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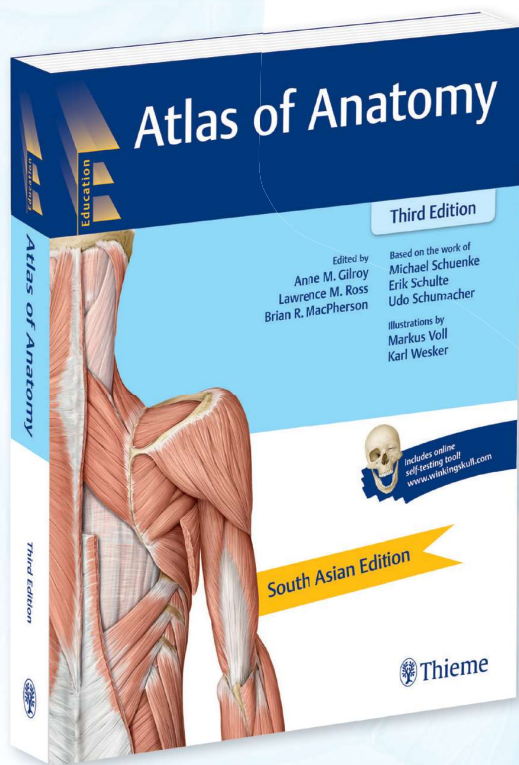
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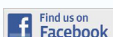
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Editorial

Annals of the National Academy of Medical Sciences (India) in a New Incarnation

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Ann Natl Acad Med Sci (India) 2019;55:1–2

It is a turning point in the journey of the National Academy of Medical Sciences (India), which is collaborating with the world renowned Thieme Medical Publishers. This partnership would not only energize our periodical but also help in making a significant impact on the national and international readership consisting of health scientists and professionals. By joining Thieme Medical Publishers, the Academy looks forward to globalization of Indian medical research and disseminating the information related to the initiatives of Ministry of Health and Family development to every corner of the country and also to an international level.

The academy wishes to inform that the new website of the journal will be www.thieme.com/anams. We wish to encourage potential authors to submit their articles at www.manuscriptmanager.net/anams.

On this occasion, it would be indeed worthwhile to inform readers about the remarkable history of the Academy, its objectives and its official journal.

The Government of India decided to establish a separate Department of Health Research in the erstwhile Ministry of Health (MoH), which is now the Ministry of Health and Family Welfare (MoHFW), and authorized it to establish institutions for Continuing Medical Education (CME) in the country. Thereafter, a society named “Indian Academy of Medical Sciences” was founded under the Department of Health Research in MoHFW, akin to the prestigious Indian Council of Medical Research (ICMR). The Memorandum of Association, at the time of the registration of the academy as the “Indian Academy of Medical Sciences,” was signed by Drs. B.K. Anand, Col. Sangham Lal, K. L. Wig, Gen. Amir Chand, S. K. Sen, V. Ramalingaswami, B.L. Taneja, and R. Viswanathan. It was registered on April 21, 1961, with the Registrar of Societies, Delhi as the “Indian Academy of Medical Sciences.”



Saroj Chooramani Gopal

Dr. Rajendra Prasad, the first president of India, honored the academy by becoming its patron-in-chief.

The first prime minister of India, Shri Jawaharlal Nehru, formally inaugurated the academy at the Sapru House on December 19, 1961. To commemorate the occasion, he was bestowed with the Honorary Fellowship of the Academy.

On November 16, 1976, the name of the society was changed to “National Academy of Medical Sciences (India).” The new name was registered at the office of the Registrar of Societies, Delhi Administration, New Delhi.

Since its inception, the objectives of the National Academy of Medical Sciences (India) are as follows:

1. Promotion of knowledge of medical sciences in India and its practical application to problems plaguing national welfare.
2. Recognizing and encouraging merit across all branches of medical sciences.
3. To ensure coordination between medical and other scientific academies, societies, associations, institutions, as well as government medical and scientific departments and services.
4. To seek help and cooperation of international agencies and national bodies of other countries.
5. To act through properly constituted National Committees in order to deal with medical scientific subjects, and undertake medical scientific work of national and international importance as the Academy may be called upon to perform by the public and the Government.
6. To publish such proceedings, journals, memoirs, transactions, and other publications as may be found desirable.

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From extreme left to right: Team Thieme along with Dr. Sunny Duttgupta (third from the left), Dr. Deep N. Srivastava (Secretary NAMS and Editor ANAMS), Dr. KK Sharma (Advisor NAMS and Editor ANAMS), Dr. Shilpa Sharma, and members of the Editorial Team, NAMS.

7. To promote and maintain a liaison between medicinal and other sciences by way of different associations and societies, and establish inter-association/inter-society partnership and collaboration.
8. To develop pattern(s) of high-level postgraduate examinations, at the all-India basis across various disciplines of medical sciences, to ensure a uniform standard of postgraduate medical qualifications, for which appropriate machinery would be required to become operational.
9. To secure and manage funds and endowments for the promotion of the objectives of the academy.
10. To undertake any other steps that may assist in, be conducive to, or be necessary for the fulfilment of the above-mentioned aims and objectives of the academy.

In 1975, the working group appointed by the Government of India recommended the setting up of a National Board of Examinations (NBE), and the responsibility of conducting high-level postgraduate examinations on an all-India basis across various disciplines of medicine was entrusted to the academy. The President of the academy was designated as the president of the NBE.

NBE, since its inception, functioned in close association with the National Academy of Medical Sciences (India), which was presided over by the president of the academy from 1975 to 1982. However, the two bodies were separated as legal entities by the MoHFW in March, 1982. The board was reconstituted and the president of the National Academy of Medical Sciences (India), who had been till then the president of the NBE, was included as one of the members of the board. Till August 1982, the postgraduate medical qualification of "MAMS" was awarded through the examination conducted by the academy. Subsequently, from February 1983 onward, the "MAMS" examinations were conducted by the NBE; instead of "MAMS," the "Diplomate of NBE (DNB)" was awarded. By a decision taken by the NAMS Council, the DNB holders of NBE are eligible to become members of NAMS and will be provided with the Scroll of "MNAMS."

The academy conducts an Annual Conference, NAMSCON, in different parts of the country to commemorate its activities

as well as disseminate recent advances in medical and health sciences by inviting a wider audience of health professionals on a regional basis. Besides, the NAMS, to maintain its mandate given by the MoHFW, acts as a promoting agency for CME to advocate "Knowledge to Action." To accomplish this, the academy provides financial support to not only organize the CMEs but also allocate financial grants to the junior and middle level bio-medical scientists to hone their research and professional skills by learning through hands-on experience in the chosen institution of eminence in the country. On their return to their native institutions, they are more equipped to take care of the health needs of their patients and health seekers.

The academy also recognizes merit and awards fellowships (FAMS) to senior scientists with an exemplary and consistent track record, and membership (MAMS) to junior and mid-level scientists among the biomedical and health professionals alike. The process of selection is conducted keeping in mind very stringent criteria, and domains are vetted at multiple levels of screening by senior experts in the field.

This year, the official journal of the academy, the *Annals* has received a facelift to reach the height of a new horizon. We hope the readers would appreciate this change and contribute to the growth of the journal and the academy in achieving its objectives. We sincerely look forward to receiving content from enthusiastic researchers and clinicians. Supported by Thieme Medical Publishers, the Academy under the aegis of Ministry of Health and Family Welfare will continue to effectively contribute towards healthcare initiatives and clinical research in the country.

Conflict of Interest

None declared.

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The help rendered by Dr. Shilpa Sharma, MNAMS, Associate Professor, Department of Pediatric Surgery, AIIMS, New Delhi by way of providing significant inputs in preparing this editorial is highly appreciated and acknowledged.

Musculoskeletal Tuberculosis: A Multifaceted Foe

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Abstract

Tuberculosis (TB) still remains a global epidemic, and India accounts for one-fourth of the world's TB burden. The incidence of extrapulmonary TB has relatively remained constant, but with the introduction of antitumor necrosis factor, there has been a surge in pulmonary and extrapulmonary TB cases presenting to rheumatologists. Musculoskeletal TB accounts for 10 to 30% of all cases of extrapulmonary TB, with spondylitis (Pott's spine) being the most common manifestation. Manifestations mimicking autoimmune spondylitis are seen in 10% of cases. Tubercular arthritis most commonly presents with large joint monoarthritis, but oligo- or polyarticular involvement is also seen. Poncet's disease is a form of reactive arthritis occurring in patients with pulmonary or extrapulmonary TB, which is rarely seen with good response to antitubercular treatment. Quite often, there is delay in the diagnosis of musculoskeletal TB due to absence of constitutional symptoms. Treatment of musculoskeletal TB involves prolonged course of antitubercular treatment, and surgical interventions are limited to special cases.

Keywords

- ▶ musculoskeletal tuberculosis
- ▶ sacroiliitis
- ▶ enthesitis

Introduction

Tuberculosis (TB) still remains a global epidemic, killing 1.4 million people annually worldwide, with more than a third of the deaths occurring in India.¹ India accounts for one-fourth of the world's TB burden, with an annual incidence of 28 lakhs and mortality of 4.8 lakhs for the year 2015.¹ The incidence of extrapulmonary TB (EPTB) has relatively remained constant at 17 to 20% in the past decade in India.¹ With the introduction of tumor necrosis factor inhibitors (TNFi) in 1999, there was a surge in both pulmonary TB (PTB) and EPTB, and an unexpectedly high number of TB cases started presenting to rheumatologists. This is particularly applicable to EPTB and disseminated TB, which showed a disproportionate increase in incidence on treatment with TNFi and other biologics. The percentage of EPTB among all TB cases in TNFi treated patients in various case series is as high as 57 to 62%.^{2,3}

Musculoskeletal TB (MSKTB) comprises around 10 to 30% of all EPTB cases.^{4,5} Diagnosis of MSKTB often becomes challenging due to its rarity at presentation and its often indolent presentation without typical features of fever and other

constitutional symptoms characteristic of TB or other infections. Furthermore, it is complicated by the fact that tissue sample may not be always be accessible in case of skeletal TB, and less than 50% cases of MSKTB have features of present or past PTB. MSKTB is acquired hematogenously, or less commonly, by direct inoculation through trauma or local spread from infected surrounding structures such as lymph nodes. Clinically, MSKTB most commonly presents as tubercular spondylitis or Pott's spine (50–60%). Other forms of presentation are osteomyelitis, septic arthritis, soft tissue abscesses, and rarely, reactive arthritis (Poncet's disease).

Spinal TB most commonly involves the lower dorsal or lumbar spine and most patients present with long-standing back pain with or without associated constitutional symptoms. This can often be confused with spondyloarthritis, especially considering the fact that sacroiliitis is seen in around 10% cases of spinal TB. Infections such as TB and brucellosis should always be kept in the diagnostic consideration when dealing with a case of unilateral sacroiliitis. Tubercular spondylitis can have extra-articular extension into surrounding structures causing soft tissue cold abscesses such as psoas abscess or retropharyngeal abscess. Tubercular spondylitis



most often starts in the anterior vertebral body and may later spread to involve posterior spinal elements and intervertebral discs. This may lead to anterior wedge vertebral collapse and gibbus formation. The more dreaded complication of neurologic involvement in the form of myelopathy, radiculopathy, or cauda equina syndrome is seen in around 30% of the cases and may need urgent intervention with stabilization of the spine. The American Spinal Injury Association (ASIA) Impairment Scale can be used to document the degree of neurodeficit in Pott's spine, with increasing severity of neurologic compromise from ASIA (E) to ASIA (A).

Apart from the vertebral body, tubercular osteomyelitis can involve other bones in the body too, especially the long bones such as femur and tibia. Tubercular osteomyelitis generally starts in the metaphysis and may spread contagiously to involve the adjacent joint such as hip or knee. Less commonly, involvement of ribs, pubic symphysis, phalanges, and skull can also be seen. Patients typically present with bone pain. Other associated symptoms may be localized swelling, abscess, or sinus formation. Although TB osteomyelitis is mostly unifocal, rarely it can present as multifocal osteoarticular TB with destructive lesions of multiple bones and joints.

Tubercular arthritis most commonly presents as large joint monoarthritis involving the hip or knee, although practically any joint of the body including sacroiliac joint, costovertebral joint, ankle, shoulder, elbow, and small joints of hand and feet can be involved. Tubercular oligo- or polyarthritis is less common and may be seen in around 10% of the cases. Tubercular arthritis can be destructive in nature if antitubercular therapy (ATT) is not instituted early in the course of disease. Prosthetic joint TB infections have rarely been reported.

Poncet's disease is a rare form of acute-onset symmetrical inflammatory oligo- or polyarthritis involving commonly the knees, ankles, elbows and wrists. It is associated with active PTB or EPTB. Poncet's disease does not involve direct TB invasion of joints and is a form of reactive arthritis, and, therefore, TB bacilli cannot be demonstrated in the involved joints. It is quite rare, and to date, around 200 cases of Poncet's disease have been reported, 70 of which are from India.⁶ Unlike tubercular arthritis, Poncet's disease is a form of nondestructive arthritis and has a very rapid response to initiation of ATT. A similar kind of presentation has also been reported in patients treated with intravesical Bacillus Calmette–Guerin for bladder malignancy.⁷

Other forms of MSKTB include mostly abscesses in the form of epidural abscess or soft tissue abscess such as psoas abscess or retropharyngeal abscess. Soft tissue and osteoarticular involvement of hand may rarely give rise to dactylitis in TB.

Imaging may guide us in suggesting a diagnosis of TB and also help in delineating the extent of disease, although there are no pathognomonic imaging findings in MSKTB. Conventional radiography may show skeletal and soft tissue changes in cases of well-established disease. In Pott's disease, radiological abnormalities start in the anterior vertebral body and progress later to involve the posterior segments of the vertebra and intervertebral discs. Changes commonly seen

on X-rays are loss of definition of bony margin, osteolysis, gibbus deformity, disc space narrowing and obliteration, and paraspinal abscess and calcification. Most cases involve lower dorsal or lumbar vertebra, with involvement of contiguous segments. Unilateral sacroiliitis can be seen in around 8 to 10% of cases. TB osteomyelitis lesions mostly appear as cystic or lytic lesions in metaphyses of long or flat bones. Tubercular bony lesions can be destructive, and the presence of lytic lesions may often lead the physician into suspicion of a malignancy.⁸ The radiographic findings in tubercular arthritis demonstrate periarticular osteopenia, peripheral osseous erosions, and joint space narrowing (Phemister's triad). A chest X-ray must always be performed in suspected cases of MSKTB to demonstrate the presence of typical lesions of PTB, if present, although less than 50% of cases show involvement of the lung. Compared with conventional radiography, magnetic resonance imaging (MRI) is much more sensitive to detect bony and soft tissue lesions. MRI helps in the early detection of TB changes in bone, with better visualization of the extent of disease and soft tissue spread. MRI has become an indispensable tool in cases of Pott's spine for the detection of spinal cord compression and neurologic involvement. Other imaging modalities such as CT scan, myelography, and scintigraphy can be used too for the detection of TB. CT scan is often used to obtain imaging-guided biopsy from the bone, synovium, or soft tissue.

The tuberculin skin test or Mantoux test is one of the oldest tests for TB and is still used as one of the screening tests for both PTB and EPTB. In rheumatological practice, it is also used as a screening test for detecting latent TB infection before the initiation of TNFi or other biologics. It is neither sensitive nor specific for the diagnosis of TB and only has an ancillary role in establishing a diagnosis of TB.

Staining of synovial fluid and other tissues for acid-fast bacilli by Ziehl–Neelsen staining has very poor sensitivity in cases of MSKTB, with being positive in only around 15 to 20% cases. Histopathology and culture are most often used for diagnosis. Synovial tissue culture demonstrates a higher sensitivity than synovial fluid culture and must be obtained in cases of suspected tubercular arthritis if synovial fluid assay is noncontributory. Histopathology of the synovium or bone may demonstrate caseating granuloma with or without acid-fast bacilli. Cultures can also be obtained from infected fluids such as synovial fluid, cold abscess, or draining sinuses. Due to the slow-growing nature of *Mycobacterium tuberculosis*, culture results may take up to 42 days to yield results.

Newer methods to help in the diagnosis of TB include interferon- γ release assays (IGRAs) and nucleic acid amplification techniques (NAATs). IGRA involves various methods that measure mononuclear cell interferon- γ production on stimulation with tubercular antigens. In India, the method used is QuantiFERON-TB Gold (Qiagen). It is also commonly used for latent TB screening and can be repeated annually in patients on biologic therapy for checking IGRA conversion. NAATs provide a rapid, sensitive, and specific method of TB diagnosis, with a sensitivity of more than 85% and a specificity of 100% in cases of MSKTB. Nested or multiplex polymerase chain reaction (PCR) methods have now mostly been replaced

in most places by Gene Xpert MTB/RIF, which, in addition to PCR, also gives information on rifampicin resistance in diagnosed cases. In spite of added advantages, availability and cost limit its use in resource-constraint countries like India.

Treatment of MSKTB involves prolonged courses of ATT, with surgical intervention reserved for special cases. The Revised National Tuberculosis Control Program (RNTCP) recommends a 2-month intensive phase with isoniazid (H), rifampicin (R), pyrazinamide, and ethambutol (E) followed by 4 months of continuation phase with HRE for newly diagnosed cases just like PTB. However, in cases of MSKTB, the continuation phase might be extended by 12 to 24 weeks depending on the clinical decision of the treating physician based on response. The RNTCP is currently moving from its thrice weekly ATT regimen to daily regimen. It has already been implemented for pediatric TB and TB with HIV. For adult TB, it has been started initially in 104 districts.⁹ Most cases of MSKTB need treatment for 9 to 15 months for complete cure of the disease. The appearance of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB has posed new challenges in their treatment and requires prolonged courses of multiple second-line agents, sometimes for 30 to 36 months.¹

Surgical intervention is reserved for patients with Pott's spine with new or progressive neurodeficit or severe spinal deformity and in cases of large or nonresponding cold abscesses. Surgery can also be performed in undiagnosed cases for large tissue samples to aid in diagnosis. A wide variety of options such as decompression, radical focal debridement, and instrumental spine stabilization exist, and it is important to individualize the procedure on a case-to-case basis. Surgical debridement may also be helpful in MDR-TB cases. In cases of peripheral arthritis, total knee or hip arthroplasty might be undertaken only after successful completion of ATT. It is advisable to perform surgery after a prolonged gap, preferably more than a year after completion of therapy. In

cases, where adequate gap cannot be given, ATT is continued for 3 months after surgery. Tissue should be obtained during surgery for TB culture, and prosthetic joint infection is uncommon if the intra-operative tissue is culture negative.

Quite often, there is delay in the diagnosis of MSKTB due to absent constitutional symptoms and its oligo- or polyarticular presentation. Awareness and high index of suspicion can avert this delay in a vast majority of patients.

Conflict of Interest

None declared.

References

- 1 Central Tuberculosis Division, Government of India. Available at: <http://tbcindia.gov.in/WriteReadData/tbIndia2017.pdf>. Accessed July 10, 2019
- 2 Keane J, Gershon S, Wise RP, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001;345(15):1098–1104
- 3 Dixon WG, Hyrich KL, Watson KD, et al; B S R B R Control Centre Consortium; BSR Biologics Register. Drug-specific risk of tuberculosis in patients with rheumatoid arthritis treated with anti-TNF therapy: results from the British Society for Rheumatology Biologics Register (BSRBR) *Ann Rheum Dis* 2010;69(3):522–528
- 4 Sharma SK, Mohan A. Extrapulmonary tuberculosis. *Indian J Med Res* 2004;120(4):316–353
- 5 Peto HM, Pratt RH, Harrington TA, LoBue PA, Armstrong LR. Epidemiology of extrapulmonary tuberculosis in the United States, 1993–2006. *Clin Infect Dis* 2009;49(9):1350–1357
- 6 Rueda JC, Crepy MF, Mantilla RD. Clinical features of Poncet's disease. From the description of 198 cases found in the literature. *Clin Rheumatol* 2013;32(7):929–935
- 7 Macía Villa C, Sifuentes Giraldo W, Boteanu A, González Lanza M, Bachiller Corral J. Reactive arthritis after the intravesical instillation of BCG. *Reumatol Clin* 2012;8(5):284–286
- 8 Tsay MH, Chen MC, Jaung GY, Pang KK, Chen BF. Atypical skeletal tuberculosis mimicking tumor metastases: report of a case. *J Formos Med Assoc* 1995;94(7):428–431

Pancreatic Incidentalomas: Review and Current Management Recommendations

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Abstract

There has been significant increase in the detection of incidental pancreatic lesions due to widespread use of cross-sectional imaging like computed tomography and magnetic resonance imaging supplemented with improvements in imaging resolution. Hence, accurate diagnosis (benign, borderline, or malignant lesion) and adequate follow-up is advised for these incidentally detected pancreatic lesions. In this article, we would review the various pancreatic parenchymal (cystic or solid) and ductal lesions (congenital or pathological), discuss the algorithmic approach in management of incidental pancreatic lesions, and highlight the key imaging features for accurate diagnosis.

Keywords

- ▶ duct
- ▶ incidentaloma
- ▶ pancreas
- ▶ pancreatic cyst

Introduction

The term “pancreatic incidentaloma” (PI) refers to those lesions in the pancreas that are diagnosed incidentally when obtaining an imaging study of the abdomen not intended to look for a pancreatic pathological process.¹ This phenomenon is not new to the pancreas and has been quite often observed in other organs such as adrenal glands, thyroid, parathyroid, pituitary, liver, prostate, and kidneys. The first case of PI was described by Kostiuk in 2001.² Incidental pancreatic cysts are frequently encountered now due to advancements and easy availability of imaging. The reported incidence of incidental cystic pancreatic lesions varies, depending on the imaging technique used. In computed tomography (CT), the prevalence varies from 0.5 to 3%, whereas in magnetic resonance imaging (MRI), this prevalence increases to 18 to 19.6%.^{3,4} In a postmortem study by Kimura et al, cysts less than 1 cm in size were detected in 24% of cases.⁵ Recent published studies have shown that the incidence of incidental PI is rising.⁶

When a PI is encountered, the first aim is to classify whether it is a pancreatic parenchymal or ductal lesion. PIs in the pancreatic parenchyma can be either cystic or solid. Incidental lesions in the main pancreatic duct (MPD) can be congenital variations or a pathological process involving the

MPD. The second aim is to further classify the lesion into benign or malignant. Various cystic and solid incidentalomas in the pancreatic parenchyma are listed in ▶Table 1.⁷

Incidental Cystic Pancreatic Tumors

Most incidental cystic pancreatic lesions are benign.^{8,9} The first step is to differentiate these cystic lesions from pancreatic pseudocysts. Serous cystadenoma, mucinous cystic lesions, and intraductal papillary mucinous neoplasms (IPMNs) account for more than 90% of primary cystic pancreatic tumors.¹⁰ Most of the patients are asymptomatic at the time of presentation. Symptomatic patients may present with abdominal pain, jaundice, weight loss, and/or recurrent episodes of pancreatitis. Morphological classification of cystic lesions of the pancreas is given in ▶Table 2.¹¹

Unilocular Cysts

Pancreatic pseudocysts are the most commonly encountered unilocular cysts. Others include mucinous and serous cystadenoma, lymphoepithelial cysts, retention cyst, developmental cyst, epidermoid cyst in intrapancreatic spleen, endometrial cyst, and cystic neuroendocrine tumor or an infectious cyst (▶Figs. 1 and 2). A unilocular lesion in

Table 1 Pancreatic incidentalomas⁷

Exocrine	Infections
Benign SCN MCN IPMN Mature cystic teratoma	<i>Mycobacterium avium</i> complex <i>Mycobacterium tuberculosis</i> Rare and atypical fungal and viral infections
Borderline Mucinous cystic tumor with moderate dysplasia Intraductal papillary mucinous tumor with moderate dysplasia SPN	Mesenchymal tumors Kaposi's sarcoma Lipoma Lymphangioma Pancreatic Castleman's disease Pancreatic hamartoma Schwannoma Teratoma
Malignant Ductal adenocarcinoma Osteoclastlike giant cell tumor Serous cystadenocarcinoma Mucinous cystadenocarcinoma IPMN Acinar cell carcinoma Pancreatoblastoma Solid pseudopapillary carcinoma Ampullary adenocarcinoma	Metastases Colon, breast, lung, lymphoma, melanoma, renal cell carcinoma
Endocrine	Nonislet cell tumors
Gastrinoma Glucagonoma GRF-secreting tumor Insulinoma PP-secreting tumor Somatostatinoma VIPoma Serotoninoma	Adenosquamous carcinoma Anaplastic tumors Colloid carcinoma Granulocytic sarcoma Leukemia Lymphoma Primitive neuroectodermal tumor
Cystic lesions	Inflammatory
Benign pancreatic cysts Dysontogenic cysts Hydatid cyst Cysticercosis LECs Pancreatic dermoid cysts Retention pancreatic cysts	Eosinophilic pancreatitis Focal pancreatitis Inflammatory myofibroblastic tumor Lymphoid hyperplasia Wegener's disease Xanthogranulomatous pancreatitis
Congenital	
Congenital cyst Epidermoid cyst in IPAS	

Abbreviations: GRF, growth hormone releasing factor; IPAS, intrapancreatic accessory spleen; IPMN, intraductal papillary mucinous adenoma; LEC, lymphoepithelial cyst; MCN, mucinous cystadenoma; PP, pancreatic polypeptide; SCN, serous cystadenoma; SPN, solid pseudopapillary tumor; VIPoma, pancreatic neuroendocrine tumor that secretes vasoactive intestinal peptide.

a patient with a clinical history of pancreatitis is almost always a pseudocyst. A lobulated unilocular cyst located in the head of the pancreas should raise the suspicion of a serous cystadenoma.

Table 2 Classification according to imaging morphology¹¹

Unilocular	Microcystic
Pseudocyst Cystic neuroendocrine tumor Unilocular serous cystadenoma Unilocular mucinous cystadenoma Retention cyst Developmental cyst Epithelial cyst Lymphoepithelial cyst Epidermoid cyst in intrapancreatic accessory spleen Endometrial cyst Infectious cyst	Serous cystadenoma Microcystic variant of ductal adenocarcinoma (very rare)
Macrocystic	Cystic transformation of the pancreas
Mucinous cystadenoma BD-IPMN Oligocystic serous cystadenoma Lymphangioma Lymphoepithelial cyst Infectious cyst Duplication cyst Mesothelial cyst	Dysontogenetic cyst Cystic fibrosis Disseminated serous cystadenoma Congenital syndromes such as von Hippel-Lindau's disease, polycystic kidney disease, Ivemark's syndrome, trisomy 13 or 15, Meckel-Gruber's syndrome
Cyst with ductal communication	Multifocal
IPMN Collections postpancreatitis as a part of disconnected duct syndrome Retention cyst/squamoid cyst	BD-IPMN Pseudocysts Serous cystadenoma Neuroendocrine tumor Developmental cyst Epithelial cyst
Solid cystic	
SPN Pancreatoblastoma Cystic metastasis Cystic degeneration in solid tumors Malignant transformation in cystic tumors Metastases Hemorrhagic pseudocyst	

Abbreviations: BD-IPMN, branch-duct intraductal papillary mucinous neoplasm; IPMN, intraductal papillary mucinous neoplasm; SPN, solid pseudopapillary tumor.

Microcystic Lesions

The most important differential diagnosis of a microcystic lesion in the pancreas is serous cystadenoma. It usually demonstrates a polycystic or microcystic pattern consisting of cysts up to 2 cm in size. They are usually lobulated. The septa and wall may show enhancement (► Fig. 3). A stellate pattern of calcification is visible in 30% of the patients and is considered a characteristic of a serous cystadenoma.¹² A rare

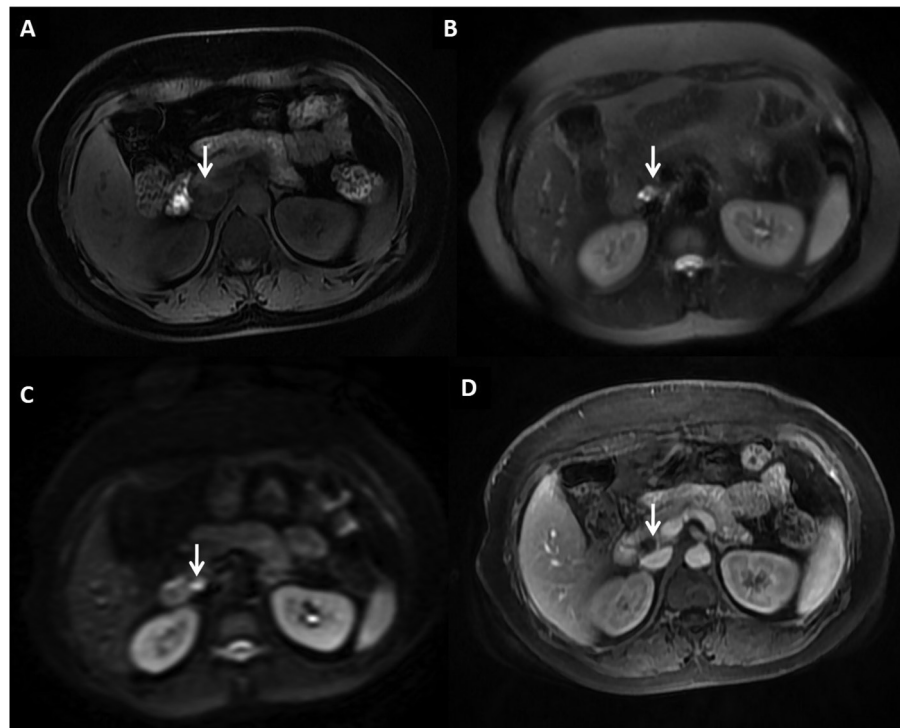


Fig. 1 A 43-year-old-female with incidentally detected lymphoepithelial cyst postcholecystectomy in the pancreas. (A) Axial T1-weighted magnetic resonance imaging showing unilocular cystic lesion, which is mildly hyperintense in the head of the pancreas (B) and hyperintense on T2-weighted images (C), showing bright signal on diffusion-weighted imaging (D) with no enhancement on T1-weighted postcontrast gradient echo images.

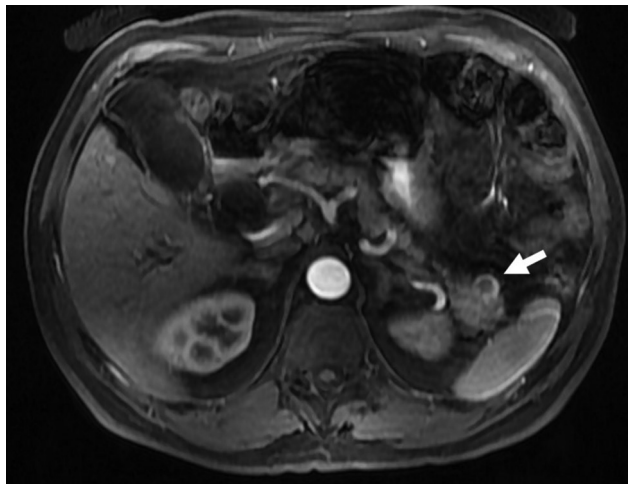


Fig. 2 A 60-year-old male with incidental cystic pancreatic neuroendocrine tumor. Axial postcontrast T1-weighted image showing incidental unilocular cystic lesion (arrow) in the tail of the pancreas, showing peripheral arterial enhancement suggestive of cystic neuroendocrine tumor.



Fig. 3 A 68-year-old-female with incidental serous cystadenoma. Axial contrast-enhanced computed tomography image showing polycystic lesion in the head of the pancreas (arrow) with thin internal septations in a case of serous cystadenoma.

differential diagnosis of a microcystic lesion in the pancreas is a microcystic variant of ductal adenocarcinoma.¹³

Macrocytic Lesions

Mucinous cystic neoplasms (cystadenomas) and branch-duct (BD) IPMNs usually present as macrocytic lesions. Mucinous cystadenomas mainly involve the body and tail of the pancreas and do not communicate with the MPD. It is important to differentiate serous from mucinous cystic neoplasm as surgery is the treatment of choice in mucinous

cystic neoplasm.¹⁴ A peripheral eggshell calcification is highly suggestive of a potentially malignant mucinous cystic neoplasm.¹⁵ Other differential diagnoses of macrocystic lesions in the pancreas are lymphangiomas, lymphoepithelial cysts, infectious cysts, mesothelial cysts, and duplication cysts.

