	0.36±0.86	0.33±0.81	1.43±2.22	3.00±2.39	1.20±1.09	<0.0001
Neutrophil/karyorrhexis, mean±SD	0.65±1.18	1.00±1.55	1.28±1.60	0.75±1.39	0.60±1.34	0.7113
Fibrinoid necrosis, mean±SD	0.29±0.73	0	0.28±0.75	0	0	0.5650
Subendothelial hyaline, mean±SD	0.81±1.05	2.00±1.55	1.43±1.27	1.35±1.28	1.20±1.30	0.0632
Interstitial inflammation, mean±SD	0.35±0.48	0.83±0.41	2.28±0.49	2.00±0	1.60±0.55	<0.0001
Chronicity index, mean±SD	1.59±1.18	5.00±2.36	2.71±1.60	5.37±0.74	3.80±1.48	<0.0001
Glomerulosclerosis, mean±SD	1.55±1.95	1.83±2.79	2.28±1.60	2.75±2.60	2.20±2.49	0.4620
Fibrous crescents, mean±SD	0	0	0.14±0.38	0.12±0.35	0	0.0025
Tubulitis, mean±SD	0.26±0.44	0.50±0.55	2.43±0.53	2.00±0	0.80±0.45	<0.0001
Tubular atrophy, mean±SD	0.43±0.49	2.17±0.41	0.85±0.38	2.00±0	1.20±0.84	<0.0001
Interstitial fibrosis, mean±SD	0.44±0.49	2.33±0.52	0.57±0.53	2.00±0	1.00±0	<0.0001
Vascular lesions, mean±SD	0.42±0.65	0.33±0.52	0.71±0.49	0.50±0.53	0.60±0.89	0.7563
Perivascular deposits, mean±SD	0.11±0.33	0	0.28±0.49	0.12±0.35	0.20±0.45	0.6376
Thrombi, mean±SD	0.05±0.23	0	0.14±0.38	0	0.20±0.45	0.4915
Vasculitis, mean±SD	0.02±0.13	0	0	0	0	0.9750
Leukocytoclastic vasculitis, mean±SD	0.01±0.09	0	0.14±0.38	0	0	0.0851
Arteriosclerosis, mean±SD	0.19±0.40	0.33±0.52	0.28±0.49	0.37±0.52	0.20±0.45	0.7343

SD: Standard deviation, ISN/RPS: International Society of Nephrology and Renal Pathology Society

Table 4: Direct immunofluorescence microscopic findings in glomeruli and tubules in different groups of tubulointerstitial nephritis in lupus nephritis

	DIF positivity and intensity	Group A (*n*=106)	Group B (*n*=6)	Group C (*n*=7)	Group D (*n*=8)	Group E (*n*=5)	*P*
Glomeruli	IgG	2.13±0.85	2.67±1.21	2.14±1.07	1.87±0.83	2.00±0.70	0.5476
	IgA	1.21±0.77	0.83±0.68	1.36±0.85	1.18±0.88	0.80±0.75	0.5677
	IgM	0.84±0.62	1.00±0.55	1.00±0.70	1.31±0.59	0.90±0.65	0.3017
	C3c	1.31±0.88	1.66±0.82	1.14±1.02	1.18±1.03	1.30±0.97	0.8547
	C1q	1.07±0.69	1.42±0.66	1.07±0.67	1.00±0.65	1.00±0.61	0.8071
	Kappa	1.44±0.82	1.75±1.08	1.07±0.88	1.81±0.92	1.20±0.45	0.3787
	Lambda	2.01±1.03	1.83±1.17	1.85±1.07	2.37±0.91	2.20±0.84	0.8273
TBM	IgG	0.31±0.81	0	1.14±1.46	1.25±1.39	0.20±0.45	0.0055
	IgA	0.19±0.57	0	0.86±1.21	0.25±0.71	0.40±0.89	0.0855
	IgM	0.11±0.39	0	0.43±0.78	0.50±0.75	0.40±0.89	0.0515
	C3c	0.16±0.54	0	0.43±0.78	0.62±1.19	0.60±1.34	0.1208
	C1q	0.19±0.59	0	0.71±0.95	0.50±0.75	0.40±0.89	0.1367

TBM: Tubular basement membrane, DIF: Immunofluorescence

Table 5: Renal outcome on follow-up of different groups of tubulointerstitial nephritis in lupus nephritis

Follow up	Group A (*n*=106), *n* (%)	Group B (*n*=6), *n* (%)	Group C (*n*=7), *n* (%)	Group D (*n*=8), *n* (%)	Group E (*n*=5), *n* (%)	*P*
CR	64 (60.3)	1 (16.6)	2 (28.5)	0	2 (40)	0.0063
PR	25 (23.5)	4 (66.4)	3 (42.8)	3 (37.5)	1 (20)	0.1466
CKD Stage 1	0	0	0	0	0	
CKD Stage 2	0	0	0	0	0	
CKD Stage 3	5 (4.7)	2 (33.3)	0	4 (50)	1 (20)	<0.0001
CKD Stage 4	2 (1.8)	0	0	1 (12.5)	0	0.3801
Relapse	8 (7.5)	0	1 (14.2)	1 (12.5)	0	0.8407
AKI	8 (7.5)	1 (16.6)	1 (14.2)	2 (25)	0	0.4282
ESRD/doubling of serum creatinine	2 (1.8)	0	0	1 (12.5)	2 (40)	0.0002
Death	0	0	0	0	0	

CR: Complete remission, PR: Partial remission, CKD: Chronic kidney disease, AKI: Acute kidney injury, ESRD: End-stage renal disease

and relapse as the markers of poor prognosis were compared [Figure 6].

In summary, Group A with the mildest TIN showed the lowest serum creatinine, highest eGFR, and lowest

incidence of hematuria. Most patients were in ISN/RPS Class II and III with lowest activity index and minimal interstitial inflammation. Chronicity index was lowest in this group with maximum incidence of CR without any death. Long-term survival was slightly <50%.

Group B patients with moderate-to-severe interstitial fibrosis and tubular atrophy (IFTA), low tubulitis, and interstitial inflammation showed the lowest incidence of hypertension and lowest 24-h urinary proteins without any active urinary sediment. Most patients were in ISN/RPS Class IV and V with the lowest incidence of cellular and fibrocellular crescents and highest IFTA without any incidence of ESRD, CKD 4, relapse, or death and more than <50% survival.

Group C depicted low IFTA, moderate-to-severe tubulitis, interstitial inflammation, and highest incidence of hypertension. Most patients were in ISN/RPS Class III, III + V, and IV + V. The highest interstitial inflammation and tubulitis were seen without the presence of any poor prognostic markers.

Group D patients showed moderate-to-severe TIN along with the highest serum creatinine, 24-h urinary protein, hematuria, active urinary sediments, and lowest eGFR. Most patients were in ISN/RPS Class IV and IV + V with the highest activity and chronicity indices and maximum incidence of cellular and fibrocellular crescents along with significantly intense IgG along the TBM. None of them showed CR.

Group E depicted all other features in mild category except interstitial inflammation or TA being moderate to severe. They had the highest incidence of ESRD, >50% survival and without any CKD 4, relapse, ATN, or death.

The survival data were compared considering ESRD, ATN, CKD 4, and relapse as the markers of poor prognosis and in Group C and Group D poor percentage survival was found.

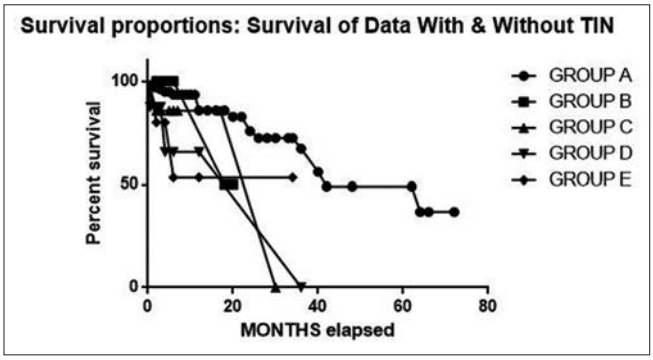


Figure 6: Cmparison of survival data of Group A, B, C, D and E patients

Glomerular and tubuinterstitial parameters are correlated within activity and chronicity indices. Glomerular activity was determined as the score after omitting the corresponding interstitial inflammation score from each activity indices. Similarly, total glomerulosclerosis and fibrous crescent were added to determine the glomerular chronicity. IF was added to TA for scoring the tubulointerstitial chronicity. The glomerular activity score and interstitial inflammation were found to have a weak positive correlation ($r = 0.31$, $P < 0.001$). The glomerular chronicity was also weakly correlated with the tubulointerstitial chronicity ($r = 0.37$, $P < 0.001$).Thus, glomerular and tubulointerstitial findings were not always representative of each other.

DISCUSSION

LN associated with TIN carries poor prognosis as observed in previous studies.[7,12] Whereas the glomerular abnormalities have attracted much attention, the immunopathogenetic mechanisms that lead to TIN in LN remain obscure. This is ironic because tubulointerstitial damage is an important poor prognostic indicator for long-term renal function, and it may be less amenable to treatment compared with glomerular lesions.

Prevalence of tubulointerstitial nephritis

In our study, the prevalence of TIN in LN is 68.9%. Many previous studies also have reported cases with significant tubulointerstitial involvement in LN.[20-23] In our study, we have found positivity for immune markers in 18.2% cases by DIF. Study by Pagni *et al.* has confirmed the presence of at least one TIN lesion in up to 60% of patients at first biopsy and 75% at repeat biopsy.[24] We have observed moderate-to-severe inflammation with scarring in 6% LN cases and without scarring in 5.3% LN cases. Moderate-to-severe scarring without inflammation are seen in 4.5% LN patients. Therefore, a comprehensive classification of LN, which should also cover TIN, is crucial for therapeutic and prognostic assessments.

Tubulointerstitial nephritis versus International Society of Nephrology and Renal Pathology Society class of lupus nephritis

This study shows that there exists different severity of TIN in LM as well as DIF, independent of different classes of LN. Patients with more TIN (Group C) belong to Class III or combined class and patients with both TIN and fibrosis (Group D) belong to Class IV or combined class. Ali and Al-Windawi have encountered the same in Class IV and in Class III LN.[25]

Correlation of glomerular activity and chronicity with tubulointerstitial nephritis

Further correlation test illustrates that tubulointerstitial lesions weakly correlate with glomerular modified NIH activity and chronicity indices in LN. Another study by Hsieh *et al*. also has noted lack of clear correlation between the magnitude of TIN with either the activity index or the glomerular histological class. They have found a correlation between TIN and the tubular components of the chronicity score, which include TA and IF.[26] In contrast to our finding, Yu *et al*. have shown good correlation of Glomerular features of activity with the degree of interstitial inflammatory cell infiltration and the glomerular chronicity with the range of TA and IF.[8] The reason may be that we have assessed the glomerular activity without considering interstitial inflammation score. However, few other studies also depict results similar to our study.[20,22] Severe glomerular involvement with milder TIN might be the early stage of LN. Otherwise tubulointerstitial lesions may occur independently with longer disease duration also. Rarely, the interstitial changes may be the major or only renal lesions of LN as reported earlier.[20-23]

Clinical and serological parameters in tubulointerstitial nephritis

Our patients with moderate-to-severe TIN (Group D) present with raised serum creatinine, high 24-h urine protein excretion, low eGFR, frequent CKD Stage 3 and poor percent survival irrespective of modified NHS/RPS class, and this observation matches with a previous study.[22] Wang *et al*. have found significant correlations between TBM immune deposits and some clinical activity indices of LN, including the serum creatinine value, serum C3 level and SLEDAI score.[27] Proteinuria may be caused by interstitial inflammatory cell infiltration and both are associated with poor prognosis.[28] According to Hsieh *et al*., the TIN lesions are associated with high serum creatinine[26] while other shown their impact on proteinuria. Furthermore, study by Pagni *et al*. have shown that mean eGFR levels are also related with TIN features, while this is not true for proteinuria.[24] In contrast to our study Hsieh *et al*. do not found any significant correlation of proteinuria with interstitial disease.[26] Our study also notes the lack of correlation of complement and anti-dsDNA with interstitial disease in LN. Others have found correlation between interstitial immune complexes and serological activity.[30] This is also seen by Ali *et al*. in a case with positive ANA, anti-dsDNA and hypocomplementemia.[25] Some studies have described that the presence of interstitial infiltrates does not correlate with the degree of interstitial immune complex deposition.[29]

Tubulointerstitial nephritis in other glomerulonephritis

Yamamoto *et al*. have already compared interstitial inflammatory cell infiltration and chronic tubulointerstitial lesions between patients with LN and those with IgA nephropathy (IgAN). They have found that IgAN with nil or mild glomerular lesions are never associated with significant interstitial inflammation and or chronic tubulointerstitial lesions. In contrast severe interstitial inflammation is seen in 36% of LN patients with nil or mild glomerular lesions.[31]

Mechanism of tubulointerstitial nephritis

Possible mechanisms of tubulointerstitial injury in experimental and human LN may be immune complex deposition,[31] albuminuria, macrophage chemokines,[32-34] and autoantibodies (anti-TBM).[35,36] Recent studies suggest activation of tubular Toll-like receptor-9 causing TIN in LN.[37,38] Anti-dsDNA antibody levels in LN cases correlate with activity index as well as glomerular and TBM immune deposits.[39] Immune deposition is associated with induction of inflammatory cytokines such as Interleukins-6. Although the mononuclear cell infiltrates have not been functionally characterized in SLE, morphologically similar cell populations in other types of interstitial nephritis have been identified as activated T cells.

Genetic and environmental factors in tubulointerstitial nephritis

The majority of our cohort is Asian and it is possible that interstitial inflammation is mostly mild in nature in this population in contrast to African American.[32,33] However, a larger, multicenter study will be needed to determine conclusively whether the renal outcomes and degree of TIN is less severe in Asian than in African American LN patients and can be explained by an increased prevalence of interstitial nephritis in this population.

Tubulointerstitial nephritis and renal outcome

Previous studies show that higher incidence of ESRD or rapid doubling of serum creatinine is associated with severe TIN which was not seen in our cases. We have observed highest ESRD in Group E patients with either moderate-to-severe interstitial inflammation or with moderate-to-severe TA. Degree of tubulointerstitial lesions could have independent prognostic value in predicting renal outcome. Furthermore, Howie has proposed that the severity of chronic TIN is a strong predictor of progression to renal failure.[40] Data obtained by the study of Hsieh *et al*. are novel and demonstrate that tubulointerstitial inflammation correlates with both renal function and renal survival.[26] Poor long-term outcomes have been particularly noted when interstitial infiltrates of mononuclear cells are still present on the repeat biopsy.

The reasons of more severe tubulointerstitial lesions causing worse renal prognosis might be attributed to irreversible chronic and sclerotic lesions, refractory to therapy. Immunosuppressive therapy consists of glucocorticoids combined with a cytotoxic drug to achieve a prompt response.[41] The high rate of renal relapse in Group C and Group D patients (13%) justifies long-term maintenance immunosuppressant. Twelve percentage Group D patients with LN and severe TIN ultimately require renal replacement therapy due to ESRD. As the interstitial inflammation is potentially reversible, the presence of severe interstitial nephritis is an important histological finding that may identify high-risk patients who would benefit from aggressive or directed therapeutic interventions and may warrant more focus in drug development and clinical trials. In some patients with repeat renal biopsy, they have achieved clinical remission with immunosuppressive treatment but the TIN and chronicity index have increased. Therefore, further therapies aimed directly at tubulointerstitial injury based on above studies should be further justified in LN.