Cyst with Solid Component (Solid Cystic)

Tumors with this morphology are solid pseudopapillary neoplasms, pancreatoblastomas, cystic metastases, cystic degeneration in solid tumors, malignant transformation in cystic tumors, and hemorrhagic pseudocysts. Cysts with

a solid component can be unilocular or multilocular and may or may not have ductal communication. Therefore, true cystic tumors with solid component as well as solid pancreatic neoplasms with a cystic component or cystic degeneration are included in this category. Most tumors in this category are malignant and should be surgically treated. MR cholangiopancreatography (MRCP) is superior to single-section helical CT to characterize these tumors.^{16,17}

Cystic Transformation of the Pancreas

Cystic transformation of the pancreas is seen in dysontogenetic cyst, cystic fibrosis, disseminated variant of serous cystadenoma, congenital syndromes such as von Hippel-Lindau's disease (VHL), autosomal dominant polycystic kidney disease (ADPKD), Ivemark's syndrome, trisomy 13 or 15, Meckel-Gruber's syndrome, and so on.^{18,19} Polycystic disease of the pancreas is also known as dysontogenetic cysts or congenital cysts of the pancreas. It is a very rare entity that may occur as a solitary cyst, polycystic disease in association with renal cysts, and liver, central nervous system, or retinal abnormalities. Pancreatic involvement in VHL is in the form of simple cysts (71%), serous cystadenomas (15%), pancreatic neuroendocrine tumors (pNETs; 10%) and rarely cystic replacement of the entire pancreas.^{20,21} Kim et al²² have shown that pancreatic cysts are five times more prevalent in patients with ADPKD with PKD2 mutation than in patients with PKD1 mutation. PKD1 has a more aggressive disease course, with an earlier age of symptom onset, end-stage renal disease, and death. Thus, the potential to discriminate PKD1 from PKD2 on MRI has important prognostic implications. MRI identification of pancreatic cysts in ADPKD significantly increases the likelihood that a PKD2 mutation is present.^{23,24}

Cyst with Ductal Communication

Tumors included in this subgroup are IPMNs, collections postpancreatitis as a part of disconnected duct syndrome, and retention cysts. IPMNs are more common in elderly males in the sixth to seventh decade of life. Three types of IPMNs may be observed: main duct, BD, and mixed variant.²⁵ Retention cysts are cystic dilatation of the pancreatic duct. It may or may not be associated with an obstructive cause such as calculi, stricture, mucin plugs, and tumors.²⁶

Multifocal Cystic Lesions

BD-IPMN, pseudocysts, serous cystadenomas, neuroendocrine tumors, developmental cysts, and epithelial cysts can be multifocal and should be considered in the differential diagnoses of multifocal cystic lesions of the pancreas.¹¹

Incidental Solid Pancreatic Tumors

The incidence of benign disease in solid pancreatic tumors suspicious of cancer ranges from 6 to 21%. Chronic pancreatitis presenting as inflammatory pancreatic mass accounts for almost 70% of the benign lesions,²⁷ with alcoholic pancreatitis being the most common cause (60%) with autoimmune pancreatitis (AIP) in up to 11% of the patients.^{28,29}

Pancreatic Adenocarcinoma

The most frequent solid lesion in the pancreas is pancreatic ductal adenocarcinoma (PDAC). Symptomatic patients present with advanced disease at the time of diagnosis (extensive local disease in ~40% and metastases in 40–55%), leaving less than 20% of patients as candidates for potentially curative resection.^{30,31} The earliest imaging finding of a PDAC before a mass becomes apparent is pancreatic ductal dilatation or pancreatic duct cutoff. On imaging, pancreatic carcinomas (PCs) are hypovascular, hypoenhancing lesions when compared with the surrounding pancreatic parenchyma. On MRI, most PDACs are hypointense on unenhanced T1-weighted sequences when compared with the surrounding pancreas and are hypointense or isointense on T2-weighted images.^{32,33} The sensitivity and specificity of fluoro-2-deoxy-d-glucose-positron emission tomography (FDG-PET) for the diagnosis of PC in patients with normal blood glucose levels range from 85 to 100% and 67 to 99%, respectively. Combination of PET and CT may offer a better accuracy.³⁴ Serum tumor markers can be helpful in differentiating benign from malignant pancreatic masses. The addition of other tumor markers such as Ca-125 does not increase the diagnostic accuracy of CA19-9 and is the gold standard marker for PDAC with sensitivity and specificity as high as 87 and 98%, respectively.³⁵

Pancreatic Neuroendocrine Tumors

Over the last decade, the wide use of imaging technology has led to the rising incidence of pNETs. pNETs are rare and account for 2 to 4% of all pancreatic neoplasms, with an incidence of 1.5 in 100,000.^{36,37} In recent years, the detection of incidentally nonfunctioning pNETs (NF-pNETs) has rapidly increased due to the widespread use of endoscopic and cross-sectional imaging. Nearly 60% secrete one or more biologically active peptides, resulting in clinical syndromes. The most frequent functioning tumors are insulinomas, gastrinomas, glucagonomas, VIPomas (a pancreatic neuroendocrine tumor that secretes vasoactive intestinal peptide), and somatostatinomas. Between 30 and 40% of pNETs are



Fig. 4 A 27-year-old female with incidental pancreatic neuroendocrine tumor. Axial contrast-enhanced computed tomography image showing incidental well-defined hypervascular lesion (arrow) in head uncinatae of the pancreas.



Fig. 5 A 45-year-old-male with intrapancreatic spleen. Axial contrast-enhanced computed tomography image showing solid enhancing lesion (arrow) in the tail of the pancreas with enhancement similar to splenic parenchyma suggestive of intrapancreatic spleen.

nonfunctioning, and this is more likely to be discovered incidentally when symptoms due to the presence of the mass are not yet obvious.³⁸

On CT scan, most pNETs are isodense or moderately hypodense masses showing good arterial enhancement (→Fig. 4). Calcification, necrosis, and cystic degeneration seem to be more common in large nonfunctioning tumors or with malignant transformation. MRI has a diagnostic sensitivity of 78 to 91%, which is equivalent to that of dynamic CT.^{39,40} On MRI, pNETs show low signal intensity on T1-weighted images and high signal intensity on T2-weighted images.^{41,42} The most important imaging differential diagnosis is intrapancreatic spleen, which shows enhancement characteristics similar to those of splenic parenchyma (→Fig. 5).

Inflammatory Pancreatic Mass

The combination of acute pancreatitis and cancer is unusual. Pancreatic cancer represents 1 to 2% of acute pancreatitis etiologies, and only 3% of cancers manifest as acute pancreatitis.⁴³ The risk of developing pancreatic cancer in patients with chronic pancreatitis is around 15 times higher than in the average population.⁴⁴ Dilatation of the pancreatic duct, and double-duct sign with both biliary and pancreatic obstruction or interruption of the pancreatic duct are unusual and should lead to considering an underlying carcinoma. Sheathing of the celiac trunk and/or mesenteric artery is seen in 30 to 60% of CT scans of adenocarcinoma.⁴⁵ But this can also be seen in AIP or IgG4 (immunoglobulin G4 related) conditions with extrapancreatic lesions in sclerosing mesenteritis and retroperitoneal fibrosis.⁴⁶

Adenocarcinoma developing in the background of chronic pancreatitis is difficult to detect on imaging. In the context of chronic pancreatitis, calcifications displaced by the mass is a pointer suggesting a coexisting PC.^{47,48} Nearly 10% of presumed PCs that are surgically operated have been composed of pseudotumoral forms of pancreatitis, nearly half of which are thought to be focal forms of AIP, involving mainly the head.^{49,50} Certain imaging criteria are helpful in

diagnosing AIP, such as both early and delayed homogeneous enhancement of the lesion close to that of normal parenchyma, peripheral pseudocapsule, a duct visible in the mass with an hourglass stenosis, absence of upstream atrophy or marked dilatation of the pancreatic duct (<4 mm), multifocal involvement, absence of vascular involvement, and presence of extrapancreatic manifestations.⁵¹

Incidental Congenital Main Pancreatic Duct Anomalies

Congenital anomalies and normal variants of the pancreas and pancreatic duct may not be detected until adulthood and are often discovered as an incidental finding in asymptomatic patients. These anomalies are considered and detected only when patients present with idiopathic pancreatitis. MRCP is the modality of choice nowadays for the assessment of congenital pancreatic anomalies since it depicts ductal anatomy rapidly and noninvasively. Anatomic variations and developmental anomalies of the pancreas and pancreatic duct include variations of the course of the pancreatic duct (descending, sigmoid, vertical, and loop-shaped course), variation of the configuration of the pancreatic duct (bifid configuration with dominant duct of Wirsung [60%], dominant duct of Santorini without divisum [1%], absent duct of Santorini, and ansa pancreatica), duplication anomalies, anomalous pancreaticobiliary ductal junction, pancreas divisum (4–14%), annular pancreas, ectopic pancreas, and pancreatic agenesis and hypoplasia of the dorsal pancreas and accessory pancreatic lobe.^{52,53}

Incidental Pathological Processes Involving the MPD

Genetic mutation associated pancreatitis (GMAP) also sometimes referred to as idiopathic painless chronic pancreatitis can present as an incidental ductal disease process while imaging for symptoms not related to the pancreas. Several gene mutations associated with chronic pancreatitis have been identified, with the most frequent involving the *CFTR* (cystic fibrosis transmembrane regulator) gene, the *SPINK1* (serine protease inhibitor, Kazal type 1) gene, and the *PRSS1* (cationic trypsinogen) gene. According to some authors, the patients with pancreatitis associated with one of these gene mutations show onset at a younger age than those with pancreatitis related to other factors, even though the diagnosis is often late compared with the appearance of symptoms. Accurate diagnosis of GMAP is important for a careful follow-up of these patients, as the risk of developing pancreatic adenocarcinoma is higher in this group than in the normal population or in patients affected by chronic pancreatitis not associated with gene mutations. On imaging, typical bull's eye pattern of stones, with a dense peripheral rim and a noncalcified radiolucent central core with stones greater than 15 mm in size, is seen.^{54,55}

pNETs expressing serotonin (carcinoid tumors) account for a small portion of neuroendocrine tumors.⁵⁶ Segmental changes in the pancreatic duct are being increasingly encountered as patients undergo abdominal imaging for

evaluation of a variety of symptoms. The two most common causes of segmental pancreatic duct dilatation and pancreatic atrophy are chronic pancreatitis and malignant neoplasms such as PDAC. In rare instances, small serotonin secretin neuroendocrine tumors (serotoninoma) can induce fibrogenesis due to production of 5-hydroxyindoleacetic acid and serotonin, leading to obstruction of the pancreatic duct.^{57,58} These tumors are often detected incidentally while imaging patients for symptoms other than pancreatic etiology.

Management of Incidental Pancreatic Lesions

Most guidelines reach the consensus that the presence of a potentially resectable solid pancreatic mass in a CT scan or endoscopic ultrasound (EUS) in an otherwise healthy patient, with no clinical or biochemical characteristics suggesting a benign condition, should prompt surgical treatment.²⁷ Management of incidentally detected pNETs is a debatable topic. Indications for surgery in pNETs are functioning pNET and

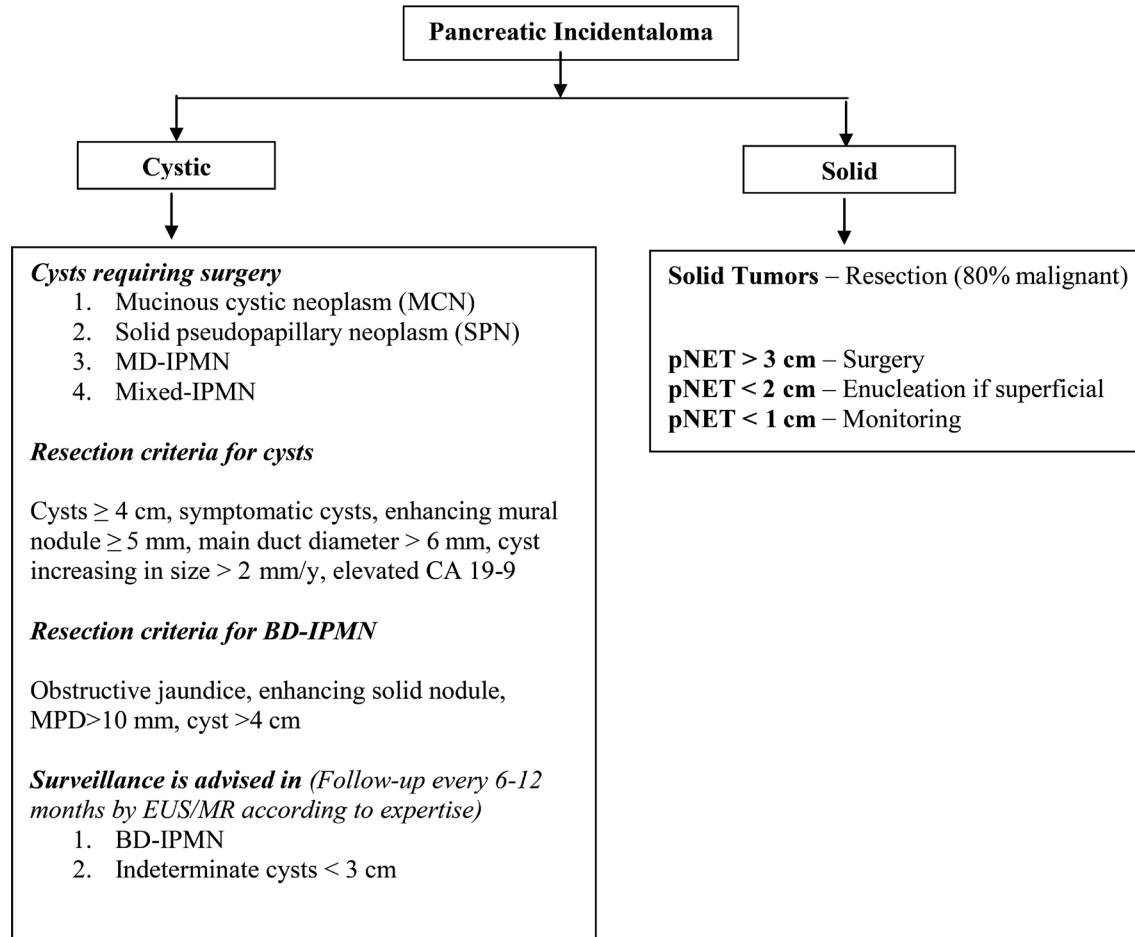


Fig. 6 Algorithm for the management of pancreatic incidentalomas.⁶¹⁻⁶⁴

Table 3 Practical tips in pancreatic incidentalomas

CT using pancreatic protocol is the imaging of choice for solid lesions
 MRI is the modality of choice in characterizing and for follow-up of cystic lesions
 EUS and fluid aspirate analysis are used for indeterminate lesions
 Solid pancreatic incidentalomas are considered malignant unless proven otherwise
 High amylase and CEA levels in fluid suggest pseudocyst and mucinous neoplasms, respectively
 Simple cysts up to 3 cm in size with no worrisome features can be safely monitored
 Surgery is considered for MCN, SPN, MD-IPMN, mixed IPMN
 Surgery is considered for cysts > 3 cm and serous cystadenoma > 4 cm
 Solid lesions up to 2 cm in size are likely to be malignant and will require surgical intervention
 Small pNETs (<1 cm) are simply monitored
 Larger pNETs (>2 cm) lesions are treated by enucleation or resection
 Generally, pancreatic incidentalomas have a better prognosis than symptomatic lesions

Abbreviations: CEA, carcinoembryonic antigen; CT, computed tomography; EUS, endoscopic ultrasound; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystadenoma; MD-IPMN, main-duct intraductal papillary mucinous neoplasm; MRI, magnetic resonance imaging; pNETs, pancreatic neuroendocrine tumors; SPN, solid pseudopapillary tumor.

NF-pNETs > 2 cm. pNET < 1 cm can be kept on monitoring, and pNETs of 1 to 2 cm should be considered for enucleation if superficial in location.⁵⁹⁻⁶¹

Various published guidelines for the management of cystic lesions recommend resection of potentially malignant tumors such as mucinous cystadenomas, solid pseudopapillary tumors, and main and mixed type of intraductal papillary mucinous neoplasms and observe benign lesions such as serous cystadenoma (< 4 cm) and BD IPMN.⁶²⁻⁶⁵ Close follow-up and surgical consideration are recommended in cases with worrisome features (symptomatic, cytology suspicious for malignancy, enhancing mural nodule < 5 mm, MPD > 6 mm, cyst size increasing > 2 mm/year, elevated CA19-9).⁶²⁻⁶⁵ The International Association of Pancreatology recommends surveillance for simple cysts < 3 cm in size. Patients with cysts < 1 cm are imaged at an interval of 2 to 3 years (CT/MRI), those with cysts 1 to 2 cm in diameter at an interval of 1 year (CT/MRI), and those with cysts of size 2 to 3 cm at an interval of 3 to 6 months (preferably endoscopic US).⁶⁴ An algorithmic approach to the management of PIs is illustrated in ►Fig. 6.

Conclusion

PIs are increasingly encountered by the radiologists today and are worrisome. These can be cystic or solid. An incidentally discovered pancreatic cystic lesion without any evidence of worrisome features and of < 2 cm is highly unlikely to cause morbidity or mortality. Around half of these lesions may eventually grow to be larger than 2 cm; therefore, adequate follow-up is advised. Solid PIs > 1 cm are potentially considered malignant, unless proven otherwise, and resection or enucleation is recommended. Key practical tips for the radiologists when dealing with PIs are given in ►Table 3.

Conflict of Interest

None declared.

References

- 1 Tsuda S, Pancreatic Incidentaloma [Internet] (updated 2013 June 06; cited 2017 April 09). Available at: <https://www.sages.org/wiki/pancreatic-incidentalmoma>. Accessed July 10, 2019
- 2 Kostiuik TS. Observation of pancreatic incidentaloma [in Russian] *Klin Khir* 2001;9(9):62–63
- 3 de Jong K, Nio CY, Hermans JJ, et al. High prevalence of pancreatic cysts detected by screening magnetic resonance imaging examinations. *Clin Gastroenterol Hepatol* 2010;8(9):806–811
- 4 Zhang XM, Mitchell DG, Dohke M, Holland GA, Parker L. Pancreatic cysts: depiction on single-shot fast spin-echo MR images. *Radiology* 2002;223(2):547–553
- 5 Kimura W, Nagai H, Kuroda A, Muto T, Esaki Y. Analysis of small cystic lesions of the pancreas. *Int J Pancreatol* 1995;18(3):197–206
- 6 Winter JM, Cameron JL, Lillemoe KD, et al. Periampullary and pancreatic incidentaloma: a single institution's experience with an increasingly common diagnosis. *Ann Surg* 2006;243(5):673–680
- 7 Pantoja M, Salazar M, Vela'zquez-Ferna'ndez D, Pancreatic incidentaloma. In: Hubbard JGH, Inabnet WB, Lo C-Y, eds.

- Endocrine Surgery: Principles and Practice (Springer Specialist Surgery Series). 1st ed. London: Springer; 2009
- 8 Fernández-del Castillo C, Targarona J, Thayer SP, Rattner DW, Brugge WR, Warshaw AL. Incidental pancreatic cysts: clinicopathologic characteristics and comparison with symptomatic patients. *Arch Surg* 2003;138(4):427–433
- 9 Allen PJ, D'Angelica M, Gonen M, et al. A selective approach to the resection of cystic lesions of the pancreas: results from 539 consecutive patients. *Ann Surg* 2006;244(4):572–582
- 10 Allen PJ, Jaques DP, D'Angelica M, Bowne WB, Conlon KC, Brennan MF. Cystic lesions of the pancreas: selection criteria for operative and nonoperative management in 209 patients. *J Gastrointest Surg* 2003;7(8):970–977
- 11 Sureka B, Bihari C, Arora A, et al. *JOP* 2016;17:452–465
- 12 Curry CA, Eng J, Horton KM, et al. CT of primary cystic pancreatic neoplasms: can CT be used for patient triage and treatment? *Am J Roentgenol* 2000;175(1):99–103
- 13 Bagci P, Andea AA, Basturk O, Jang KT, Erbarut I, Adsay V. Large duct type invasive adenocarcinoma of the pancreas with microcystic and papillary patterns: a potential microscopic mimic of non-invasive ductal neoplasia. *Mod Pathol* 2012;25(3):439–448
- 14 Horvath KD, Chabot JA. An aggressive resectional approach to cystic neoplasms of the pancreas. *Am J Surg* 1999;178(4):269–274
- 15 Mathieu D, Guigui B, Valette PJ, et al. Pancreatic cystic neoplasms. *Radiol Clin North Am* 1989;27(1):163–176
- 16 Taouli B, Vilgrain V, Vullierme MP, et al. Intraductal papillary mucinous tumors of the pancreas: helical CT with histopathologic correlation. *Radiology* 2000;217(3):757–764
- 17 Klöppel G, Kosmahl M. Cystic lesions and neoplasms of the pancreas. The features are becoming clearer. *Pancreatol* 2001;1(6):648–655
- 18 Nygaard KK, Walters W. Polycystic disease of the pancreas (dysontogenetic cysts): report of a case with partial pancreatectomy. *Ann Surg* 1937;106(1):49–53
- 19 Rodrigues-Pinto E, Pereira P, Cunha R, Macedo G. Pancreatic dysontogenetic cysts. *Rev Esp Enferm Dig* 2014;106(7):477–478
- 20 Hammel PR, Vilgrain V, Terris B, et al. Pancreatic involvement in von Hippel-Lindau disease. The Groupe Francophone d'Etude de la Maladie de von Hippel-Lindau. *Gastroenterology* 2000;119(4):1087–1095
- 21 van Asselt SJ, de Vries EG, van Dullemen HM, et al. Pancreatic cyst development: insights from von Hippel-Lindau disease. *Cilia* 2013;2(1):3
- 22 Kim JA, Blumenfeld JD, Chhabra S, et al. Pancreatic cysts in autosomal dominant polycystic kidney disease: prevalence and association with PKD2 gene mutations. *Radiology* 2016;280(3):762–770
- 23 Harris PC, Torres VE. Polycystic kidney disease. *Annu Rev Med* 2009;60:321–337
- 24 Chapman AB, Devuyt O, Eckardt KU, et al; Conference Participants. Autosomal-dominant polycystic kidney disease (ADPKD): executive summary from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int* 2015;88(1):17–27
- 25 Palmucci S, Trombatore C, Foti PV, et al. The utilization of imaging features in the management of intraductal papillary mucinous neoplasms. *Gastroenterol Res Pract* 2014;2014:765451
- 26 Ren F, Zuo C, Chen G, et al. Pancreatic retention cyst: multi-modality imaging findings and review of the literature. *Abdom Imaging* 2013;38(4):818–826
- 27 Wolfson D, Barkin JS, Chari ST, et al. Management of pancreatic masses. *Pancreas* 2005;31(3):203–217
- 28 Steer ML, Waxman I, Freedman S. Chronic pancreatitis. *N Engl J Med* 1995;332(22):1482–1490
- 29 Finkelberg DL, Sahani D, Deshpande V, Brugge WR. Autoimmune pancreatitis. *N Engl J Med* 2006;355(25):2670–2676

- 30 McMahan PM, Halpern EF, Fernandez-del Castillo C, Clark JW, Gazelle GS. Pancreatic cancer: cost-effectiveness of imaging technologies for assessing resectability. *Radiology* 2001; 221(1):93–106
- 31 Nichols MT, Russ PD, Chen YK. Pancreatic imaging: current and emerging technologies. *Pancreas* 2006;33(3):211–220
- 32 Saisho H, Yamaguchi T. Diagnostic imaging for pancreatic cancer: computed tomography, magnetic resonance imaging, and positron emission tomography. *Pancreas* 2004;28(3):273–278
- 33 Lopez Hänninen E, Amthauer H, Hosten N, et al. Prospective evaluation of pancreatic tumors: accuracy of MR imaging with MR cholangiopancreatography and MR angiography. *Radiology* 2002;224(1):34–41
- 34 Kalra MK, Maher MM, Boland GW, Saini S, Fischman AJ. Correlation of positron emission tomography and CT in evaluating pancreatic tumors: technical and clinical implications. *Am J Roentgenol* 2003;181(2):387–393
- 35 Cwik G, Wallner G, Skoczylas T, Ciecanski A, Zinkiewicz K. Cancer antigens 19-9 and 125 in the differential diagnosis of pancreatic mass lesions. *Arch Surg* 2006;141(10):968–973
- 36 Yao JC, Hassan M, Phan A, et al. One hundred years after “carcinoid”: epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* 2008;26(18):3063–3072
- 37 Bettini R, Boninsegna L, Mantovani W, et al. Prognostic factors at diagnosis and value of WHO classification in a mono-institutional series of 180 non-functioning pancreatic endocrine tumours. *Ann Oncol* 2008;19(5):903–908
- 38 Brentjens R, Saltz L. Islet cell tumors of the pancreas: the medical oncologist’s perspective. *Surg Clin North Am* 2001;81(3):527–542
- 39 Kaltsas GA, Besser GM, Grossman AB. The diagnosis and medical management of advanced neuroendocrine tumors. *Endocr Rev* 2004;25(3):458–511
- 40 Debray MP, Geoffroy O, Laissy JP, et al. Imaging appearances of metastases from neuroendocrine tumours of the pancreas. *Br J Radiol* 2001;74(887):1065–1070
- 41 Thoeni RF, Mueller-Lisse UG, Chan R, Do NK, Shyn PB. Detection of small, functional islet cell tumors in the pancreas: selection of MR imaging sequences for optimal sensitivity. *Radiology* 2000;214(2):483–490
- 42 Semelka RC, Custodio CM, Cem Balci N, Woosley JT. Neuroendocrine tumors of the pancreas: spectrum of appearances on MRI. *J Magn Reson Imaging* 2000;11(2):141–148
- 43 Gambill EE. Pancreatitis associated with pancreatic carcinoma: a study of 26 cases. *Mayo Clin Proc* 1971;46(3):174–177
- 44 McKay CJ, Glen P, McMillan DC. Chronic inflammation and pancreatic cancer. *Best Pract Res Clin Gastroenterol* 2008;22(1):65–73
- 45 Megibow AJ, Bosniak MA, Ambos MA, Beranbaum ER. Thickening of the celiac axis and/or superior mesenteric artery: a sign of pancreatic carcinoma on computed tomography. *Radiology* 1981;141(2):449–453
- 46 Luetmer PH, Stephens DH, Fischer AP. Obliteration of periarterial retropancreatic fat on CT in pancreatitis: an exception to the rule. *Am J Roentgenol* 1989;153(1):63–64
- 47 Frampas E, Morla O, Regenet N, Eugène T, Dupas B, Meurette G. A solid pancreatic mass: tumour or inflammation? *Diagn Interv Imaging* 2013;94(7-8):741–755
- 48 Kim T, Murakami T, Takamura M, et al. Pancreatic mass due to chronic pancreatitis: correlation of CT and MR imaging features with pathologic findings. *Am J Roentgenol* 2001;177(2):367–371
- 49 Kajiwara M, Gotohda N, Konishi M, et al. Incidence of the focal type of autoimmune pancreatitis in chronic pancreatitis suspected to be pancreatic carcinoma: experience of a single tertiary cancer center. *Scand J Gastroenterol* 2008;43(1):110–116
- 50 Manfredi R, Graziani R, Cicero C, et al. Autoimmune pancreatitis: CT patterns and their changes after steroid treatment. *Radiology* 2008;247(2):435–443
- 51 Learn PA, Grossman EB, Do RK, et al. Pitfalls in avoiding operation for autoimmune pancreatitis. *Surgery* 2011;150(5):968–974
- 52 Türkvtan A, Erden A, Türkoğlu MA, Yener Ö. Congenital variants and anomalies of the pancreas and pancreatic duct: imaging by magnetic resonance cholangiopancreatography and multidetector computed tomography. *Korean J Radiol* 2013;14(6):905–913
- 53 Schulte SJ. Embryology, normal variation, and congenital anomalies of the pancreas. In: Stevenson GW, Freeny PC, Margulis AR, Burhenne HJ, eds. *Margulis and Burhenne’s Alimentary Tract Radiology*, 5th ed. St. Louis, MO: Mosby 1994;1039–1051
- 54 Graziani R, Frulloni L, Cicero C, et al. Bull’s-eye pattern of pancreatic-duct stones on multidetector computed tomography and gene-mutation-associated pancreatitis (GMAP) *Radiol Med (Torino)* 2012;117(8):1275–1286
- 55 Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. *N Engl J Med* 1998;339(10):653–658
- 56 Wilson RW, Gal AA, Cohen C, DeRose PB, Millikan WJ. Serotonin immunoreactivity in pancreatic endocrine neoplasms (carcinoid tumors) *Mod Pathol* 1991;4(6):727–732
- 57 Druce M, Rockall A, Grossman AB. Fibrosis and carcinoid syndrome: from causation to future therapy. *Nat Rev Endocrinol* 2009;5(5):276–283
- 58 Kawamoto S, Shi C, Hruban RH, et al. Small serotonin-producing neuroendocrine tumor of the pancreas associated with pancreatic duct obstruction. *Am J Roentgenol* 2011;197(3):W482–8
- 59 Stratilatovas E, Sangaila E, Cicen S. Radical surgery for large-sized slow-growing neuroendocrine tumor of the pancreas. *J BUON* 2002;7(4):381–383
- 60 Franko J, Feng W, Yip L, Genovese E, Moser AJ. Non-functional neuroendocrine carcinoma of the pancreas: incidence, tumor biology, and outcomes in 2,158 patients. *J Gastrointest Surg* 2010;14(3):541–548
- 61 Karatzas T, Dimitroulis D, Charalampoudis P, Misiakos EP, Vasileiadis I, Kouraklis G. Management of cystic and solid pancreatic incidentalomas: a review analysis. *J BUON* 2013;18(1):17–24
- 62 Hol L, Bruno MJ, Cahen DL. Follow-up of asymptomatic pancreatic cysts in clinical practice: a vignette questionnaire. *Pancreatol* 2016;16(3):416–422
- 63 Vege SS, Ziring B, Jain R, Moayyedi P; Clinical Guidelines Committee; American Gastroenterology Association. American Gastroenterological Association Institute guideline on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. *Gastroenterology* 2015;148(4):819–822
- 64 Tanaka M, Fernández-Del Castillo C, Kamisawa T, et al. Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. *Pancreatol* 2017;17(5):738–753
- 65 Del Chiaro M, Verbeke C, Salvia R, et al; European Study Group on Cystic Tumours of the Pancreas. European experts consensus statement on cystic tumours of the pancreas. *Dig Liver Dis* 2013;45(9):703–711

Nanoceria and Its Biomedical Relevance

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Abstract

Keywords

- ▶ cerium oxide nanoparticles
- ▶ antioxidant
- ▶ reactive oxygen species
- ▶ inflammatory diseases

Nanoceria is a nanosized particle preparation of cerium oxide. It shows mixture of cerium in the 3+ and 4+ states on the nanoparticle surface, giving it interesting redox properties. Nanoceria shows effective biological antioxidant properties, which makes it a great candidate for biomedical applications. Many studies have shown promising results on therapeutic potential of nanoceria in diseases like cancer, diabetes, atherosclerosis, and neurodegenerative diseases. Meanwhile, other studies explored biodistribution and toxicity of nanoceria. This review article describes nanoceria, its relevant biomedical applications, and adverse effects, based on previously reported studies.