Finally, the modified ISN/RPS classification of LN based on glomerular lesions is not always parallel to the TIN. Addition of activity and chronicity indices describing the TIN component becomes essential to predict the survival and disease prognosis in LN. Further correlation of TBM deposits with TIN may predict more precise disease outcome in future. It has to be determined in future whether IF and TA should be considered separately or combined into one parameter (as in the Oxford classification for IgAN) and whether making a distinction between interstitial inflammations in areas with or without IF has any clinical significance.

CONCLUSIONS

The incidence of significant TIN is 6% in our cases and it is associated with high NIH activity and chronicity indices irrespective of the modified ISN/RPS class of LN. TIN is independent of the changes in the glomerular compartment. When both inflammation and fibrosis are moderate to severe in degree, significantly raised serum creatinine level, low eGFR, and high 24-h urinary protein excretion are seen. Urinary RBC and active sediments are also present. Percentage of survivability of patients with CKD Stage 3 is grave. No patients show CR. Statistically significant high interstitial and tubular inflammation without IFTA is also associated with poor percentage of survivability due to highest incidence of relapse and significant hypertension.

Limitations

There are some limitations in this study:

1. Although it is a prospective study, there is some heterogeneity in the treatment that may have influenced the histological and DIF features and outcome
2. The sample size of patients with unparalleled glomerular and tubulointerstitial lesions as well as sample size of patients with moderate-to-severe TIN was not large enough to draw a valid conclusion
3. The current study lacks investigation on pathogenic mechanism of tubulointerstitial injury in LN. Therefore, a further well-designed prospective study is required.

Ethics

This study is approved by the Institutional ethical committee. All the participants gave informed consent in writing at the beginning of the study.

Financial support and sponsorship

Funding is provided by our Institution.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ginzler EM, Dooley MA, Aranow C, Kim MY, Buyon J, Merrill JT, *et al.* Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. N Engl J Med 2005;353:2219-28.
2. Appel GB, Contreras G, Dooley MA, Ginzler EM, Isenberg D, Jayne D, *et al.* Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. J Am Soc Nephrol 2009;20:1103-12.
3. Flanc RS, Roberts MA, Strippoli GF, Chadban SJ, Kerr PG, Atkins RC. Treatment for lupus nephritis. Cochrane Database Syst Rev 2004;1:CD002922.
4. Korbet SM, Schwartz MM, Evans J, Lewis EJ, Collaborative Study Group. Severe lupus nephritis: Racial differences in presentation and outcome. J Am Soc Nephrol 2007;18:244-54.
5. Appel GB, Radhakrishnan J, D'Agati V. Secondary glomerular disease. In: Brenner BM, editor. Brenner & Rector's The Kidney. 8th ed. Philadephia: W.B. Saunders; 2008. P. 1067-146.
6. D'Agati V. Renal disease in systemic lupus erythematosus, mixed connective tissue disease, Sjogren's syndrome, and rheumatoid arthritis. In: Jennette JC, Olson JL, Schwartz MM, *et al.* (eds). Heptinstall's Pathology of the Kidney. 6th ed. Philadephia: Lippincott-Raven; 2007. p. 517-612.
7. Park MH, D'Agati V, Appel GB, Pirani CL. Tubulointerstitial disease in lupus nephritis: Relationship to immune deposits, interstitial inflammation, glomerular changes, renal function, and prognosis. Nephron 1986;44:309-19.
8. Yu F, Wu LH, Tan Y, Li LH, Wang CL, Wang WK, *et al.* Tubulointerstitial lesions of patients with lupus nephritis classified by the 2003 International Society of Nephrology and Renal Pathology Society system. Kidney Int 2010;77:820-9.
9. Bajema IM, Wilhelmus S, Alpers CE, Bruijn JA, Colvin RB, Cook HT, *et al.* Revision of the International Society of Nephrology/Renal Pathology Society classification for lupus nephritis: Clarification of definitions, and modified National Institutes of Health activity and chronicity indices. Kidney Int 2018;93:789-96.
10. Hochberg MC. Updating the American College of Rheumatology

revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.
11. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum 1992;35:630-40.
12. Austin HA 3rd, Boumpas DT, Vaughan EM, Balow JE. Predicting renal outcomes in severe lupus nephritis: Contributions of clinical and histologic data. Kidney Int 1994;45:544-50.
13. Hill GS, Delahousse M, Nochy D, Mandet C, Bariéty J. Proteinuria and tubulointerstitial lesions in lupus nephritis. Kidney Int 2001;60:1893-903.
14. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD, *et al*. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. Arthritis Care Res (Hoboken) 2012;64:797-808.
15. Wang J, Hu W, Xie H, Zhang H, Chen H, Zeng C, *et al*. Induction therapies for class IV lupus nephritis with non-inflammatory necrotizing vasculopathy: Mycophenolate mofetil or intravenous cyclophosphamide. Lupus 2007;16:707-12.
16. Ginzler EM, Dooley MA, Aranow C, Kim MY, Buyon J, Merrill JT, *et al*. Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. N Engl J Med 2005;353:2219-28.
17. Chan TM, Tse KC, Tang CS, Lai KN, Li FK. Long-term outcome of patients with diffuse proliferative lupus nephritis treated with prednisolone and oral cyclophosphamide followed by azathioprine. Lupus 2005;14:265-72.
18. Wang HY, Cui TG, Hou FF, Ni ZH, Chen XM, Lu FM, *et al*. Induction treatment of proliferative lupus nephritis with leflunomide combined with prednisone: A prospective multi-center observational study. Lupus 2008;17:638-44.
19. KDIGO clinical practice guideline for glomerulonephritis. Kidney Int Suppl 2011 2012;2:142.
20. Mori Y, Kishimoto N, Yamahara H, Kijima Y, Nose A, Uchiyama-Tanaka Y, *et al*. Predominant tubulointerstitial nephritis in a patient with systemic lupus nephritis. Clin Exp Nephrol 2005;9:79-84.
21. Hayakawa S, Nakabayashi K, Karube M, Arimura Y, Soejima A, Yamada A, *et al*. Tubulointerstitial immune complex nephritis in a patient with systemic lupus erythematosus: Role of peritubular capillaritis with immune complex deposits in the pathogenesis of the tubulointerstitial nephritis. Clin Exp Nephrol 2006;10:146-51.
22. Omokawa A, Wakui H, Okuyama S, Togashi M, Ohtani H, Komatsuda A, *et al*. Predominant tubulointerstitial nephritis in a patient with systemic lupus erythematosus: Phenotype of infiltrating cells. Clin Nephrol 2008;69:436-44.
23. Hodgin JB, Whelan J, Markowitz GS, Stokes MB. Giant cell tubulitis with immune complex deposits in a patient with lupus nephritis. Am J Kidney Dis 2009;53:513-7.
24. Pagni F, Galimberti S, Galbiati E, Rebora P, Pietropaolo V, Pieruzzi F, *et al*. Tubulointerstitial lesions in lupus nephritis: International multicentre study in a large cohort of patients with repeat biopsy. Nephrology (Carlton) 2016;21:35-45.
25. Ali A, Al-Windawi S. Tubulointerstitial lupus nephritis. J Nephropathol 2013;2:75-80.
26. Hsieh C, Chang A, Brandt D, Guttikonda R, Utset TO, Clark MR. Predicting outcomes of lupus nephritis with tubulointerstitial inflammation and scarring. Arthritis Care Res (Hoboken) 2011;63:865-74.
27. Wang H, Xu J, Zhang X, Ren YL, Cheng M, Guo ZL, *et al*. Tubular basement membrane immune complex deposition is associated with activity and progression of lupus nephritis: A large multicenter Chinese study. Lupus 2018;27:545-55.
28. Hiramatsu N, Kuroiwa T, Ikeuchi H, Maeshima A, Kaneko Y, Hiromura K, *et al*. Revised classification of lupus nephritis is value in predicting outcome with an indication of the proportion of glomeruli affected by chronic lesions. Rheumatology (Oxford) 2008;47:702-7.
29. Hill GS, Delahousse M, Nochy D, Rémy P, Mignon F, Méry JP, *et al*. Predictive power of the second renal biopsy in lupus nephritis: Significance of macrophages. Kidney Int 2001;59:304-16.
30. Hunter MG, Hurwitz S, Bellamy CO, Duffield JS. Quantitative morphometry of lupus nephritis: The significance of collagen, tubular space, and inflammatory infiltrate. Kidney Int 2005;67:94-102.
31. Yamamoto T, Nagase M, Hishida A, Honda N. Interstitial inflammatory and chronic tubulointerstitial lesions in lupus nephritis: Comparison with those in IgA nephropathy. Lupus 1993;2:261-8.
32. Zheng L, Sinniah R, Hsu SI. Renal cell apoptosis and proliferation may be linked to nuclear factor-kappaB activation and expression of inducible nitric oxide synthase in patients with lupus nephritis. Hum Pathol 2006;37:637-47.
33. Zheng L, Sinniah R, Hsu SI. Pathogenic role of NF-kappaB activation in tubulointerstitial inflammatory lesions in human lupus nephritis. J Histochem Cytochem 2008;56:517-29.
34. Ferraccioli G, Romano G. Renal interstitial cells, proteinuria and progression of lupus nephritis: New frontiers for old factors. Lupus 2008;17:533-40.
35. Yung S, Tsang RC, Sun Y, Leung JK, Chan TM. Effect of human anti-DNA antibodies on proximal renal tubular epithelial cell cytokine expression: Implications on tubulointerstitial inflammation in lupus nephritis. J Am Soc Nephrol 2005;16:3281-94.
36. Tan Y, Yu F, Yang H, Chen M, Fang Q, Zhao MH. Autoantibodies against monomeric C-reactive protein in sera from patients with lupus nephritis are associated with disease activity and renal tubulointerstitial lesions. Hum Immunol 2008;69:840-4.
37. Anders HJ, Vielhauer V, Eis V, Linde Y, Kretzler M, Perez de Lema G, *et al*. Activation of toll-like receptor-9 induces progression of renal disease in MRL-Fas (lpr) mice. FASEB J 2004;18:534-6.
38. Benigni A, Caroli C, Longaretti L, Gagliardini E, Zoja C, Galbusera M, *et al*. Involvement of renal tubular Toll-like receptor 9 in the development of tubulointerstitial injury in systemic lupus. Arthritis Rheum 2007;56:1569-78.
39. Termaat RM, Assmann KJ, van Son JP, Dijkman HB, Koene RA, Berden JH. Antigen-specificity of antibodies bound to glomeruli of mice with systemic lupus erythematosus-like syndromes. Lab Invest 1993;68:164-73.
40. Howie J, Turhan N, Adu D. Powerful morphometric indicator of prognosis in lupus nephritis, QJM Int J Med 2003;96:411-20.
41. Houssiau FA, Vasconcelos C, D'Cruz D, Sebastiani GD, Garrido Ed Ede R, Danieli MG, *et al*. Immunosuppressive therapy in lupus nephritis: The euro-lupus nephritis trial, a randomized trial of low dose versus high-dose intravenous cyclophosphamide. Arthritis Rheum 2002;46:2121-31.

Original Article

Aldehyde dehydrogenase 1 and CD44 serve as prognostic markers in patients with breast cancer

Ayan Pradhan, Diptendra Kumar Sarkar

Department of General Surgery, Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India

Abstract

Background and Objective: Breast cancer is the second leading cause of cancer-related death in women globally, and its prevalence is rising quickly, particularly in low- and middle-income nations. Despite significant advancements in treatment options, a small percentage of individuals with advanced-stage breast cancer have a dismal prognosis. The most extensively utilised markers for identifying breast cancer stem cells are ALDH1 and CD44 (BCSCs).

The goal of this study was to look into the expression of ALDH1 and CD44 in breast carcinoma and see if there was any correlation with other clinicopathological factors to see if they might be used to predict prognosis in patients with breast cancer.

Methods: This study comprised 30 women with breast cancer who were undergoing mastectomy. Immunohistochemistry (IHC) labelling with an ALDH1, CD44 primary antibody was used to assess ALDH1, CD44 levels in paraffin-embedded tissues. The percentage of positive cells was used to assess the expression level, which was then associated with clinicopathological characteristics.

Results: Out of 30 patients, 23 (76%) had CD44 positive; out of 30 patients, 21 had CD44 positivity (70 percent). ALDH1 expression was linked to the number of lymph nodes, while CD44 expression was linked to tumour size.

Conclusions: In breast cancer, ALDH1 and CD44 expression acts as an independent prognostic indicators. However, bigger population-based prospective patient trials are needed to confirm these findings.

Keywords: Aldehyde dehydrogenase 1, breast cancer, CD44, immunohistochemistry

Address for correspondence: Prof. Diptendra Kumar Sarkar, Department of General Surgery, Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India.
E-mail: diptendra3@gmail.com
Submitted: 22-Mar-2022, **Revised:** 10-Aug-2022, **Accepted:** 11-Aug-2022, **Published:** 23-Nov-2022.

INTRODUCTION

Breast cancer is a worldwide disease that is the second biggest cause of female cancer-related death. In India, one out of every two patients diagnosed with breast cancer dies. Invasive breast cancer is the most common type of cancer in women worldwide, accounting for 23% of all malignancies in women.[1] Breast cancer is now the most frequent cancer in India, as well as in the major metropolitan metropolis in the eastern region of the country, where the current study took place. Distant metastasis is the most common cause of death. Various prognostic markers and a number of expensive radiological technologies have been used to diagnose metastatic breast cancer.[2] Breast cancer is a heterogeneous illness, as evidenced by the fact that

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/amsr
	DOI: 10.4103/amsr.amsr_15_22

How to cite this article: Pradhan A, Sarkar DK. Aldehyde dehydrogenase 1 and CD44 serve as prognostic markers in patients with breast cancer. Ann Med Sci Res 2022;1:131-5.

phenotypically comparable breast tumours have differing clinical presentations and disease severity, in addition to systemic gene expression pattern analyses. Breast tumours are classified into at least five molecular subtypes, each with distinct races/ ethnicities, risk factors distribution, prognosis, therapeutic treatment responsiveness, clinical outcomes, and relapse-free survival rates.[3] These are luminal cell-like tumours, which are further subdivided into luminal A and B, both of which express Oestrogen receptor (ER) and have profiles similar to those of normal luminal cells of the breast glands, basal cell like (BCL) phenotype ER and progesterone receptor (PgR)-negative tumours with genes normally expressed by basal/myoepithelial cells], HER2-enriched (Human epidermal growth factor receptor 2, Cancer stem cells, according to the cancer stem cell (CSC) theory, are a subgroup of tumour cells that have the ability to self-renew and differentiate, resulting in a heterogeneous tumour cell population.