Introduction

Cerium is a rare earth metal having atomic number 58, belonging to lanthanide series. Unlike most rare earth metals, cerium can exist in both 3+ and 4+ states and thus, oxides of cerium exist as both CeO₂ and Ce₂O₃.^{1,2} Cerium oxide nanoparticles (nanoceria) show mixture of cerium in the 3+ and 4+ states on their surface, giving them interesting redox properties. Nanoceria is being widely used in fields of chemicals, cosmetics, mechanical polishing/planarization, corrosion protection, solar cells, fuel oxidation catalysis, and automotive exhaust treatment.^{1,2} Other than these applications, nanoceria also displays many biorelevant activities—mimicking superoxide dismutase (SOD), catalase, peroxidase, oxidase, and phosphatase, and scavenging hydroxyl radicals, nitric oxide radicals, and peroxyxynitrite. These biorelevant activities of nanoceria can be used in pharmacological agents, drug delivery, and bioscaffolding.¹

Synthesis of Nanoceria

Numerous techniques, such as green synthesis, hydrothermal, solvothermal, aqueous precipitation, reversed micelles, thermal decomposition, and flame spray methods have been reported to synthesize nanoceria, while maintaining control of its size and properties.^{1–3} The synthesized nanoceria can be bare or wrapped with a coating of protective substances that can be hydrophilic or hydrophobic.³ Naked nanoceria has poor water solubility, and thus limited biological

applications. Polymer coating of nanoceria enhances its stability, biocompatibility, and water solubility.² For biological use, biocompatible nanoceria has been systematically synthesized in pure water or with coating/functionalization of polyethylene glycol, dextran, polyacrylic acid, cyclodextrin, glucose, and so on.^{3,4} Methods of nanoceria preparation or synthesis are important because they determine the solubility, size, surface condition, charge, structural arrangement, and morphology of nanoparticles, thus affecting their properties, including catalytic activities.³

Mechanism of Action

The basis for activities of nanoceria is the thermodynamic efficiency of redox-cycling between 3+ and 4+ states on their surface and their unique ability to take up and release oxygen.¹ Nanoceria could have a dual role as an oxidation catalyst and reduction catalyst, depending on the reaction conditions and surrounding microenvironment. The cerium atom on surface of nanoceria has the ability to easily and drastically adjust its electronic configuration to fit its immediate environment, and thus contributing to redox and antioxidant properties.³ The Ce³⁺/Ce⁴⁺ valence switching capacity of nanoceria makes it an SOD mimic.³ Nanoceria could also act as catalase mimic in a redox-state-dependent manner, and higher levels of cerium in the +4 state exhibit higher activity.³ Nanoceria can also show pro-oxidant properties at lower pH and high concentrations, and it has shown potential toxicity based on synthesis method, concentration, and exposure time.⁵

Biomedical Applications

Increased oxidative stress has been found to be associated with many neurodegenerative and chronic inflammatory diseases like Alzheimer's, Parkinsonism, Rheumatoid Arthritis (RA), Ischemic stroke, and diabetes etc. Due to its antioxidant properties, nanoceria has been studied extensively for treatment of these disorders.^{3,6} Nanoceria shows neuroprotective effects by protecting against free radical/ reactive oxygen species (ROS) mediated injuries. Nanoceria administration in rat model of Parkinsonism (6-OHDA-induced) resulted in partial neuroprotection against disturbances in motor performance, partially through their antioxidant and antiapoptotic effects.⁷ Many studies have also confirmed antibacterial activity of nanoceria against *Pseudomonas aeruginosa*, *E. coli*, *B. subtilis*, *Shewanella oneidensis* and *Pseudokirchneriella supcapitata*.²

Diabetes is also believed to be associated with oxidative stress and beneficial effects of nanoceria have been demonstrated in diabetic rats.⁸ In another study, nanoceria treatment significantly reduced glucose levels and diabetogenesis in streptozocin induced diabetic Swiss mice. In addition, cytokines (IL-6 and TNF- α and p65-NF- κ B) expression were diminished by nanoceria treatment, whereas the nuclear factor erythroid 2-related factor 2 (Nrf2) expression was enhanced, indicating the role of modulation of NF- κ B/Nrf2 pathway. Nanoceria also exhibited promising superoxide dismutase 1 mimetic and antiapoptotic activity in these diabetic mice.⁹

Nanoceria has also been shown to reduce retinal degeneration by reducing ROS generation in rat retinal cells.¹⁰ The alginate-gelatin injectable hydrogel loaded with oligo-chitosan coated nanoceria showed good biocompatibility and high potential in protecting cells from apoptosis, angiogenesis, and production of proinflammatory cytokines in age related macular degeneration cellular models.¹¹

Increased ROS production in synovium causes chronic inflammation and contributes to rheumatoid arthritis. Nanoceria and manganese ferrite nanoparticles anchored to mesoporous silica nanoparticles showed synergistic effects by scavenging ROS and promoting recruitment of anti-inflammatory macrophages in joints of rat RA model.¹² Nanoceria also showed therapeutic potential by preventing valvular calcification mediated by ROS related damage,¹³ cardio-protective effects by preventing myocardial remodelling,¹⁴ and attenuating ischemia reperfusion induced hepatic injury in rats.¹⁵

Antioxidant and anti-inflammatory properties of nanoceria were found beneficial in cisplatin induced nephrotoxicity. As nanoceria ameliorated oxidative stress by showing a reduction in levels of malondialdehyde, increased levels of endogenous antioxidants, reduced glutathione and catalase, and decreased levels of proinflammatory cytokines.¹⁶ In vivo studies in mice with induced liver toxicity (by carbon tetrachloride [CCl₄]) showed that nanoceria administered mice exhibit findings similar to mice treated with N-acetyl cystine (NAC), a common therapeutic to reduce oxidative stress.¹⁷

Nanoceria has also shown potential for treatment of smoking-related diseases by exhibiting ability to protect against cigarette smoke extract (CSE)-induced oxidative stress and inflammation in cultured rat H9c2 cardiomyocytes. Results

indicated that nanoceria can inhibit CSE-induced cell damage via inhibition of ROS generation, NF- α B activation, inflammatory gene expression, and antioxidant depletion.¹⁸ Another study showed anti-inflammatory and antioxidant effects of nanoceria in both healthy rats, and rats with pneumonia.¹⁹

Many studies have shown that nanoceria is toxic to cancer cells.³ Nanoceria induces ROS mediated damage, lipid peroxidation, apoptosis, and membrane leakage in cancer cells, but not in healthy/normal cells.³ This has been attributed to cancer cells having a more acidic cytosolic pH than normal cells because of higher glycolysis and lactate production.³ Although, results from few studies are conflicting. Nanoceria treatment to human monocytic leukemia cells (THP-1) resulted in reduction of ROS but cytotoxicity was not observed.²⁰ A study in broncho-alveolar carcinoma-derived A549 cells showed that uptake of the nanoceria resulted in slight change of the cell cycles, i.e., more cells stayed in the G1 phase but the cell viability was not significantly altered.²¹

When functionalized with anticancer molecules, nanoceria demonstrate synergistic toxic effects in cancer cells.^{3,22,23} Protective effects of nanoceria on healthy cells, while killing glioma cancer cells have been reported.²⁴ Nanoceria abolished toxic effects of anticancer drug (doxorubicin) on human dermal fibroblasts.²⁵

The multi-enzyme-like properties of nanoceria have been successfully used for biological detection and analysis, e.g., colorimetric immunoassays, enzyme-linked immunosorbent assay (ELISA), and biosensors etc.^{3,26-28}

Biodistribution

In vivo analysis of the biological distribution of nanoceria administered to mice perorally (PO), intravenously (IV), or intraperitoneally (IP) showed that the IV and IP administration leads to most extensive and cumulative nano-deposition while PO administration led to excretion of greater than 95% nanoceria within 24 hours. Organ deposition for IV and IP mice was highest in the spleen followed by the liver, lungs, and kidneys. Nanoceria was excreted through feces all administration routes.¹⁷ Study in *C. elegans* showed that positively charged nanoceria is significantly more toxic and accumulated to a greater extent than the neutral and negatively charged nanoceria.²⁹

Toxicity and Adverse Effects

Cerium is not found in the human body and there are no known clearance mechanisms for it. This implies that exposure to cerium would lead to systemic toxicity.¹ Nanoceria is successfully up taken by cells in both normal and diseased states through multiple routes. In most cases of in vitro intracellular assays, nanoceria was reported to exhibit positive effects (such as scavenging ROS) and was regarded as a promising biomaterial for biomedical applications. However, several reports suggested that the uptake of nanoceria could induce oxidative stress and DNA damage, apoptosis, dephosphorylation of various substrates, aberrant cell signaling, and alterations in the transcriptional and posttranslational levels.^{3,30-32}

In vivo analyses of the biological effect of different sizes of nanoceria have been performed by per-oral, intravenous or intraperitoneal administration to laboratory animals.^{3,33} There are few studies showing that exposure of animals to nanoceria resulted in significant lung responses, including lung inflammation, cytotoxicity, lung injury, alveolar macrophage functional changes, induction of phospholipidosis, and release of proinflammatory and fibrotic cytokines. Cerium is also linked to fibrosis of the heart, and nanoceria was shown to induce myocardial fibroblast proliferation and collagen deposition in rats.

Due to the extensive use, nanoceria is getting released to the environment and humans are getting exposed, mostly via inhalation.² Nanoceria is used as a diesel fuel catalyst, and thus can be emitted into air, resulting in exposure to humans by inhalation. A recent study investigated the acute (24 hours) effect of intratracheal (IT) instillation of nanoceria on pulmonary and systemic inflammation, oxidative stress and thrombosis in mice. Results showed that acute pulmonary exposure to nanoceria induced pulmonary and systemic inflammation, oxidative stress, and thrombosis in vivo.³⁴ Nanoceria administration has shown to result in elevated WBC counts after IV and IP administration in mice,¹⁷ and hepatic injury with oxidative stress in rats after single vascular infusion.³⁵

Above data necessitates careful optimization of applications and synthesis parameters to generate nontoxic nanoceria that are based on the treatment strategy being used, and further exploration of biochemical effects of nanoceria.^{1,3}

Conflict of Interest

None declared.

References

- Dhall A, Self W. Cerium oxide nanoparticles: a brief review of their synthesis methods and biomedical applications. *Antioxidants* 2018;7(8):E97
- Rajeshkumar S, Naik P. Synthesis and biomedical applications of Cerium oxide nanoparticles-A Review. *Biotechnol Rep (Amst)* 2017;17:1–5
- Xu C, Qu X., Cerium oxide nanoparticle: a remarkably versatile rare earth nanomaterial for biological applications. *NPG Asia Materials* 2014;6(3):e90
- Caputo F, Mameli M, Sienkiewicz A, et al. A novel synthetic approach of cerium oxide nanoparticles with improved biomedical activity. *Sci Rep* 2017;7(1). Doi: 10.1038/s41598-017-04098-6
- Yokel RA, Hussain S, Garantziotis S, Demokritou P, Castranova V, Cassee FR. The Yin: An adverse health perspective of nanoceria: uptake, distribution, accumulation, and mechanisms of its toxicity. *Environ Sci Nano* 2014;1(5):406–428
- Naz S, Beach J, Heckert B, et al. Cerium oxide nanoparticles: a 'radical' approach to neurodegenerative disease treatment. *Nanomedicine (Lond)* 2017;12(5):545–553
- Hegazy MAE, Maklad HM, Elmonsif DAA, Elnozhy FY, Alqubiea MA, Alenezi FA. The possible role of cerium oxide (CeO₂) nanoparticles in prevention of neurobehavioral and neurochemical changes in 6-hydroxydopamine-induced parkinsonian disease. *Alexandria Journal of Medicine* 2017;53(4):351–360
- Pourkhalili N, Hosseini A, Nili-Ahmadabadi A, et al. Biochemical and cellular evidence of the benefit of a combination of cerium oxide nanoparticles and selenium to diabetic rats. *World J Diabetes* 2011;2(11):204–210
- Khurana A, Tekula S, Godugu C. Nanoceria suppresses multiple low doses of streptozotocin-induced Type 1 diabetes by inhibition of Nrf2/NF-κB pathway and reduction of apoptosis. *Nanomedicine (Lond)* 2018;13(15):1905–1922
- Chen J, Patil S, Seal S, McGinnis JF. Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides. *Nat Nanotechnol* 2006;1(2):142–150
- Wang K, Mitra RN, Zheng M, Han Z. Nanoceria-loaded injectable hydrogels for potential age-related macular degeneration treatment. *J Biomed Mater Res A* 2018;106(11):2795–2804
- Kim J, Kim HY, Song SY, et al. Synergistic oxygen generation and reactive oxygen species scavenging by manganese ferrite/ceria co-decorated nanoparticles for rheumatoid arthritis treatment. *ACS Nano* 2019;13(3):3206–3217
- Xue Y, St Hilaire C, Hortells L, Phillippi JA, Sant V, Sant S. Shape-specific nanoceria mitigate oxidative stress-induced calcification in primary human valvular interstitial cell culture. *Cell Mol Bioeng* 2017;10(5):483–500
- Kumari P, Saifi MA, Khurana A, Godugu C. Cardioprotective effects of nanoceria in a murine model of cardiac remodeling. *J Trace Elem Med Biol* 2018;50:198–208
- Manne N, Arvapalli R, Graffeo VA, et al. Prophylactic treatment with cerium oxide nanoparticles attenuate hepatic ischemia reperfusion injury in sprague dawley rats. *Cell Physiol Biochem* 2017;42(5):1837–1846
- Saifi MA, Sangomla S, Khurana A, Godugu C. Protective effect of nanoceria on cisplatin-induced nephrotoxicity by amelioration of oxidative stress and pro-inflammatory mechanisms. *Biol Trace Elem Res* 2019;189(1):145–156
- Hirst SM, Karakoti A, Singh S, et al. Bio-distribution and in vivo antioxidant effects of cerium oxide nanoparticles in mice. *Environ Toxicol* 2013;28(2):107–118
- Niu J, Wang K, Kolattukudy PE. Cerium oxide nanoparticles inhibit oxidative stress and nuclear factor-κB activation in H9c2 cardiomyocytes exposed to cigarette smoke extract. *J Pharmacol Exp Ther* 2011;338(1):53–61
- Serebrovska Z, Swanson RJ, Portnichenko V, et al. Anti-inflammatory and antioxidant effect of cerium dioxide nanoparticles immobilized on the surface of silica nanoparticles in rat experimental pneumonia. *Biomed Pharmacother* 2017;92:69–77
- Patel P, Kansara K, Singh R, et al. Cellular internalization and antioxidant activity of cerium oxide nanoparticles in human monocytic leukemia cells. *Intl J Nanomed* 2018;13(T-NANO 2014 Abstracts):39–41
- Zhou X, Wang B, Chen Y, Mao Z, Gao C. Uptake of cerium oxide nanoparticles and their influences on functions of A549 cells. *J Nanosci Nanotechnol* 2013;13(1):204–215
- Xu C, Lin Y, Wang J, et al. Nanoceria-triggered synergetic drug release based on CeO(2)-capped mesoporous silica host-guest interactions and switchable enzymatic activity and cellular effects of CeO(2). *Adv Healthc Mater* 2013;2(12):1591–1599
- Sack M, Alili L, Karaman E, et al. Combination of conventional chemotherapeutics with redox-active cerium oxide nanoparticles—a novel aspect in cancer therapy. *Mol Cancer Ther* 2014;13(7):1740–1749
- Sack-Zschau M, Bader S, Brenneisen P. Cerium oxide nanoparticles as novel tool in glioma treatment: an. *Vitro study J Nanomed Nanotechnol* 2017;8(6) 474
- Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol Rev* 2004;56(2):185–229
- Song HP, Jang JY, Bae SH, Choi SB, Yu BJ, Kim MI. Convenient colorimetric detection of thrombin via aptamer-mediated

- inhibition and restoration of the oxidase activity of nanoceria. *J Nanosci Nanotechnol* 2018;18(9):6570–6574
- 27 Ni P, Xie J, Chen C, et al. Spectrophotometric determination of the activity of alkaline phosphatase and detection of its inhibitors by exploiting the pyrophosphate-accelerated oxidase-like activity of nanoceria. *Mikrochim Acta* 2019;186(5):320
- 28 Liao H, Liu Y, Chen M, Wang M, Yuan H, Hu L. A colorimetric heparin assay based on the inhibition of the oxidase mimicking activity of cerium oxide nanoparticles. *Mikrochim Acta* 2019;186(5):274
- 29 Collin B, Oostveen E, Tsyusko OV, Unrine JM. Influence of natural organic matter and surface charge on the toxicity and bioaccumulation of functionalized ceria nanoparticles in *Caenorhabditis elegans*. *Environ Sci Technol* 2014;48(2):1280–1289
- 30 Könen-Adıgüzel S, Ergene S. In vitro evaluation of the genotoxicity of CeO₂ nanoparticles in human peripheral blood lymphocytes using cytokinesis-block micronucleus test, comet assay, and gamma H2AX. *Toxicol Ind Health* 2018;34(5):293–300
- 31 Eskandari N, Nejadi Babadaei MM, Nikpur S, et al. Biophysical, docking, and cellular studies on the effects of cerium oxide nanoparticles on blood components: in vitro. *Int J Nanomedicine* 2018;13:4575–4589
- 32 Hussain S, Al-Nsour F, Rice AB, et al. Cerium dioxide nanoparticles induce apoptosis and autophagy in human peripheral blood monocytes. *ACS Nano* 2012;6(7):5820–5829
- 33 Yokel RA, Tseng MT, Dan M, et al. Biodistribution and biopersistence of ceria engineered nanomaterials: size dependence. *Nanomedicine (Lond)* 2013;9(3):398–407
- 34 Nemmar A, Al-Salam S, Beegam S, Yuvaraju P, Ali BH. The acute pulmonary and thrombotic effects of cerium oxide nanoparticles after intratracheal instillation in mice. *Int J Nanomedicine* 2017;12:2913–2922
- 35 Tseng MT, Lu X, Duan X, et al. Alteration of hepatic structure and oxidative stress induced by intravenous nanoceria. *Toxicol Appl Pharmacol* 2012;260(2):173–182

Time Trends in Prevalence of Anemia in Preschool Children in India

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Abstract

Introduction Anemia is a major public health problem in Indian children. India introduced iron-folic acid supplementation for preschool children in the 1970s. In 1990, the component of detection and treatment of anemia was added. It is important to assess the impact of these programs on the prevalence of anemia in preschool children.

Materials and Methods Prevalence of anemia in preschool children at the national and state level was tabulated from National Family Health Survey (NFHS) 2, 3, and 4 national and state reports. Raw data from NFHS 2, 3, 4; District Level Household Survey (DLHS) 2 and 4; and Annual Health Survey and its Clinical Anthropometric and Biochemical component (AHS CAB) were analyzed to find out the changes in mean Hb, prevalence of anemia, and frequency distribution of Hb.

Results Compared with NFHS 2, there was a 5% increase in prevalence of anemia, 0.3 g/dL fall in mean Hb levels, and a small shift to the left in frequency distribution of Hb levels in NFHS 3. There was a 10% reduction in prevalence of anemia in NFHS 4 as compared with NFHS 3. Comparison between DLHS 2, DLHS 4, and AHS CAB showed that there was a 15 to 20% reduction in prevalence of anemia, approximately 1 g/dL increase in mean Hb and a shift to the right in distribution of Hb levels.

Conclusion There has been some improvement in Hb levels in preschool children in the last decade, but prevalence of anemia continues to be very high. Effective implementation of the comprehensive package of interventions recommended in National Iron Plus Initiative (NIPI) guidelines is urgently needed to achieve rapid and sustained reduction in anemia.

Keywords

- ▶ dietary intake of iron
- ▶ dietary intake
- ▶ iron fortified iodized salt
- ▶ test and treat strategy

Introduction

India has recognized the prevalence of anemia among preschool children as a major public health problem. Research studies in India had documented that poor iron stores in infants born to anemic mothers, poor dietary intake of iron and folate, and poor bioavailability of iron from Indian dietaries were the major factors responsible for high prevalence of anemia; infections like malaria in endemic areas aggravated the preexisting anemia. Intervention programs to address major factors responsible for anemia were initiated in the 1970s of the last century. Applied nutrition program focused on homestead vegetable cultivation for increasing

consumption of vegetables for improving iron and folate intake. Health sector interventions aimed at early detection and effective treatment of malaria and hook worm infection to reduce anemia in preschool children. However, the coverage under these interventions programs was very low as rural and urban nutrition and primary health care institutions had not been developed. Given the infrastructural constraints, screening all children and detection and treatment of anemic children was not possible. Therefore, the country embarked on identifying preschool children and providing all of them with iron and folic acid (IFA) supplementation. Available limited data from 1970 to 1999 based on surveys conducted by the National Nutrition Monitoring Bureau and

small scale research studies showed that the coverage under IFA supplementation in preschool children was low due to erratic availability and poor distribution of iron folic acid syrup as well as pediatric iron folic acid tablets; compliance with supplementation was poor because when children had initial minor gastro-intestinal side effects, the health system could not provide needed supportive supervision and reassurance.

By 1990s, most of the primary health care institutions in urban and rural areas were functional and it was expected that the coverage under IFA supplementation in pre-school children will improve. To monitor progress, coverage, and impact of IFA supplementation were incorporated as a part of all the national health and nutrition surveys. Data on these two aspects are now available from National Family Health Survey (NFHS 2 [1998–99]),¹ and NFHS 3 [2005–2006],² and 4 (2015),³ National Nutrition Monitoring Bureau (NNMB) micro-nutrient survey (2003),⁴ District Level Household Survey (DLHS 2 [2002–04],⁵ and DLHS 4 [2013–14]),⁶ and Clinical Anthropometric and Biochemical (CAB) component of the Annual Health Survey (AHS; 2014–15).⁷ All these surveys showed that the coverage under iron-folic acid (IFA) supplementation in preschool children continues to be very low (< 10%) in many states. Erratic availability and distribution of iron folic acid syrup as well as pediatric iron-folic acid tablets continue to be the major factors responsible for low coverage. Over the last four decades there has been:

- Some improvement in socio-economic status, household food security, and dietary intakes.
- Steady if slow reduction in under-nutrition rates in preschool children.
- Improvement in access to health care and reduction in malaria and hook worm infestation.

It is important to find out whether there has been any improvement in Hb levels and reduction in prevalence of anemia in preschool children in the last two decades, so that appropriate modifications can be made in the ongoing intervention programs for combating anemia.

Materials and Methods

Data on prevalence of anemia in the period between 1998 and 2016 at national and state level are available from NFHS 2, 3, and 4 national and state reports, DLHS-2 report, and DLHS-4

and AHS CAB fact sheets. NFHS surveys used Hemocue method for estimation of Hb; NNMB, DLHS, and AHS CAB used cyanmethaemoglobin method for Hb estimation. There have been several publications indicating that there were differences in Hb levels estimated by cyanmethaemoglobin method and Hemocue method.^{8–11} Therefore, the trends on changes in Hb were calculated separately for the NFHS series and AHS/DLHS series.

Data on prevalence of anemia in preschool children in India and different states were tabulated from the reports of the NFHS 2, 3, and 4. Raw data from NFHS 2, 3, and 4 were obtained from Demographic and Health Survey Program ICF International. The mean Hb levels in under-three in NFHS 2, under-three and under-five children in NFHS 3, and under-five in NFHS 4 were computed and compared. Frequency distribution of Hb in under-three children in NFHS 2 and 3 and under-five children in NFHS 3 and 4 were computed and compared.

DLHS 2 covered all the states and UTs; AHS CAB covered 9 poorly performing states (AHS CAB states), while DLHS4 covered 21 states and UTs (DLHS 4 states). The raw data of DLHS 2, 4, and AHS CAB were analyzed. Prevalence of anemia and the mean Hb levels in preschool children in DLHS 2 and 4 and AHS CAB were computed. The mean and frequency distribution of Hb and prevalence of anemia in preschool children in DLHS 2 were compared with the mean and frequency distribution of Hb and prevalence of anemia in preschool children in DLHS 4 and AHS CAB in the respective states.

Results

The age group and number of preschool children surveyed, blood sample collected, and valid Hb results available in different surveys are shown in ► **Table 1**. Prevalence of anemia at national level in preschool children was high in NFHS 2, 3, and 4 (► **Fig. 1**).

There was a 5% increase in prevalence of anemia in the 6 to 35 months age group in NFHS 3 as compared with NFHS 2. There was a 10% reduction in the prevalence of anemia in under-five children in NFHS 4 as compared with NFHS 3. There were substantial inter-state differences in the prevalence of anemia in all the three surveys. Prevalence of anemia in the Southern and Western states was lower as compared with the Central and Northern states both in under-three and under-five and across

Table 1 Number of children surveyed

Survey	Age of children surveyed (months)	Total number of children	Blood sample taken	Valid Hb
DLHS 2	6–71	313,646	195,193	173,684
NFHS 2	6–35	28,662	27,268	27,261
NFHS 3	6–59	48,084	35,851	35,844
NFHS 4	6–59	259,627	209,496	209,295
DLHS 4	6–59	71,707	48,896	44,494
AHS CAB	6–59	148,307	75,324	73,278

Abbreviations: AHS CAB, Annual Health Survey Clinical Anthropometric and Biochemical component; DLHS, District Level Household Survey; NFHS, National Family Health Survey.

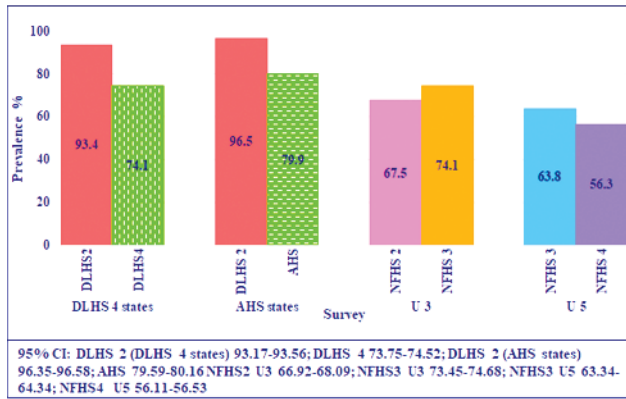


Fig. 1 Time trends in prevalence of anemia (DLHS 2, 4 AHS, and NFHS 2, 3, 4).

all the three surveys. Prevalence of anemia in under-three children was higher in NFHS 3 as compared to NFHS 2 in most but not all states. Prevalence of anemia in under-five children was lower in NFHS 4 as compared with NFHS 3 in all states though the magnitude of reduction varied between states.

Mean Hb levels (**Fig. 2**) in:

- Under-three children were lower in NFHS 3 as compared with NFHS 2.
- Under-five children in NFHS 3 was lower as compared with NFHS 4.
 - Frequency distribution of Hb:
 - In under-three children in NFHS 3 was to the left of Hb in under-three in NFHS 2 (**Fig. 3**).
 - In under-five children in NFHS 3 was to the left of the under-five children in NFHS 4 (**Fig. 3**).

Prevalence of anemia in under-five children in DLHS 4 states in DLHS 2 and 4, and prevalence of anemia in AHS CAB states in DLHS 2 and AHS CAB is shown in **Fig. 1** Prevalence of anemia in under-five children in AHS CAB states were higher as compared with the DLHS 4 states. There was a 20% reduction in the prevalence of anemia in DLHS 4 states and a 15% reduction in AHS CAB states as compared with the DLHS 2.

Mean Hb in DLHS 4 and AHS CAB states is shown in **Fig. 2**. The mean Hb levels in DLHS 4 states were higher as compared with AHS CAB states in both time periods. As compared with Hb levels in DLHS 2, there was an improvement in mean Hb of approximately 1.3 g/dL in the DLHS 4 states and 1 g/dL AHS CAB states.

Inter-state differences in prevalence of anemia in under-five children in AHS CAB and DLHS 4 states are shown in

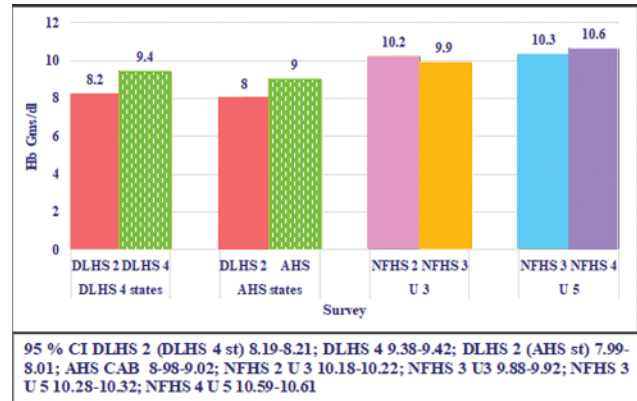


Fig. 2 Time trends in mean Hb (DLHS 2, 4 AHS CAB NFHS 2, 3, and NFHS 4).

Fig. 4. There were substantial inter-state differences in the prevalence of anemia in all the three surveys. Prevalence of anemia was lower in DLHS 4 states as compared with AHS CAB states at both the time points (**Fig. 4**). In all states except Uttarakhand, prevalence of anemia was lower in DLHS 4 and AHS CAB.

Frequency distribution of Hb in under-five children in DLHS 2 was compared with Hb distribution in DLHS 4 states and AHS states, respectively. There was a clear shift to the right in the frequency distribution of Hb both in DLHS 4 states and AHS states (**Fig. 5**).

Discussion

NFHS 2, 3, and 4, NNMB micro-nutrient survey, DLHS 2, 4, and AHS CAB survey have all undertaken Hb estimation between 1999 and 2015. Data from the NFHS surveys suggest that there was a 5% increase in prevalence of anemia between NFHS 2 and 3 and a 10% reduction in prevalence of anemia between NFHS 3 and 4. The reason for the higher prevalence of anemia in NFHS 3 is not clear. This might at least in part be due to the problems in the method (Hemocue) used for Hb estimation.⁸⁻¹¹ On the other hand as compared with DLHS 2, both DLHS 4 and AHS CAB (in their respective states) show substantial reduction in prevalence of anemia, increase in mean Hb, and shift to the right in frequency distribution of Hb. It is reassuring to note that despite continued poor coverage under IFA supplementation program there has been an improvement in Hb status of preschool children. Over the last four decades, there has been substantial improvement in per capita income and reduction in poverty and improvement

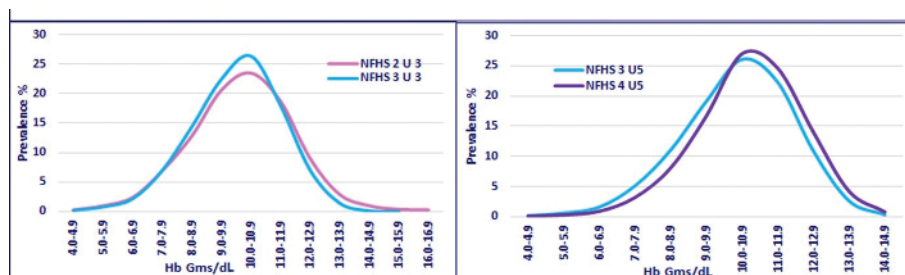


Fig. 3 Time trends in frequency distribution of Hb in under-three (NFHS 2, 3) and in under-five children (NFHS 3, 4). NFHS, National Family Health Survey; Hb, hemoglobin.

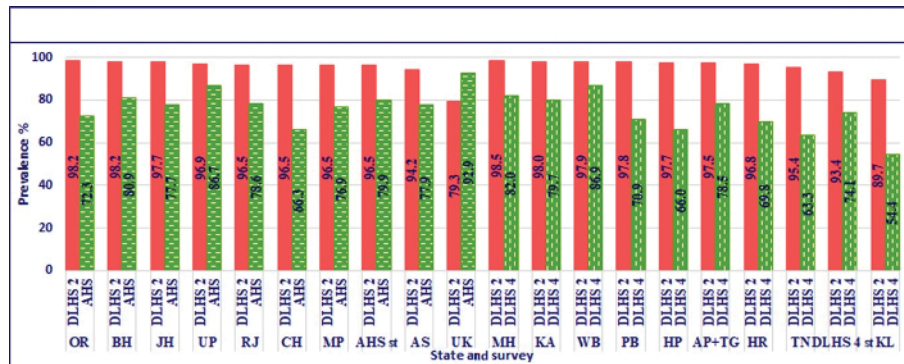


Fig. 4 Inter-state variation in prevalence of anemia (DLHS 2, 4 and AHS CAB). OR, Orissa; BH, Bihar; JH, Jharkhand; UP, Uttar Pradesh; RJ, Rajasthan; CH, Chhattisgarh; MP, Madhya Pradesh; UK, Uttarakhand; MH, Maharashtra; KA, Karnataka; WB, West Bengal; PB, Punjab; HP, Himachal Pradesh; AP + TG, Andhra Pradesh + Telangana; HR, Haryana; TN, Tamil Nadu; KL, Kerala.

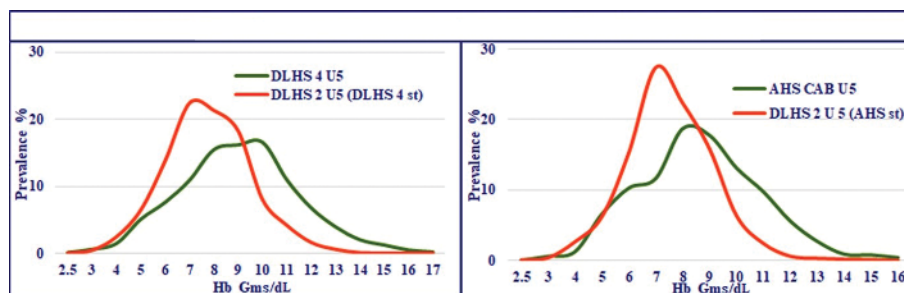


Fig. 5 Frequency distribution of Hb (DLHS 2, 4 and AHS CAB). DLHS, District Level Household Survey; AHS CAB, Annual Health Survey Clinical Anthropometric and Biochemical component; Hb, hemoglobin.

in household food security; access to health care for malaria and hook worm infestation has improved and there has been a slow but steady decline in under-nutrition rates. It is possible that the observed reduction in anemia might be part of the overall improvement in nutrition and health status of preschool children.