ALDH1A1 is a detoxification enzyme that converts aldehydes to carboxylic acids.[4] It is found in high amounts in hematopoietic and neural stem cells, as well as in the epithelium of the testes, brain, eye, liver, and kidneys.[5] It is known to metabolize and detoxify chemotherapeutics like cyclophosphamide,[6] and is thus expected to contribute to hematopoietic stem cells' intrinsic chemotherapeutic resistance. CD44, a nonkinase transmembrane glycoprotein, is overexpressed in a variety of cell types, including CSCs, and frequently shows alternatively spliced variants that may play a role in cancer genesis and progression. The major ligand for CD44 is hyaluronan, which binds to CD44 and activates CD44 cell signaling pathways promoting cell proliferation, boosting cell survival, modifying cytoskeletal alterations, and improving cellular motility.[7] Based on existing understanding and data, it supports the notion that ALDH1A1 and CD44 are both cell surface indicators, and ALDH1A1 activity is the most accurate way for identifying and isolating CSC-like cells within breast cancer populations.[7] In this study, we have used immunohistochemistry (IHC) to detect breast CSCs in mastectomy specimens from patients with breast carcinoma and compared the expression of stem cell markers ALDH1A1 and CD44 to standard prognostic factors, as well as highlighted its potential utility as a surrogate biomarker.

MATERIALS AND METHODS

Reagents

Except for antihuman CD44 (clone 4B12), ALDH (clone C6/144B), EnVisionTM FLEX Mini Kit, High pH (DAB + chromogen), and EnVisionTM FLEX Target Retrieval Solution, all reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) (Dako, Glostrup, Denmark).

Population studied

Between the period of February and November 2019, 30 female patients with primary breast carcinoma were enrolled on a random basis who visited the Comprehensive Breast Clinic and Breast Cancer Research Unit, IPGME and R/SSKM Hospital, Kolkata, West Bengal, India.

Histopathology

Hematoxylin and eosin (H and E) were used to analyze the overall histopathology of formalin-fixed paraffin-embedded (FFPE) tissues that were sectioned (3 m), mounted on poly L-lysine coated slides, and examined for overall histopathology. The cellular infiltrate was graded semi-quantitatively as 1 = mild, 2 = moderate, 3 = severe, and 4 = extremely severe (1 = 0–25 cells/mm^2; 2 = 26–50 cells/mm^2; 3 = 51–75 cells/mm^2; 4 = >75 cells/mm^2) (Mukherjee *et al.*, 2015). Five fields were manually counted at ×40 under a light microscope (EVOS FL Cell Imaging System, Waltham, MA, USA), and the average was calculated.

The size of breast cancer tumors was assessed after they were fixed in 10% neutral-buffered formalin for 24 h. After that, the tumor was embedded in paraffin and sectioned, and the lymph nodal status and grade were identified. For IHC, paraffin sections of tumors were deparaffinized and hydrated with xylene, 100% ethanol, a phosphate buffer (10 mM), (pH 7.4), and 0.138 M saline containing 2.7 (mM) KCl, followed by washes with xylene, 100% ethanol, and a phosphate buffer (10 mM), (pH 7.4), and 0.138 M saline containing Antigen retrieval was carried out using antigen retrieval buffer that had been diluted (DAKO Corp.). 3% hydrogen peroxide was used to inhibit endogenous peroxidase. After that, the slides were rinsed in PBS and incubated for 1 h at 4°C with 10% normal horse serum, followed by the primary antibody (mouse anti-CD44 and ALDH antibody).

Immunohistochemistry

FFPE slices were deparaffinized in xylene and rehydrated in sliding grades of alcohol (100%–70%) and distilled water for IHC. After heat-induced epitope retrieval at pH 6 or pH 9, the slides were incubated for 1 h with appropriate dilutions of primary antibody (1:100 for ALDH and CD44), washed with Tris-buffered saline containing 0.05% Tween-20 (0.02M, pH 7.4, TBS-T), and incubated with EnVisionTM G | 2 System/AP-Rabbit/Mouse (Permanent Red) or EnVisionTM FLEX.

Ethics statement

The study was approved by the School of Tropical Medicine's Institutional Ethics Committee and the Institute of Post Graduate Medical Education and Research's Institutional Ethics Committee, and all experiments were carried out in conformity with relevant standards and regulations. Individuals or their legally authorized representative (if under the age of 18) signed a written informed consent form. Informed consent was also obtained to publish patient confidentiality information/images in an online open-access publication after respecting patient confidentiality.

Statistical analysis

Results were expressed as median (Interquartile range) and data analyzed between groups using the Kruskal–Wallis test, followed by Dunn's multiple comparison test for nonparametric data, while paired data were analyzed using a *t*-test (for parametric data). The correlation was by Pearson's correlation for parametric data and Spearman's rank correlation for nonparametric data using GraphPad Prism software (version-5.0, GraphPad Software Inc., La Jolla, CA, USA), with $P < 0.05$ being statistically significant. After this, the significant values and ratios are to be correlated with the molecular subtypes of breast cancer and the NPI score of the tumor for the purpose of prognostication.

Table 1: Study Population

Parameters	Patients with breast cancer (*n*=30)
Age range (years), median (IQR)	32–37, 51.50 (43-60)
Sex	Female
Tumor size	3.657±0.27
Grade	1.57±0.12
NPI	4.60±0.24
ALDH1	75.93±5.67
CD44	70.08±8.82

IQR: Interquartile range, NPI: Nottingham prognostic index, ALDH1: Aldehyde dehydrogenase 1

RESULTS

Aldehyde dehydrogenase 1 protein expression in tumor cells and tumor tissues

Aldehyde dehydrogenase 1 (ALDH1) is a particular marker for detecting, isolating, and tracking human breast cancer. IHC labeling with an ALDH1A1 primary antibody was used to assess the levels of ALDH1 in paraffin-embedded tumor tissues from 30 breast cancer patients, and the expression level of ALDH1 was measured in terms of the percentage and intensity of positive cells. Within tumor tissues, markedly diverse ALDH1 staining was found in the cytoplasm of tumor cells [Figure 1 and Table. 1]. ALDH1 expression was found in 23 of the 30 breast cancer cases studied (76.66%). Only a few cases had moderate-to-strong expression, whereas the majority had only favorable expression. ALDH1-positive cells were found in 10% of the cases (3 cases), 13.33% of the cases (4 cases), and 76.66% of the cases (23 cases).

CD44 protein expression in tumor cells and tumor tissues

CD44 is a nonkinase, single-span transmembrane glycoprotein family that is found in embryonic stem cells as well as connective tissues and bone marrow at varying levels. CD44 expression is also elevated in cancer cell subpopulations and is used as a molecular marker for CSCs. CD44 is a unique marker that can be used to identify, isolate, and track human breast cancer. IHC staining with CD44 primary antibody was used to assess the levels of CD44 in paraffin-embedded tumor tissues from 30 breast cancer patients, and the expression level of CD44 was measured in terms of the percentage and intensity of positive cells [Figure 2 and Table 1].CD44 expression was found in 21 of the 30 breast cancer cases studied (70%). Only a few cases had moderate-to-strong expression, whereas the majority had only favorable expression. CD44-positive cells were found in 13% of cases (4 cases), 16.66% of cases (5 cases), and 70% of cases (23 cases).

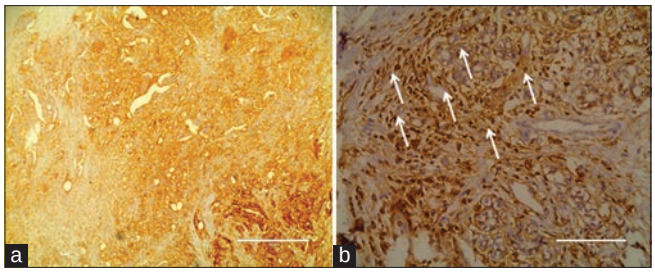


Figure 1: ALDH1 immunohistochemistry in formalin-fixed, paraffin-embedded breast cancer tissues. (a) ×10 magnification of ALDH 1 positive staining in tumor cells. (b) Local positive ALDH 1 staining in tumor cells at a magnification of ×40. ALDH1: Aldehyde dehydrogenase 1

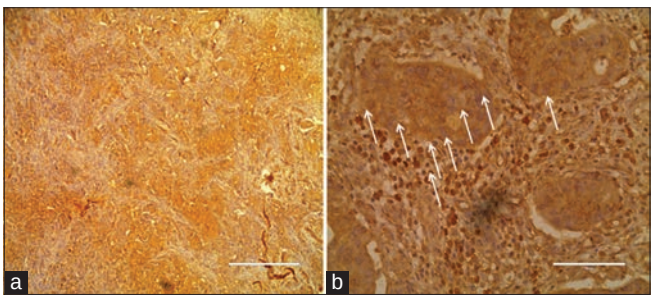


Figure 2: CD44 immunohistochemistry in formalin-fixed, paraffin-embedded breast cancer tissues. (a) ×10 of CD44 positive staining in tumor cells. (b) CD44 positive staining in tumor cells at a magnification of ×40

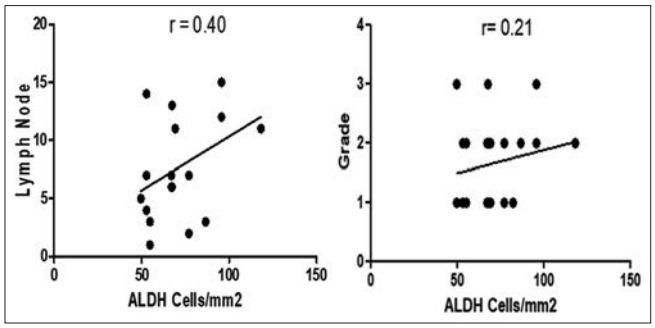


Figure 3: Correlation between ALDH versus Lymph node and ALDH versus tumor Grade in patients with breast cancer

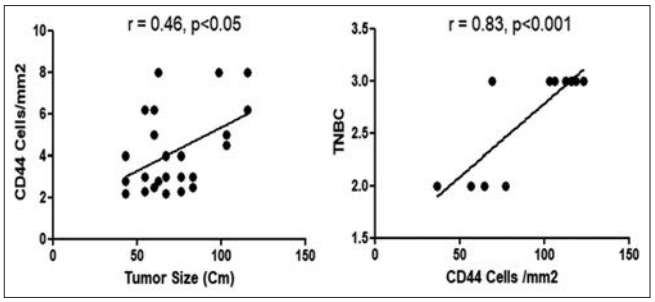


Figure 4: Correlation between CD44 versus Lymph node and CD44 versus tumor Grade in patients with breast cancer

Aldehyde dehydrogenase 1 expression and clinic pathological characteristics in patients with breast cancer were correlated

To explore the potential significance of ALDH1 and CD44 as a prognostic marker, we investigated the correlation between ALDH1 and CD44 protein with clinicopathological characteristics in patients with breast cancer. The expression of ALDH1 correlated positively with lymph node (r =0.40, Figure 3) and grade (r=0.21, Figure 3). Whereas the expression of CD44 protein in tumour as measured by immunohistochemical staining was strongly linked with tumour size (r =0.46, p<0.05, Figure 4) and triple negative breast cancer (r =0.83, p<0.05, Figure. 4). Other clinicopathological factors such as age, grade, and lymph node status, however, had no significant connection with CD44 expression.

DISCUSSION

ALDH1, which plays a role in early stem cell differentiation by oxidizing retinol to retinoic acid, has been proposed as a strong candidate for CSCs in breast tissue. ALDH1 is one of the CSC markers that can be utilized as an independent prognostic indicator in node-positive breast cancer, according to several studies.[8] Because ALDH1 is one of the main enzymes involved in ethanol metabolism and is found in the human breast epithelium, a role comparison with the other enzymes is required. These enzymes are thought to play a key part in the carcinogenesis process. ALDH1 expression was found in the majority of cases in our investigation, which is consistent with the findings of Sarkar *et al.* and Zhong *et al.*[4,9] who found ALDH1 expression in 76% of cases. Our data show that ALDH1 expression is more common in an Indian population's breast cancer series, compared to 19% and 30% in two separate Caucasian populations documented by others.[10] Our findings also show a link between lymph node status and overall health. These findings show that ALDH1+ cancer cells are overrepresented in breast carcinoma tissue. These disparities may be due to the variety of detection techniques and samples under analysis, especially as more than 90% of the cases evaluated in our study were invasive carcinoma of no particular kind.

CD44 is a nonkinase, single-span transmembrane glycoprotein family that is expressed on embryonic stem cells as well as other cell types such as connective tissue and bone marrow at varying levels.[11,12] CD44 expression is also elevated in cancer cell subpopulations and is used as a molecular marker for CSCs.[13] CD44 is encoded by 19 exons in humans, with 10 of these exons remaining constant throughout all isoforms. The ten constant exons encode the standard version of CD44 (CD44s). Alternative splicing produces CD44 variant isoforms (CD44 v), which contain the ten constant exons and any combination of the remaining nine variant exons.[14,15] Hyaluronic acid (HA), an important component of the extracellular matrix expressed by stromal and cancer cells, is the major ligand for CD44.[16] HA interacts with the CD44 ligand binding domain, causing conformational changes that allow adaptor proteins or cytoskeletal components to bind to intracellular domains, resulting in cell proliferation, adhesion, migration, and invasion.[17,18] CD44s and CD44v isoforms play overlapping and separate roles in the body. Additional binding sites in CD44v isoforms improve CD44 interaction with molecules in the microenvironment.[19] CD44v isoforms can function as co-receptors by binding/sequestering growth factors on the cell surface and delivering them to their respective receptors.[20] Cancer cells that undergo an epithelial-to-mesenchymal transition (EMT) develop stem cell-like characteristics and express more CD44.[21] Furthermore, cancer cells that have an EMT pattern are more aggressive and resistant to treatment.[22] CD44s and isoforms' clinicopathological roles in carcinogenesis suggest that CD44 could be a molecular target for cancer therapy.[23] Furthermore, CD44's significance in retaining stemness and the activity of CSCs in tumor regeneration after therapy suggests that CD44 could be a useful prognostic marker. CD44 expression was found in 70% of breast cancer cases in our investigation, which agrees

with the findings of.[24] Furthermore, we discovered a strong association between CD44 protein expression and tumor size in this investigation, which is consistent with earlier findings,[24] suggesting that CD44 protein expression may play a predictive function in patients with breast cancer.