There have been debates how far prophylactic IFA supplementation can reduce prevalence of anemia in countries with low iron intake and high prevalence of anemia. The WHO expert groups had consistently recommended oral iron supplementation as public health intervention for improving Hb and iron status and reducing the prevalence of anemia in preschool children. Systematic reviews of the randomized clinical trials with iron folic acid supplementation in preschool children have shown that even in situations where prevalence of anemia was high, iron folic acid supplementation for 3 months or longer resulted in improvement in mean Hb ($-0.5-1$ g/dL) and ferritin levels.^{12,13} Improvement was higher in daily supplementation as compared with biweekly or weekly supplementation, but compliance with daily supplementation was more difficult to maintain on long-term basis. Current WHO guidelines¹⁴ recommend daily IFA supplementation for 3 months every year in settings where prevalence of anemias is 40% or higher. For infants and young children (6–23 months) iron syrup containing 10 to 12 mg of elemental iron (strong recommendation, moderate quality of evidence) and for children aged 24 to 59 months, 30 mg of elemental iron as drops/syrup or tablets (strong recommendation, very low quality of evidence) are to be given. The WHO guidelines note that unlike IFA supplementation in

school age children, the evidence base for the recommendations regarding preschool children is inadequate and emphasize the need for research studies documenting:

- Optimal dose, schedule and duration of iron supplementation.
- The effect of different doses and durations of iron supplementation on severity, prevalence of anemia.
- Safety of long-term iron supplementation in children who are iron sufficient.

Countries with relatively lower prevalence of anemia have expressed concerns over potential adverse health consequences due to iron overload following IFA supplementation. WHO guidelines recommend that if the prevalence of anemia is 20 to 40%, intermittent regimens of iron supplementation can be considered. Malaria-endemic countries concern over safety of IFA supplements has been allayed based on the WHO data which have shown that iron supplementation did not increase the risk of clinical malaria or death in areas where regular malaria-surveillance and treatment services are provided.

Program managers in India accept that low and erratic availability of IFA syrup/drops and tablets is the major factor responsible for the current low coverage under IFA supplementation. Sorting out these supply chain related problems is an essential prerequisite for effective implementation of the IFA supplementation program and once this is achieved there will be reduction in prevalence of anemia. There is, however, a persistent worry about ability to ensure optimal coverage under program conditions for 3 to 6 year children year after

year. Following IFA supplementation, not all anemic children become nonanemic; in children with low dietary intake of iron, there can be deterioration in Hb levels once supplementation is stopped.¹⁵ In the Indian context with low iron intake and 60 to 70% prevalence of anemia in preschool children, it is important to undertake operational research studies to document:

- Optimal duration of supplementation—3 or 6 months/year.
- The improvement in Hb and iron stores following IFA supplements.
- The proportion of anemic children who become nonanemic after supplementation.
- Sustainability of the supplementation program under service conditions.
- Changes in Hb and iron stores 6 months after stopping of the supplementation.

All the Hb surveys indicate that though there has been some reduction in the prevalence of anemia, currently prevalence of anemia across all states of the country is unacceptably high. There is an urgent need to achieve a faster and sustained reduction in prevalence of anemia in preschool children. WHO¹⁴ guidelines as well as the NIPI¹⁶ guidelines recommend that as and when preschool children access health care, Hb estimation should be done and anemic children given appropriate treatment. One of the essential prerequisites for this strategy is accurate estimation of Hb. NIPI guidelines recommend Hb estimation using Hb color card or Sahli's Haemoglobinometer.¹⁶ Neither is accurate enough for correctly grading anemia and monitoring improvement with therapy. Hemocue has been advocated for use as it does not require accurate pipetting and results are immediately available; but it is expensive and not accurate.⁸⁻¹¹ Experience with DLHS 2,4, and AHS CAB indicate that dried blood spot indirect cyanmethaemoglobin method is the most appropriate method for Hb estimation in community and primary health care settings. Auxiliary nursing midwives (ANMs) and laboratory technicians (LTs) can be trained for finger prick blood collection, depositing 20µL of blood on filter paper, drying it and putting in a plastic zip lock bag. All primary health centers have a LT; most of them have a colorimeter; so they can do Hb estimation by cyanmethaemoglobin method. Given the importance of anemia and need to operationalize 'test and treat strategy' to achieve more rapid decline in anemia, it is essential to invest time in training ANMs in finger prick blood collection and technicians in Hb estimation by cyanmethaemoglobin method. Once the accurate estimation of Hb is available at all levels of care, it might be possible to effectively implement the 'test and treat strategy' and achieve more rapid reduction in prevalence of anemia.

Experience from long-term supplementation programs across countries have shown, it is difficult to sustain supplementation programs year after year; personnel required for distributing, counseling, and monitoring supplementation programs make them expensive. In this context, WHO¹⁴ advocates use of food fortification with iron. NIPI¹⁶ guidelines

envisage improving the Hb status and reducing the prevalence of anemia using a multi-pronged strategy. The strategy envisages:

- Improvement in dietary intake of vegetables and inexpensive fruits rich in iron and folic acid;
- increase in use of iron fortified iodized salt (DFS) to begin with through the hot cooked meal in the Integrated Child Development Service (ICDS) anganwadi centers and progressively leading to improved access to and use of DFS at household level;
- improved coverage under biweekly iron folic acid supplementation to under-five children through Village Health and Nutrition Days and home visits by ASHA; and,
- 'test and treat strategy' for early detection and treatment of anemia.

Effective implementation of the comprehensive package of interventions recommended in the NIPI guidelines is urgently needed to achieve rapid and sustained reduction in anemia.

Conclusion

Data from national surveys using accurate time tested methods for Hb estimation have shown that, in India there has been some improvement in Hb levels in preschool children in the last decade, but prevalence of anemia continues to be unacceptably high.

Data from the Indian surveys have shown the importance of using accurate method of Hb estimation to monitor improvement in Hb over time. It is essential to use accurate method of Hb estimation in large scale national surveys across the world, as the world embarks on monitoring improvement in Hb over time to assess progress toward sustainable development goals (SDG) target for anemia.

Funding

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Conflict of Interest

None declared.

Acknowledgments

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References

- 1 IIPS, National Family Health Survey (NFHS) 2. Available at: <http://rchiips.org/nfhs/nfhs2.shtml>. Accessed January 18, 2018
- 2 IIPS, National Family Health Survey (NFHS) 3. Available at: <http://rchiips.org/nfhs/nfhs3.shtml>. Accessed January 18, 2018
- 3 IIPS, National Family Health Survey (NFHS) 4 Fact sheets. Available at: http://rchiips.org/nfhs/factsheet_NFHS-4.shtml. Accessed January 18, 2018

- 4 NNMB, PREVALENCE OF MICRONUTRIENT DEFICIENCIES NNMB technical report 22 (2003). Available at: <http://nnmbindia.org/NNMB%20MND%20REPORT%202004-Web.pdf>. Accessed January 18, 2018
- 5 IIPS, DLHS-2 - District Level Household & Facility Survey. Available at: <http://rchiips.org/PRCH-2.html>. Accessed January 18, 2018
- 6 IIPS, DLHS-4 - District Level Household & Facility Survey. Available at: <http://rchiips.org/DLHS-4.html>. Accessed January 18, 2018
- 7 RGI, Annual Health Survey: CAB component. Available at: <http://www.censusindia.gov.in/2011census/hh-series/cab.html>. Accessed January 18, 2018
- 8 Mohanram M, Ramana Rao GV, Sastry JG. A comparative study on prevalence of anaemia in women by cyanmethemoglobin and hemocue methods. *Indian J Community Med* 2002;27(2):58-61
- 9 Kapoor SK, Kapil U, Dwivedi SN, Anand K, Pathak P, Singh P. Comparison of HemoCue method with cyanmethemoglobin method for estimation of hemoglobin. *Indian Pediatr* 2002;39(8):743-746
- 10 Bhaskaram P, Balakrishna N, Radhakrishna KV, Krishnaswamy K. Validation of hemoglobin estimation using Hemocue. *Indian J Pediatr* 2003;70(1):25-28
- 11 Pathak P, Kapoor SK, Dwivedi SN, Singh P, Kapil U. Comparison of hemoglobin estimates from filter paper cyanmethemoglobin and HemoCue methods. *Indian J Community Med* 2004;29(3):149
- 12 Thompson J, Biggs BA, Pasricha SR. Effects of daily iron supplementation in 2- to 5-year-old children: systematic review and meta-analysis. *Pediatrics* 2013;131(4):739-753
- 13 Pasricha SR, Hayes E, Kalumba K, Biggs BA. Effect of daily iron supplementation on health in children aged 4-23 months: a systematic review and meta-analysis of randomised controlled trials. *Lancet Glob Health* 2013;1:e77-e86
- 14 WHO, Guideline: Daily Iron Supplementation to Infants and Children Guideline. Geneva: World Health Organisation; 2016
- 15 Duque X, Martinez H, Vilchis-Gil J, et al. Effect of supplementation with ferrous sulfate or iron bis-glycinate chelate on ferritin concentration in Mexican schoolchildren: a randomized controlled trial. *Nutr J* 2014;13(1):71
- 16 MoHFW - GOI: Guidelines for Control of Iron Deficiency Anaemia. Available at: http://www.pbnrhm.org/docs/iron_plus_guidelines.pdf. Accessed September 23, 2017

Immobilization of Urease on DEAE-Cellulose Strips for One Step Urea Detection

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Abstract

Urease isolated from pigeonpeas was immobilized on 1 cm × 6 cm diethylaminoethyl (DEAE)-cellulose strips. The optimum immobilization (55% activity) was observed at 4°C, with a protein concentration of 1 mg per strip at pH 6.5. Immobilized strips stored at 4°C showed an increased shelf-life and there was no leaching of enzyme from the immobilized strips for 6 months. Enzyme could be eluted on application of 0.2 M KCl in buffer, which showed that the binding of the enzyme was tight on DEAE-cellulose strips. These strips when used along with dye, phenol red (coimmobilized) gave different shades from light yellow to dark magenta depending upon the concentration of urea. The ease of immobilization of urease on DEAE-cellulose strips and easy availability of pigeonpeas urease described in the present study makes it a suitable product for potential applications in urea diagnostics.

Keywords

- ▶ diethylaminoethyl-cellulose paper strips
- ▶ urease
- ▶ urea
- ▶ immobilization
- ▶ analytical application

Introduction

Estimation of urease is frequently performed in the medical as the metabolic function of the kidney is reflected in the concentration of urea in blood or urine.¹ Urease (urea amidohydrolase, EC 3.5.1.5) catalyzes the hydrolysis of urea yielding ammonia and carbon dioxide.^{2,3} It has now become possible to decompose urea using artificial kidneys containing immobilized urease (Japanese Patent Publications numbers 36751/85 and 17467/86). In food industry, urea is undesirable, particularly in biologically fermented food products, such as beer, wine, soy sauce, or sake. For these applications, neutral urease obtained from seeds of plant bean species such as *canavalia ensiformis*, or from microorganisms such as *eubacterium aerofaciens* and *P. mirabilis* are very useful and yielding good results.⁴ There is ongoing approach to explore cheap sources of urease, which are readily available and have better physico-chemical properties.

The utilization of any enzyme is driven by its versatility, regio-, chemo- and enantio-selectivity while operating under mild conditions making any process environmentally compatible.⁵ Success of an enzyme's utilization in industries depends on its reuse. Enzyme immobilization is one of the best ways to reuse the enzymes. The anchoring of an enzyme

to a solid insoluble support should be straightforward and cost-efficient. The key factor determining the success or failure of immobilization depends on the methodology used for enzyme immobilization.⁶ One of the serious problems for the industrial application of the technology of immobilization is the diffusional limitations to the transport of substrate and product.⁷ This limitation arises from the steric hindrance of the solid matrix to the free diffusion of substrates and products toward or away from the catalytic site of the immobilized enzymes.

Urease from pigeonpea was purified and characterized from our laboratory⁸ and can be bound on DEAE-cellulose strips at neutral pH. The ready availability of urease, its low cost, and the ease of its immobilization on cellulose paper strips described in the present study, makes it a product for future applications in therapeutics and diagnostics.

Materials and Methods

Materials

Urease was isolated from dehusked pigeonpeas procured from agriculture shops from the local market. Urea (enzyme grade), ammonium chloride, and Tris were obtained from Sisco Research Laboratories (Mumbai, India). Nessler's

reagent and phenol red were obtained from HiMedia Laboratories (Mumbai, India). Trichloroacetic acid was obtained as 20% solution from Loba Chemie (India). DEAE-cellulose paper (DE 81) from Whatman International (Maidstone, Kent, United Kingdom) was a generous gift from Professor PM Dey, School of Biological Sciences, University of London, United Kingdom. All solutions were prepared in Milli Q water (Millipore, United States).

Urease Purification

Urease was extracted in 0.1 M Tris-acetate buffer, pH = 6.8 and purified 200 fold to electrophoretic homogeneity, from dehusked pigeonpeas according to the method described from this laboratory.⁸ The specific activity of the purified enzyme was 5×10^3 units/mg of protein, with approximately 10% yield.

Protein Assay

Protein content was measured using the method of Lowry et al.,⁹ using bovine serum albumin as standard protein.

Urease Assay

For measurement of urease activity, ammonia liberated on incubating the enzyme with urease in a fixed time period at an enzyme saturating concentration of urease was determined using Nessler's reagent. The golden brown color produced was measured spectrophotometrically at 405 nm. The amount of ammonia liberated in the test solution was calculated by calibrating the reagent with standard ammonium

chloride solution. An enzyme unit was defined as the amount of enzyme required to liberate 1 μ mole of ammonia per minute under test conditions (0.1 M urea, 0.05 M Tris-acetate buffer, pH = 7.3 at 37°C).

Immobilization on Paper Strips and Assay

The DEAE-cellulose paper was cut into 1 cm \times 6 cm strips and prewashed with Tris-acetate buffer (0.1 M, pH = 6.5) and dried using a hair drier. Strips were dipped in a solution of urease 1 mg/mL and 0.6 mg/mL phenol red and dried again and stored in capped tubes till used at 4°C. By doing so, urease and dye were coimmobilized on the DEAE-cellulose strips. These strips were then dipped in different concentrations of urea (0.1–2 g/L). This yields different colors on strips from light yellow to deep magenta. Response time for this was only 30 seconds. Such a strip chart developed can be then used for assay of urea in a blood serum samples.

Storage Stability

DEAE-cellulose strips (1 cm \times 6 cm) were stored at 4°C for several months and used, when required.

Results and Discussion

The immobilization of urease on DEAE-cellulose strips under different conditions of immobilization was studied. The results are obtained on the percentage immobilization with varying protein concentration and optimal volume

Table 1 The optimal condition testing for DEAE-cellulose paper strips (1 cm \times 6 cm)

Condition	Volume of urease loaded (μ L)	Protein conc. (mg/mL)	Incubation (h)	Immobilization (%)
Protein concentration (mg/mL)				
0.1	80	–	24	45
0.2	80	–	24	48
0.5	80	–	24	50
1.0	80	–	24	55
1.5	80	–	24	52
Volume of enzyme loaded (μL)				
10	–	1	24	40
20	–	1	24	45
40	–	1	24	50
80	–	1	24	55
100	–	1	24	53
Incubation (h)				
6	80	1	–	45
12	80	1	–	50
24	80	1	–	55
48	80	1	–	45
60	80	1	–	38

Abbreviation: DEAE, diethylaminoethyl.

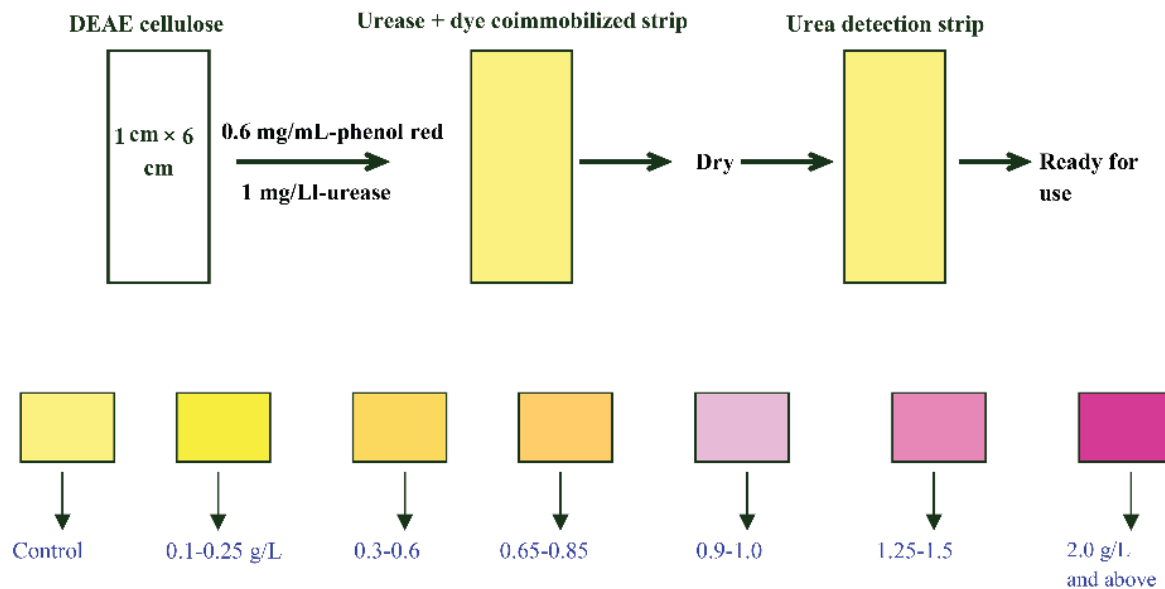


Fig. 1 The one step urea detection: the top panel shows the preparation of the urease strip and the bottom panel shows the color chart upon addition of different concentrations of urea.

loading were studied and are summarized in **Table 1**. clearly showed that optimum immobilization was obtained at 1 mg protein per strip at 4°C. Immobilization percentage reduced above or below 1 mg of protein/strip. Optimal loading of volume was 80 µL and the optimum incubation time was 24 hours. A pH of 6.5 was maintained for immobilization so that urease is tightly bound to DEAE-cellulose. The shelf-life of immobilized strips at 4°C increased to about 6 months (data not shown). It may be noted here that during urease purification from pigeonpeas, a DEAE-cellulose chromatography was an essential step and the urease adsorbed could be eluted only by applying a KCl gradient, which confirmed that urease is tightly bound to DEAE-cellulose.⁸

Highly purified urease was successfully immobilized on DEAE-cellulose paper strips. The percentage retention was 55% and the binding was tight. This was confirmed by elution of the enzyme by using 0.2 M KCl in buffer. No enzyme was eluted when strips were washed with buffer alone. The levels of urea in the clinical range are easily detectable by this method. The urease strips are highly specific for detection of urea present in serum. Earlier studies have clearly showed that urease has an absolute specificity for its substrate urea.⁸ Strips give different shades of color depending upon the concentration of urea present in the serum samples. It has a color range from light yellow to dark magenta. Normal values of urea in blood/serum are 0.15 to 0.40 g/L and the dynamic range is 0.5 to 2 g/L. The color chart of urea strip was prepared and is shown in **Fig. 1**. The control strip showed pale yellow color. Though many different methods are available for urea assay, present method provides a simple and fast detection based on the color matching with the strip.

Immobilization led to stabilization of urease on the strip with a shelf-life of 6 months at 4°C. Similar storage stability

was observed earlier when urease was immobilized on gold nanoparticles; 4 however, cost-wise DEAE-cellulose paper strips are much less expensive. We also immobilized urease on glutaraldehyde-activated chitosan beads; however, getting uniform sized chitosan beads was a problem and the shelf-life was approximately 1 month.¹⁰ Therefore, these cellulose strips may have potential market for urea assay. Furthermore, detection of urea in milk samples is important to detect 'adulteration in milk' in several parts of western Uttar Pradesh. These strips may be useful for detection of urea in 'synthetic milk' and thus to check its adulteration.

Conflict of Interest

None declared.

Acknowledgments

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References

- 1 Das N, Kayastha AM, Malhotra OP. Immobilization of urease from pigeonpea (*Cajanus cajan* L.) in polyacrylamide gels and calcium alginate beads. *Biotechnol Appl Biochem* 1998;27(1):25-29
- 2 Andrews RK, Blakeley RL, Zerner B. Urea and urease. *Adv Inorg Biochem* 1984;6:245-283
- 3 Mobley HLT, Island MD, Hausinger RP. Molecular biology of microbial ureases. *Microbiol Rev* 1995;59(3):451-480
- 4 Dwevedi A, Routh SB, Yadav AS, Singh AK, Srivastava ON, Kayastha AM. Response surface analysis of nano-ureases from

- canavalia ensiformis and cajanus cajan. *Int J Biol Macromol* 2011;49(4):674–680
- 5 Hanefeld U, Gardossi L, Magner E. Understanding enzyme immobilisation. *Chem Soc Rev* 2009;38(2):453–468
 - 6 Xiao QG, Tao X, Zou HK, Chen JF. Comparative study of solid silica nanoparticles and hollow silica nanoparticles for immobilization of lysozyme. *Chem Eng J* 2008;137:38–44
 - 7 Prakasham RS, Devi GS, Laxmi KR, Rao CS. Novel synthesis of ferric impregnated silica nanoparticles and their evaluation as a matrix for enzyme immobilization. *J Phys Chem* 2007;111:3842–3847
 - 8 Dwevedi A, Kayastha AM. Optimal immobilization of β -galactosidase from Pea (PsBGAL) onto Sephadex and chitosan beads using response surface methodology and its applications. *Bioresour Technol* 2009;100(10):2667–2675
 - 9 Das N, Kayastha AM, Srivastava PK. Purification and characterization of urease from dehusked pigeonpea (*Cajanus cajan* L) seeds. *Phytochemistry* 2002;61(5):513–521
 - 10 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193(1):265–275
 - 11 Kayastha AM, Srivastava PK. Pigeonpea (*Cajanus cajan* L.) urease immobilized on glutaraldehyde-activated chitosan beads and its analytical applications. *Appl Biochem Biotechnol* 2001;96(1-3):41–53

The Experiences of Medical Graduates to Peer Teaching in a Large Group

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Abstract

Medical education is persisting to be chiefly structured around faculty authority and didactic lectures. This upholds idiosyncratic spirited milieu rather than the two-way ones desirable for the relevance in current clinical practice. The present study was set to refurbish the at hand scenario by the assimilation of active learning strategy as seminars in human anatomy curriculum of medical undergraduate program. The underlying purpose of this study was to evaluate the inclusion of varied modalities of active learning stratagem. The aim was also to construct an interactive two-way classroom prospects for thorough understanding, conceptualizing, problem solving, and utilizing student oriented presentations to elucidate multifarious subject concepts in an easy and de novo approach. The study was conducted on First Professional MBBS students in the Department of Anatomy at the Institute by a seminar activity for active comprehension followed by student feedback. A qualitative and quantitative analysis was done where close-ended questions were concerned with the usefulness of the activity and significant aspects related to the understanding of anatomy. The scores for student feedback were graded in a five-point Likert's scale. The institutional experience of facilitators of this tertiary care institution and their efforts in successful implementation of seminar activity have set an example and responsibility for the medical educators all over the globe to use more and more of such instructional approaches.

Keywords

- ▶ anatomy
- ▶ seminar activity
- ▶ active learning
- ▶ feedback

Introduction

The key aspect in student-centered learning is well illustrated by various workers by apprentice activities. The major emphasis is always on the experiences attained during and preceding the schedule. The progression is the major concern instead of the final learning outcome. The literature is also suggestive of handing over the mode of learning to be chosen by learners themselves or in conciliation with the educator.¹ The studies many a time accentuated on the significance of the use of various means of assessment employed and the reason behind it. It is also important to note that medical students are expected to learn, comprehend, and execute the learned skills in a limited training.² It was reported that the ways of assessment shall be altered by moderators for a better learning experience. Such ideology is still relevant after

so many years.³ Still there are scores of instances showing employment of poor assessment stratagem in medical education curriculum.

In today's constantly evolving era, education is also showing marked alterations. The contemporary cohort of learners wishes delightful atmosphere, self-command, apparent prospects and expositions, personal empathy with the mentors, integrity, and unconstrained utilization of technology.⁴⁻⁶

The innovative trend incorporates teaching with extensive utilization of active learning practices so as to augment the performance.⁷ A well-known fact is that learning and participation are in each other's pocket.⁸ Such studies by eminent workers worldwide optimized the facilitators and persuade them to accustom and modify their instructional archetype in accordance to the cohort of neologist learners.⁶

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In medical education, many facilitators are harmonizing specifically amid the core aim for assessment as comprehension control and assessment as integral component of the learning progression. Nowadays, the major emphasis is on creating learning environment that stresses on student-centered ideas and promotes soaring activity amongst students. With this, we were intended to plan a mode of assessment that would come out as an unambiguous tool for learning, and also provide the facilitators with an unswerving basis for righteousness while allocating marks. In the present study, we shall be elucidating and describing our institutional experience along with drawing some conclusions and determining whether this innovation regarding the use of seminars would be well received and effective for student's teaching-learning of anatomy.

The rationale of this study was to appraise the inclusion of varied modalities of active learning stratagem. It included encouraging camaraderie, enhancing interpersonal and communication skills, and commencing and formulating a system for disseminating intricate medical concepts in an accessible uncomplicated approach for learners to employ in the future clinical scenario in the anatomy course of the undergraduate medical curriculum.

Materials and Methods

A study was conducted on First Professional MBBS students in the department of Anatomy at the All India Institute of Medical Sciences, Jodhpur, by a seminar activity for the active comprehension followed by the student feedback. The anatomy curriculum for undergraduates comprises both small-group (up to 12 students) cadaveric dissection sessions and large-group lecture classes of 1 to 2 hours each.

In an endeavor to sever the monotony and persuade the apprentices with dynamic learning, an activity of student seminar was conducted. The activity comprised two elements, a 10 minutes presentation by student, followed by 5 minutes for the postpresentation discussions.

In the introductory assembly, students were made well-versed with the active learning strategies which were included as an activity instead of proverbial lectures. As an enticement other than the benefits of individual learning and the skill development that would place them in better stead with future colleagues and patients, students were also briefed that involvement in the exercise would harvest part of their final evaluation.

Student Presentations

Faculty members prepared a list of topics appropriate for the presentation out of whole anatomy curriculum. Each student was allotted a topic according to their roll numbers and this list was displayed 2 weeks before starting the activity. In these 2 weeks, students prepared their topics and guidance was given by the allotted supervising faculty member. The activity was performed in the hours scheduled for anatomy lectures and practicals only. Thus there was no rescheduling of timetable of other subjects in the First Professional course. The students for the presentation were randomly selected by the

sweepstake system. On each day of activity, a random student was asked to pick up blinded roll numbers from the given box. These picked-up students were to present their topics on that particular day. The sessions were moderated by at least three faculty members always at any point of time along with all the in-house residents during whole activity. Students were called to the podium and asked to summarize the topic allotted in stipulated slot. The student presenters were instructed to use various props and modes of choice as models, bones, charts, or blackboard to elaborate and elucidate the topic. They were discouraged to use the electronic modes and pre-prepared printed matter. The reason to do so was to encourage them to be creative and learn the diagrams as well.

Postpresentation Discussions

Subsequent to the presentation, the session was made open for the audience to carry out the discussion. Stipulated time was assigned for this section also. Audience students were encouraged to ask questions regarding the concerned topics and were permitted to highlight the faults in the presentation in a constructive mannerism. Moreover, at the same time the ideas were invited to rectify them. The students were expected to stress upon the missed points, in case, by the presenter. The participant students were allotted marks by all the moderating faculties. In addition, the provision of scoring marks was there for the audience students too depending on the queries and the valid points rose. The final comment and suggestion was by the faculty regarding the topic, way of presentation, flaws, and strengths etc. At the end, applause was done to motivate and encourage the presenters for their efforts.

The marks allotted to each student by various faculties including its presentation and active participation in others sessions was tabulated and a final score was obtained.

At the end of a fortnight long activity of the seminar, a pre-tested questionnaire was disseminated among the students about the worth of this educational activity. It comprised both closed- and open-ended questions. Close-ended questions were concerned with the usefulness of the activity and significant aspects related to the understanding of anatomy and were graded in a five-point Likert's scale.

The quantitative data were entered and analyzed through SPSS for Windows version 16. The qualitative data were assessed through thematic analysis.

Observation and Results

Subsequent to the seminar activity, the students were given a questionnaire to be filled. All the 90 participants voluntarily participated with consent. They were given necessary instructions and requested to fill the questionnaire. It was completed and returned with full keenness.

The mean age of our sample was 18.5 (\pm 1.5) years and it consisted of 69 (76.66%) male and 21 (23.33%) female students.

The outcome of students' retort to the feedback form is represented in ► **Fig. 1**. Analysis of statistical feedback data from the seminar-activity questionnaire was done using the Excel program. It was presented graphically as well as accounted in descriptive manner.

S. No.		Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1.	Were Seminars Informative a. For speakers b. For audience	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
2.	Retaining information was better through seminars	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3.	Preparation required more than a week's time	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4.	Peers were of help in the preparation	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5.	Declaration of schedule and topics well ahead helped in preparing better	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6.	One should read the topics presented by others after the seminar also	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.	It is a good way of revision	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8.	It is a good source of knowledge of topics	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9.	Sessions were open to ideas and interactions	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10.	Improved hesitation of public speaking and highlighted the shortcomings	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11.	Question and answer session were of importance	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12.	Drawing of diagrams were improved by it	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
13.	Seminars are of importance regarding a. Long-questions b. Short-questions c. Viva-voce	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
14.	Motivational aspect a. Increased the interest in topics and subject b. Motivation to learn	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
15.	Improved facilitation of learning by different methodology than didactic lectures	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
16.	Helped in relating text book knowledge to clinical aspect in open discussions	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
17.	In-depth interactions cleared doubts	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
18.	Direct prompt feedback from faculty has improved a. Speaker b. Audience c. Both	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
19.	Role of strictness of attendance	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
20.	Overall rating of seminars	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Fig. 1 Feedback questionnaire given to the students.

The majority of students agreed that this seminar was informative and useful and encouraged creativity and teamwork. Moreover, most of them agreed that skills related to presentation, counseling, and evidence-based medicine were also enhanced.

A good number (40%) of students agreed that the seminars were informative and a learning experience for speakers,

whereas around 58.8% of them considered it to be just an average medium of the audience. In contrast, 82.22% found the retaining of information difficult through the activity (► Fig. 2).

Very less of the fraction considered it to take more than a week time for preparation and around 30% found the peer group a great help in preparing the topic for presentation.

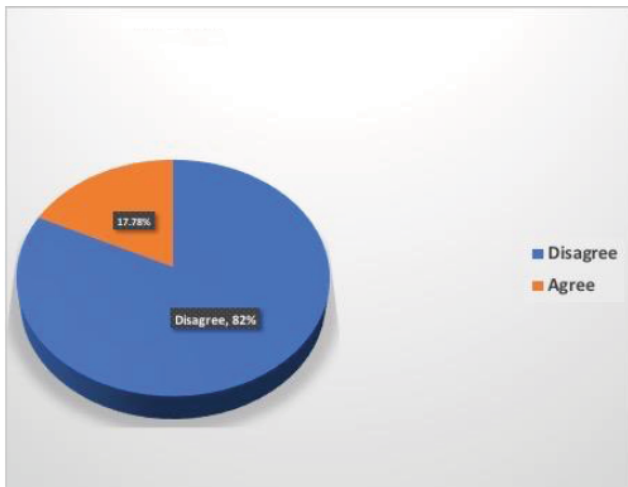


Fig. 2 Chart showing retaining information was better through seminars.

They also favored the declaration of schedule and topics well ahead which helped them in preparing the topics better. Not many of the students (31.1%) were interested in reading the topics presented by others even after the seminars. Acceptability of this novel method as a model for revision and as a source of knowledge was found less among the students. For a larger group (46.66%), the post seminar session were open to ideas and interactions. Question and answer session were of importance and drawing of diagrams (31.11%) was also reportedly found to be improved. Out of whole census, 65.55% reported to have improved the hesitation of public speaking and also found that their shortcomings were highlighted in a productive mannerism (► **Fig. 3**).