CONCLUSION

To summarize, ALDH1 and CD44 serve as independent prognostic indicators for patients with breast cancer. To confirm these findings, more research with a Bigger cohort, standardized and well-matched controls will be required.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Mazhar D, Ang R, Waxman J. COX inhibitors and breast cancer. Br J Cancer 2006;94:346-50.
2. Ma F, Li H, Li Y, Ding X, Wang H, Fan Y, *et al.* Aldehyde dehydrogenase 1 (ALDH1) expression is an independent prognostic factor in triple negative breast cancer (TNBC). Medicine (Baltimore) 2017;96:e6561.
3. Ranger GS, Salhab M, Mokbel K. The role of cyclooxygenase-2 in breast cancer: Review. Breast Cancer Res Treat 2008;109:189-98.
4. Sarkar P, Basu K, Sarkar P, Chatterjee U, Mukhopadhyay M, Choudhuri MK, *et al.* Correlations of aldehyde dehydrogenase-1 (ALDH1) expression with traditional prognostic parameters and different molecular subtypes of breast carcinoma. Clujul Med 2018;91:181-7.
5. O'Conor CJ, Chen T, González I, Cao D, Peng Y. Cancer stem cells in triple-negative breast cancer: A potential target and prognostic marker. Biomark Med 2018;12:813-20.
6. Yoshioka T, Umekita Y, Ohi Y, Souda M, Sagara Y, Sagara Y, *et al.* Aldehyde dehydrogenase 1 expression is a predictor of poor prognosis in nodepositive breast cancers: A long-term follow-up study. Histopathology 2011;58:608-16.
7. Chen C, Zhao S, Karnad A, Freeman JW. The biology and role of CD44 in cancer progression: Therapeutic implications. J Hematol Oncol 2018;11:64.
8. Panigoro SS, Kurnia D, Kurnia A, Haryono SJ, Albar ZA. ALDH1 cancer stem cell marker as a prognostic factor in triple-negative breast cancer. Int J Surg Oncol 2020;2020:7863243.
9. Zhong Y, Lin Y, Shen S, Zhou Y, Mao F, Guan J, *et al.* Expression of ALDH1 in breast invasive ductal carcinoma: An independent predictor of early tumor relapse. Cancer Cell Int 2013;13:60.
10. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, *et al.* ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007;1:555-67.
11. Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 2001;189:54-63.
12. Domev H, Amit M, Laevsky I, Dar A, Itskovitz-Eldor J. Efficient engineering of vascularized ectopic bone from human embryonic stem cell-derived mesenchymal stem cells. Tissue Eng Part A 2012;18:2290-302.
13. Yin T, Wang G, He S, Liu Q, Sun J, Wang Y. Human cancer cells with stem cell-like phenotype exhibit enhanced sensitivity to the cytotoxicity of IL-2 and IL-15 activated natural killer cells. Cell Immunol 2016;300:41-5.
14. Screaton GR, Bell MV, Bell JI, Jackson DG. The identification of a new alternative exon with highly restricted tissue expression in transcripts encoding the mouse Pgp-1 (CD44) homing receptor. Comparison of all 10 variable exons between mouse, human, and rat. J Biol Chem 1993;268:12235-8.
15. Prochazka L, Tesarik R, Turanek J. Regulation of alternative splicing of CD44 in cancer. Cell Signal 2014;26:2234-9.
16. Banerjee S, Modi S, McGinn O, Zhao X, Dudeja V, Ramakrishnan S, *et al.* Impaired synthesis of stromal components in response to minnelide improves vascular function, drug delivery, and survival in pancreatic cancer. Clin Cancer Res 2016;22:415-25.
17. Ponta H, Sherman L, Herrlich PA. CD44: From adhesion molecules to signalling regulators. Nat Rev Mol Cell Biol 2003;4:33-45.
18. Zöller M. CD44: Can a cancer-initiating cell profit from an abundantly expressed molecule? Nat Rev Cancer 2011;11:254-67.
19. Yamano S, Gi M, Tago Y, Doi K, Okada S, Hirayama Y, *et al.* Role of deltaNp63(pos)CD44v(pos) cells in the development of N-nitroso-tris-chloroethylurea-induced peripheral-type mouse lung squamous cell carcinomas. Cancer Sci 2016;107:123-32.
20. Orian-Rousseau V, Chen L, Sleeman JP, Herrlich P, Ponta H. CD44 is required for two consecutive steps in HGF/c-Met signaling. Genes Dev 2002;16:3074-86.
21. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, *et al.* The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008;133:704-15.
22. Zhao S, Chen C, Chang K, Karnad A, Jagirdar J, Kumar AP, *et al.* CD44 expression level and isoform contributes to pancreatic cancer cell plasticity, invasiveness, and response to therapy. Clin Cancer Res 2016;22:5592-604.
23. Li L, Hao X, Qin J, Tang W, He F, Smith A, *et al.* Antibody against CD44s inhibits pancreatic tumor initiation and postradiation recurrence in mice. Gastroenterology 2014;146:1108-18.
24. Ricardo S, Vieira AF, Gerhard R, Leitão D, Pinto R, Cameselle-Teijeiro JF, *et al.* Breast cancer stem cell markers CD44, CD24 and ALDH1: Expression distribution within intrinsic molecular subtype. J Clin Pathol 2011;64:937-46.

Original Article

Assessment of deoxyribonucleic acid damage in exfoliated bladder cells and its prognostic implication in urinary bladder cancer patients

Piyush Gupta, Kalyan Banerjee[1], Amlan Ghosh[1], Dilip Kumar Pal

Department of Urology, Institute of Post Graduate Medical Education and Research, [1]Department of Life Sciences, Presidency University, Kolkata, West Bengal, India

Abstract

Background: In this era where when one has to look for prognostic and diagnostic methods for evaluating tumors not only they are invasive but very much expensive also which is not at all easy for everyone to opt for. In our study we have tried to look for Deoxyribonucleic Acid (DNA) damage in exfoliated bladder cells which is obtained from a simple urine test and tried to correlate the damage with tumour stage and grade obtained from bladder biopsy.

Aim: To assess Deoxyribonucleic Acid (DNA) damage in Bladder tumour and it's extrapolation on exfoliated bladder cells and to study association of DNA damage markers with diseases prognosis if any.

Study and Design: It is analytical cross sectional study done in tertiary care centre in Kolkata, West Bengal, India.

Material and Method: After fulfilling inclusion and exclusion criterion we evaluated 70 patients with Bladder space occupying lesion (SOL) to study the DNA damage on exfoliated bladder cells by Fast Halo method and Gamma Histone Analysis and studied the correlation between them if any and also Histopathological Findings.

Statistical Analysis Used: All statistical tests were done using GraphPad PRISM (version 7, 2016) and SPSS 20 for Windows (IL, USA). Association was determined by Student t-test and correlation study was performed by Spearman's correlation coefficient.

Results: It was concluded that DNA damage assessed by Fast Halo method done on exfoliated bladder cells statistically correlates with the findings of DNA damage assessed by Gamma Histone analysis and there was a positive correlation with tumour grade and stage.

Conclusion: DNA damage assessed by Fast Halo method correlates well with the finding of DNA damage assessed by Gamma Histone analysis and both these findings were consistent with the findings obtained by Histopathology.

Keywords: Exfoliated bladder cells, fast-halo assay, gamma histone analysis

Address for correspondence: Dr. Dilip Kumar Pal, Department of Urology, Institute of Post Graduate Medical Education and Research, Kolkata - 700 020, West Bengal, India.
E-mail: urologyipgmer@gmail.com
Submitted: 25-Aug-2022, **Revised:** 09-Sep-2022, **Accepted:** 10-Sep-2022, **Published:** 23-Nov-2022.

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/amsr
	DOI: 10.4103/amsr.amsr_47_22

How to cite this article: Gupta P, Banerjee K, Ghosh A, Pal DK. Assessment of deoxyribonucleic acid damage in exfoliated bladder cells and its prognostic implication in urinary bladder cancer patients. Ann Med Sci Res 2022;1:136-41.

INTRODUCTION

Bladder cancer is stated as the seventh-most common cancer diagnosed in the male population globally, whereas it comes down to tenth when we talk about both genders.[1] Due to variability and differences in different diagnostic practices available in different countries, treatment, and risk factors, the worldwide age-standardized incidence rate for both genders; mortality rates may vary.

One of the major causative factors includes tobacco inhalation and any kind of occupational exposure to poly-arylamines for this disease.[2,3] The carcinogens, such as arylamines, undergo metabolic transformations, and such transformations form activated deoxyribonucleic acid (DNA) adducts, which play a major role in carcinogenesis.[4]

Although environmental factors play an important role in the development of any cancer, the genetic and epigenetic constituency of an individual determines the incidence of the diseases.

A list of chemical compounds can damage the DNA of living cells profoundly. If not repaired, or if they are produced in excessive amounts, can initiate a cascade of biological effects at the cellular, organ, individual, and finally at the community and population level, the most prominent consequence being carcinogenesis.[5]

DNA lesions and the repair mechanisms, which maintain the integrity of genomic DNA, are important in preventing carcinogenesis and, thus, its progression. Interestingly, mutations that occur in DNA repair mechanisms are associated with cancer predisposition syndromes. Moreover, these mechanisms maintain the genomic integrity of cancer cells.

There are numerous strategies with inherent advantages and disadvantages that can be used for the evaluation of DNA damage and repair. Following DNA damage, cellular responses are initiated that allow the cell to repair the damage through a number of mechanisms.[6] Therefore, DNA repair proteins are invariably important biomarkers for predicting the response of tumors to genotoxic stress and the prognosis of patients with more accuracy. This highlights the importance of detecting and quantifying DNA damage.

Our study is extensively based on analyzing DNA damage in exfoliated bladder cells using fast-halo assay (FHA) and to analyze DNA damage in exfoliated bladder cells using immunohistochemical analysis and finally confirm the diagnosis by histopathology and studying the correlation among these three and its diagnostic and prognostic implication of DNA damage markers, if exists.

MATERIALS AND METHODS

From October 2019 to December 2021, data of all bladder cancer patients with their demographic profile, previous medical and surgical history, and smoking habits were tabulated in Excel sheet and were thoroughly examined.

Our inclusion criterion included all those patients who presented with bladder mass and were further evaluated by radiological imaging techniques such as ultrasound (USG) and computed tomography (CT) scan to detect the extent of tumor and followed by Transurethral resection of bladder tumor and histopathologically proven transitional cell carcinoma. Our exclusion criterion included all those patients who had received radiotherapy and all those who were not willing to participate in the study.

Later on, part of freshly operated bladder cancer tissues, normal tissue, urine sample, and ethylenediaminetetraacetic acid blood sample from case were collected.

Assessment of DNA breakage in bladder exfoliated cells was done by two methods, i.e. FHA, an improvement of the alkaline halo assay technique originally developed by Sestili *et al*.[7] for the detection of single-strand DNA breakage, following the procedure of Sestili *et al*.,[8] and results are expressed after calculating the nuclear diffusion factor (NDF), which represents the ratio between the total area of the halo plus the nucleus and that of the nucleus.

Moreover, another method being immunohistostaining of gamma histone, using suitable primary antibody (Rabbit Polyclonal IgA antibody [Cat No. SC842]) and secondary

Table 1: Distribution of patients according to stage, involvement of muscle, and grade

	n
Stage	
Ta	8
T1	42
T2	14
T3	4
T4	2
Depending on muscle invasion	
NMIBC	50
MIBC	20
Grade	
Low	27
High	43

MIBC: Muscle invasive bladder cancer, NMIBC: Non-MIBC

antibody (Goat Anti-Rabbit [Cat No. BBSAB01A]) according to the manufacturer's protocol, and scoring was done according to Perrone *et al*.[9]

The samples were then sent for pathological examination and further histopathological grade and stage at the pathology department of our institute.

RESULTS

This is a prospective study of 70 patients with urinary bladder tumor, where assessment of DNA damage was made using two different techniques, and the association of DNA damage markers was studied with diseases prognosis, if any.

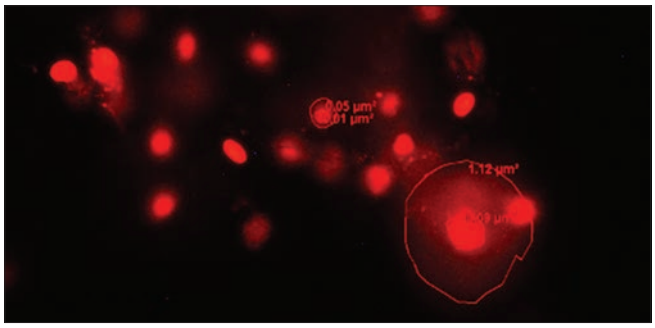


Figure 1: Fast-halo images of DNA damage in exfoliated bladder cells. Larger the halo area corresponding to greater DNA damage. Results are expressed after calculating the NDF values.

$$NDF = \frac{\text{Total area of halo + nucleus}}{\text{Total area of nucleus}}$$

DNA: Deoxyribonucleic acid, NDF: Nuclear diffusion factor

Of 70 patients with a median age of 62 years with a range of 41–81 years, 54 (77%) patients were male and 16 (23%) patients were female, and 46 (66%) patients were smoker. Fifty patients had nonmuscle invasive bladder cancer (NMIBC), and the rest 20 had muscle-invasive bladder cancer (MIBC), of which 27 had low-grade disease and 43 had high-grade disease [Table 1].

When samples were analyzed for DNA damage with fast-halo method [Figure 1] and gamma histone expression analysis method, it was noted that with NDF, 18 patients had low NDF expression, 18 had intermediate, and the rest 34 showed high NDF expression.

Similarly, 17 patients showed low gamma histone expression, 24 showed intermediate, and the rest 29 expressed high gamma histone score [Table 2].

Association of NDF and gamma histone expression with respect to MIBC and NMIBC with low-grade

Table 2: Distribution of patients according to Nuclear Diffusion Factor and Gamma Histone expression score

Distribution of patients according to Nuclear Diffusion Factor (NDF) Finding (quartile division)		
Grade of Expression	**Frequency**	**Percentage (%)**
Low NDF expression (Q1)	18	26
Intermediate NDF expression (Q1-Q2)	18	26
High NDF expression (>Q2)	34	48
Distribution of patients according to Gamma Histone expression score		
Grade of Expression	**Frequency**	**Percentage (%)**
Low Gamma Histone (0-2)	17	24
Intermediate Gamma Histone (3-5)	24	34
High Gamma Histone (6-7)	29	42

NDF: Nuclear Diffusion Factor

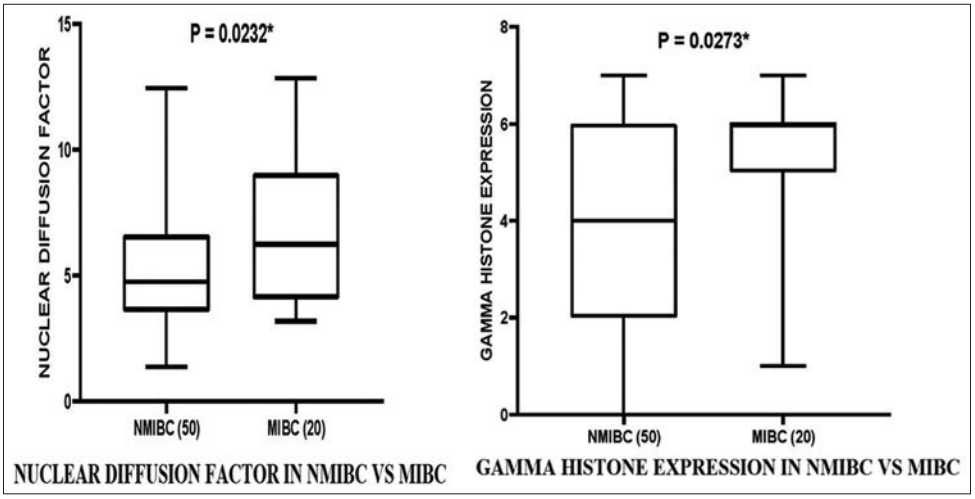


Figure 2: Box and whisker plot shows association of NDF with NMIBC and MIBC, and it was seen that there is a higher value of NDF (higher DNA damage) with MIBC compared to NMIBC with $P = 0.0232$, and another box and whisker plot showing association of gamma histone expression on MIBC and NMIBC, and it was seen that expression of gamma histone is highly associated with MIBC compared to NMIBC having $P = 0.0273$, which is statistically significant. NDF: Nuclear diffusion factor, MIBC: Muscle invasive bladder cancer, NMIBC: Non-MIBC, DNA: Deoxyribonucleic acid