The students found the seminars to be of help in practical examination in answering the questions at time of viva voce (29%). Moderate percentage agreed for it to be helpful of importance regarding long and short questions. Regarding the motivational aspect, again most students found it just to be a not good to fair medium to enhance the interest in topics and their motivation to learn. Around more than half participants (60%) were in support of collateral methodology other than didactic lectures in curriculum (► **Fig. 4**).

Such presentations by the peer group also found to help them in relating text book knowledge to clinical aspect in open discussions (34.44%). A mixed response was found regarding the impact of direct prompt feedback from faculty in the improvement (► **Fig. 5**).

Interesting was to observe that ample number of students were in disagreement (25.55%) with the mandatory attendance during whole activity by the facilitators. Still students also found it to be very good (18.88%) and excellent (24.44%) way to expose as maximum as possible participants in activity. Overall, by and large, big assemblage (41.11%) of students found this activity useful for their learning.

Students were also asked open-ended questions regarding the role of seminar activity. One question was "What did you like BEST about this seminar?" In informal discussion, students responded to this question. The affirmative theme that was highlighted included an augmented intensity of interest

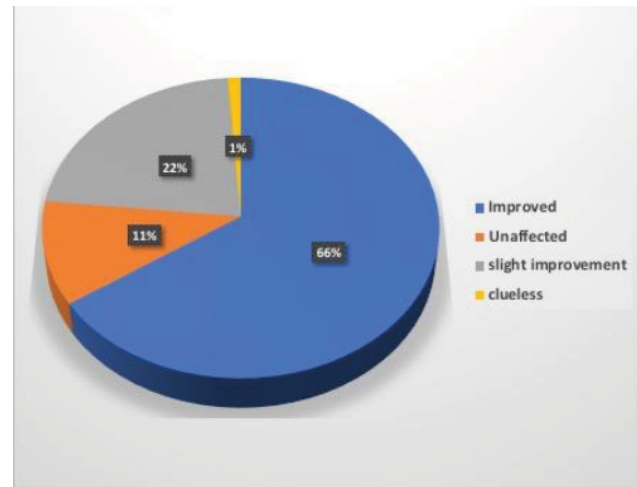


Fig. 3 Chart showing opinion regarding effect on public speaking.

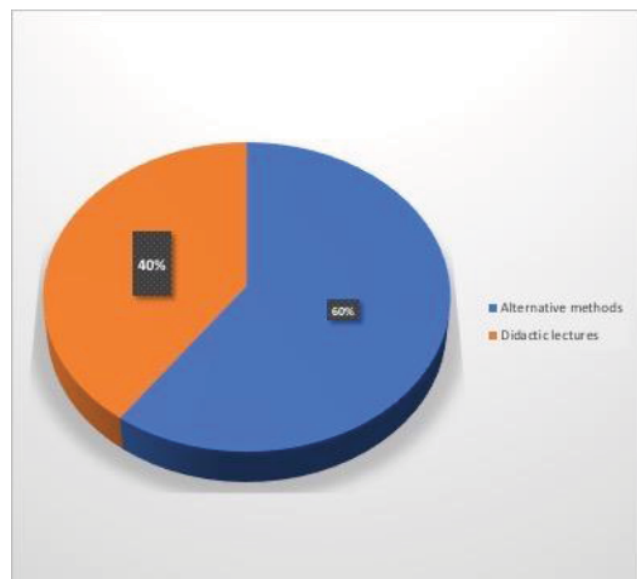


Fig. 4 Chart showing improved facilitation of learning by different methodology than didactic lectures.

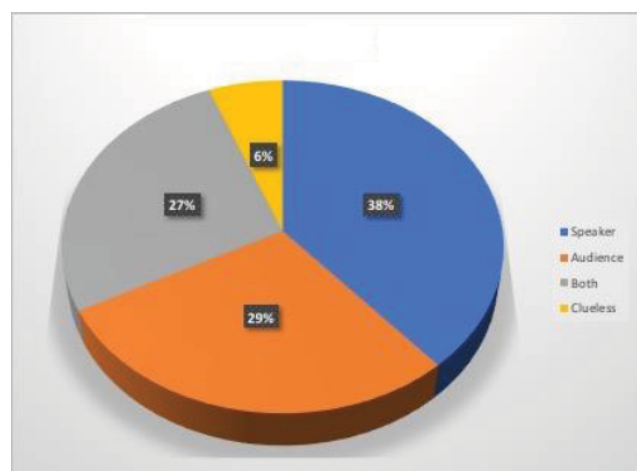


Fig. 5 Chart showing direct prompt feedback from faculty has improved.

in their studies and the break from the boredom and repetitiveness of lectures in a monotonous manner throughout the curriculum. It was also emphasized that such commotion was distinctive by being concurrently ingenious, tranquil and on the whole educational for the students.

Regarding the question of LEAST liked about the seminars, students were bit hesitant initially to answer. But still after gaining the confidence of them by explaining them the utility of this particular opinion in the whole activity, the few responded to this question. The unswerving theme stressed in this was the appraisal or prompt feedback offered by the faculty at the closing stages of each presentation. Students found that the faculty was contemptuous of their efforts and in few cases, open criticism was not taken in a constructive manner. The students already in low esteem and public speaking skills found it not to be of any help as it sometimes happening to lower their confidence even more. The major fact behind this was that few students took the critique in a personalized way.

It was also the opinion that the faculty apparently took this entire activity as a serious scholastic activity; while for few students, acceptability for a novel approach was non-palatable. This disparity of insight may have rooted a divergence and in turn was the reason behind major off-putting citations.

Discussion

A futuristic envision for an advanced panel approach to the patient management in clinical set-up has motivated the educators for active participation of medical students in their personal learning and training. This encourages and endows with prospects for the thinking skills development and interpersonal skills desired to function efficiently in the new setting. Such exercises with student involvement accentuate assemblage activities for the medical students. Active learning utilizes approaches that deal with a greater range of individual learning modes and encourages the efficient team work and interpersonal skills during the progression.

It is most important for the curriculum planners to understand the objectives of the course and the basic requisite of the learners. This comprehension will help them in choosing diverse approaches that can be exercised to involve students in the self-learning. Out of wide variety of active learning, which includes model making, demonstrations, extempore, use of simulators, acts, painting exercises, problem solving, case studies, and learning through games etc., the student led seminars is also an imperative strategy. Worldwide literature has revealed that these approaches are extensively used nowadays as novel method of teaching-learning in medical education.

During the practical hands-on in dissection hall, it is simple to engross students in dialogue and active learning. Whereas, the greater part of theory is covered under didactic lectures. It is apparent that the tedium and bleakness with continuous one-way communication makes the students uninterested in this lecture format of teaching-learning.^{9,10} In the analysis of student feedback, the integration of active learning approaches was appreciated by the students.¹¹ Still

in contrast, the major issue raised was the time of activity in curriculum as it was selected at the end before exams. They found it good for revision but it was a high time for their self-study too.¹² The literature mining made a valid point of various benefits of dynamic, student-centered learning in comparison to conventional didactic lecture. It is noteworthy that no single method is complete and merits of didactic lecture can also not be denied.^{13,14} In other words, there lies a difference in envisaging crisis and really doing something to solve it, the approach of active learning offers an occasion for the learners to do something to decipher the problem.⁵

Few students also had opinion of such activities to be a misuse of their high time before examination. They also have a view that for few topics, stipulated time was either insufficient or their way of elucidation needed more time to cover-up complete topic. This is not unusual. It is evident and well-known finding that learners are unwilling in incorporating such active learning modes as their preset mind is unable to consider such tangential ways positively in the scholastic setting.^{4,5,15,16} The passive existences during didactic lectures is rather an easy way for nonserious students and even for majority as students in India are habituated of learning through such ways only.

Transforming the conventional form of seminar by addition of questionnaires, test sessions, role plays, and group discussions with seminars have found to enhance the students' attentiveness, zeal, and motivation; hence, accentuating the learning spirit.¹⁷

The notion of peer involved experimental learning is further reinforced by socio-cultural learning model, which elucidates how involvement and interaction with peers aids students in acquiring knowledge and comprehension.¹⁸

The present study and its successful implementation have set an example and responsibility for the medical educators all over the globe to the more and more use of such instructional approaches. The notion of considering the lateral activities as not the serious academic teaching-learning should be changed. From prehistoric times, medical education is renowned for its blending of classical and novel strategies which keep the educators as well as learners as well at ease. Also for the mass of students who are apprehensive of their performance in routine exam format, get a fair chance for improving the grades and scores.

Conclusion

Inclusion of active learning process enhances not only students understanding of subject but also their set of skills that benefits them in their clinical practice in future career. Although the outcome of study is preliminary, by and large limited by the results of a single exercise for one academic session, the student-led seminar activity may have the potential to improve training for medical undergraduates; the challenge presented here can be justified further with auxiliary exploration.

Conflict of Interest

None declared.

References

- 1 Boud D. Assessment and learning: contradictory or complementary. In: Knight P, ed. *Assessment and Learning in Higher Education*. London: Taylor & Francis; 1995: 35–48
- 2 Hernández-Torrano D, Ali S, Chan CK. First year medical students' learning style preferences and their correlation with performance in different subjects within the medical course. *BMC Med Educ* 2017;17(1):131
- 3 Fowler G. Postmodernism: this changes everything! *J Stud Cent Learn* 2003;1(2):87–95
- 4 Gomathi KG, Shaafie IA, Venkatramana M. Student-led seminars as a teaching-learning method-effectiveness of a modified format. *South East Asian J Med Educ* 2014;8:82–84
- 5 Parekh M, Munjappa H, Shinde S, Vaidya S. Student perceptions on activity-based learning in physiology. *Natl J Physiol Pharm Pharmacol* 2018;8:590–593
- 6 Nelson C. Student diversity requires different approaches to college teaching, even in math and science. *Am Behav Sci* 1996;40(2):165–175
- 7 Wlodkowski RJ. Fostering motivation in professional development programs. *New Dir Adult Contin Educ* 2003;98:39–47
- 8 Rao M. The rapid-response: a break during lecture. *Adv Physiol Educ* 2006;30(2):95
- 9 Prober CG, Heath C. Lecture halls without lectures—a proposal for medical education. *N Engl J Med* 2012;366(18):1657–1659
- 10 Terenzini E, Pascarella P. Living with myths: undergraduate education in America, change. *Magazine of Higher Learning* 1994;26(1):28–32
- 11 Wolff M, Wagner MJ, Poznanski S, Schiller J, Santen S. Not another boring lecture: engaging learners with active learning techniques. *J Emerg Med* 2015;48(1):85–93
- 12 Jaarsma AD, Dolmans DD, Muijtjens AM, Boerboom TT, van Beukelen P, Scherpbier AJ. Students' and teachers' perceived and actual verbal interactions in seminar groups. *Med Educ* 2009;43(4):368–376
- 13 Russell IJ, Hendricson WD, Herbert RJ. Effects of lecture information density on medical student achievement. *J. Med Educ* 1984;59(11 Pt 1):881–889
- 14 Padgett RD, Johnson MP, Pascarella ET. First-generation undergraduate students and the impacts of the first year of college: some additional evidence. *J Coll Student Dev* 2012;53(2):243–266
- 15 Panitz T. Why more teachers do not use student centered learning techniques and policies needed to encourage positive changes. *J Stud Cent Learn* 2003;1(2):55–60
- 16 Spruijt A, Jaarsma ADC, Wolfhagen HAP, van Beukelen P, Scherpbier AJ. Students' perceptions of aspects affecting seminar learning. *Med Teach* 2012;34(2):e129–e135
- 17 Palappallil DS, Sushama J, Ramnath SN. Effectiveness of modified seminars as a teaching-learning method in pharmacology. *Int J Appl Basic Med Res* 2016;6(3):195–200
- 18 de Menezes S, Premnath D. Near-peer education: a novel teaching program. *Int J Med Educ* 2016;7:160–167

Breast Cancer in Indian Women: Genetic Risk Factors and Predictive Biomarkers

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Abstract

Breast cancer is the most common cancer among Indian women with a significant increase in incidence in young women. To identify risk factors for breast cancer in young women, study of BRCA1 and BRCA2 germ line mutations was done in a cohort of 204 Indian breast cancer patients. The study showed a total of 18 mutations in 2.94% of the tested patients, 44% BRCA1 and 78% BRCA2 mutations were found unique to the Indian population. Association of low penetrance genes mainly CYP17, VDR gene and AR-CAG repeat polymorphisms with breast cancer risk showed CYP17 A2 and VDR Poly-A L as high risk alleles, the risk of developing breast cancer among women carrying three high-risk alleles is 4.68 (95% confidence interval [CI]: 0.77–28.0; *p* for trend = 0.10) compared with women carrying none. CYP17 A2 allele was also found associated with development of breast cancer at young age and can also serve as a target for therapy. Betel quid chewing has been found as a significant and independent risk factor for developing breast cancer in North East Indian women which induces genetic alterations leading to breast carcinogenesis. Studies to assess the predictive role of various tumor markers showed that expression of p-glycoprotein in pretreatment biopsy predicts a poor clinical response to neoadjuvant chemotherapy (NACT) in patients having locally advanced breast cancer. The chemotherapy-induced toxicity (vomiting and alopecia) correlated significantly with clinical and immunohistochemical response (reduction in bcl2/bax ratio) and were found to be a cost-effective and reliable predictor of response to NACT. Androgen receptor (AR) has been identified as independent predictive marker for response to NACT in locally advanced breast cancer cases and can serve as novel therapeutic target for triple negative breast cancers.

Keywords

- breast cancer
- genetic risk factors
- predictive biomarkers

Introduction

Breast cancer is currently the most common cancer among Indian women in majority of urban cancer registries, viz. Delhi, Mumbai, Bangalore, Chennai, Ahmadabad, and Thiruvanthapuram (age-adjusted incidence rate [AAR] ranges between 33 and 41 per 100,000 women), and has rapidly

overtaken cervical cancer in frequency.¹ An analysis of the time trends of breast cancer incidence in India by estimating annual percentage change (APC) in AAR for a 5-year calendar period showed an increase in APC ranging between 0.5 and 2% at different registries (Ahmadabad, Bangalore, Chennai, Mumbai, Nagpur, and Pune). Analysis of APC for age of women by estimating change in age-specific incidence rates

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(ASR) revealed a 4.24% increase in the younger age group women (15–34 years) compared with 1.6 and 0.8% in age groups of 35 to 44 and 45 to 54 years, respectively, suggesting a significant increase in breast cancer incidence mainly in the younger age group of patients during the last decade.²

These data demonstrate the magnitude of the current health problem associated with breast cancer in the Indian population. Understanding its pathogenesis, morphological features, and epidemiological factors including various risk factors, family history etc., holds a great promise for the treatment, early detection, and prevention of this cancer. A study had been undertaken to describe some of the clinico-pathological features of the breast cancer cases seen at Safdarjung Hospital: a tertiary level hospital in the city of Delhi.³ A detailed analysis of 578 breast cancer cases showed the average age of the patient at presentation to be 47.9 years, 57.9% patients were found below 50 years of age. A positive family history had been found in 22.4% cases, more in patients under 45 years of age. Tumor, node, and metastasis (TNM) staging revealed 78.6% patients presented at locally advanced stage (III B in 36.1%, III A in 27.1%, and II B in 16.3%) and metastasis (IV) was found in 7.9% cases.³ Since high percentage of Indian women developed breast cancer at young age (< 50 years) and presented at locally advanced stage, this study had been undertaken to identify the genetic risk factors for detection of breast cancer at early stage and predictive biomarkers to predict response to therapy for proper management.

Genetic Risk Factors for Breast Cancer in Indian Women

Genetic susceptibility to cancer is triggered in several ways; the best understood causal mechanism being due to inactivating germ line mutations in tumor suppressor and DNA repair genes, which lead to accumulation of mutations in oncogenes and cell-cycle checkpoints that are required for uncontrolled cell division. About 5 to 10% of breast and ovarian cancers occur as a result of highly penetrant germ line mutations.

Two major breast cancer susceptibility genes are BRCA1 (MIM 113705, gene bank accession number U14680) and BRCA2 (MIM 600185, gene bank accession number U43746), located on long arms of chromosomes 17 and 13, respectively, and both apparently function as tumor suppressor genes.^{4,5} Mutations in the BRCA1 and BRCA2 genes were first reported in conjunction with their identification in 1994

and 1996.^{6–8} Germline mutations in the BRCA1 or BRCA2 tumor-suppressor genes are strong predictors of breast and/or ovarian cancer development. Mutation prevalence varies among different ethnic groups and may be influenced by the potential modifying effects of the patient's own genetic and environmental background.

Prevalence of BRCA Genes Mutations in North Indian Population

A large number of distinct mutations in the BRCA1 and BRCA2 genes have been reported worldwide. To investigate the role of these inherited susceptibility genes in breast cancer risk among Indian women, study of distribution and the nature of BRCA1 and BRCA2 germ line mutations and polymorphisms in a cohort of 204 Indian breast cancer patients and 140 age-matched controls was undertaken at National Institute of Pathology, New Delhi.⁹

All coding regions and exon–intron boundaries of the BRCA1 and BRCA2 genes were screened by hetero duplex analysis followed by direct sequencing of detected variants.

In total, 18 genetic alterations were identified (► Fig. 1). Three deleterious frame-shift mutations (185delAG in exon 2; 4184del4 and 3596del4 in exon 11) were identified in BRCA1, along with one missense mutation (K1667R), one 5'UTR alteration (22C>G), three intronic variants (IVS10–12delG, IVS13+2T>C, IVS7+38T>C), and one silent substitution (5154C>T). Similarly, three pathogenic protein-truncating mutations (6376insAA in exon 11, 8576insC in exon 19, and 9999delA in exon 27) along with one missense mutation (A2951T), four intronic alterations (IVS2+90T>A, IVS7+75A>T, IVS8+56C>T, and IVS25+58insG), and one silent substitution (1593A>G) were identified in BRCA2 gene. Four previously reported polymorphisms (K1183R, S1613G, and M1652I in BRCA1, and 7470A>G in BRCA2) were detected in both controls and breast cancer patients. Of these, six were pathogenic protein truncating mutations. In addition, several variants of uncertain clinical significance were identified. Among these are two missense variants, one alteration of a consensus splice donor sequence, and a variant that potentially disrupts translational initiation.

Of these 18 mutations, six clearly deleterious sequence variants were detected in 2.94% of the tested patients, four of the nine BRCA1 (44%) and seven of the nine BRCA2 mutations (78%) are found unique to the Indian population and are distributed throughout the exons of BRCA1 and BRCA2 genes.

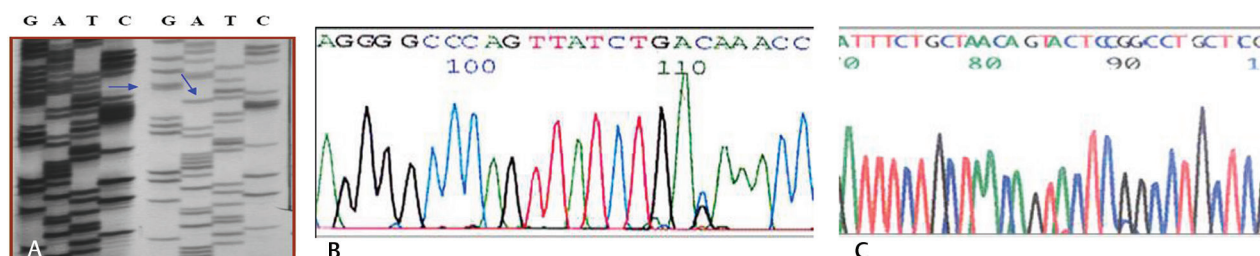


Fig. 1 BRCA1 and BRCA2 mutational analysis (A) BRCA1 185 del AG by manual sequencing (B) electropherogram showing BRCA1 22C>G mutation (C) electro pherogram showing BRCA2 8576 insC by automated sequencing.

The prevalence of BRCA1/2 mutations in our Indian patient series appeared to be low compared with other Asian countries but is comparatively similar to that reported from Shanghai, China. However, a significant proportion of women who had breast cancer diagnosed at age ≤ 40 years without any family history were also carriers (all variants: 14.2%, known deleterious mutations: 3.8%), which is comparable to other Asian countries. In addition, the present study is in agreement with the findings from a pilot study done on a small independent group of 20 breast cancer patients where 3 out of 5 cases with mutations in BRCA1/2 had early onset disease.¹⁰ Thus, it is reasonable to postulate that women with early-onset disease without family history are likely to have a disease associated alteration of the BRCA1 or BRCA2 gene. In the present study, the identified mutations account for a comparatively small proportion of the familial risk of breast/ovarian cancer (all variants: 11.7%, known deleterious mutations: [2.9%]).

Contribution of Low Penetrance Genes to Breast Cancer Susceptibility in Indian Women

Contribution of mutations in highly penetrant genes BRCA1/2 to only 5 to 10% of total breast cancer cases suggests that there are other unidentified genes involved, which may also play role in conferring susceptibility to develop breast cancer at larger scale. Analysis of genetic risk of cancer has shown that most nonhereditary, sporadic cancers develop in genetically predisposed individuals and this predisposition is the result of several low penetrance genes rather than single high penetrant gene mutation. Several classes of low penetrance genes polymorphisms with potential functional significance have been evaluated, including genes involved in (1) carcinogen metabolism (GST family), (2) estrogen metabolite biosynthesis (CYP17), (3) steroid hormone receptor activation (VDR, AR), (4) DNA damage response and replication checkpoint (CHEK2), (5) DNA repair (XRCC1), and (6) alcohol metabolism (ADH3). Because endogenous steroid hormone exposure is known to influence breast cancer risk, genes responsive to such hormones are currently being considered as plausible candidates for low-risk breast cancer genes. Steroid hormones viz. estrogen, progesterone, androgen, and vitamin D are key factors in the development and growth of tumors in hormone dependent tissues specially breast. According to some studies, genetic polymorphisms in genes related to the biosynthesis and metabolism of hormones are associated with an altered risk of breast cancer and other types of cancer. Studies have been undertaken to assess the possible association of low penetrance genes mainly CYP 17, VDR gene, and AR-CAG repeat polymorphisms with breast cancer risk in North Indian women.

CYP17 Gene Polymorphism and Its Association with High-Risk North Indian Breast Cancer Patients

Hormonal risk is one of the nongenetic factors that may contribute to the disease etiology. Several clinical, epidemiological, and experimental studies have proved that estrogen

and progesterone play a major role in the growth of normal breast tissue,¹¹ and breast cancer risk is strongly related to exposure to these endogenous steroid hormone levels,¹² specially estrogen.¹³ Because cumulative exposure to circulating estrogen has been linked to increased risk of breast cancer, interest has been focused on the enzymes regulating the biosynthetic and metabolic pathways of these hormones. The estrogen-forming ovary expresses the CYP17 gene,¹⁴ which codes for the cytochrome P450c17a enzyme and mediates both steroid activities and functions at key branch points in human steroid genesis.¹⁵ A single polymorphism, T > C, in the CYP17 gene creates an additional Sp I- type promoter site (CCACC), which is associated with increased estrogen level.¹⁶ The base pair change also creates a recognition site for the MspA1 digestion of a polymerase chain reaction (PCR) product that has been used to designate two alleles, A1 and A2, arbitrarily. It has been reported that women who are heterozygous or homozygous for the CYP 17 A2 allele show comparatively higher serum levels of estradiol than do women with an A1/A1 genotype.¹⁷

To investigate the influence of genetic polymorphism of CYP17 on breast cancer susceptibility mainly in high-risk Indian breast cancer patients, targeting early onset breast cancer and familial cases, a total of 242 histopathologically confirmed breast cancer (106 early onset, 80 late onset, and 56 familial) and 212 age-matched controls were investigated for CYP 17 gene polymorphism by PCR-RFLP method at National Institute of Pathology, New Delhi.¹⁸ The genotypes identified were assigned as homozygous wild type (A1A1), heterozygous variant (A1A2), and homozygous variant (A2A2; ►Fig. 2). We additionally investigated association of CYP17 polymorphisms with expression of estrogen receptor. The results showed significantly increased ($p = 0.001$) frequency of heterozygous (A1A2) and homozygous (A2A2) CYP17 genotypes in breast cancer patients (94.3%) than in controls (80.3%). A highly statistically significant increased risk in carriers of homozygous A2 allele was found in young patients ($p < 0.001$) in comparison with patients having late onset condition ($p = 0.260$). However, no significant association between the genotype and breast cancer risk was observed among women with strong family history. Further, data had showed that patients (80.6%) with at least one A2 allele exhibit ER-independent cell proliferation, although statistical significance could not be established ($p = 0.160$).

VDR Polymorphism(s) and Breast Cancer Risk in North Indians

Identification of 1, 25 (OH)₂-D₃ and VDR as components of a signaling network that affects breast tissue proliferation, differentiation, and apoptosis raises the possibility that Vitamin D may play a protective role against mammary transformation and common VDR gene variants may be associated with the risk of breast cancer.¹⁹⁻²¹ This study examined the association between VDR (ApaI/TaqI/Poly-A) genotype breast cancer risk in North Indian population. Genotypes for polymorphism at 3' end of VDR gene were studied in 160 cases and 140 control subjects using PCR-RFLP method to determine Apa-I and

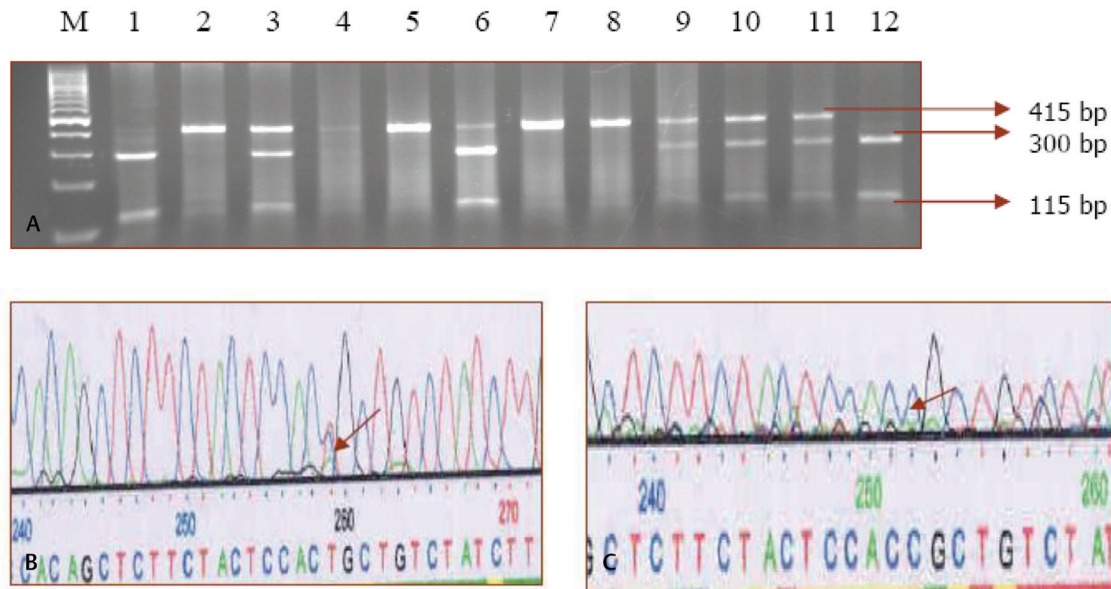


Fig. 2 (A) MspAI restriction fragments of CYP17 alleles separated by agarose gel electrophoresis. Lane 1: 100 bp ladder, 1 to 12 are samples; Lane 2, 7, and 8: homozygous A1A1 (415bp); Lane 3, 9, 10, and 11: heterozygous A1A2 (415bp, 300bp, 115bp); Lane 1, 6, and 12: homozygous A2A2 (300 bp). (B, C): Electropherogram showing heterozygous (B) and homozygous (C) A2 alleles of CYP17 T>C polymorphism.

Taq-I polymorphisms and fragment analysis to determine variable length poly-A micro satellite repeats.²²

Patient's having long poly-A repeats ($\chi^2 = 87.03$, $df = 2$, $p = < 0.001$) and homozygous L allele (odds ratio [OR]: 26.97, 95% CI: 12.27–59.17) showed a significant association with disease while no significant association was found with VDR Apa-I ($\chi^2 = 1.00$, $df2$, $p = 0.60$), and Taq-I polymorphism ($\chi^2 = 0.35$, $df2$, $p = 0.83$). The results indicate that VDR poly-A genotypic variants confer susceptibility to breast cancer with increasing number of long (L) poly-A allele, while Apa-I polymorphism may modify the individual susceptibility to disease. Analysis of the VDR genotype in relation to covariates has revealed that the LL genotype is significantly associated with high grade breast cancer suggesting that this VDR variant might be associated with disease progression.

The genotype also mediates an increased risk of breast cancer in women with early onset compared with late onset. We also observed significant associations between disease and genotype AATTLL (OR: 5.09; 95% CI: 1.23–20.80; $p = 0.02$). The decrease in risk in terms of odds ratio from 5.09 to 2.08 even with single (a) in genotype AaTTLL may indicate the protective role of the same in the presence of susceptible (L) allele.

AR-CAG Repeat Polymorphism and Breast Cancer Risk in North Indians

The endogenous steroid hormones (estrogen, progesterone, and androgen) exposure is known to influence breast cancer risk, hence genes responsive to such hormones are currently being considered as plausible candidates for low-risk breast cancer genes. Considerably, little is known about the biological role and clinical significance of androgen and its receptor (AR) expression in breast cancer.^{23,24} AR protein, functions as

a transcription factor (TF) that regulate the transactivation of hormone responsive genes and is thus of specific interest. The exon 1 of AR gene contains trinucleotide repeat polymorphism, CAG (encoding for polyglutamine) which flank the N-terminal domain of the AR protein, where the transactivation activity resides. Remarkably, a CAG trinucleotide repeat is also a target for multiple RNA binding proteins, which have functional impact on AR protein function.^{6,7} Sparse epidemiologic data suggest that a long AR-CAG repeat yielding a less active AR may be associated with increased risk of breast cancer.²⁵ Polymorphisms in AR-CAG repeat have been intensively studied as determinant of susceptibility to prostate cancer in Indian population^{26,27}; however, its association with breast carcinoma in Indian population is not yet explored.

To determine the role of AR-CAG repeats in risk of breast cancer and their association with response to NACT, genotyping of the AR-CAG repeat region was done in 70 patients and 80 healthy aged matched female controls.²⁸ Genotype carriers with ≥ 20 CAGn showed decrease of AR mRNA expression, however, no association could be established with the risk ($p < 0.47$). In patients with locally advanced breast cancer, higher AR mRNA expression was seen prior to NACT in responders ($p < 0.02$), which decreased significantly after NACT ($p < 0.014$). Although, expansion of the CAGn in the AR gene did not show any association with breast cancer risk, however, significant correlation was found with response to NACT in locally advanced breast cancer (LABC) patients.

Multigenic Model of Breast Cancer Susceptibility

Considering CYP17 A2 and VDR poly- (A) L as high risk alleles, a multigenic model of breast cancer susceptibility was developed to identify women carrying a combination of

alleles, which put them at a relatively higher risk to develop breast cancer. The risk among women carrying three high-risk alleles was 4.68 (95% CI: 0.77–28.0; p for trend = 0.10) compared with those who carried none. The conditional logistic regression analysis revealed that the heterozygous TC genotype for CYP17 and AR1AR2 of AR imparted significantly fourfold risk for the breast cancer risk, in comparison to the referent genotype TT and AR1AR1 (adjusted ORs: 3.705 [1.236–11.106], $p < 0.019$ and 4.391 [1.324–14.557], $p < 0.016$, respectively). Gene–gene interaction showed that the combinations TC*AA, TC*Aa, TC*aa, and CC*Aa imparted significantly four- to 15-fold more risk for the breast cancer (4.377 [1.159–16.520], $p = 0.029$; 4.041 [1.092–14.956], $p = 0.036$; 15.071 [0.975–232.81], $p = 0.052$; 4.151 [1.053–16.371], $p = 0.042$, respectively; ►Table 1). These findings suggest that breast cancer risk has a strong genetic component and support the theory that the underlying mechanism of “complex trait” can be understood using a multigenic model of low penetrant genes. Genes involved in hormone synthesis and metabolizing pathway play significant role in breast cancer development.

Study of Environmental and Genetic Risk Factors for Breast Cancer in North East India

The several fold difference in incidence of breast cancer between different geographical regions suggest that environmental factors also influence breast cancer risk significantly. Among the identified environmental risk factors in general for cancers, tobacco exposure has been reported as the leading preventable risk factor.²⁹ The Northeast districts of India have the highest incidence of cancers associated with both smoking and smokeless tobacco. The mean age for tobacco use initiation in this region is 18.5 years and the prevalence of tobacco use is estimated as 41% that includes a large number of female chewers too apart from male smokers.³⁰ Epidemiological perspective suggests an increased risk associated with exposure to genotoxic agents during breast development, as the undifferentiated ductal elements of the breast are more susceptible to the action of genotoxins early in life.³¹ Environmental genotoxic stress like tobacco smoke and smokeless tobacco contain

polycyclic aromatic hydrocarbons (PAHs), tobacco-specific nitrosamines, nitrosamino acids, aldehydes, metals, aromatic and heterocyclic amines, and other genotoxic carcinogens.^{32,33} The concomitant use of betel quid also leads to a 50-fold increase in reactive oxygen species generated.³⁴

The role of tobacco exposure and polymorphisms in detoxification enzymes in breast cancer risk was analyzed using samples collected from patients registered at Dr. B Borooah Cancer Institute, Guwahati. Polymorphisms in five gene variants, GSTT1, GSTM1, GSTP1, TP53 and CYP17, and four environmental exposure variables (tobacco smoking, tobacco chewing, betel quid chewing, and alcohol) were analyzed in 117 breast cancer cases and 174 cancer free controls.³⁵ Multifactor dimensionality reduction (MDR) identified betel quid chewing as the single main risk factor and women with betel quid chewing history had five times the risk of developing breast cancer (OR: 4.78 [2.87–8.00], $p = 0.001$). In logistic regression analysis, GSTT1 null and GSTM1 null genotypes conferred 41% (0.59 [0.34–1.03] 0.06) and 55% (0.58 [0.30–1.02] 0.05) reduced risk to breast cancer, respectively. However, the risk increased in women with GSTP1 variant G allele, which conferred 1.43 times ([0.96–2.11] 0.07) more risk to breast cancer. MDR analysis revealed, betel quid chewing to be the single factor imparting the main effect (testing accuracy of 0.6851 and cross validation consistency 10/10, $p = 0.05$). The Figure below depicts the interactions between nine attributes from the MDR analysis via a graphical representation of a “dendrogram” (►Fig. 3). It shows betel quid chewing, GSTT1 and GSTM1 on a separate branch imparting their independent effects to breast cancer risk. The study highlights betel quid chewing as a significant and independent risk factor for developing breast cancer in NE region.

Association of DNA Repair and Cell Cycle Gene Variations with Breast Cancer Risk

Polymorphisms in DNA repair and cell cycle genes contribute to increased breast cancer risk. Their association and interaction in relation to betel quid and tobacco chewing habits needs exhaustive multi-analytical investigation to explain breast cancer predisposition due to DNA damage.

Table 1 Genotypes for high-risk breast cancer

	Code (CYP17, VDR, AR)	No. of cases (%) (n = 70)	No. of controls (%) (n = 80)	OR (95% CI)	p-Value
No putative high risk genotype	000	2 (2.8)	3 (3.7)	Ref.	–
One putative high risk genotype	001, 002, 010, 2020, 100, 200	13 (18.5)	27 (33.7)	0.72 (0.12–4.06)	1.0
Two putative high risk genotype	011, 012, 021, 022, 110, 120, 201, 202, 210, 220	30 (42.8)	42 (52.5)	1.07 (0.20–5.69)	1.0
Three putative high risk genotype	111, 112, 122, 211, 212, 221, 222	25 (37.5)	8 (10.0)	4.68 (0.77–28.0)	0.1

Abbreviation: OR, odds ratio.

Note: 0: A1A1, SS, <20<20 CAG repeat; 1: A1A2, SL, <20≥20 CAG repeat; 2: A2A2, LL, ≥20≥20 CAG repeat.

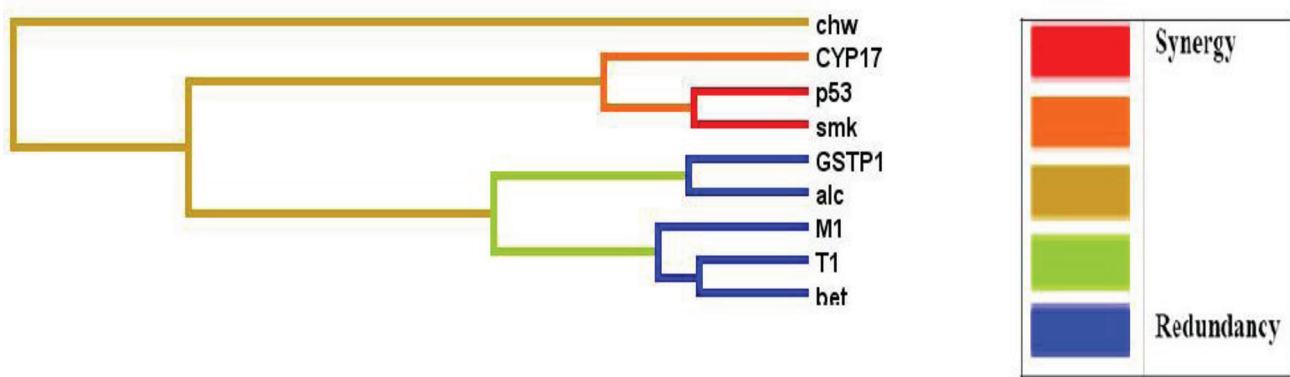


Fig. 3 Interaction dendrogram for the breast cancer dataset: graphical representation of interactions between nine attributes (GST1 (T1), GSTM1 (M1), GSTP1, CYP17, TP53 (p53), tobacco smoking (smk), tobacco chewing (chw), betel quid chewers (bet) and alcohol consumption (alc)) from the multifactor dimensionality reduction analysis using an ‘interaction dendrogram’.

Polymorphism in TP53–72Arg>Pro, RAD51–135G>C, BRCA2, and CCND1–G870A were examined in 204 BC cases and 217 controls from Northeast Indian population.³⁶ Multifaceted analytic approaches were used to explore relationships between polymorphisms, tobacco history, and breast cancer susceptibility. Betel quid chewing was identified as the predominant risk factor. CCND1-AA and dominant model showed protection toward breast cancer in BQC (betel-quid chewer) and NBQC (nonbetel-quid chewer). TP53-Pro/Pro genotype showed protection toward breast cancer in NBQC. RAD51-C allele was associated with breast cancer risk in BQC. Two BQC cases had BRCA2 8415G >T:K2729N mutation in exon18.

The hierarchical interaction graph showed (►Fig. 4A), large independent effect of betel quid chewing (9.38%) among environmental factors for total sample set. A strong interaction (1.32%) was seen between RAD51 and TP53. MDR analysis showed best 4 locus model in NBQC. Interaction diagram concurred the interactions between TP53 and RAD51 (1.32%) with independent effect (1.89%) of CCND1 in NBQC (►Fig. 4B). In CART analysis, BQC with CCND1 GG genotype were at risk followed by combination of BQC, CCND1, no smoking, alcohol. Risk was also observed in BQC, CCND1, no smoking, nonalcohol, TP53 combination and BQC, CCND1, no smoking, nonalcohol, TP53 (►Fig. 5). NBQC group showed risk with combination of NBQC and TP53.

These data indicate that common genetic variations in DNA repair and cell cycle genes contribute toward breast cancer risk. In addition, different predisposition was observed amongst BQC and NBQC breast cancer patients rendering dissimilar susceptibility toward breast cancer. The elevated risk for breast cancer in BQC may be attributable to betel quid carcinogens and minor roles of BRCA2 mutation and C allele of RAD51. Whereas NBQC could be at slightly lower risk for breast cancer due to the protection offered by the Pro/Pro-TP53 form. CCND1 polymorphism conferred protection irrespective of the betel quid chewing status.

Genomic Alterations in Breast Cancer Patients in Betel Quid and Non Betel Quid Chewers

Tobacco chewing with BQ results in increased exposure (1,000 mg/day) to carcinogenic tobacco-specific nitrosamines

(TSNAs). N'-nitrosornicotine (NNN), 4- (N-methyl-N-nitrosamino)-1- (3-pyridyl)-1-butanone (NNK). Nitrosoanabasine (NAB), N-nitrosodimethylamine, and N-nitrosodiethylamine have been detected in saliva of BQ with tobacco chewers³⁷ and are known to induce mammary tumors in rodents and anaphase bridges via DNA double stranded breaks causing genomic imbalances in human cells.^{36,38} Examination of genomic alteration due to tobacco carcinogens depicts gain on chromosomes 6 and 8, and losses on chromosomes 11 and 14 in mouse lung adenocarcinomas³⁹ and gains of 1p and 3q in patients with tobacco exposure history in head and neck squamous cell carcinomas.⁴⁰ Although the literature suggests role of BQ carcinogens in mediating genomic alterations, there is no cause and effect evidence suggesting its role in breast carcinogenesis.

To elucidate the role of betel quid carcinogens in breast carcinogenesis, we investigated genomic alterations in breast cancer patients from North East India with and without BQ chewing history. Study of copy number variations and pathway networks using SNP array and ingenuity pathway analysis (IPA) was done in 26 BQC and 17 NBQC breast cancer patients.⁴¹ BQC tumors showed significantly ($p < 0.01$) higher number of alterations, as compared with NBQC tumors, $48 \pm 17\%$ versus 32 ± 25 respectively. Incidence of gain in fragile sites in BQC tumors were significantly higher ($p < 0.001$) as compared with NBQC tumors. The chromosomal regions 7q33 and 21q22.13 were significantly ($p < 0.05$) associated with BQC tumors, while regions 19p13.3–19p12 and 20q11.22 were significantly associated with NBQC tumors. Gene ontology (GO) and network analysis showed that genes associated with BQC regions, 7q33 and 21q22.1 were enriched for oxidoreductase ($p < 0.001$) and aldo-ketoreductase activity ($p = 0.015$) in contrast to G-protein coupled receptor protein signaling pathway ($p = 0.005$) and cell surface receptor linked signal transduction ($p = 0.012$) for 19p13.3–19p12 and 20q11.22 regions associated with NBQC. IPA analysis for BQC associated regions revealed one top network (score 20) “drug metabolism, molecular transport, nucleic acid metabolism” encompassing genes like AKR1B1, AKR1B10, AKR1B15, ERG, ETS2 (►Fig. 6A). IPA analysis for NBQC genes revealed two top networks (score 29) “molecular transport, nucleic

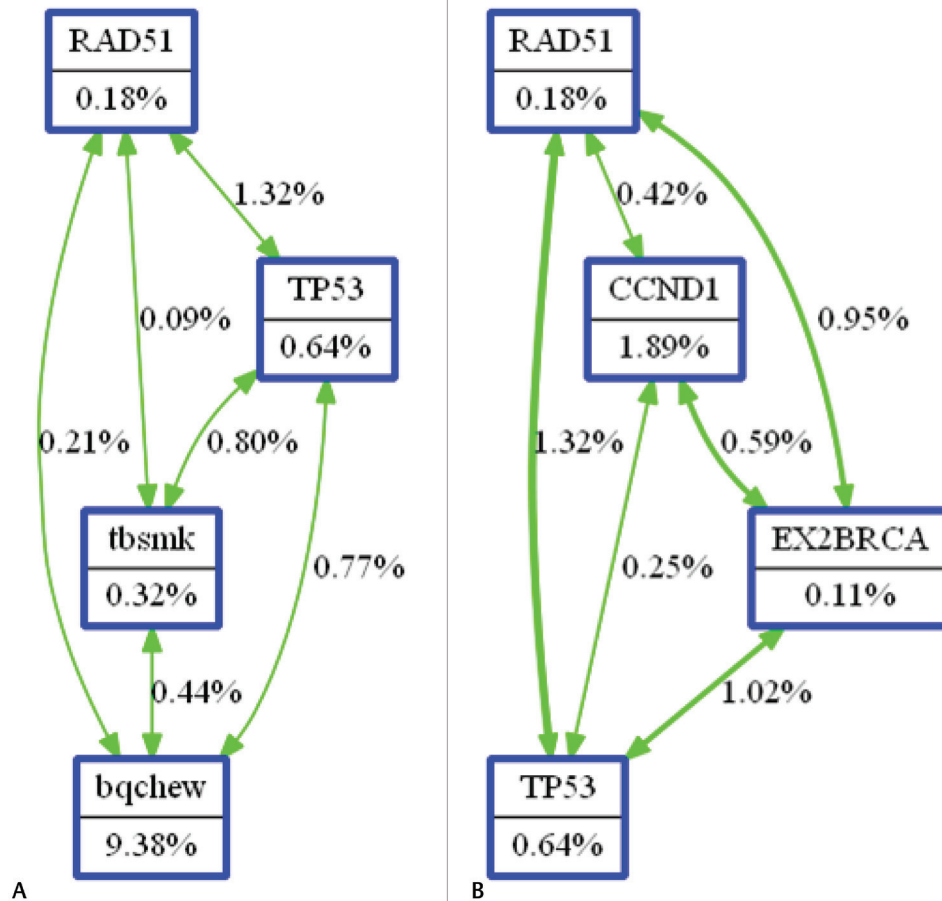


Fig. 4 Interaction dendrogram using orange software for the (A) total dataset and (B) the NBQC dataset. tbsmk, tobacco smoking; bqchew, betel-quid chewing.

acid metabolism, small molecule biochemistry” and “cellular development, embryonic development, organismal development” (→Fig. 6B) encompassing genes like RPN2, EMR3, BLCAP and VAV1, NNAT and MUC16, respectively.

Twenty-seven common regions of gain were illustrated between BQC and NBQC tumors. Both groups exhibited gain on chromosomes 1q, 5p, 7p, 8q, 12q, 16p, 17q, and 20q. Enrichment and IPA of genes associated with these regions show activation of protein kinase activity ($p = 0.009$) and cell junction ($p = 0.01$). IPA analysis revealed three top networks, (→Fig. 7). Network 1 functions in cellular movement, connective tissue development and function, cellular assembly and organization (score 43) with key role played by PTK2. Network 2 functions in cell-to-cell signaling and interaction, tissue development, organismal injury and abnormalities (score 43) with RPN2, EMR3, VAV1, NNAT, and MUC16 important genes. Network 3 functions in cell morphology, cellular assembly and organization, and cellular compromise (score 32) with key roles played by MYC and YWHAZ. Among all the tumor associated canonical pathways enriched were GNRH signaling ($p = 2.92E-04$), cAMP-mediated signaling ($p = 3.60E-04$), protein kinase A signaling ($p = 3.77E-04$), CXCR4 signaling ($p = 4.99E-03$), molecular mechanisms of cancer ($p = 8.58E-03$), phospholipase C signaling ($p = 1.01E-02$), RAR activation ($p = 3.16E-02$), and ILK signaling ($p = 4.21E-02$).

The study shows that breast cancer associated with betel quid chewing exemplifies genetic alterations differing from those observed in the nonbetel quid chewer. Several genetic changes are shared in both tumor groups considered as crucial in breast cancer progression.

Predictive Tumor Markers in Breast Cancer in Indian Women

Majority of breast cancer patients in India present at locally advanced stage (IIIB, IIIA, and IIB) and standard of care for these patients is NACT followed by surgery in the form of modified radical mastectomy and three more cycles of adjuvant chemotherapy.^{42,43} NACT facilitates local as well as distant control of the disease and provides an in vivo chemosensitivity test for a particular regime.⁴⁴ It is vital to predict response to chemotherapy to tailor the regime in a particular patient for an optimum response and to avoid chemotoxicity in a nonresponder. The prediction would help in avoiding the toxicity induced by an ineffective chemotherapeutic regime in a nonresponder and would also help in the planning of an alternate regime. Studies had been undertaken to assess the predictive role of various tumor markers viz. p-glycoprotein, p53, apoptotic markers, mismatch repair genes, androgen receptor (AR) gene, and

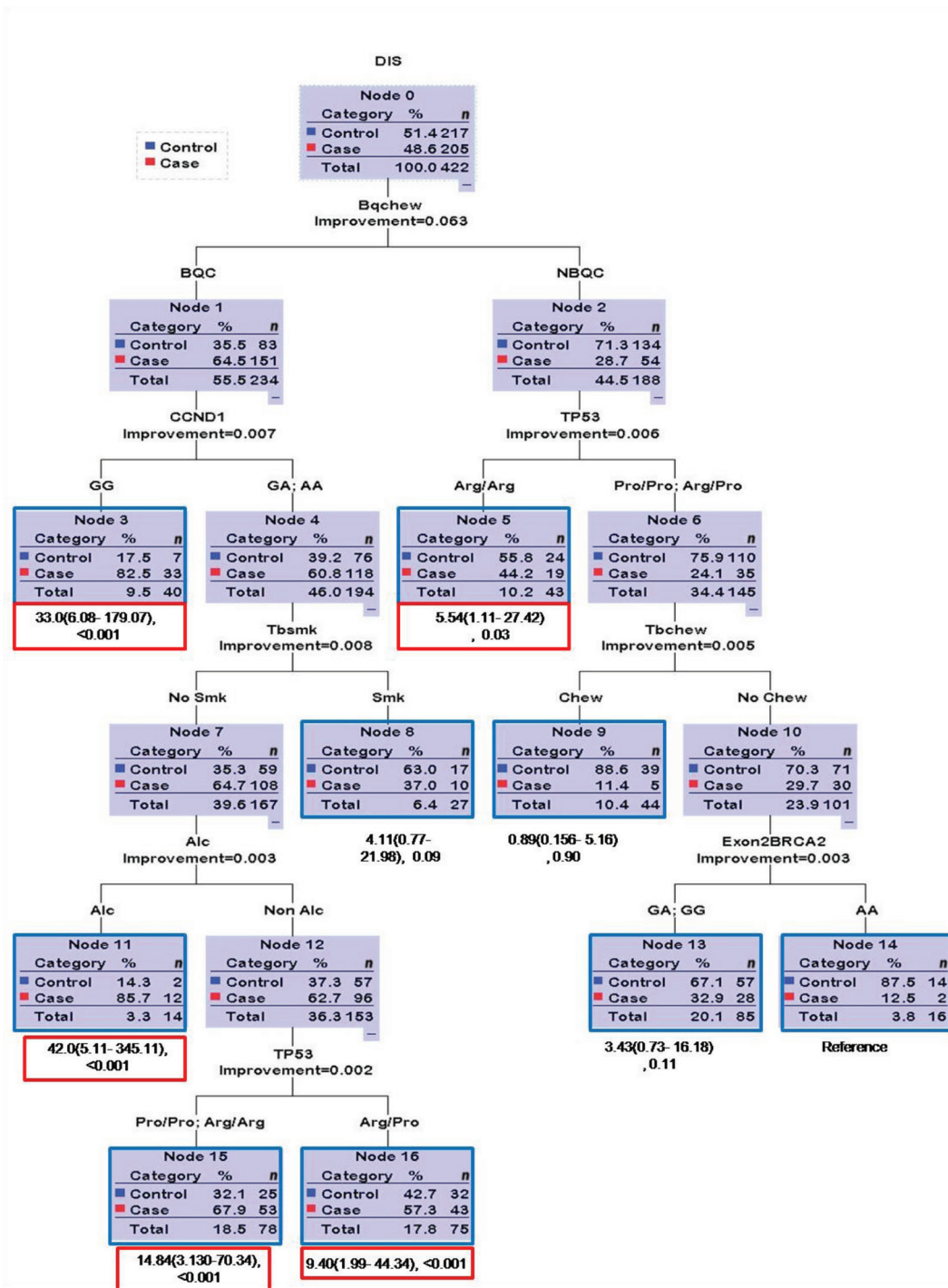


Fig. 5 Classification and regression tree (CART) analysis for the DNA repair and cell cycle genes and environmental risk factors. Terminal nodes are bordered thick blue. Red bordered odd ratio boxes values are <0.05. BQC, betel-quid chewer; NBQC, non betel-quid chewer; smk, smoking; tb, tobacco; alc, alcohol.

type 1 growth factor receptor family genes in search for an ideal predictor.

Role of p-glycoprotein Expression in Predicting Response to NACT in Breast Cancer

Development of resistance to chemotherapeutic agents is a major problem and one of the mechanisms considered

responsible is the expression of 170-kDa membrane glycoprotein (usually referred to as p-170 or p-glycoprotein), which is encoded by multidrug resistance (MDR1) gene.⁴⁵ The expression of p-glycoprotein at initial presentation has been found to be associated with refractoriness to chemotherapy and poor outcome.⁴⁶ Against this background, a prospective study was conducted by studying expression of p-glycoprotein in pretreatment biopsy of tumor tissue to ascertain whether pretreatment detection of p-glycoprotein

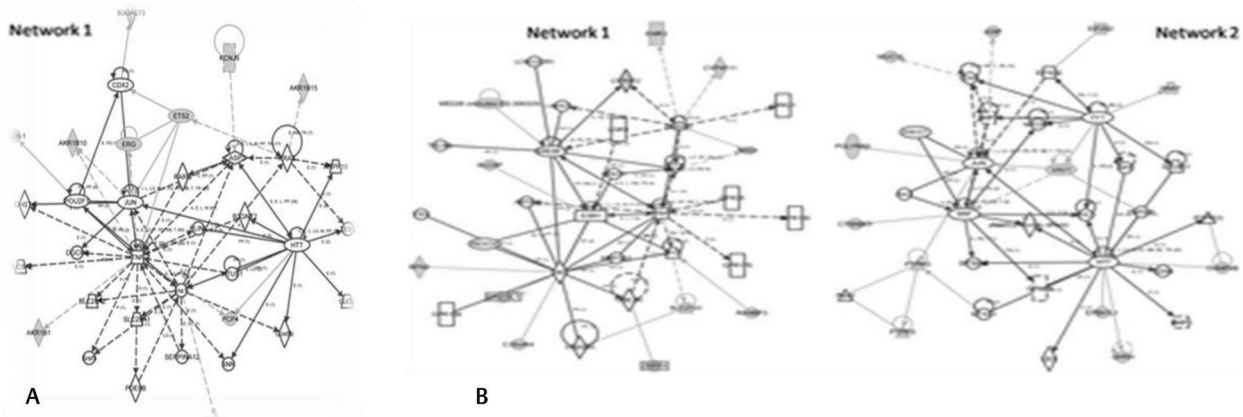


Fig. 6 (A) BQC Network 1 drug metabolism, molecular transport, nucleic acid metabolism. (B) NBQC networks: molecular transport, nucleic acid metabolism, small molecule biochemistry (Network 1) and cellular development, embryonic development, organismal development (Network 2).

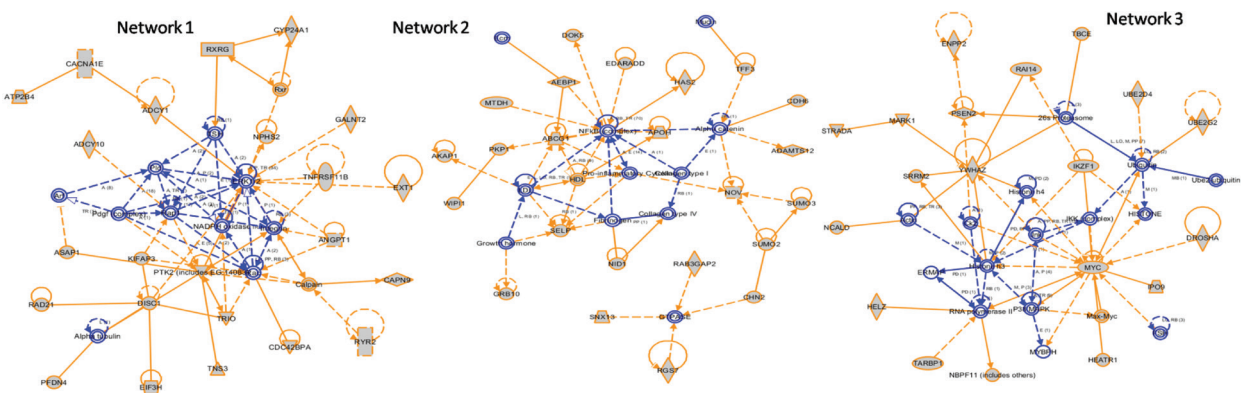


Fig. 7 Common networks: cellular movement, connective tissue development and function, cellular assembly and organization (Network 1), cell-to-cell signaling and interaction, tissue development, organismal injury and abnormalities (Network 2), cell morphology, cellular assembly and organization, cellular compromise (Network 3).

expression could be utilized as a reliable predictor of response to NACT in patients of locally advanced breast cancer (LABC). Fifty cases of locally advanced breast cancer were subjected to trucut biopsy and the tissue samples were evaluated for expression of p-glycoprotein, estrogen receptor (ER) and progesterone receptor (PR) by immunohistochemistry (IHC).⁴⁷ The response to NACT was assessed clinically and by using ultrasound after three cycles of FAC (fluorouracil, adriamycin, and cyclophosphamide) regime. The clinical response was correlated with both the pre and post chemotherapy p-glycoprotein expression. A significant correlation was observed between positive p-glycoprotein expression and poor clinical response rates ($p < 0.05$). Significant increase in expression of p-glycoprotein was seen after three cycles of NACT, 52% before initiation of NACT increased to 73.5% after NACT. The chemotherapy induced p-glycoprotein positivity observed in the study could possibly explain the phenomenon of acquired chemoresistance and may also serve as an intermediate end point in evaluating drug response particularly if the adjuvant therapy is planned with the same regime.

The study concluded that pretreatment p-glycoprotein expression predicts and indicates a poor clinical response to NACT.

Role of p53 and Apoptotic Markers in Predicting Response to NACT in Breast Cancer

The pathway associated with tumor cell death is mainly apoptosis or programmed cell death and chemotherapeutic drugs like DNA-damaging agents act on rapidly multiplying cells including both the tumor and the normal cells by following this pathway.^{48,49} This could account for both the response and toxic effects. Absence or decreased apoptosis has been found to be associated with chemo resistance. The change in expression of apoptotic markers (Bcl-2 and Bax proteins) brought about by various chemotherapeutic regimens has been used to identify drug resistance in the tumor cells.⁵⁰ A prospective clinical study was conducted to assess whether chemotherapy induced toxic effects could serve as reliable predictors of apoptosis or response to neoadjuvant chemotherapy in patients with locally advanced breast cancer. Fifty cases of locally advanced breast cancer after complete routine and metastatic work up were subjected to trucut biopsy and the tissue evaluated immunohistochemically for apoptotic markers (Bcl-2/Bax ratio).⁵¹ Three cycles of NACT using FAC regime (5-fluorouracil, adriamycin, cyclophosphamide) were

given at 3 weekly intervals and patients assessed for clinical response as well as toxicity after each cycle. Modified radical mastectomy was performed in all patients 3 weeks after the last cycle and the specimen were re-evaluated for any change in the Bcl-2/Bax ratio. The clinical response, immunohistochemical response and the drug-induced toxicity were correlated and compared. Immunohistochemical response was defined as decrease in the Bcl-2/Bax ratio.

Clinical response including the reduction in the tumor size and axillary lymph node status was observed in 70% of patients and was found to be statistically significant ($p < 0.0001$). There were no patients in the N0 group and 29.4% of the N1 patients were down staged to N0, while 70.6% remained N1. In patients with N2 disease, 7.7% were down staged to N0 status, while 46.2% were down staged to N1 status, and 46.2% did not show any response. Change in immunohistochemical expression (IHC response) was observed in 60% patients, which was statistically significant ($p = 0.008$). Correlation between IHC and clinical response was also found to be statistically significant ($p < 0.0001$).

Acute vomiting was observed in 63.3% patients. Vomiting was found in 81% clinical responders ($p = 0.002$) and 78% IHC responders which was statistically significant ($p = 0.04$). Alopecia was observed in 86% clinical responders ($p = 0.000$) and 94% IHC responders ($p = 0.000$). Leucopenia was observed in only 14% and 17% of clinical and IHC responders respectively and was found to be an insignificant predictor of response in the present study. When multiple toxicities were correlated with the clinical and IHC response, 46.7% of patients had both acute vomiting and alopecia. 67% clinical responders ($p = 0.001$) had both vomiting and alopecia. 72% immunohistochemical responders ($p = 0.001$) had both vomiting and alopecia.

A significant positive correlation was observed between the presence of vomiting ($r = +0.558$), alopecia ($r = +0.802$) and response to NACT. A significant negative correlation was observed between the absence of side effects and poor response to NACT. The chemotherapy-induced toxicity was observed to be a cost effective and reliable predictor of response to NACT.⁵¹

Correlation of Mismatched Repair Genes with Response to NACT in Breast Cancer

The DNA mismatch repair (MMR) pathway is an important postreplicative repair process. It is involved in the maintenance of genomic stability and MMR genes have therefore been named the proofreaders of replicating DNA.⁵² These genes repair the replicative errors of DNA and are thus imperative for genomic stability.⁵³

The MMR genes have been found to be involved in promoting cytotoxicity, apoptosis, p53 phosphorylation, and cell cycle arrest following exposure to exogenous DNA damaging agents. Loss of MMR function prevents the correction of replicative errors leading to instability of the genome, and can be detected by polymorphisms in microsatellites nucleotide repeat sequences scattered in whole genome. This phenomenon, known as microsatellite instability (MSI), is a hallmark of MMR dysfunction and can be used as a marker of MMR

dysfunction in various malignancies.⁵⁴ An alternative method for detection of MMR dysfunction is to test the expression of protein products of the MMR genes by IHC, as mutations in these genes lead to reduced or absent expression of their gene products. Correlation between losses of MMR function, histopathological, and behavioral parameters of the tumor with response to chemotherapy in breast cancers may be of value in predicting response to NACT.

Thirty-one cases of locally advanced breast carcinoma were studied to assess the correlation between MMR dysfunction, clinicopathological parameters and clinical response to NACT.⁵⁵ Analysis for immunohistochemical expression for four MMR protein products MLH1, MSH2, MSH6, and PMS2 was done in the pre NACT trucut biopsy specimen and after three cycles of NACT with CAF (cyclophosphamide, adriamycin, 5-fluorouracil) regimen, in the modified radical mastectomy specimen. Seventeen patients (54.83%) showed down staging of the tumor size and axillary lymph node status in response to NACT including 2 patients with complete pathological response ($p = 0.000$).

A significant correlation was observed between expression of MLH1 and MSH2 with histological grade ($p = 0.048$ and 0.038 respectively). Tumors with decreased expression of MLH1 and MSH2 showed poor differentiation. Combination of loss of expression of MMR proteins with histological grade may serve as a predictor of aggressive behavior and poor outcome. Cases with high post NACT expression of PMS2 were poor responders to chemotherapy. MSH6 was the most frequently altered MMR gene, with loss of expression in 48% patients; high expression is associated with poor response to NACT.

Role of AR in Breast Cancer

AR belongs to a family of intracellular steroid hormone receptors that function as ligand dependent transcription factor which regulates target gene expression. The full length AR protein is a 110 kDa phosphoprotein, which mediates its physiological functions by binding to its ligand testosterone or after its conversion to 5α -dihydrotestosterone (DHT) by 5α -reductases.⁵⁶⁻⁵⁸ AR has an N-terminal transactivation domain, which contains the poly-glutamine (CAG) repeat sequence, a DNA-binding domain (DBD) having two C4 type zinc fingers, a hinge region, and a C-terminal ligand-binding domain (LBD), which gets activated upon binding to androgens. Androgen binding to C-terminal of AR leads to the dissociation of chaperone proteins and dimerization of AR leading to a conformational change whereby its nuclear localization signal (NLS) is exposed. Exposed NLS then aids in the translocation of AR to the nucleus, where it binds to androgen-response elements (AREs) present in the promoters of several target genes in a tissue-specific manner. In the nucleus, AR recruits many other proteins, such as general transcription factors and RNA polymerase to activate androgen-responsive genes.⁵⁹ Traditionally, ER and PR are known to be the prominent players in the progression and development of breast cancer. Recent evidence suggests that AR antagonizes ER function and plays an antiproliferative role in

ER+ breast cancers whereas it plays a significant role in facilitating tumor cell growth in an androgen-dependent manner in an ER-/AR+ breast cancers.^{60,61}

Role of AR as Independent Predictor for Response to NACT in Locally Advanced Breast Cancers

The clinical significance of AR in breast cancer has been studied by correlating its expression with clinicopathological parameters, other steroid receptors (ER and PR), and growth factors receptors (EGFR and CD105) in 100 cases by IHC.⁶² Risk ratio (RR) along with 95% confidence interval (CI) was estimated to assess the strength of association between the markers and clinicopathological characteristics. Categorical principal component analysis (CATPCA) was applied to obtain new sets of linearly combined expression, for their further evaluation with clinicopathological characteristics. In 31 cases presenting with locally advanced breast cancer (LABC), the expression of AR, ER, PR, EGFR, and CD105 correlated with response to NACT. The results indicate the association of AR+ ($p = 0.001$) and AR+/EGFR- ($p = 0.001$) with the therapeutic response to NACT in LABC patients. The AR expression exhibited maximum sensitivity, specificity and likelihood ratio of positive and negative test for response to NACT.