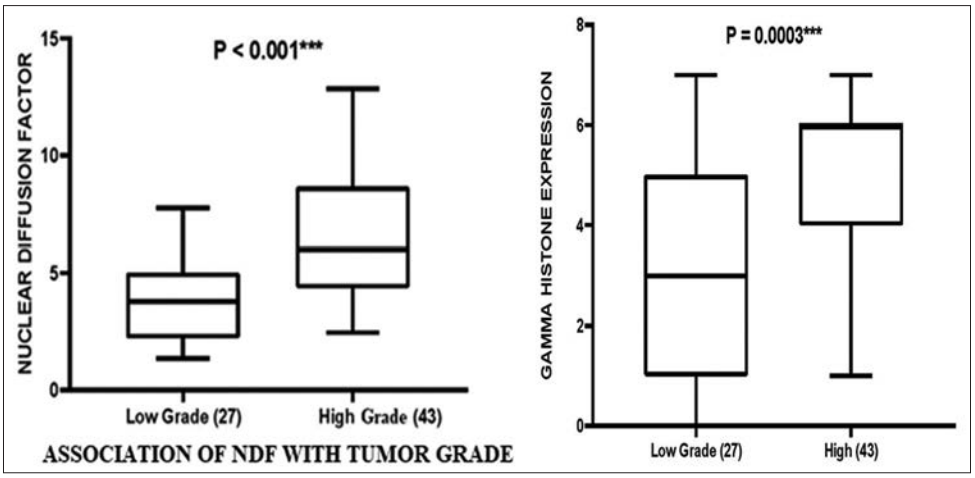


Figure 3: Box and whisker plot shows association of NDF with grade of diseases and it was seen that there is a higher value of NDF (higher DNA damage) with high-grade diseases compared to low grade with $P < 0.001$, and another box and whisker plot showing association of gamma histone expression with grade of diseases, and it was seen that expression of gamma histone is highly associated with high-grade diseases compared to low-grade diseases having $P = 0.0003$, which is statistically very significant. NDF: Nuclear diffusion factor, DNA: Deoxyribonucleic acid

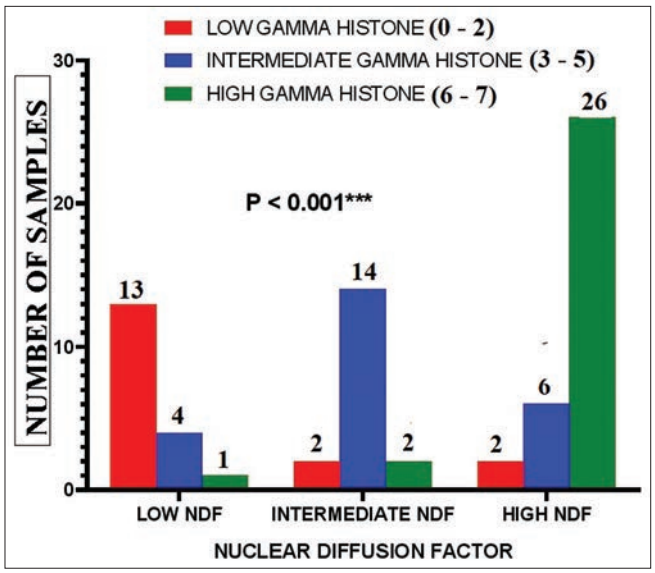


Figure 4: Histogram shows the different grades of expression of gamma histone as well as NDF. It was seen that low grade of NDF in exfoliated bladder cells was corresponding with low grade of gamma histone expression on exfoliated bladder cells, and similar pattern was seen with intermediate and high grades as well, corresponding with each other with $P < 0.001$, which is statistically significant. NDF: Nuclear diffusion factor

and high-grade diseases was studied, and it was found statistically significant [Figures 2 and 3].

Furthermore, when the correlation was studied between NDF and gamma histone, it showed positive correlation, which was statistically very significant having $P < 0.001$ [Figures 4 and 5].

DISCUSSION

We have already mentioned that the urinary bladder is invariably one of the most common cancers; the seventh-most common in developed and the ninth-most common worldwide, with around 4,29,800 new cases diagnosed in 2012, which accounts for nearly 3% of the total.[1] We have also seen the relation of tobacco intake and occupational exposure. Besides, arsenic in drinking water, infection to Schistosoma,[10] and type II diabetes mellitus[11] can also cause the diseases. Several variations at the gene and protein level have been reported independently; however, the molecular alterations caused by these carcinogens; correlated with the progression and prognosis of the diseases has not been reported in detail. Therefore, genetic and molecular progression knowledge is required for a better treatment of the diseases.

The DNA of every cell is continuously damaged by endogenous and exogenous sources, such as replication or by metabolic by-products (e.g. reactive oxygen species). In addition, factors, such as ultraviolet light, ionizing radiation, various genotoxic drugs, and environmental toxins, are capable to induce DNA lesions.[12] In contrast to other biomolecules, which are degraded and newly synthesized after alteration, DNA does not have such a constant recycling process. Instead, a variety of lesion-specific DNA damage response (DDR) mechanisms exists to restore DNA integrity. During the last decade, numerous studies uncovered diverse molecular DDR mechanisms, which have been extensively reviewed previously.[12,13]

Following DNA damage, cellular responses are initiated, and this allows the cell to repair the damage through a variety of mechanisms.[6] Therefore, DNA repair proteins are invariably important biomarkers for predicting the response of tumors to genotoxic stress and the prognosis of patients with more accuracy. This highlights the

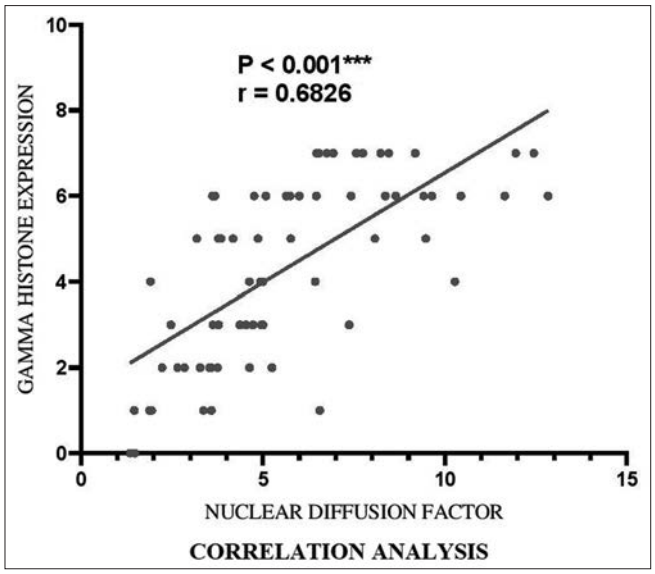


Figure 5: Scatter diagram showing positive correlation (r = 0.6826) between NDF and gamma histone expression on exfoliated bladder cells with $P < 0.001$, which is statistically very significant. NDF: Nuclear diffusion factor

importance of detecting and quantifying DNA damage. There are a number of strategies that allow the investigation of these underlying mechanisms. These techniques may be separated into two perspectives: techniques for detecting DNA damage and techniques for evaluating the underlying repair mechanism.[14]

One of the most severe types of DNA damage is DNA double-strand breaks. They are either repaired by classical or alternative nonhomologous end-joining or by homologous recombination.[15] Nonetheless, DNA damage accumulates throughout the lifetime and induces chromatin alterations in different cell types, such as tissue-specific stem cells. This may be the driving force of aging as well as for the development of numerous diseases, like malignancy.[16,17] Defect within DDR pathways has been reported to be involved in tumorigenesis and premature aging.[18,19] Several techniques can be applied to analyze DNA damage and corresponding DDR in primary human cells.

Our study is extensively based on analyzing DNA damage in exfoliated bladder cells using FHA, and to analyze DNA damage in exfoliated bladder cells using immunohistochemical analysis and finally confirm the diagnosis by histopathology and studying the correlation among these three and its diagnostic and prognostic implication of DNA damage markers, if exists.

Till now, no work was done on bladder-exfoliated cells to assess DNA damage using FHA though little work was done to assess the DNA damage on bladder tumor tissue using other methods.

Similar technique of assessing DNA damage using fast-halo method on buccal epithelial cells (BEC) was done by Mondal *et al.* in 2011 and published a study for the assessment of DNA damage by comet assay and FHA in BEC of Indian women chronically exposed to biomass smoke. A total of 161 premenopausal women aged 20 and 40 years from rural areas of West Bengal, a state in eastern India, were enrolled in the study. Analysis of DNA damage on BEC was performed by a buccal cell model of single-cell gel electrophoresis (comet assay) and by FHA. Comet assay and FHA data of the study showed significant DNA damage in BEC of relatively young and never-smoking women of rural India who were engaged in cooking exclusively with highly polluting biomass, because they cannot afford cleaner fuel.[20]

Our results were also strikingly similar as there was a positive correlation between DNA damage assessed by fast halo in bladder-exfoliated cells and DNA damage assessed by gamma histone expression on exfoliated bladder cells.

CONCLUSION

- We all know very well that the most initial step in the cascade of developing carcinoma is the DNA damage that has already occurred in the respective cell or tissue. Urinary bladder cancer is no different.
- In our study, we have assessed DNA damage in exfoliated bladder cells using the FHA method on patient's urine sample, which was easy, least cumbersome, and quick method.
- We conclude that DNA damage assessed by fast-halo method done on exfoliated bladder cells very well correlates with DNA damage assessed by gamma histone analysis method on exfoliated bladder cells.
- It was seen that when both these findings were verified with histopathological stage and grade of tumor, there was also positive correlation of higher DNA damage with high tumor grade and muscle invasive stage.
- The findings we have got using a simple patient's urine sample which could give us the enough information regarding the grade and stage of diseases.
- The information being consistent with the findings obtained from another method which was invasive and cumbersome though very little work has been done on the former method
- Our sample size was very limited, and in the past, a very few studies were undertaken on this subject, this being our limiting factor. Having said that more

intensive studies are required in the same regard to establish and further support this method.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Parkin DM. The global burden of urinary bladder cancer. Scand J Urol Nephrol Suppl 2008;42:12-20.
2. Clavel J, Cordier S, Boccon-Gibod L, Hemon D. Tobacco and bladder cancer in males: Increased risk for inhalers and smokers of black tobacco. Int J Cancer 1989;44:605-10.
3. Risch A, Wallace DM, Bathers S, Sim E. Slow N-acetylation genotype is a susceptibility factor in occupational and smoking related bladder cancer. Hum Mol Genet 1995;4:231-6.
4. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, *et al*. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 2013;63:234-41.
5. Moustacchi E. DNA damage and repair: Consequences on dose-responses. Mutat Res 2000;464:35-40.
6. Cao LL, Shen C, Zhu WG. Histone modifications in DNA damage response. Sci China Life Sci 2016;59:257-70.
7. Sestili P, Brambilla L, Cantoni O. Rotenone and pyruvate prevent the tert-butylhydroperoxide-induced necrosis of U937 cells and allow them to proliferate. FEBS Lett 1999;457:139-43.
8. Sestili P, Martinelli C, Stocchi V. The fast halo assay: An improved method to quantify genomic DNA strand breakage at the single-cell level. Mutat Res 2006;607:205-14.
9. Perrone F, Suardi S, Pastore E, Casieri P, Orsenigo M, Caramuta S, *et al*. Molecular and cytogenetic subgroups of oropharyngeal squamous cell carcinoma. Clin Cancer Res 2006;12:6643-51.
10. Gutiérrez J, Jiménez A, de Dios Luna J, Soto MJ, Sorlózano A. Meta-analysis of studies analyzing the relationship between bladder cancer and infection by human papillomavirus. J Urol 2006;176:2474-81.
11. Newton CC, Gapstur SM, Campbell PT, Jacobs EJ. Type 2 diabetes mellitus, insulin-use and risk of bladder cancer in a large cohort study. Int J Cancer 2013;132:2186-91.
12. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001;411:366-74.
13. Shaheen M, Allen C, Nickoloff JA, Hromas R. Synthetic lethality: Exploiting the addiction of cancer to DNA repair. Blood 2011;117:6074-82.
14. Figueroa Gonzalez G, Perez Plasencia C. Strategies for the evaluation of DNA damage and repair mechanisms in cancer. Oncol Lett 2017;13:3982-8.
15. Mladenov E, Magin S, Soni A, Iliakis G. DNA double-strand-break repair in higher eukaryotes and its role in genomic instability and cancer: Cell cycle and proliferation-dependent regulation. Semin Cancer Biol 2016;37-38:51-64.
16. Hoeijmakers JH. DNA damage, aging, and cancer. N Engl J Med 2009;361:1475-85.
17. Schuler N, Rübe CE. Accumulation of DNA damage-induced chromatin alterations in tissue-specific stem cells: The driving force of aging? PLoS One 2013;8:e63932.
18. Phillips ER, McKinnon PJ. DNA double-strand break repair and development. Oncogene 2007;26:7799-808.
19. Ambrose M, Gatti RA. Pathogenesis of ataxia-telangiectasia: The next generation of ATM functions. Blood 2013;121:4036-45.
20. Mondal NK, Bhattacharya P, Ray MR. Assessment of DNA damage by comet assay and fast halo assay in buccal epithelial cells of Indian women chronically exposed to biomass smoke. Int J Hyg Environ Health 2011;214:311-8.

Original Article

Acute fulminant rhino-orbito-cerebral mucormycosis: Our experience with open approach in the COVID era

Souvik Adhikari, Partha Sundar Biswas[1], Alangkar Saha[1]

Associate Professor of Plastic Surgery, [1]Department of Dentistry, Institute of Post Graduate Medical Education & Research, Kolkata, India

Abstract

Objectives: Rhino-orbito-cerebral mucormycosis (ROCM) is a quite rare and an extremely aggressive infection which can cause profound destruction of tissues. A high index of suspicion is required to diagnose and treat this infection. Although mostly occurring in diabetic or immunosuppressed patients, a sudden surge of these patients has been observed in the COVID era. Mortality is extremely high in neglected cases and can range from 40%–85% or even more. In this article, we aim to highlight patient demographics, clinical features, diagnosis, management, and outcome of fulminant ROCM in patients managed jointly by plastic surgeons and maxillofacial surgeons by the open approach.

Materials and Methods: Between May 2021 and August 2021, there was a sudden upsurge of mucormycosis patients admitted in our institute (IPGME and R, Kolkata). Less severe fulminant forms of the disease were managed by the department of otorhinolaryngology by endoscopy, whereas fulminant cases with extensive involvement were managed by the department of plastic surgery in conjunction with the department of maxillofacial surgery. The surgical management involved open access to the involved tissues and resection under direct vision combined with appropriate medical management.