Correlation of expression of steroid (AR) and type 1 growth receptor genes (EGFR, ERBB2, and ERBB3) and gene associated with metabolism of chemotherapeutic drugs (MDR1), before and after therapy, was studied in LABC patients with response to treatment.⁶³ Significant correlation was found with high levels of pre NACT AR gene expression ($p = 0.016$) with responders, which decreased following NACT ($p = 0.008$). Hence, AR upregulation can serve as a useful predictive marker for response to NACT. A significant posttherapeutic increase in the expression levels of EGFR and MDR1 gene in responders ($p = 0.026$ and $p < 0.001$) as well as in nonresponders ($p = 0.055$ and $p = 0.001$) suggests that expression of these genes changes during therapy but they do not have any impact on tumor response, whereas a post-therapeutic reduction was observed in AR in responders. The study indicated an independent predictive role of AR with response to NACT.

Study of AR Signaling in Breast Cancer

AR has been implicated in the development and progression of breast and prostate cancers. Therefore, understanding the regulatory mechanisms of the functioning of AR in ER-/AR+ breast cancer will provide many novel targets for the purpose of therapeutic intervention.

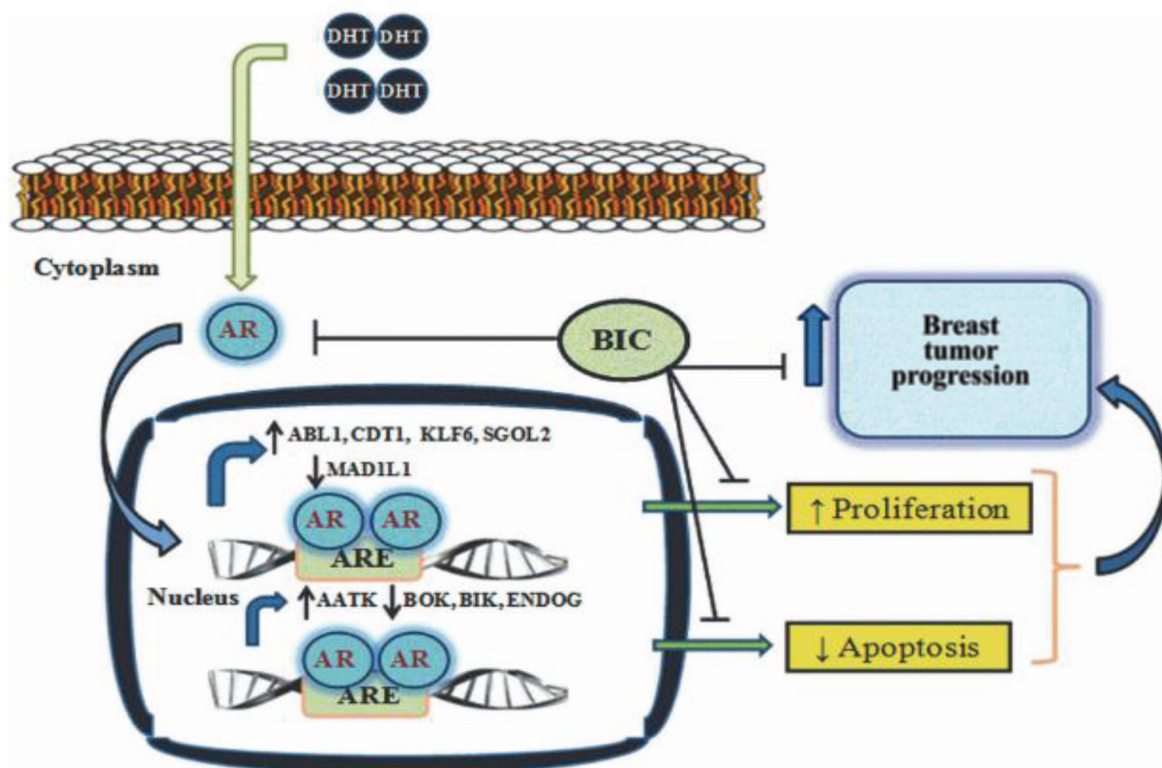


Fig. 8 Proposed model shows AR regulation of the breast tumor progression. On DHT stimulation AR translocates into the nucleus and bind to its cognate androgen response elements (AREs). Inside the nucleus, AR upregulates the expression of ABL1, CDT1, KLF6, and SGOL2, while it represses MAD1L1 expression to induce cell proliferation. At the same it induces AATK expression and down regulates the expression of BOK, BIK, and ENDOG to decrease apoptosis and promote breast cancer progression. Bicalutamide (BIC) reverses the effect of AR on cell cycle and apoptosis by binding and preventing its activation. Due to its ability to negate the effects of AR, bicalutamide can be used to block breast cancer progression.

To get the molecular insights into AR signaling in breast cancer.⁶⁴ We identified 75 AR targets having prominent roles in cell cycle, apoptosis, and metabolism by using bioinformatics tools and validated 10 genes as AR targets by studying the regulation of these genes in MDA-MB-453 cell line on stimulation by androgens like 5 α -DHT using RT-qPCR and ChIP assay. Our experimental data showed that treatment of breast cancer cells with DHT promotes cell proliferation and decreases apoptosis while treatment with antiandrogen like bicalutamide was able to reverse the effect of DHT, thereby demonstrating the application of bicalutamide along with taxanes as novel therapy for the treatment of triple-negative breast cancers (TNBCs), which are positive for downstream AR signaling (► Fig. 8). To understand how AR influences DNA binding, an exhaustive, rigid-body docking between individual ARE and DNA binding domain (DBD) of the AR protein was performed. To decipher the DNA-protein contact points between the AR-DBD and ARE(s), five genes namely, AATK, CDT1, KLF6, LIPH, and SGOL2 were selected on the basis of TF search score and fold enrichment in ChIP-qPCR experiments for the purpose of interaction studies. Our data showed that novel residues K567, K588, R591, and K592 are involved in the process of DNA binding and make important contributions in increasing the stability of DNA-protein complexes. To verify these specific DNA-protein interactions, electrostatic energy term calculations for each residue were determined using the linearized Poisson-Boltzmann equation.

Conclusion

These studies showed the occurrence of breast cancer at younger age in Indian women with significant annual percentage increase in incidence at younger age. The significant number of cases showing family history indicates genetic component playing a major role in the etiology. This study suggests one of the several possibilities with respect to genetic predisposition in the North Indian population. First, there may be a significant proportion of BRCA1/2 mutations that are large germ line rearrangements, which would not have been detected by the method of mutation screening employed. Second, it is possible that there are some unknown genes, which may contribute more significantly to familial breast carcinoma in this population than do BRCA1 and BRCA2. CYP17 A2 allele gene polymorphism plays a significant role for development of breast cancer in Indian women at young age and can also serve as a target for therapy. Due to association of A2 allele of CYP17 gene with increased estrogen level, the steroid hydroxylases are turning out to be potential new targets for drug development. Instead of inhibiting the action of steroid hormones at the level of their receptors by using antihormones, CYPs may help to develop new inhibitors of hydroxylase enzymes.

Betel quid chewing has been shown as a significant and independent risk factor for developing breast cancer in NE region. Although common genetic variations in DNA repair and cell cycle genes contribute toward breast cancer risk, BQC are at an elevated risk for breast cancer attributable to betel quid carcinogens with minor roles of BRCA2 mutation

and C allele of RAD51. Breast carcinogenesis associated with betel quid chewing show genetic alterations different from those observed in the nonbetel quid chewers. Several genetic changes are shared in both tumor groups considered as crucial in breast cancer progression.

Expression of p-glycoprotein in pretreatment biopsy predicts a poor clinical response to NACT in patients having LABC. The chemotherapy-induced toxicity (vomiting and alopecia) correlated significantly with clinical and immunohistochemical response (reduction in Bcl2/Bax ratio) and were found to be a cost-effective and reliable predictor of response to NACT. Alteration in expression of MMR proteins was found mainly in high histological grades and predicts aggressive behavior and poor response to NACT in patients with LABC. Androgen receptor has been identified as independent predictive marker for response to NACT in locally advanced breast cancer cases. It can serve as novel therapeutic target for TNBCs. Bicalutamide (anti androgen) can be used along with other chemotherapeutic drugs as novel therapy for the treatment of TNBCs, which are positive for downstream AR signaling.

Note

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Conflict of Interest

None declared.

References

- 1 National Cancer Registry Programme. Three Year Report of Population Based Cancer Registries 2012–2014. Bangalore: Indian Council of Medical Research (ICMR); 2016
- 2 Murthy NS, Chaudhry K, Nadayil D, Agarwal UK, Saxena S. Changing trends in incidence of breast cancer: Indian scenario. *Indian J. Cancer* 2009;46(1):73–74
- 3 Saxena S, Rekhi B, Bansal A, Bagga A, Chintamani, Murthy NS. Clinico-morphological patterns of breast cancer including family history in a New Delhi hospital, India—a cross-sectional study. *World J Surg Oncol* 2005;3:67
- 4 Hall JM, Lee MK, Newman B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 1990;250(4988):1684–1689
- 5 Wooster R, Neuhausen SL, Mangion J, et al. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12–13. *Science* 1994;265(5181):2088–2090
- 6 Miki Y, Swensen J, Shattuck-Eidens D, et al; BRCA SG. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994;266(5182):66–71
- 7 Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene BRCA2. *Nature* 1995;378(6559):789–792

- 8 Tavtigian SV, Simard J, Rommens J, et al. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. *Nat Genet* 1996;12(3):333–337
- 9 Saxena S, Chakraborty A, Kaushal M, et al. Contribution of germline BRCA1 and BRCA2 sequence alterations to breast cancer in Northern India. *BMC Med Genet* 2006;7:75
- 10 Saxena S, Szabo CI, Chopin S, et al. BRCA1 and BRCA2 in Indian breast cancer patients. *Hum Mutat* 2002;20(6):473–474
- 11 Lupulescu A. Estrogen use and cancer incidence: a review. *Cancer Invest* 1995;13(3):287–295
- 12 Pike MC, Pearce CL, Wu AH. Prevention of cancers of the breast, endometrium and ovary. *Oncogene* 2004;23(38):6379–6391
- 13 Feigelson HS, Henderson BE. Estrogens and breast cancer. *Carcinogenesis* 1996;17(11):2279–2284
- 14 Hanukoglu I. Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis. *J Steroid Biochem Mol Biol* 1992;43(8):779–804
- 15 Brentano ST, Picado-Leonard J, Mellon SH, Moore CC, Miller WL. Tissue-specific, cyclic adenosine 3',5'-monophosphate-induced, and phorbol ester-repressed transcription from the human P450c17 promoter in mouse cells. *Mol Endocrinol* 1990;4(12):1972–1979
- 16 Carey AH, Waterworth D, Patel K, et al. Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17. *Hum Mol Genet* 1994;3(10):1873–1876
- 17 Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FZ, Henderson BE. Cytochrome P450c17 α gene (CYP17) polymorphism is associated with serum estrogen and progesterone concentrations. *Cancer Res* 1998;58(4):585–587
- 18 Chakraborty A, Murthy NS, Chintamani C, et al. CYP17 gene polymorphism and its association with high-risk north Indian breast cancer patients. *J Hum Genet* 2007;52(2):159–165
- 19 Colston KW, Chander SK, Mackay AG, Coombes RC. Effects of synthetic vitamin D analogues on breast cancer cell proliferation in vivo and in vitro. *Biochem Pharmacol* 1992;44(4):693–702
- 20 Hansen CM, Frandsen TL, Br nner N, Binderup L. 1 α , 25-dihydroxyvitamin D3 inhibits the invasive potential of human breast cancer cells in vitro. *Clin Exp Metastasis* 1994;12(3):195–202
- 21 James SY, Mackay AG, Colston KW. Effects of 1,25 dihydroxyvitamin D3 and its analogues on induction of apoptosis in breast cancer cells. *J Steroid Biochem Mol Biol* 1996;58(4):395–401
- 22 Chakraborty A, Mishra AK, Soni A, et al. Vitamin D receptor gene polymorphism(s) and breast cancer risk in north Indians. *Cancer Detect Prev* 2009;32(5-6):386–394
- 23 Nicol s D az-Chico B, Germ n Rodr guez F, Gonz lez A, et al. Androgens and androgen receptors in breast cancer. *J Steroid Biochem Mol Biol* 2007;105(1-5):1–15
- 24 Gonzalez LO, Corte MD, Vazquez J, et al. Androgen receptor expression in breast cancer: relationship with clinicopathological characteristics of the tumors, prognosis, and expression of metalloproteases and their inhibitors. *BMC Cancer* 2008;8:149
- 25 Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res* 1994;22(15):3181–3186
- 26 Mittal RD, Mishra D, Mandhani AK. Role of an androgen receptor gene polymorphism in development of hormone refractory prostate cancer in Indian population. *Asian Pac J Cancer Prev* 2007;8(2):275–278
- 27 Mishra D, Thangaraj K, Mandhani A, Kumar A, Mittal R. Is reduced CAG repeat length in androgen receptor gene associated with risk of prostate cancer in Indian population? *Clin Genet* 2005;68(1):55–60
- 28 Chintamani, Kulshreshtha P, Chakraborty A, et al. Androgen receptor status predicts response to chemotherapy, not risk of breast cancer in Indian women. *World J Surg Oncol* 2010;8:64
- 29 Terry PD, Rohan TE. Cigarette smoking and the risk of breast cancer in women: a review of the literature. *Cancer Epidemiol Biomarkers Prev* 2002;11(10 Pt 1):953–971
- 30 Mudur G. India has some of the highest cancer rates in the world. *BMJ* 2005;330(7485):215
- 31 Williams JA, Phillips DH. Mammary expression of xenobiotic metabolizing enzymes and their potential role in breast cancer. *Cancer Res* 2000;60(17):4667–4677
- 32 Boffetta P, Hecht S, Gray N, Gupta P, Straif K. Smokeless tobacco and cancer. *Lancet Oncol* 2008;9(7):667–675
- 33 Lash TL, Bradbury BD, Wilk JB, Aschengrau A. A case-only analysis of the interaction between N-acetyltransferase 2 haplotypes and tobacco smoke in breast cancer etiology. *Breast Cancer Res* 2005;7(3):R385–R393
- 34 Anantharaman D, Chaubal PM, Kannan S, Bhisey RA, Mahimkar MB. Susceptibility to oral cancer by genetic polymorphisms at CYP1A1, GSTM1 and GSTT1 loci among Indians: tobacco exposure as a risk modulator. *Carcinogenesis* 2007;28(7):1455–1462
- 35 Kaushal M, Mishra AK, Raju BS, et al. Betel quid chewing as an environmental risk factor for breast cancer. *Mutat Res* 2010;703(2):143–148
- 36 Narayan S, Jaiswal AS, Kang D, Srivastava P, Das GM, Gairola CG. Cigarette smoke condensate-induced transformation of normal human breast epithelial cells in vitro. *Oncogene* 2004;23(35):5880–5889
- 37 Nair U, Bartsch H, Nair J. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: a review of agents and causative mechanisms. *Mutagenesis* 2004;19(4):251–262
- 38 Luo LZ, Werner KM, Gollin SM, Saunders WS. Cigarette smoke induces anaphase bridges and genomic imbalances in normal cells. *Mutat Res* 2004;554(1-2):375–385
- 39 Herzog CR, Desai D, Amin S. Array CGH analysis reveals chromosomal aberrations in mouse lung adenocarcinomas induced by the human lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Biochem Biophys Res Commun* 2006;341(3):856–863
- 40 Singh B, Wreesmann VB, Pfister D, et al. Chromosomal aberrations in patients with head and neck squamous cell carcinoma do not vary based on severity of tobacco/alcohol exposure. *BMC Genet* 2002;3:22
- 41 Kaushal M, Mishra AK, Sharma J, et al. Genomic alterations in breast cancer patients in betel quid and non betel quid chewers. *PLoS One* 2012;7(8):e43789
- 42 Deo SV, Bhutani M, Shukla NK, Raina V, Rath GK, Purkayasth J. Randomized trial comparing neo-adjuvant versus adjuvant chemotherapy in operable locally advanced breast cancer (T4b N0-2 M0) *J Surg Oncol* 2003;84(4):192–197
- 43 Charfare H, Limongelli S, Purushotham AD. Neoadjuvant chemotherapy in breast cancer. *Br J Surg* 2005;92(1):14–23
- 44 Heys SD, Chaturvedi S. Primary chemotherapy in breast cancer: The beginning of the end or the end of the beginning for the surgical oncologist? *World J Surg Oncol* 2003;1(1):14
- 45 Gerlach JH, Kartner N, Bell DR, Ling V. Multidrug resistance. *Cancer Surv* 1986;5(1):25–46
- 46 Schneider J, Bak M, Efferth T, Kaufmann M, Mattern J, Volm M. P-glycoprotein expression in treated and untreated human breast cancer. *Br J Cancer* 1989;60(6):815–818
- 47 Chintamani, Singh JP, Mittal MK, et al. Role of p-glycoprotein expression in predicting response to neoadjuvant chemotherapy in breast cancer—a prospective clinical study. *World J Surg Oncol* 2005;3:61
- 48 Ellis PA, Smith IE, Detre S, et al. Reduced apoptosis and proliferation and increased Bcl-2 in residual breast cancer following preoperative chemotherapy. *Breast Cancer Res Treat* 1998;48(2):107–116
- 49 Kerr JF, Winterford CM, Harmon BV. Apoptosis. Its significance in cancer and cancer therapy. *Cancer* 1994;73(8):2013–2026

- 50 Frassoldati A, Adami F, Banzi C, Criscuolo M, Piccinini L, Silingardi V. Changes of biological features in breast cancer cells determined by primary chemotherapy. *Breast Cancer Res Treat* 1997;44(3):185–192
- 51 Chintamani SV, Singhal V, Singh JP, Lyall A, Saxena S, Bansal A. Is drug-induced toxicity a good predictor of response to neo-adjuvant chemotherapy in patients with breast cancer?—a prospective clinical study. *BMC Cancer* 2004;4:48
- 52 Edelman W, Yang K, Umar A, et al. Mutation in the mismatch repair gene Msh6 causes cancer susceptibility. *Cell* 1997;91(4):467–477
- 53 Yamada M, O'Regan E, Brown R, Karran P. Selective recognition of a cisplatin-DNA adduct by human mismatch repair proteins. *Nucleic Acids Res* 1997;25(3):491–496
- 54 Fink D, Aebi S, Howell SB. The role of DNA mismatch repair in drug resistance. *Clin Cancer Res* 1998;4(1):1–6
- 55 Chiaravalli AM, Furlan D, Facco C, et al. Immunohistochemical pattern of hMSH2/hMLH1 in familial and sporadic colorectal, gastric, endometrial and ovarian carcinomas with instability in microsatellite sequences. *Virchows Arch* 2001;438(1):39–48
- 56 Shi WF, Leong M, Cho E, et al. Repressive effects of resveratrol on androgen receptor transcriptional activity. *PLoS One* 2009;4(10):e7398
- 57 Brinkmann AO, Trapman J. Prostate cancer schemes for androgen escape. *Nat Med* 2000;6(6):628–629
- 58 Shang Y, Myers M, Brown M. Formation of the androgen receptor transcription complex. *Mol Cell* 2002;9(3):601–610
- 59 Coffey K, Robson CN. Regulation of the androgen receptor by post-translational modifications. *J Endocrinol* 2012;215(2):221–237
- 60 Doane AS, Danso M, Lal P, et al. An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. *Oncogene* 2006;25(28):3994–4008
- 61 Hickey TE, Robinson JL, Carroll JS, Tilley WD. Minireview: The androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene? *Mol Endocrinol* 2012;26(8):1252–1267
- 62 Mishra AK, Agrawal U, Negi S, et al. Expression of androgen receptor in breast cancer & its correlation with other steroid receptors & growth factors. *Indian J Med Res* 2012;135(6):843–852
- 63 Singh LC, Chakraborty A, Mishra AK, et al. Study on predictive role of AR and EGFR family genes with response to neoadjuvant chemotherapy in locally advanced breast cancer in Indian women. *Med Oncol* 2012;29(2):539–546
- 64 Mehta J, Asthana S, Mandal CC, Saxena S. A molecular analysis provides novel insights into androgen receptor signalling in breast cancer. *PLoS One* 2015;10(3):e0120622

Efficacy of Phenylephrine in Preventing Hemodynamic Responses of Oxytocin during Elective Cesarean Section: A Randomized, Double-Blind, Controlled Trial

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Abstract

This study compared hemodynamic changes and occurrence of complications following oxytocin administration with a prior injection of phenylephrine 100 µg or normal saline during elective cesarean section. Sixty-six healthy term parturients with uncomplicated, singleton pregnancy undergoing elective cesarean section under spinal anesthesia were studied. They received either intravenous phenylephrine 100 µg or normal saline before oxytocin 3 IU was administered over 30 seconds. Oxytocin dose was repeated depending on the adequacy of uterine tone. There was no significant change in systolic, diastolic, and mean arterial pressures during the initial 3 minutes following oxytocin administration in the phenylephrine group but a significant fall in mean and diastolic pressures in the saline group. Heart rate did not change significantly, and no significant complications occurred in either of the groups. To conclude, phenylephrine 100 µg administered before oxytocin injection maintained hemodynamic parameters better than normal saline injection during elective cesarean section.

Keywords

- ▶ oxytocin
- ▶ phenylephrine
- ▶ cesarean section
- ▶ hemodynamic changes
- ▶ blood pressure

Introduction

Uterotonics are the drugs that initiate and maintain adequate uterine contractility after placental delivery, thus reducing blood loss from the site of placental attachment. Among the various agents available in clinical practice, oxytocin is used as the first line drug for prophylaxis and treatment of uterine atony.¹ It is routinely administered during cesarean section and is known to decrease the incidence of postpartum hemorrhage by up to 40%.² However, use of intravenous (IV) oxytocin may be associated with adverse hemodynamic effects such as tachycardia, hypotension, and electrocardiography (ECG) changes.³

Traditionally, large doses of IV oxytocin, that is, 5 to 10 IU, were used.⁴ Recent studies have proven the effectiveness of low-dose oxytocin boluses ranging from 1 to 3 IU.^{3,5–8} However, even this low dose can cause hypotension and tachycardia;

doses between 0.5 and 3 IU have been shown to produce hypotension in 20 to 30% of patients.³

Phenylephrine, an α -adrenergic receptor agonist, is now established as the vasopressor of choice to prevent and treat postspinal hypotension during cesarean section.⁹ The most commonly used bolus dose is 100 µg. It results in increased blood pressure with an associated reflex decrease in heart rate (HR). Thus, phenylephrine might prove beneficial for the prevention of hypotension and tachycardia associated with oxytocin administration.

Current literature has not established the best effective phenylephrine dose for the prevention of oxytocin-induced hypotension and tachycardia. Dyer et al in 2009 demonstrated that phenylephrine 80 µg with oxytocin 2.5 IU could obtund but not abolish the adverse hemodynamic effects of oxytocin and suggested further research to find the most effective dose and timing of phenylephrine administration.⁶

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Rumboll et al in 2015 found that prior administration of IV phenylephrine 50 µg did not prevent hypotension and tachycardia caused by a slow bolus of 3 IU oxytocin.⁷ As phenylephrine administration in a dose of 50 µg was found to be ineffective⁷ and the dose of 80 µg was partially effective in abolishing oxytocin-induced hemodynamic effects,⁶ it was hypothesized that administration of phenylephrine 100 µg just before oxytocin injection would be effective in preventing oxytocin-induced hypotension and tachycardia. Hence, this study was conducted with the aim of evaluating the efficacy of phenylephrine 100 µg in preventing hemodynamic responses of 3 IU oxytocin during elective cesarean section. The objectives were to study and compare the changes in HR and blood pressure and the occurrence of complications following oxytocin administration with a prior injection of phenylephrine 100 µg or normal saline during elective cesarean section.

Materials and Methods

This was a prospective, randomized, double-blind, placebo-controlled trial conducted after obtaining approval from the institutional ethics committee. The recruitment was conducted from November 2015 to February 2017, and a written informed consent was obtained from all the patients. The trial was registered prospectively at www.ctri.nic.in.

A total of 66 healthy term parturients carrying an uncomplicated, singleton pregnancy who were planned for elective cesarean section under spinal anesthesia were included. The study excluded the following patients: those in active labor; those with ruptured amniotic membranes; those with maternal complications such as preeclampsia, diabetes mellitus, cardiovascular disease, and cerebrovascular disease; those with known risk factors for postpartum hemorrhage such as multiple gestation, abnormal placentation, uterine fibroid, macrosomia, hydramnios, history of postpartum hemorrhage, and uterine atony; and those with contraindications for spinal anesthesia such as infection in lumbar area, coagulation abnormalities, autonomic neuropathy, spinal deformities, other neurological diseases, and hypovolemia due to any cause.

The patients were randomly divided into two groups of 33 each using computer-generated random number table, group P and group NS, as per the administered drug. Sealed envelopes were prepared according to the random number allocation to maintain allocation concealment. In group P, phenylephrine 100 µg in 1-mL volume was injected before oxytocin administration; whereas, in group NS, 1-mL saline was injected before oxytocin.

The patients fasted for at least 8 hours and received aspiration prophylaxis in the form of ranitidine and metoclopramide before being shifted to the operating room. In the operating room, baseline maternal HR and noninvasive blood pressure (NIBP) were recorded with a wedge placed under the right buttock. IV coloadung with 15 mL/kg of Ringer's lactate solution was started. Subarachnoid block was performed with a midline approach at L2–L3 or L3–L4 vertebral interspace using a 25-gauge spinal needle, and 2.2 or 2.0 mL

of hyperbaric 0.5% bupivacaine was injected if the patient's height was ≥ 150 cm or < 150 cm, respectively.

Intraoperative monitoring included continuous ECG, HR, NIBP, and pulse oximetry. HR and NIBP were noted before giving spinal anesthesia and then every minute after spinal injection until 10 minutes after delivery of baby. Hypotension was defined as a fall of $\geq 20\%$ from the baseline mean arterial pressure (MAP) and was treated with phenylephrine 100 µg. Hypertension was defined as a rise of more than 20% from the baseline MAP. IV glycopyrrolate 0.2 mg was administered at HR < 60 bpm with hypotension or HR < 45 bpm irrespective of MAP value.

At the time of umbilical cord clamping, the test drug was administered. Following this, all the patients received a slow injection of oxytocin 3 IU diluted to 5 mL over 30 seconds. The readings of HR and NIBP just before the injection of test drug were considered as the baseline values for subsequent changes. Thereafter, HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), and MAP were monitored every minute for the next 10 minutes. Patients who received phenylephrine for the treatment of hypotension within 2 minutes before delivery or whose MAP at the time of delivery was more than 20% from initial baseline value were excluded. In this situation, the same randomization code was used for the next patient.

Uterine tone was graded 3 minutes after delivery as adequate or inadequate by the obstetrician. If inadequate, a rescue dose of oxytocin 3 IU was given slowly. Two such rescue doses were given at 3-minute interval, if required. An infusion of oxytocin at a rate of 0.08 IU/minute was started after 10 minutes of initial oxytocin injection. If uterine tone was unsatisfactory despite three oxytocin boluses, carboprost injection was administered in consultation with the obstetrician.

Intraoperative blood loss was estimated by the volume of blood in the suction bottle and the number of soaked sponges. The timings of skin incision, uterine incision, and delivery of baby; uterine tone at 3 minutes; intraoperative requirement of uterotonics; intraoperative blood loss; and any complications observed, such as nausea, vomiting, and ECG changes were recorded.

The primary outcome measure was peak changes in MAP and HR during the initial 3 minutes following oxytocin administration, whereas the secondary outcomes included changes in SBP and DBP and any complications observed after delivery.

Sample Size Calculation

Sample size was calculated based on the primary outcomes, that is, peak changes in HR and MAP following oxytocin administration. Considering standard deviation (SD) of peak change in MAP after oxytocin administration to be around 10%⁶ and taking 10% change as clinically significant, the sample size required at 90% power and 2.5% level of significance was 27 in each group. Similarly, for peak HR changes, considering SD of change to be around 20%⁶ and taking 20% change in peak HR as clinically significant, the sample size again turned out to be 27 per group. To compensate for possible

exclusions after randomization, 20% of this number was added and therefore 33 patients were studied in each group. Because of two primary outcomes, that is, HR and MAP, the type I error was reduced to 2.5%.

Statistical Analysis

The statistical analysis was performed using SPSS statistical software (version 20.0). The data were presented as mean (SD) or as median (interquartile range). Unpaired *t*-test was used to compare demographic profile, other patient characteristics, baseline hemodynamic parameters, various time intervals, phenylephrine dose and time before delivery, and hemodynamic variables at different time points before and after delivery. Dunnett's test and Tukey's test were applied to analyze the peak changes in various hemodynamic parameters. Value of $p < 0.05$ was considered significant.

Results

A total of 78 patients met the inclusion criteria, of which 66 patients were included for the trial. The CONSORT

(Consolidated Standards of Reporting Trials) flow diagram is shown in ►Fig. 1.

Demographic profile, various intraoperative time intervals, and other patient variables such as gestational age, fluid till delivery, phenylephrine dosage and time of the last dose before delivery, spinal to cord clamp interval, and baseline hemodynamic parameters were comparable in the two groups (►Table 1).

Similar treatment was provided for the management of hypotension, with 15 patients in group NS and 17 in group P developing hypotension and receiving phenylephrine boluses before delivery. Thus, both groups were comparable till the time of intervention, that is, test drug administration at cord clamping.

The uterine tone assessed after 3 minutes of initial oxytocin administration was adequate in all but three patients in each group. The total dose of oxytocin administered was comparable in both the groups (►Table 1). None of the patients required carboprost administration.

The estimated blood loss was significantly higher in patients receiving phenylephrine than those receiving normal saline (►Table 1).

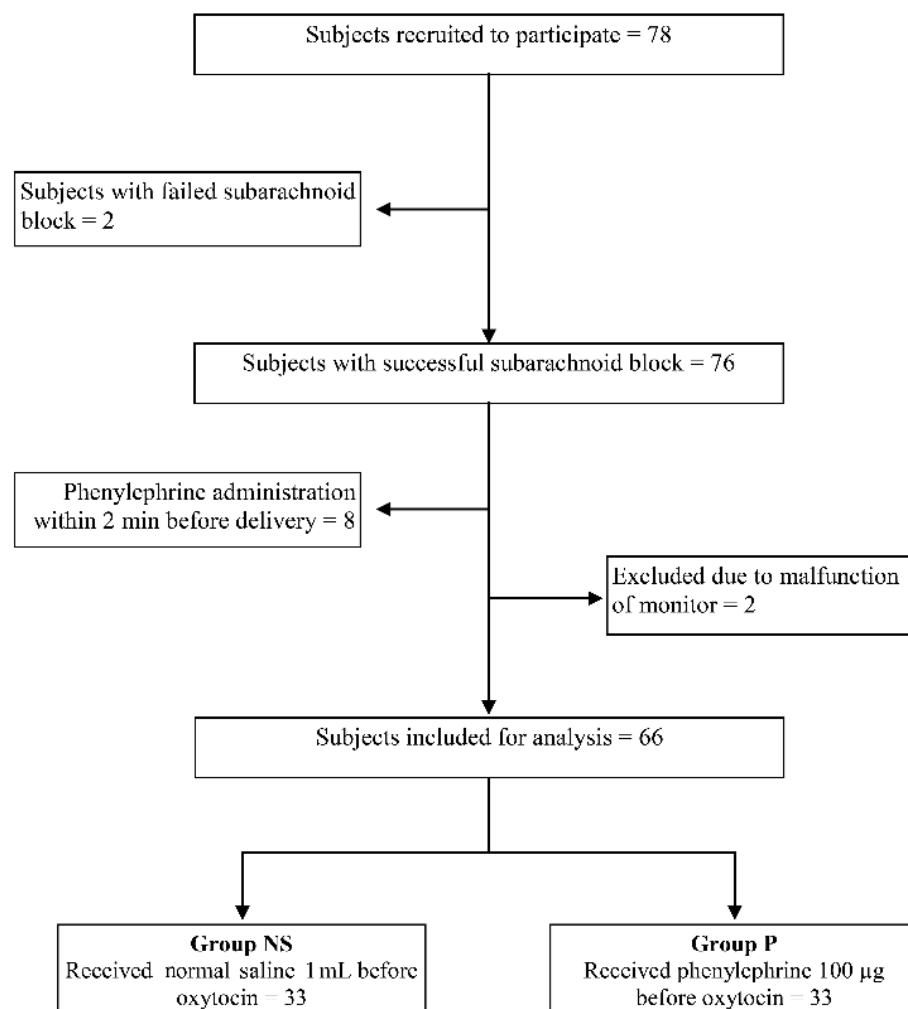


Fig. 1 Consolidated Standards of Reporting Trials flow diagram.