Results: In total, we operated on eight cases of fulminant ROCM of which 4 patients were male and 4 females. Apart from this, two male patients and one female patient were also planned for extensive debridement surgery but the same could not be performed because of poor anesthetic risk. Biopsies were taken in all of these patients. Therefore, out of a total of 11 patients with fulminant ROCM, 9 patients did not survive taking the mortality rate in fulminant cases to 82%.

Conclusion: ROCM can be an extremely difficult disease to treat and is associated with a very high mortality rate in fulminant cases. Early diagnosis coupled with adequate debridement and systemic amphotericin B has the prospect of salvaging patients with this dreaded disease.

Keywords: COVID, diabetes, fulminant mucormycosis, mucormycosis, orbital mucormycosis

Address for correspondence: Dr. Souvik Adhikari, Department of Plastic Surgery, Institute of Post Graduate Medical Education and Research, 244 A J C Bose Road, Kolkata - 700 020, West Bengal, India.
E-mail: souvikadhikari@yahoo.com
Submitted: 10-Aug-2022, **Revised:** 22-Sep-2022, **Accepted:** 23-Sep-2022, **Published:** 23-Nov-2022.

INTRODUCTION

The Zygomycetes class of fungi causing zygomycosis is divided into two orders: Mucorales and Entomophthorales. Of these, the Mucorales are responsible for infections labeled as mucormycosis.[1] Paultauf described the first case of zygomycetes[2] while Baker first proposed the term "mucormycosis."[3]

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/amsr
	DOI: 10.4103/amsr.amsr_44_22

How to cite this article: Adhikari S, Biswas PS, Saha A. Acute fulminant rhino-orbito-cerebral mucormycosis: Our experience with open approach in the COVID era. Ann Med Sci Res 2022;1:142-9.

Mucormycosis is an opportunistic infection usually occurring in immunocompromised patients which can be life-threatening because of its aggressive nature. The most common fungi being implicated in causing infections belong to Rhizopus, Mucor, and Absidia with rhino-orbito-cerebral mucormycosis (ROCM) being most commonly caused by Rhizopus.[4] ROCM is the most common type of mucormycosis, and recently, there has been a huge surge of ROCM cases in patients who have recovered from COVID-19 infection.[5]

Most infections occur in patients who are immunocompromised or are diabetic as hyperglycemia coupled with ketoacidosis and low oxygen tension in the blood provide an excellent medium for fungal growth because of the presence of the enzyme ketone reductase in the fungus.[6] The hallmark of this infection is necrosis with minimal inflammation.[7]

Other predisposing factors implicated in the causation of this infection include the use of steroids and anti-neoplastic drugs, hepatic cirrhosis, chronic renal failure, bone marrow, and other organ transplants among others. The infection usually starts in the paranasal sinuses spreading to the orbits as well as the cranium. Cerebral involvement usually occurs via breach of the cribriform plate and through the orbital apex.[8]

Stage I of the disease involves only the nasal mucosa and paranasal sinuses (sino-nasal disease) while Stage II involves the orbit (rhino-orbital disease) and the most advanced or Stage III has cerebral involvement (orbito-cerebral disease) occurring via the ophthalmic artery or superior orbital fissure or cribriform plate.[8]

The diagnosis of the disease is usually by tissue biopsy followed by staining with GMS or H/E stains. Recently, more accurate diagnosis can be done by the polymerase chain reaction (PCR) systems.[9] The disease usually requires an magnetic resonance imaging (MRI) evaluation to delineate the extent of the disease including sinus involvement, to demonstrate blood vessel and orbital involvement as well as to evaluate the cranial extensions which can help in planning the extent of surgery.[10]

Treatment of the disease is by prompt institution of systemic antifungal therapy usually with liposomal amphotericin B instead of the deoxycholate type because of its greater effectivity (67% vs. 39% in one group of patients)[11] followed by extensive debridement. The patient can later be shifted to oral posaconazole. Surgical debridement needs to be early and aggressive coupled with local instillation of amphotericin B solution because the fungus causes thrombosis of blood vessels such that systemic antifungals are not able to penetrate into the poorly perfused tissues.[12] Despite this, the mortality rate can be pretty high for this dreaded infection and can range from 40% to 85% or even more.

In this article, we aim to highlight the patient demographics, clinical features, diagnosis, management, and outcome of extensive fulminant ROCM in our patient group. This experience may help in formulating the lines of treatment in further epidemics of this dreaded disease in case the same occur in future in conjunction with further COVID waves.

MATERIALS AND METHODS

Incidence

Between May 2021 and August 2021, there was a sudden upsurge of mucormycosis patients admitted in our institute. This was more so in the first 2 months and the trend matched with an upsurge of COVID-19-positive cases recorded in our state in the second wave of the pandemic. As the number of COVID-19 cases started declining, so did the mucormycosis cases.

Till the 1st week of August 2021, there were a total of 91 patients with acute fulminant mucormycosis admitted in the Institute of Post Graduate Medical Education and Research (IPGME and R), Kolkata, and of these, two patients were diagnosed with mucormycosis of the chest while 89 presented with ROCM. Patients with chest mucormycosis were managed jointly by the department of chest and cardiothoracic surgeries while less severe ROCM patients were managed by the department of otorhinolaryngology by endoscopic maneuvers. Fulminant cases of ROCM demonstrated by rapidly progressing disease with other systemic features and toxic appearance of the patients which had extensive involvement and were not amenable to endoscopic management were managed by the department of plastic surgery in conjunction with the department of maxillofacial surgery. Assistance was also requested from the departments of ophthalmology and neurosurgery.

Of 89 patients with ROCM, a total of 11 patients were diagnosed as extensive fulminant cases of ROCM requiring surgery through open approach and were therefore referred to us.

Management protocol

A standard management protocol was devised by the mucormycosis team created by the institute comprising

specialists in medicine, otorhinolaryngology, plastic surgery, maxillofacial surgery, neuromedicine, endocrinology, and ophthalmology. The standard operating procedure was drawn up by the Institutional Multidisciplinary Mucormycosis Review Board. The institutional ethical committee approval was obtained for the study and informed consent was obtained from every patient and their family members.

Immediately on admission, based on the clinical features and clinical suspicion, systemic amphotericin B was started in all patients even if no tissue biopsy was available. It needs mention at this stage that owing to the sudden upsurge of mucormycosis cases all over the country, liposomal amphotericin B was in acutely short supply; therefore, all of these patients received the deoxycholate form of amphotericin B in the dose of 0.5–1 mg/kg/day depending on patient tolerance. MRI was done in every patient. Biopsies were taken in all patients, but surgery was possible in only eight of them. The rest three were categorized as very poor anesthetic risks and could not be subjected to surgical debridement.

Of these 11 patients with fulminant ROCM, nine patients did not survive taking the mortality rate in extensive fulminant cases to 82%. Two patients were discharged in the stable condition with advice on continuing oral posaconazole.

Case series

Our first patient was a 64-year-old female who was diabetic and post-COVID and had been on steroids for 2 weeks. She presented with a very short history of 3 days with blindness of both eyes and respiratory distress. The right eyelid was edematous with chemosis of the right eye. A midface degloving incision was used to perform a right total maxillectomy with right orbital exenteration with subtotal maxillectomy on the left side. The nasal septum was gangrenous and had to be removed. Craniotomy was also done to debride a portion of the frontal lobe. Postoperatively, the patient expired after 4 days with refractory hypotension [Figure 1a].

The second patient was a 38-year-old male who had no evidence of COVID and had a history of diabetes and steroid use who presented with blindness of the right eye and cerebral symptoms. A right total maxillectomy with right orbital exenteration was done followed by craniotomy and clearance of frontal sinuses and frontal lobes. Culture revealed mixed growth of Mucorales and Aspergillus. The patient developed left lung consolidation following surgery and a bronchoscopy was done which revealed casts in the bronchus. The patient expired 7 days after surgery from refractory hypoxia and hypokalemia [Figure 1b].

Figure 1: Spectrum of clinical manifestations in fulminant mucormycosis. (a) Extensive involvement of facial tissues including bones with destruction of the nasal septum (arrow). (b) Craniotomy in a patient with rhinocerebral mucormycosis shows pus emanating from the frontal sinus (arrow)– culture demonstrated mixed growth of aspergillus and mucorales. (c) Extensive soft tissue involvement in a patient with mucormycosis– culture revealed mixed growth of mucorales and *Staphylococcus aureus*. (d) Coverage of defect of left orbit with a temporalis flap and skin graft

The third patient was a 62-year-old male who was a diabetic patient post-COVID infection who presented with symptoms of ophthalmoplegia left eye and upper gingival sinuses. An infrastructure maxillectomy was done but the patient expired 3 days after surgery with refractory hypotension and hypokalemia.

The fourth patient was a 52-year-old male who was also a diabetic and active COVID patient with a history of steroid use who presented with blindness of the left eye. A total maxillectomy of the left side with left orbital exenteration was done and the patient continued with amphotericin B. He had a coverage of the orbital defect 23 days after surgery with a temporalis flap. The patient was eventually discharged after a total of 48 days [Figure 1d].

The fifth patient was a 50-year-old male who presented with ophthalmoplegia and blindness of his right eye and soft-tissue involvement of the right cheek. He was also having active COVID at presentation. Biopsy was taken and radical surgery was planned, but the patient expired 3 days postadmission from severe hypoxia.

Our sixth patient was a 52-year-old female of post-COVID who presented with ophthalmoplegia of the left eye with gangrenous changes noted in the left cheek. She was severely toxic. A left infrastructure maxillectomy with

debridement of the left cheek was done. Culture revealed mixed growth of Mucorales and *Staphylococcus aureus*. The patient expired 5 days after surgery from severe metabolic acidosis and hypoxia [Figure 1c].

The seventh patient was a 46-year-old female who was a diabetic and an active COVID patient at presentation. She presented with an eschar over her left cheek and involvement of bilateral maxillae evident on MRI. Bilateral infrastructure maxillectomy with removal of the nasal septum was done. The patient expired 4 days after surgery from refractory hypotension.

Our eighth patient was a 61-year-old female who was a diabetic and had active COVID infection at presentation and was on steroids. She was toxic, disoriented and gradually passed into coma. She had right eye proptosis with ptosis and eventually expired from COVID pneumonia and acute respiratory distress syndrome.

The ninth patient was a 51-year-old male who was a post-COVID patient with a history of steroid use. He developed upper gingival sinuses followed by right maxillary pain and swelling with right pleural effusion. An infrastructure maxillectomy was done followed by pleurocentesis after a few days. He was eventually discharged after a hospital stay of 51 days.

The tenth patient was a 60-year-old male patient who was a diabetic and on steroids with no evidence of COVID who presented with ophthalmoplegia on the left side, an eschar inferior to the lower eyelid and erythema of the left cheek. He was planned for surgery but developed sudden pneumonia and MODS and expired despite all efforts.

The last patient was a 46-year-old female who was a case of active COVID with ROCM right side and was on steroids. She was initially managed conservatively and was then operated. A total maxillectomy with lid sparing orbital exenteration was done on the right side. The frontal sinuses were cleared through the inferior approach. Postoperatively, the patient had hypokalemia and hypoalbuminemia and ultimately expired 23 days after surgery.

RESULTS AND ANALYSIS

There were in total 11 patients with extensive fulminant ROCM in our case series, of which 6 patients were male and 5 were female [Table 1]. The mean age of male patients and the standard deviation was 52.17 ± 7.8. The mean age of female patients and the standard deviation was 53.8 ± 7.49. The mean age of all patients combined and the standard deviation was 52.9 ± 7.7. Out of five female patients, diabetes was present in 4 (80%) and of 6 male patients, diabetes was present in 4 (66.67%). Overall, diabetes was present in 8 out of 11 patients (72.72%).

With regard to COVID state, a diagnosis of COVID was made using reverse transcription-PCR for each patient (9 out of 11) and the mean computed tomography value with standard deviation was 23.65 ± 2.3559. Of 5 female patients, two had recovered from COVID infection while three were diagnosed with COVID at the time of presentation. In male patients, however, two patients did not have any evidence of COVID infection, two patients had recovered from COVID, while 2 were having active COVID at the time of presentation. Taken together, 9 out of 11 patients had an association with COVID infection (81.8%). 3 out of 5 female patients had a history of steroid use and 4 out of 6 male patients similarly had a history of steroid use which therefore translated into seven patients out of 11 (63.6%).

Surgery could be contemplated in 8 out of 11 patients and in the rest 3 only biopsy could be done. Surgery in our hands comprised of a midface degloving in all cases followed by radical debridement till bleeding surfaces were encountered. In four patients, unilateral orbital exenteration was performed. Two patients underwent craniotomy, but both of them expired in the postoperative period. Debridement of soft tissues of the cheek was done in cases presenting with an eschar over the cheek. Two patients had temporalis flap cover with split-thickness skin grafting to cover the orbital defects. Overall, two patients in our series with fulminant mucormycosis survived taking the mortality rate to 82%.

With regard to infection characteristics, in all patients with fulminant ROCM, paranasal sinuses were involved [Table 2]. In all cases, there was some degree of ocular involvement with 1 patient having mild chemosis only. The symptoms included chemosis, ophthalmoplegia, and in extreme cases, blindness among others. Ocular involvement was unilateral in 10 cases (right 5, left 5) and in one case was bilateral with bilateral blindness. Blindness was noted in seven cases. When culture was done, the results revealed growth of Mucorales in all cases, but in two patients, there was mixed culture with concomitant growth of Aspergillus in one and Staph aureus in the other. None of these mixed culture positive patients survived.

MRI was done in every patient [Table 3]. Conspicuous among MRI findings was the involvement of pterygopalatine

Table 1: Patient characteristics, interventions and outcome

Age	Gender	COVID state	Diabetes	Steroid use	Surgery	Outcome
64	Female	Post-COVID	Yes	Yes	Yes, bilateral maxillectomy+Rt orbital exenteration+temporalis flap+craniotomy	Death
38	Male	No	Yes	Yes	Yes, maxillectomy+Rt orbital exenteration+craniotomy	Death
62	Male	Post-COVID	Yes	No	Yes, maxillectomy	Death
52	Male	Active COVID	Yes	Yes	Yes, maxillectomy+orbital exenteration; reconstruction later with temporalis flap	Discharged in stable state
50	Male	Active COVID	No	No	No, only biopsy	Death
52	Female	Post-COVID	Yes	No	Yes, maxillectomy, cheek debridement	Death
46	Female	Active COVID	Yes	No	Yes, bilateral maxillectomy, cheek debridement	Death
61	Female	Active COVID	Yes	Yes	No, only biopsy	Death
51	Male	Post-COVID	No	Yes	Yes, maxillectomy, pleurocentesis	Discharged in stable state
60	Male	No	Yes	Yes	No, only biopsy	Death
46	Female	Active COVID	No	Yes	Yes, maxillectomy+orbital exenteration	Death

Table 2: Infection characteristics

Patient number	Paranasal sinuses involvement	Ocular involvement	Blindness	Cerebral involvement	Culture
1	Yes	Yes, bilateral	Yes, bilateral	Yes	Mucorales
2	Yes	Yes, right	Yes, right	Yes	Mucorales+Aspergillus
3	Yes	Yes, left	No	No	Mucorales
4	Yes	Yes, left	Yes, left	Yes	Mucorales
5	Yes	Yes, right	Yes, right	Yes	Mucorales
6	Yes	Yes, left	No	Yes	Mucorales+Staph aureus
7	Yes	Yes, left	Yes	Yes	Mucorales
8	Yes	Yes, right	Yes, right	Yes	Mucorales
9	Yes	Yes, right	No	No	Mucorales
10	Yes	Yes, left	No	Yes	Mucorales
11	Yes	Yes, right	Yes, right	Yes	Mucorales

Table 3: Magnetic resonance imaging findings of patients

Patient number	Paranasal sinus involvement				Pterygopalatine fossa involvement	Orbital cavity involvement	Cavernous sinus involvement	Cerebral involvement
	Maxillary	Ethmoidal	Frontal	Sphenoidal				
1	BL	BL	Right	Right	BL	BL	No	Frontal
2	Right	Right	Right	Right	Right	Right	Right	Frontal
3	Left	Left	No	Left	Left	Left	No	No
4	Left	Left	Left	No	Left	Left	No	Temporal
5	Right	Right	Right	Right	Right	Right	Right	Frontal, temporal
6	Left	Left	No	Left	Left	Left	Left	Frontal, temporal
7	BL	Left	Left	Left	BL	Left	No	Frontal
8	Right	Right	Right	Right	Right	Right	Right	Frontal, temporal
9	Right	Right	No	No	Right	No	No	No
10	Bilateral	Left	Left	Left	Left	Left	Left	Frontal
11	Right	Right	Right	Right	Right	Right	Right	Frontal

BL: Bilateral

fossa in every patient (9 unilateral and 2 bilateral). Cavernous sinus involvement was noted in six patients. Cerebral involvement was noted in nine cases with predominant involvement of the frontal (72.7% cases) and temporal (36.36% cases) lobes. This ranged from cortical edema and space-occupying lesions in seven cases to necrosis and abscess formation in two cases. However, in these patients with cranial involvement, craniotomy was done in only two patients. In the rest, expectant management was advocated with intravenous amphotericin B as their intraoperative parameters precluded extensive cranial surgery. Soft-tissue involvement varied and in some cases was extensive.

DISCUSSION

Mucormycosis, till recently, was a rare fungal infection seen mostly among immunosuppressed patients having a high mortality rate even in treated patients. However, there has been a surge of mucormycosis cases in the Indian subcontinent seen primarily during the second wave of the COVID-19 pandemic.[13] It is unclear why there has been a sudden surge of mucormycosis cases in the COVID pandemic and that too in the Indian subcontinent. There was a high proportion of diabetic patients in our study overall and maybe diabetes, combined with COVID might have led to some immune

dysregulation which paved the way for this extremely fatal infection.[14] However, this too cannot explain the problem as a very high proportion of COVID patients in the second wave was diabetic but did not suffer from ROCM, even though Mucorales spores are ubiquitous in the environment. Rampant unjustified steroid use in COVID patients might also be a factor.[8] COVID-19 infection leads to an increased serum ferritin level in patients which also provides an ideal environment for the propagation and dissemination of the fungi.[15]

ROCM is the most common form of the disease, although other systemic affections have also been noted. In patients who have presented late in fulminant cases, the mortality can be very high.[16] In our study of extensive fulminant ROCM, the mortality rate was 82%. A very high degree of suspicion is needed to diagnose the disease early and institute prompt management as this is the best chance to save the life of the patient. We encountered 11 patients with extensive fulminant ROCM in this short period of time and there were a number of factors which contributed to this late presentation of patients. First of all, clinicians have not been frequently exposed to ROCM as it is quite a rare disease; therefore, the degree of suspicion was less. It was only when the disease took an aggressive course were the patients referred to us from the peripheral hospitals. Second, many patients were treated rampantly with steroids when they had symptoms associated with COVID infection which might have led to increased incidence of the disease and possibly a fulminant course. Third, patients with COVID-positive status were isolated and COVID symptoms were given more importance when early ROCM might have been missed; therefore, the disease progressed into an advanced stage.

ROCM usually evolves through three stages: in the first stage, the nose and paranasal sinuses are involved from where the infection spreads to the orbit as the second stage. In the third stage it spreads to the brain. It can be classified into three types: granulomatous, chronic invasive, and acute fulminant.[17] Acute fulminant cases are rapidly progressing, extremely destructive in nature and are associated with a high mortality rate in patients. Accordingly, we included only those patients in our study who had a rapidly progressive disease were associated with systemic symptoms in addition to extensive local destructive manifestations and could not be managed by endoscopic maneuvers only. In all of these patients where surgery was possible (eight cases), we performed open surgery through the midface degloving incision for debridement. In three cases, only biopsy could be taken and the patients ultimately succumbed to the disease.

Suspected cases of ROCM were started on intravenous amphotericin B on admission and the biopsy was taken and subjected to KOH mount immediately. Open or endoscopic biopsy was performed in all cases and the specimens were subjected to KOH mount and histopathologic examination. Broad aseptate hyphae was most commonly found in the KOH mount and was confirmed by histopathology. In addition to this, MRI was done in every patient to determine the extent of the disease.

MRI was important as it depicted the extent of infection in the patients. A particular finding in the MRI of all patients was involvement of the pterygopalatine fossa which was bilateral in a few cases [Figure 2a]. This is a significant finding since the pterygopalatine fossa has extensive communication with the nasal cavity, masticator space, inferior orbital fissure, cavernous sinus, middle cranial fossa, and nasopharynx among others, thereby leading to a spread of infection.

MRI also depicted the extent of soft-tissue involvement and therefore guided the surgeon regarding the needed debridement to achieve clear margins [Figure 2b]. It also demonstrated the involvement of the paranasal sinuses and in many cases demonstrated sinus involvement even when it was not clinically appreciated [Figure 2c]. It also showed the involvement of critical structures as well as the extent of cranial involvement which helped in surgical planning [Figure 2d]. Cerebral involvement varied from edema in early cases to direct invasion and in extreme cases, abscess formation. Craniotomy was advocated only in those patients having abscess formation because of the

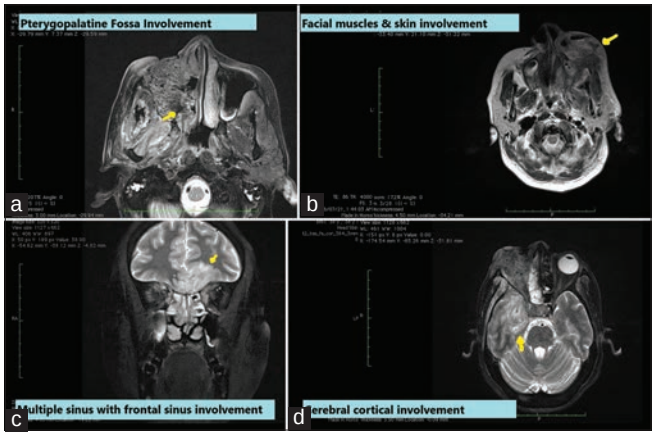


Figure 2: Various MRI manifestations in fulminant mucormycosis. (a) Extensive paranasal sinus involvement with involvement of the pterygopalatine fossa apparent in MRI. (b) Extensive involvement of facial muscles and skin apparent in MRI. (c) Involvement of multiple paranasal sinuses with frontal sinus (arrow) evident in MRI. (d) Direct cerebral cortex involvement evident in MRI. MRI: Magnetic resonance imaging

morbid nature of this surgery which enhanced the risk as these patients were severely toxic.

In the eight patients we operated on, we attempted to perform debridement to clear margins. Debridement was carried out till healthy, bleeding tissues appeared as the fungi cause thrombosis of blood vessels leading to tissue necrosis.[18] Craniotomy was performed in two patients but in the rest with cerebral involvement, craniotomy was decided against because of the very poor general condition of the patients which precluded intensive and prolonged surgery. The duration of surgery varied from 85 min to 310 min (higher duration for craniotomy patients). Immediate coverage of one orbital cavity with a temporalis muscle flap was done in only one patient and in no other patient was immediate reconstruction contemplated. Postoperatively, amphotericin B therapy was continued.

The postoperative management of patients with fulminant ROCM can be a problem because they have multiple comorbidities such as diabetes, hypertension, and so on. In addition, amphotericin B can cause renal toxicity but still needs to be continued. In all of our patients, hypokalemia developed following amphotericin B treatment despite intensive potassium supplementation. However, despite intensive surgical and medical management, the mortality rate in fulminant ROCM can be pretty high and in our series, it was 82%. Therefore, early diagnosis and management is actually the key to successful management of this dreaded disease.

Future directions

COVID-19 has resulted in an upsurge of mucormycosis in patients belonging to the Indian subcontinent. It is unclear why patients were spared during the first wave as the parallel rise and fall of this uncommon disease matched with the second wave of the COVID infection. It is also not clear why patients from a particular geographic location suffered from this dreaded disease. Further molecular and genetic analysis needs to be undertaken to highlight the risk factors in a more definitive way as we are still in the risk of encountering further waves of COVID-19 infection in the future with unknown, mutated strains. In addition, for epidemics in future where larger studies can be performed, serum ferritin, D-dimer levels and COVID antibody levels of patients can be evaluated to note if these have any correlation with fulminant mucormycosis infections.

CONCLUSION

Acute fulminant ROCM is a rare infection which usually occurs in immunosuppressed patients but which has shown an upsurge in the Indian subcontinent in the COVID era. It is extremely important that physicians dealing with COVID patients are educated to look for signs of this dreaded disease as early diagnosis and combined medical and surgical treatment is the only way to salvage patients from this dreaded disease. Once the disease assumes a fulminant course, mortality rates are extremely high and even intensive medical and surgical therapy at that stage might not be enough to prevent the death of the patient.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initial s will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Avendano MV, Conteras SA, Dhoble CY, Camacho AR, Medina M, Amari KR. A rare case of subcutaneous zygomycosis on the neck of an immunocompetent patient. Case Rep Intern Med 2015;2:57-62.
2. Paultauf A. Mycosis mucorina. Path Arch Anat 1885;102:543-64.
3. Baker RD. Mucormycosis; a new disease? J Am Med Assoc 1957;163:805-8.
4. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, *et al*. Epidemiology and outcome of zygomycosis: A review of 929 reported cases. Clin Infect Dis 2005;41:634-53.
5. Raut A, Huy NT. Rising incidence of mucormycosis in patients with COVID-19: Another challenge for India amidst the second wave? Lancet Respir Med 2021;9:e77.
6. Artis WM, Fountain JA, Delcher HK, Jones HE. A mechanism of susceptibility to mucormycosis in diabetic ketoacidosis: Transferrin and iron availability. Diabetes 1982;31:1109-14.
7. Schwartz JN, Donnelly EH, Klintworth GK. Ocular and orbital phycomycosis. Surv Ophthalmol 1977;22:3-28.
8. Wali U, Balkhair A, Al-Mujaini A. Cerebro-rhino orbital mucormycosis: An update. J Infect Public Health 2012;5:116-26.
9. Hammond SP, Bialek R, Milner DA, Petschnigg EM, Baden LR, Marty FM. Molecular methods to improve diagnosis and identification of mucormycosis. J Clin Microbiol 2011;49:2151-3.
10. Herrera DA, Dublin AB, Ormsby EL, Aminpour S, Howell LP. Imaging findings of rhinocerebral mucormycosis. Skull Base 2009;19:117-25.
11. Gleissner B, Schilling A, Anagnostopolous I, Siehl I, Thiel E. Improved outcome of zygomycosis in patients with hematological diseases? Leuk Lymphoma 2004;45:1351-60.
12. Blitzer A, Lawson W, Meyers BR, Biller HF. Patient survival factors in paranasal sinus mucormycosis. Laryngoscope 1980;90:635-48.
13. Dubey S, Mukherjee D, Sarkar P, Mukhopadhyay P, Barman D,

Bandopadhyay M, *et al.* COVID-19 associated rhino-orbital-cerebral mucormycosis: An observational study from Eastern India, with special emphasis on neurological spectrum. Diabetes Metab Syndr 2021;15:102267.
14. Sen M, Honavar SG, Bansal R, Sengupta S, Rao R, Kim U, *et al.* Epidemiology, clinical profile, management, and outcome of COVID-19-associated rhino-orbital-cerebral mucormycosis in 2826 patients in India – Collaborative OPAI-IJO Study on mucormycosis in COVID-19 (COSMIC), Report 1. Indian J Ophthalmol 2021;69:1670-92.
15. Abe F, Inaba H, Katoh T, Hotchi M. Effects of iron and desferrioxamine on Rhizopus infection. Mycopathologia 1990;110:87-91.
16. Baldin C, Ibrahim AS. Molecular mechanisms of mucormycosis-the bitter and the sweet. PLoS Pathog 2017;13:e1006408.
17. deShazo RD, O'Brien M, Chapin K, Soto-Aguilar M, Gardner L, Swain R. A new classification and diagnostic criteria for invasive fungal sinusitis. Arch Otolaryngol Head Neck Surg 1997;123:1181-8.
18. Jiang N, Zhao G, Yang S, Lin J, Hu L, Che C, *et al.* A retrospective analysis of eleven cases of invasive rhino-orbito-cerebral mucormycosis presented with orbital apex syndrome initially. BMC Ophthalmol 2016;16:10.

Case Report

Chronic urinary tract infection by biofilm-producing *Mycobacterium abscessus* following a posttraumatic laparotomy wound infection

Nupur Pal, Raja Ray, Nishar Akhtar, Prasanta Kumar Maiti

Department of Microbiology, Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India

Abstract

Mycobacterium abscessus is an emerging hospital-acquired infection, mostly found after surgical procedures and instrumentations, but urinary isolate of *M. abscessus* is rarely reported. Here we present a case of an immunocompetent individual where *M.abscessus* was isolated from a post laparotomy wound on the abdomen which recovered after treatment both clinically and microbiologically. Subsequently, *M.abscessus* was also isolated from urine when the patient presented with chronic cystitis, not responding to conventional antibiotics. Further management was planned to be done in the light of biofilm. Further management was planned to be done in the light of biofilm.

Keywords: Biofilm, chronic UTI, *Mycobacterium abscessus*

Address for correspondence: Dr. Nupur Pal, Associate Professor, Department of Microbiology, Institute of Post Graduate Medical Education and Research, 244 A.J.C.Bose Road, Kolkata - 700 020, West Bengal, India.
E-mail: docnupur80@gmail.com
Submitted: 12-Jul-2022, **Revised:** 10-Aug-2022, **Accepted:** 11-Aug-2022, **Published:** 23-Nov-2022.

INTRODUCTION

Nontuberculous mycobacteria (NTM) is an emerging pathogen in both immunocompromised and immunocompetent patients. The rising incidence and prevalence of NTM disease are becoming a major health problem in recent years.[1] After diagnosing clinically and microbiologically, one should consider the clinical significance of such findings before resorting to therapy because, besides the limited options, treatment is lengthy and varies with species, sometimes, situations become more complicated by biofilm formation and therefore poses a challenge. Proper awareness among clinicians and timely diagnosis is crucial for the appropriate management of such cases.

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/amsr
	DOI: 10.4103/amsr.amsr_39_22

CASE REPORT

A 45-year-old businessman had an accidental fall and sustained blunt trauma over the lower abdomen following which he developed peritonitis due to a small perforation in the rectosigmoid junction. Exploratory laparotomy was done with the right paramedian incision below the umbilicus for the repair of that posttraumatic perforation along with appendectomy under general anesthesia. Within 1 month, he developed a stitch abscess and had secondary suturing. However, later on, chronic discharging sinus was developed on the surgical scar, which did not respond to prolonged empirical antibiotics therapy [Figure 1a]. The patient was sent to the microbiology department. Serosanguinous discharge

How to cite this article: Pal N, Ray R, Akhtar N, Maiti PK. Chronic urinary tract infection by biofilm-producing *Mycobacterium abscessus* following a posttraumatic laparotomy wound infection. Ann Med Sci Res 2022;1:150-2.

was collected, and ZN stain and AFB culture were done. Growth appeared on LJ media after 5 days of incubation and it was morphologically different from *M. tuberculosis* [Figure 1b]. TBAg MPT-64 test (ICT) was negative. *Mycobacterium abscessus* was identified by conventional methods such as growth in MacConkey agar and 5% NaCl and negative nitrate reduction.[2] Pus was also sent outside for DNA sequencing which confirmed *M. abscessus*. The Patient was treated with clarithromycin, ethambutol and levofloxacin which is the current choice of therapy for localized disease caused by *M.chelonae* and *M. abscessus*.[3] The discharging sinus was healed after a 3-month course of the above combination therapy. After an interval of 5 months, the same patient again appeared with a repeated history of urinary incontinence and cloudy urine, not responding to conventional antibiotics. There was no history of urinary instrumentation or catheterization. His routine blood tests (complete blood count, fasting blood sugar, urea, and creatinine) were within normal limits, HIV was nonreactive, and his chest X-ray appeared normal. Multiple urinalyses demonstrated abundant neutrophils but culture showed neither bacterial nor fungal growth. No anatomical abnormality of the urinary tract was detected by ultrasonographic examination. Cystoscopic examination was conducted to rule out any iatrogenic minor/minute injury inflicted upon the urinary tract during exploratory laparotomy about 8 months back and and no abnormality was detected. Five consecutive first morning urine samples were sent from the urology outpatient department for *M. tuberculosis* and NTM species identification. Rapidly growing mycobacteria (RGM) was isolated on 3rd day of culture, and *M. abscessus* was identified by both conventional and molecular methods [Figure 2a]. The Patient was again treated withsame drugs for six months, but the symptoms persisted. The strain was tested for biofilm by 96-well microtiter plate assay[4] and found to be a strong biofilm producer [Figure 2b]. Repeat cystoscopy was suggested to note microbiofilm colonization such as pods on the bladder wall along with bladder wash with appropriate antimicrobial agents through catheter and lock for a reasonable period of time before flushing.[5] Now, the patient is under follow-up.

DISCUSSION

NTM is a large diverse group of environmental organisms, ubiquitous in water and soil, and known to produce soft-tissue infections following surgical procedures but rarely cause UTI.[1,6] Genitourinary tuberculosis is the second most common (27%) extra pulmonary tuberculosis , but urinary infections caused by nontuberculous mycobacteria are rarely reported.[7] The risk of infection with *M. abscessus* in hospitals, especially in our setting, remains high because of the lack of individual and collective hygiene. Our patient developed post laparotomy wound infection by *M. abscessus* following traumatic gut perforation. He was apparently immunocompetent. However that may be due to nosocomially acquired during laparotomy or implanted during trauma, but the pathogenesis of urinary tract infection by NTM was poorly understood. As both were the same species, though strain identity was not performed, it could not be explained how it was transmitted and why UTI developed even after successful treatment of the initial lesion. This may be due to unnoticed injury in the urinary tract during trauma or surgery, NTM implantation during manipulations, or autoinfection by the patient himself. Micropod-like multiple small biofilm colonization such as pods on bladder wall was reported by Anderson *et al.* which might be a cause of treatment refractoriness in this case.[5] During NTM treatment for postlaparotomy wound infection, only planktonic forms were removed from the bladder but persisted as a biofilm. A very high-resistant

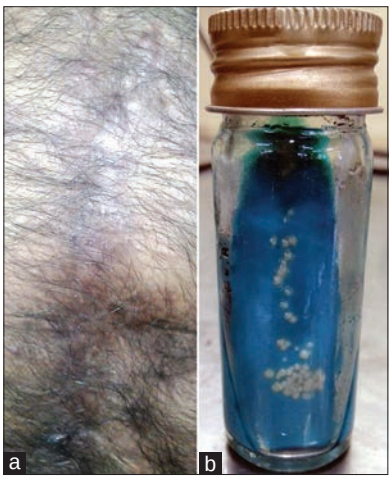


Figure 1: (a) Chronic discharging sinus from laparotomy wound (b) Growth of rapid grower mycobacteria on LJ media after 5 days of incubation

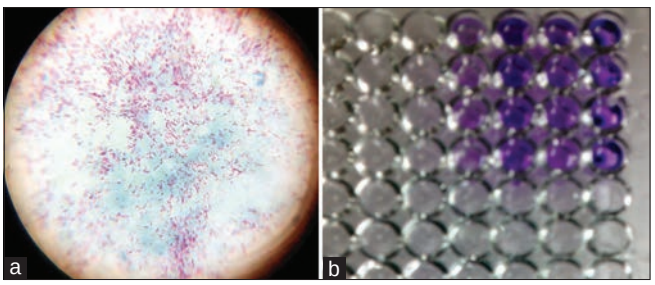


Figure 2: (a) Acid fast bacilli on ZN stain from growth on LJ media (b) *in vitro* biofilm production of *Mycobacterium abscessus* in 96 µl well method

subpopulation may remain as dormant persisters within the biofilm, it may be one of the causes of recurrence in this case.[8]

CONCLUSION

A definitive diagnosis of clinically suspected RGM infections can be made by culture of the organism, especially those unresponsive to conventional antibiotics. Biofilm formation should be suspected in case of standard treatment unresponsiveness, and an appropriate biofilm intervention approach should be considered for total eradication.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initial s will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

RNTCP fund (RNTCP OR Project).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, *et al.* An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 2007;175:367-416.
2. Set R, Shastri J. Laboratory aspects of clinically significant rapidly growing mycobacteria. Indian J Med Microbiol 2011;29:343-52.
3. Bastian S, Veziris N, Roux AL, Brossier F, Gaillard JL, Jarlier V, *et al.* Assessment of clarithromycin susceptibility in strains belonging to the *Mycobacterium abscessus* group by erm(41) and rrl sequencing. Antimicrob Agents Chemother 2011;55:775-81.
4. Coffey BM, Anderson GG. Biofilm formation in the 96-well microtiter plate. Methods Mol Biol 2014;1149:631-41.
5. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. Intracellular bacterial biofilm-like pods in urinary tract infections. Science 2003;301:105-7.
6. Sharma P, Vazquez Guillamet LJ, Miljkovic G. Atypical mycobacterial infection after abdominoplasty overseas: A case report and literature review. Case Rep Infect Dis 2016;2016:3642567.
7. Huang CT, Chen CY, Chen HY, Chou CH, Ruan SY, Lai CC, *et al.* Genitourinary infections caused by nontuberculous mycobacteria at a university hospital in Taiwan, 1996-2008. Clin Microbiol Infect 2010;16:1585-90.
8. Singh R, Ray P, Das A, Sharma M. Enhanced production of exopolysaccharide matrix and biofilm by a menadione-auxotrophic *Staphylococcus aureus* small-colony variant. J Med Microbiol 2010;59:521-7.

A case of multifocal hepatic hemangioma in a newborn

A 10-day-old, 2800 g, male infant presented with progressively increasing abdominal distension and respiratory distress since birth. The baby was dyspneic on admission with preductal oxygen saturation between 80% and 85% in room air. The baby was immediately put on noninvasive respiratory support which stabilized his breathing. On further examination, there was massive, firm, and tender hepatomegaly. Chest X-ray revealed pulmonary edema and a provisional diagnosis of congestive heart failure (CHF) was made. To evaluate the cause of CHF, few routine investigations and imaging were ordered. Abdominal ultrasonography revealed massive hepatomegaly with multiple hypoechoic nodules covering the entire liver. Echocardiography revealed biventricular dysfunction and moderate pulmonary arterial hypertension (peak systolic pulmonary pressure 56 mmHg) with bidirectional shunt through a patent foramen ovale. Differential diagnoses of the liver pathology could be either multifocal hepatic hemangioma, metastatic neuroblastoma, or hepatoblastoma. Serum alpha-fetoprotein was mildly elevated, making malignant disease unlikely.[1] To confirm the liver pathology, triple-phase contrast computed tomography (CT) imaging of the abdomen was performed which revealed the diagnosis.

Precontrast CT revealed multiple hypodense nodules in the liver [Figure 1]. After contrast injection, the arterial phase showed intense peripheral enhancement [Figure 2] and the portal venous phase revealed progressive centripetal fill-in of the hepatic nodules [Figure 3]. The above CT imaging was suggestive of hepatic hemangioma.[2,3] Further echocardiography revealed a difference in the aortic diameter before and after the origin of the celiac artery, which was also suggestive of high-flow lesions [Figure 4].[3] Histopathological diagnosis was not considered, there was a chance of hemorrhage and the imaging was typical of hepatic hemangioma. However, there was no cutaneous manifestation. Such diffuse lesions could also explain the CHF and subsequent pulmonary hypertension from ventricular dysfunction.[4]

The infant was started on propranolol and prednisolone. Fluid restriction and diuretics controlled cardiac failure. Repeat echocardiography after a week revealed improvement in ventricular function and reduction of pulmonary pressure.

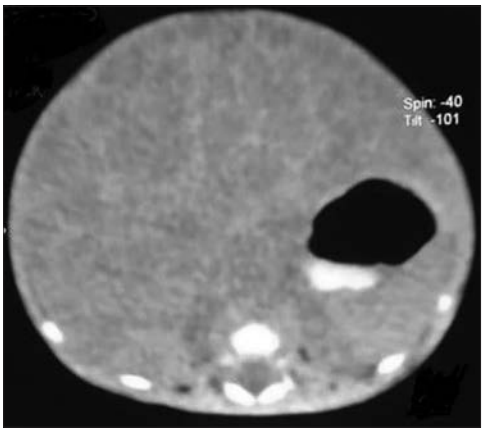


Figure 1: Precontrast CT image showing multiple hypodense hepatic nodules. CT: Computed tomography

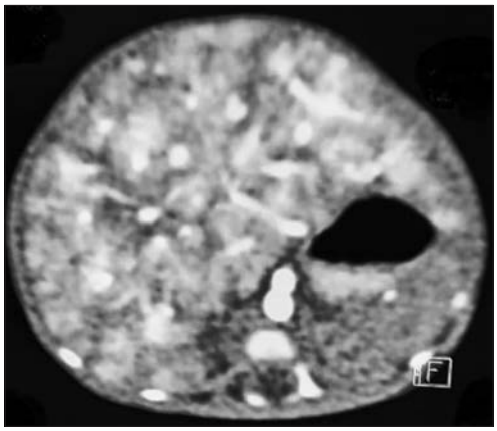


Figure 2: Arterial phase of contrast CT showing hyperintense hepatic nodules with peripheral enhancement. CT: Computed tomography

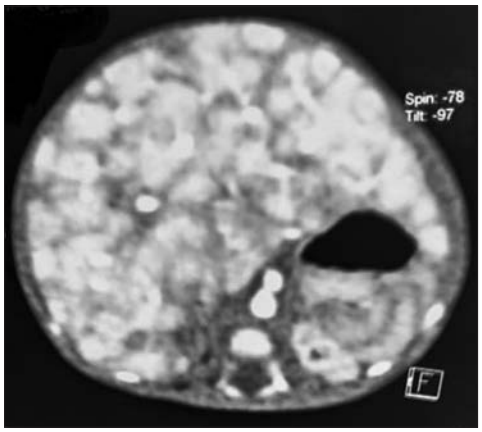


Figure 3: Portal venous phase of contrast CT showing centripetal fill-in of the hepatic nodules. CT: Computed tomography

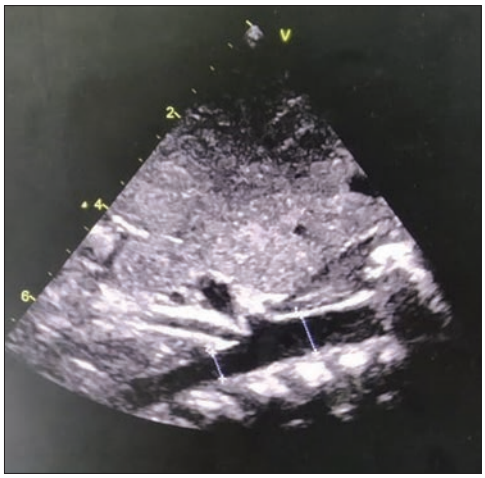


Figure 4: Difference in aortic diameter proximal (7 mm) and distal (5 mm) to the origin of the celiac artery suggestive of a high-flow lesion

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initial s will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Somnath Pal, Rajib Loshan Bora, Tanima Roy, Syamal Sardar, Suchandra Mukherjee

Department of Neonatology, Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India

Address for correspondence: Dr. Somnath Pal, Department of Neonatology, Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India.
E-mail: somnathpal1983@gmail.com

Submitted: 15-Jul-2022, **Revised:** 20-Sep-2022, **Accepted:** 21-Sep-2022, **Published:** 23-Nov-2022.

REFERENCES

1. Sari N, Yalçin B, Akyüz C, Haliloglu M, Büyükpamukçu M. Infantile hepatic hemangioendothelioma with elevated serum alpha-fetoprotein. Pediatr Hematol Oncol 2006;23:639-47.
2. Tsai MC, Liu HC, Yeung CY. Efficacy of infantile hepatic hemangioma with propranolol treatment: A case report. Medicine (Baltimore) 2019;98:e14078.
3. Kassarjian A, Zurakowski D, Dubois J, Paltiel HJ, Fishman SJ, Burrows PE. Infantile hepatic hemangiomas: Clinical and imaging findings and their correlation with therapy. AJR Am J Roentgenol 2004;182:785-95.
4. Zhang XT, Ren WD, Song G, Xiao YJ, Sun FF, Wang N. Infantile hepatic hemangiomas associated with high-output cardiac failure and pulmonary hypertension. BMC Cardiovasc Disord 2019;19:216.

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/amsr
	DOI: 10.4103/amsr.amsr_40_22

How to cite this article: Pal S, Bora RL, Roy T, Sardar S, Mukherjee S. A case of multifocal hepatic hemangioma in a newborn. Ann Med Sci Res 2022;1:153-4.

Editor-in-Chief Prof.(Dr.) Dilip Kumar Pal, Printed and Published by Wolters Kluwer India Private Limited on behalf of Institute of Post Graduate Medical Education and Research and printed at Nikeda Art Printers Pvt. Ltd., Kanjur Ind Est, Quarry Rd, Near Mangatram Petrol Pump, Bhandup (W), Mumbai, and published at A-202, 2nd Floor, The Qube, C.T.S. No.1498A/2 Village Marol, Andheri (East), Mumbai - 400 059, India.