Table 1 Demographic and other patient variables

	Group NS (n = 33)	Group P (n = 33)	p-Value
Age (years)	26.1 ± 3.8	25.4 ± 2.9	0.421
Weight (kg)	60.6 ± 5.6	62.7 ± 7.4	0.180
Height (cm)	151.0 ± 3.9	152.1 ± 3.2	0.220
Period of gestation (weeks)	38.2 ± 1.1	38.0 ± 1.1	0.435
Fluid till delivery (mL)	722.1 ± 63.0	737.9 ± 77.1	0.366
Total phenylephrine before delivery (µg)	160.0 ± 82.8	170.6 ± 99.0	0.746
Time of last phenylephrine dose before delivery (minutes)	6.9 ± 2.8	5.5 ± 1.9	0.097
Total oxytocin (IU)	3.5 ± 1.2	3.9 ± 1.6	0.294
Blood loss (mL)	506.1 ± 90	563.6 ± 95.4	0.014 ^a
Spinal to cord clamp interval (minutes)	11.1 ± 4.4	10.4 ± 3.5	0.480
Baseline HR (beats/min)	88.1 ± 13.7	92.4 ± 14.8	0.225
Baseline MAP (mmHg)	94.1 ± 7.0	91.2 ± 7.1	0.096
Baseline SBP (mmHg)	123.8 ± 9.8	121.3 ± 10.0	0.300
Baseline DBP (mmHg)	76.2 ± 8.5	71.7 ± 9.8	0.050
Hypotension value (mmHg)	75.3 ± 5.6	73.0 ± 5.6	0.096
Hypertension value (mmHg)	112.6 ± 8.6	108.9 ± 8.4	0.083

Abbreviations: DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure.

Note: Values are presented as mean ± standard deviation.

^aStatistically significant.

Hemodynamic Changes

The HR, MAP, SBP, and DBP values did not differ significantly between the two groups at any time point from just before drug administration till up to 10 minutes after delivery of the baby. The trends of HR and MAP are shown in ►Figs. 2 and 3.

►Table 2 shows peak changes in hemodynamic variables within 3 minutes after oxytocin administration. The peak changes in HR within 3 minutes of oxytocin administration remained statistically similar between as well as within both groups ($p = 0.333$).

The peak changes in MAP as well as DBP showed a statistically significant fall in group NS within the initial 3 minutes after oxytocin administration ($p = 0.000$). These changes were not significant within group P. On intergroup comparison, the difference could not achieve statistical significance (►Table 2).

Although the fall in SBP values in group NS appeared to be large, this change was statistically insignificant within the group ($p = 0.054$). The peak change within group P was also not significant. However, on intergroup analysis, the peak change in SBP was found to be significantly higher in group NS than in group P (►Table 2).

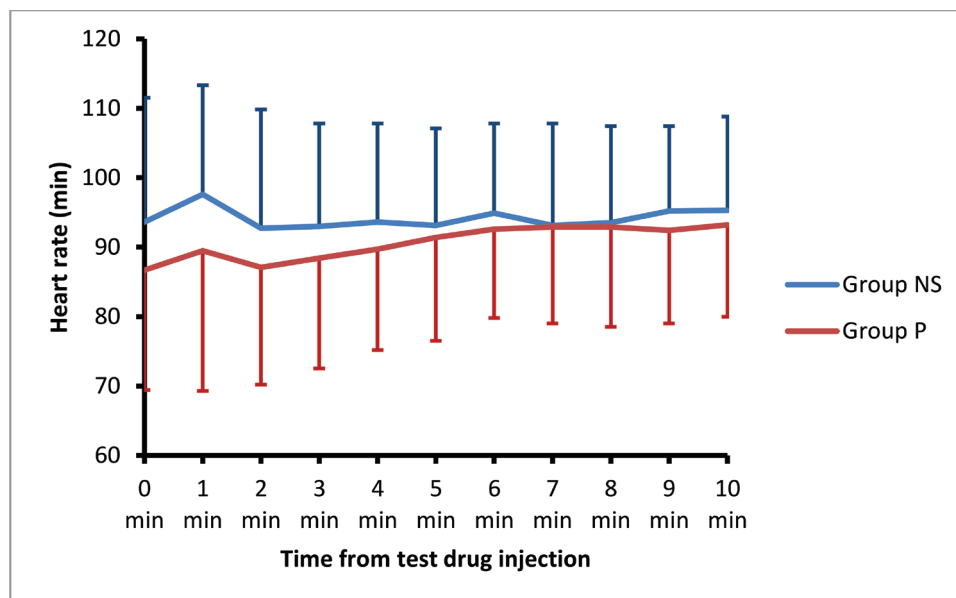


Fig. 2 Heart rate trends after test drug and oxytocin administration.

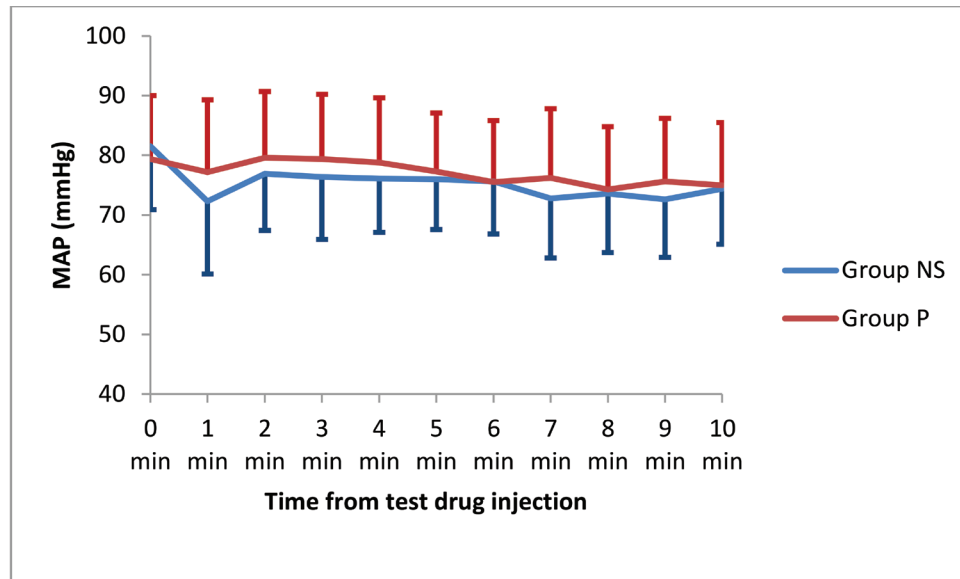


Fig. 3 Mean arterial pressure trends after test drug and oxytocin administration.

Table 2 Peak changes in hemodynamic variables within 3 minutes after oxytocin administration

	Group NS			Group P			Intergroup p-value
	Mean at oxytocin injection	Peak value	Peak change	Mean at oxytocin injection	Peak value	Peak change	
Heart rate (beats/minutes)	93.6 ± 17.9	95.8 ± 18.6	+2.18	86.73 ± 17.3	88.5 ± 20.1	+1.79	0.078
Mean BP (mm Hg)	81.5 ± 10.6	70.6 ± 11.0	-10.9	79.4 ± 10.6	78.0 ± 14.3	-1.4	0.053
Systolic BP (mm Hg)	113.2 ± 14.0	103.9 ± 15.1	-9.3	111.1 ± 13.0	113.0 ± 16.4	+1.9	0.043
Diastolic BP (mm Hg)	60.3 ± 11.4	51.1 ± 12.7	-9.2	57.8 ± 10.1	56.0 ± 15.5	-1.8	0.386

Abbreviation: BP, blood pressure.

Note: Values are presented as mean ± standard deviation.

Complications

Two patients in group NS and none in group P developed nausea during the study period. No ECG changes or other complications were noted in any patient in either group during the study period.

Discussion

The results of this study demonstrated that phenylephrine 100 µg injected just before the administration of oxytocin 3 IU prevented a significant fall in MAP and DBP during the subsequent 3-minute period when compared with baseline values; however, the intergroup difference in peak change within 3 minutes following oxytocin administration in patients receiving phenylephrine or saline pretreatment was statistically significant only for SBP. No significant effect on HR could be seen with or without phenylephrine administration.

Earlier, oxytocin was used in high doses of 5 to 10 IU bolus. However, use of such high bolus doses for prophylaxis of uterine atony has been questioned.^{5,10} Higher doses of oxytocin are probably not necessary because the concentration

of myometrial receptors reaches its peak at term under the influence of estrogen. Therefore, lower doses may be equally effective and appear to be associated with fewer and less severe maternal side effects. Tsen and Balki proposed a “rule of threes” for the administration of uterotonics.¹⁰ The same protocol was followed in this study. The total oxytocin requirement was found to be low and comparable in both groups. It was 3.5 ± 1.2 IU in group NS and 3.9 ± 1.6 IU in group P ($p = 0.294$).

Oxytocin is known to cause tachycardia when administered as IV bolus in conventional doses of 5 to 10 IU.³ The incidence reduces with lower doses but even these lower doses have been seen to cause adverse hemodynamic effects.³ However, in this study, the administration of oxytocin did not cause any clinically significant tachycardia in either of the groups. This could be because we used a low dose of oxytocin 3 IU administered as a slow IV bolus over 30 seconds.

Phenylephrine being a direct α -adrenergic agonist increases the peripheral vascular resistance, thus preventing oxytocin-induced hypotension.⁶ This is evident in our results as the peak changes in blood pressure values were clinically significant in only group NS.

Our results are supported by the work of Dyer et al who studied hemodynamic effects of coadministration of phenylephrine 80 µg with oxytocin 2.5 IU during spinal anesthesia for elective cesarean delivery.⁶ The mean peak percentage change in MAP was 2.99% in group P and -28.9% in group NS. They concluded that phenylephrine obtunded the hemodynamic effects of oxytocin.

Rumboll et al used phenylephrine 50 µg or saline before injecting oxytocin 3 IU and observed a mean peak percentage change in SBP to be -16.9% in the phenylephrine group and -19.0% in the saline group ($p = 0.44$).⁷ They concluded that phenylephrine 50 µg was not effective in preventing oxytocin-induced hypotension. The low dose of phenylephrine used by Rumboll et al was probably the reason behind the lack of efficacy in their study.

Recently, Gangadharaiah et al studied the effects of coadministration of phenylephrine 50 µg, phenylephrine 75 µg, or saline with oxytocin on the prevention of hypotension during cesarean section.¹¹ They demonstrated a significant fall in MAP after oxytocin infusion in all the three groups; however, the magnitude of fall was minimum with phenylephrine 75 µg. HR remained comparable with no incidence of bradycardia in any of the groups. Their study design was different from ours in many aspects. They included patients undergoing both elective and emergency cesarean sections. The oxytocin requirements and hemodynamic responses may be quite different in emergency cesareans. They assessed uterine tone only at the end of uterine closure, whereas we assessed it every 3 minutes and administered additional uterotonics accordingly. Their method of oxytocin administration was also different from ours.

In this study, patients in active labor and those with ruptured membranes were excluded as these patients may have been exposed to oxytocin before coming for lower segment cesarean section, resulting in oxytocin receptor desensitization.¹² This may lead to higher oxytocin requirements and greater use of second-line uterotonics. Phenylephrine was also used before delivery to treat any hypotensive episodes. As this could influence the hemodynamic parameters following oxytocin administration, the patients receiving phenylephrine within 2 minutes before delivery were excluded. Eight such patients were excluded because of this reason. Repeat phenylephrine injection at cord clamping could have led to exaggerated hypertension; hence, it was decided to exclude and not administer the test drug at the time of cord clamping to the patients whose MAP at the time of delivery was more than 20% from the initial baseline value. However, no patient in either group had MAP more than 20% from the initial baseline value at the time of delivery. Two patients in group NS had to be excluded, as the vital sign monitor developed an error during the conduct of the study, resulting in some missed values of HR and blood pressure. The estimated blood loss was found to be more in group P. Although this difference was statistically significant ($p = 0.014$), it did not appear to be clinically significant.

This study has certain limitations. First, the intraoperative blood loss was measured by the volume of blood collected in the suction bottle and the number of soaked sponges. This was not a very accurate method of blood loss calculation due to mixing of liquor in the suction bottle at the time of delivery. However, it served good as a rough estimate. Second, the study included only elective cesarean sections. Therefore, the results cannot be generalized and may not be extrapolated to patients undergoing emergency cesarean sections.

To conclude, phenylephrine 100 µg administered before oxytocin injection 3 IU over 30 seconds maintains hemodynamic parameters better than normal saline injection during elective cesarean section.

Conflict of Interest

None declared.

References

- 1 World Health Organization, WHO recommendations for the prevention and treatment of postpartum haemorrhage. 2012. Available at: apps.who.int/iris/bitstream/10665/75411/1/9789241548502_eng.pdf. Accessed April 30, 2016
- 2 Fortner CL, Manley ES, Jr, Woodbury RA. Effects of synthetic oxytocin with and without preservatives upon coronary blood flow in the dog. *J Pharmacol Exp Ther* 1969;165(2):258-266
- 3 Butwick AJ, Coleman L, Cohen SE, Riley ET, Carvalho B. Minimum effective bolus dose of oxytocin during elective cesarean delivery. *Br J Anaesth* 2010;104(3):338-343
- 4 Mockler JC, Murphy DJ, Wallace EM. An Australian and New Zealand survey of practice of the use of oxytocin at elective caesarean section. *Aust N Z J Obstet Gynaecol* 2010;50(1):30-35
- 5 Dyer RA, Butwick AJ, Carvalho B. Oxytocin for labour and caesarean delivery: implications for the anaesthesiologist. *Curr Opin Anaesthesiol* 2011;24(3):255-261
- 6 Dyer RA, Reed AR, van Dyk D, et al. Hemodynamic effects of ephedrine, phenylephrine, and the coadministration of phenylephrine with oxytocin during spinal anesthesia for elective cesarean delivery. *Anesthesiology* 2009;111(4):753-765
- 7 Rumboll CK, Dyer RA, Lombard CJ. The use of phenylephrine to obtund oxytocin-induced hypotension and tachycardia during caesarean section. *Int J Obstet Anesth* 2015;24(4):297-302
- 8 Kovacheva VP, Soens MA, Tsen LC. A Randomized, double-blinded trial of a "rule of threes" algorithm versus continuous infusion of oxytocin during elective cesarean delivery. *Anesthesiology* 2015;123(1):92-100
- 9 Ngan Kee WD. Prevention of maternal hypotension after regional anaesthesia for caesarean section. *Curr Opin Anaesthesiol* 2010;23(3):304-309
- 10 Tsen LC, Balki M. Oxytocin protocols during cesarean delivery: time to acknowledge the risk/benefit ratio? *Int J Obstet Anesth* 2010;19(3):243-245
- 11 Gangadharaiah R, Duggappa DR, Kannan S, et al. Effect of co-administration of different doses of phenylephrine with oxytocin on the prevention of oxytocin-induced hypotension in caesarean section under spinal anaesthesia: a randomised comparative study. *Indian J Anaesth* 2017;61(11):916-922
- 12 Kimura T, Saji F, Nishimori K, et al. Molecular regulation of the oxytocin receptor in peripheral organs. *J Mol Endocrinol* 2003;30(2):109-115

Use of Fluoridated Dentifrices among Children: Are We in the Right Direction?

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Abstract

Introduction Use of topical fluorides in dentifrices has always been an important tool in prevention of dental caries in young children. Due to the easy availability of various low and high fluoride dentifrices, the parents have no clear understanding about their correct age-appropriate use in children. This study was undertaken to evaluate and understand the trend and current practices among the end user.

Materials and Methods A total of 173 children aged 4 to 6 years were enrolled in the study from schools located in two different geographical areas of the Chandigarh city; group 1 ($n = 90$) from a *peri-urban slum cluster*: (Govt. Primary School, Indira Colony, Mani Majra, $n = 51$); Govt. Middle School, Mani Majra ($n = 39$); group 2 ($n = 83$) from an *urban private city school* (Ankur, Punjab University, Sector 14) using cluster sampling method. Two examiners using type IV examination examined all the children aged 4 to 6 years present using a preinstructed close-ended questionnaire. Data were analyzed using SPSS Software Version 25 (SPSS Inc., Chicago, IL, United States).

Results The data regarding the knowledge of fluoride in pastes showed that 85% of the children were using high fluoride pastes, 10% were using nonfluoride pastes, and 5% were using low fluoride pastes. In group 1, none of the users were aware about the benefits/risk of using fluoride toothpastes and only 22% were aware in group 2. Only 27% of children in group 1 dispensed the correct amount of dentifrice for this age group i.e., a pea head size versus 67% in group 2; a half brush length was dispensed by 61.5% in group 1 and 28% in group 2, and just a smudge by 11.5% in group 1 and 5% in group 2. Forty one percent children in both the groups had a history of having intentionally consumed the toothpaste. The toothpaste was dispensed to the child by parent in 89% of cases in group 2 and only 50% in group 1 and 88% parents claimed to always supervise the child while tooth brushing versus only 53% in group 1. Majority of the respondents', i.e., 97% in group 1 and 63% in group 2 had never been explained about the correct method of use of fluoride paste in children.

Conclusion Knowledge about fluoridated toothpastes is low among the population. The children in peri-urban slums areas are exposed to the high fluoride pastes from

Keywords

- ▶ fluoride dentifrices use
- ▶ very young children
- ▶ trend

very early in life and there is no other toothpaste which is brought home except for those which are commonly used among the members. In the city schools; however, a small percentage of population uses low fluoride pastes in children, possibly due to a greater awareness and access to information, but has no clear idea about their limitations and benefits of age-appropriate use. Till appropriate guidelines are available for the country, a safe practice to follow is tailoring individual need based protocol. The children in peri-urban slums areas need to be educated more on the health practices and importance of use of fluoride dentifrices and the children in the city schools need to be guided more on the age appropriate use of high and low fluoride dentifrices.

Introduction

Prevention of oral diseases is an important aspect of Pediatric Dentistry. Topical fluorides from water, dietary sources, professional applications, etc. have a well-proven role in prevention and progression of dental caries in children.¹ Dentifrices are the most widely used source of topical fluorides for children and have caused the largest impact on oral health, since their inception in early 1960s. Fluoride in the dentifrices acts by decreasing the rate of enamel demineralization and by enhancing the remineralization of an early carious lesion.^{2,3} Presence of fluoride in the tooth also increases the resistance of the remineralized areas to secondary acid attack.^{2,3} The use of fluorides in dentistry, however, has always been considered a two-edged sword with the cariostatic effect of fluoride being well proved, but not without the risk of causing fluorosis in the developing dentition.⁴⁻⁶ When used in very young children with the rising incidence of early childhood caries (ECC), fluoride as a preventive modality of dental caries, however, has become indispensable because of its cost effectiveness.⁷

Fluoride as a mineral is required in our body in very low quantities of around 0.05 to 0.07 mg/kg body weight to exert its desirable effects.⁸ The risk of fluorosis occurs when the amount of fluoride ingested exceeds the amount required during tooth formation/development.⁴ An inadvertent consumption of fluoridated toothpastes in a young child with developing teeth has been associated with mild fluorosis as it remains an important source of ingested fluoride.^{9,10}

To prevent these sequelae, and also to deliver the benefit of fluoride to children, low fluoride pastes with concentration of fluoride almost half or one fourth of that present in most conventional pastes had been devised and marketed.¹¹⁻¹⁵ The different guidelines have taken the safety issues in consideration but concerns however, have been raised regarding the questionable efficacy of these low fluoride pastes.¹⁶⁻¹⁹

With the easy availability and marketing of various low and high fluoride toothpastes in the market²⁰ one does not know the current practices among the end user, which is helpful in formulating new guidelines or reinforcing the current ones. This study was undertaken to evaluate and understand the trend among 4 to 6 years old children regarding use of fluoride pastes in the city.

Materials and Methods

A total of 173 children aged 4 to 6 years were enrolled in the study from schools located in two different geographical areas of the Chandigarh city; group 1 ($n = 90$) from a *peri-urban slum cluster*: (Govt. Primary School, Indira Colony, Mani Majra, $n = 51$); Govt. Middle School, Mani Majra ($n = 39$); group 2 ($n = 83$) from an *urban private city school* (Ankur, Punjab University, Sector 14) using cluster sampling method. Ethical clearance from the Institute's ethics committee was obtained prior to initiation of the study. The school authorities were contacted prior to any interaction with the children for their consent, and an appropriate time suitable for recording of the questionnaires in the presence of at least one parent was decided. Two examiners using type IV examination examined all the children aged 4 to 6 years present. The examiners were calibrated (A.K. and M.K.) before the start of the study. A preinstructed close-ended questionnaire regarding the use and practices of fluoride paste among children including the routine oral health practices was prepared and with at least one parent of each child within the school premises.

Data were analyzed using SPSS Software Version 25 (SPSS Inc., Chicago, IL, United States). Chi-square analysis was used to find the significance of the cross tabulation of counts of two or more variables. Student *t*-test and analysis of variance was used to find the significance of the cross tabulation of a variable with the mean of another variable.

Results

The data recorded were mainly categorized as (1) routine oral health practices and (2) practices pertaining to the use of dentifrices (→ **Table 1**).

Routine Oral Health Practices

Majority of the children in group 1 either brushed their teeth only once daily (54%) or were irregular brushers (34.4%). Only 5.5% brushed twice daily and the remaining had not started brushing till now. Whereas in group 2, 79.4% brushed at least once daily and 13.3% brushed twice daily. The irregular brushers were 7.3%. Ninety-eight percent of children in group 2 were using the pediatric sized brush versus 72%

Table 1 Distribution of parameters recorded pertaining to use of fluoride dentifrices among the two groups

Questions	Group 1 responses	Group 2 responses	p-Value
Q1. Frequency of tooth brushing			$p < 0.001$
Once daily	49	66	
Twice daily	5	11	
Thrice daily	31	6	
Not started	5	0	
Q2. Average sugar exposures per day			$p < 0.001$
Less than three times	40	54	
3–5 times	23	27	
More than 5 times	22	2	
Q3. Has the child been introduced to dental floss?			$p = 0.075$
Never seen or heard	84.2%	84.14	
Aware	15.8%	15.84	
Q4. Type of toothbrush used			$p < 0.001$
Adult size	15	2	
Pediatric size	61	81	
Not specific	9	0	
Q5. Type of toothpaste used			$p < 0.001$
Fluoridated	56	71	
Non fluoridated	12	3	
Low fluoride	0	7	
Did not know	11	0	
Do not use	7	2	
Q.6 Are you aware about fluoridated toothpastes?			$p < 0.001$
No	85	65	
Yes	0	18	
Q7. Amount of toothpaste dispensed			$p < 0.001$
Half brush length or more	48	23	
Pea head size	21	54	
Just a smudge	9	4	
Q8. Method of dispensing toothpaste			$p < 0.001$
Every time by parent	39	72	
Parent/child	18	8	
Child	14	1	
By child under supervision	7	0	
Q9. Is the child able to spit out after brushing?			$p = 0.067$
Yes, fully	42	64	
Partially	33	16	
Never tries/unable	3	1	
Q10. Has the child ever intentionally consumed toothpaste?			$p = 0.421$
Do not know	7	2	
Yes	32	33	
No	46	48	

continued

Table 1 (continued)

Questions	Group 1 responses	Group 2 responses	p-Value
Q11. Have you ever been explained the correct use of toothpaste by a health care provider?			
Yes	1	9	p < 0.001
Other sources	2	22	
No	82	52	
Q12. Do you supervise your child while toothbrushing?			
Always	45	73	p < 0.001
Partially	19	9	
Never, does on its own	21	1	

in group 1. Majority of children in group 1 had more than five times sugar intake per day (26%) versus very few (2.4%) in group 2. Twenty seven percent in group 1 and 32.5% in group 2 had sugar exposures between 3 to 5 per day. Sugar exposures less than three times a day were present in 47% in group 1 and 65% in group 2.

Awareness of Dental Floss

Only 15% of children in both the groups were aware of dental floss and knew it should be customarily used along with tooth brushing every day. This may be due to the fact that dental floss is not as well marketed as other oral hygiene aids in India and that there is a lack of awareness regarding dental floss among the general population. So, it can be recommended that dental flossing should be taught at school level.

Types of Toothbrushes

It was seen that around 71% in group 1 and 97% of group 2 children uses pediatric size of tooth brush and 17% in group 1 and 2% in group 2 uses adult size tooth brush. It may be because of nonavailability or lack of knowledge among the parents; however, it should be kept in mind that such children become more prone to the trauma due to toothbrush injury.

Practices Pertaining to Use of Dentifrices

The data regarding the knowledge of fluoride in pastes showed that 85% of the children were using high fluoride pastes, 10% were using nonfluoride pastes, and 5% were using low fluoride pastes. In the group 1, none of the users were aware about the fluoride toothpastes and only 22% were aware in the group 2. Only 27% of children in group 1 dispensed the correct amount of dentifrice for this age group i.e., a pea head



Fig. 1 The rice grain and pea head size of toothpastes.²⁴

size versus 67% in group 2; a half brush length was dispensed by 61.5% in group 1 and 28% in group 2, and just a smudge by 11.5% in group 1 and 5% in group 2. Forty-one percent children in either group had a history of having intentionally consumed the toothpaste. The toothpaste was dispensed to the child by parent in 89% of cases in group 2 and in only 50% in group 1 and 88% parents claimed to always supervise the child while tooth brushing versus only 53% in group 1. Majority of the respondents' i.e., 97% in group 1 and 63% in group 2 had never been explained about the correct method of use of fluoride paste in children.

Discussion

Fluoride remains the cornerstone of the noninvasive management of noncavitated caries lesions and the use of fluoride toothpaste is generally recognized as the main reason for the decline in caries in industrialized countries over the last four decades. It is the only nonprescription toothpaste additive proven to prevent dental caries.

One of major concern in having fluoridated toothpaste in the vicinity of a young child is inadvertent ingestion leading to toxicity. Taking the average amount of toothpaste in a tube to be 100 g, and the average amount of fluoride concentration to be 1,000 ppm in our country (as per drug and cosmetic act, 1940),²¹ the total amount of fluoride, which is available, is 100 mg (1,000 ppm = 1mg/g). The safely tolerated dose (STD) of fluoride is 8 to 16 mg/kg body weight.²² Taking the average body weight of a 5-year-old child to be 20 kg, the STD is between than 160 to 320 mg of fluoride, which remains in very safe limits. The probable toxic dose (PTD) is 5 mg/kg of body weight,²² that is 100 mg of fluoride. The child, therefore, may show symptoms of toxicity only if almost the entire paste is inadvertently ingested. Second, there have been concerns about the occurrence of fluorosis in the permanent dentition due to early use of fluoride pastes.²³ The American Academy of Pediatric Dentistry, therefore, recommends²⁴ use of only a smudge or rice grain size (► Fig. 1)²⁴ of fluoridated paste (0.125 g) dispensed each time in children up to 3 years of age to reduce the risk of fluorosis due to ingestion of fluoride from the paste. For children up to 6 years of age, a pea size of the toothpaste is to be dispensed (0.25 g). These children ingest approximately 30 to 40% of the paste dispensed.

The European Academy of Pediatric Dentistry recommends²⁵ a use of pea size of 1,000 ppm fluoride toothpaste (0.25 g) for children between 2 to–6 years. Therefore, when using fluoridated toothpaste (1,000 ppm) for a young child, if appropriate instructions are followed, there is minimal risk to the child of developing mild fluorosis or toxicity.

Overall, in this study, it is seen that knowledge about fluoridated toothpastes is low among the population. Some parents have not heard about the low fluoride pastes and avoid tooth brushing with pastes due to risk of ingestion, whereas others who have heard about the low fluoride pastes continue to use it even beyond two years and have no clear idea about when to stop its use. The children in peri-urban slums areas are exposed to the high fluoride pastes from very early in life and there is no other which is brought home except for those for common use among the members. In the city schools; however, a small percentage of population uses low fluoride pastes in children, possibly due to a greater awareness and access to information, but was found to have no clear idea about their limitations and benefits of age appropriate use. Lima et al. in a cross sectional observation study on tooth brushing habits of Brazilian schoolchildren aged 3 to–4 years found 42% children from high socioeconomic status (SES) and 2.7% children from low SES to be using toothpaste without fluoride or below 1000 ppm fluoride. There was a lot of difference in the oral health practices between two groups in the present study.²⁶ The number of sugar exposure children is quite high and knowledge about brushing twice daily and introduction to flossing still remains low. It is imperative, therefore, to guide the population about the need for an age appropriate use of fluoride pastes and also instruct them about their safe practice. The children in peri-urban slums areas need to be educated more on the health practices and importance of use of fluoride dentifrices and the children in the city schools need to be guided more on the age appropriate use of high and low fluoride dentifrices. One limitation of the study is that the toothpastes marketed without any known concentration of fluoride were recorded as fluoride free and actual fluoride levels in these tooth pastes were not assessed as this was beyond the scope of the present investigation.

Conclusion

Till appropriate guidelines are available for the country, a safe practice to follow is tailoring individual need based protocol. In a city like Chandigarh, where the water fluoride levels are only 0.3 ppm and there is no other source of fluoride apart from the dietary fluoride, topical fluorides remain an important caries preventive tool. Since the risk of consumption is high till 2 years and the diet of the child is strictly under parental control reducing the number of sugar exposure, a low fluoride dentifrice can be used. Also, the most esthetically vulnerable teeth—the maxillary incisors, remain at a risk of being affected with enamel hypomineralization between 22 to 25 months. Beyond two years, the child undergoes individual choices about food, the risk of increased sugar

exposure and more frequent snacking increases; a high fluoride paste with brushing under strict parental supervision is thus advisable.

Conflict of Interest

None declared.

References

- 1 Adair SM. Evidence-based use of fluoride in contemporary pediatric dental practice. *Pediatr Dent* 2006;28(2):133–142
- 2 Ericsson SY. Cariostatic mechanisms of fluorides: clinical observations. *Caries Res* 1977;11(Suppl 1):2–41
- 3 Duggal MS, Toumba KJ, Amaechi BT, Kowash MB, Higham SM. Enamel demineralization in situ with various frequencies of carbohydrate consumption with and without fluoride toothpaste. *J Dent Res* 2001;80(8):1721–1724
- 4 Ekambaram M, Itthagarun A, King NM. Ingestion of fluoride from dentifrices by young children and fluorosis of the teeth—a literature review. *J Clin Pediatr Dent* 2011;36(2):111–121
- 5 Wong MC, Glenn AM, Tsang BWK, Lo ECM, Worthington HV, Marinho VCC. Using a fluoridated supplement with a high fluoride concentration in children aged under 6 years may increase the risk of fluorosis. *Evidence-based dentistry* 2010;11(1):6–9
- 6 Kaminsky LS, Mahoney MC, Leach J, Melius J, Miller MJ. Fluoride: benefits and risks of exposure. *Crit Rev Oral Biol Med* 1990;1(4):261–281
- 7 Marinho VCC, Higgins JPT, Sheiham A, Logan S. Fluoride toothpastes for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev* 2003; (1):CD002278
- 8 Farkas CS, Farkas EJ. Potential effect of food processing on the fluoride content of infant foods. *Sci Total Environ* 1974;2(4):399–405
- 9 Naccache H, Simard PL, Trahan L, et al. Factors affecting the ingestion of fluoride dentifrice by children. *J Public Health Dent* 1992;52(4):222–226
- 10 Bentley EM, Ellwood RP, Davies RM. Fluoride ingestion from toothpaste by young children. *Br Dent J* 1999;186(9):460–462
- 11 Beltrán ED, Szpunar SM. Fluoride in toothpastes for children: suggestion for change. *Pediatr Dent* 1988;10(3):185–188
- 12 Horowitz HS. The need for toothpastes with lower than conventional fluoride concentrations for preschool-aged children. *J Public Health Dent* 1992;52(4):216–221
- 13 Pessan JP, Alves KMRP, Ramires I, et al. Effects of regular and low-fluoride dentifrices on plaque fluoride. *J Dent Res* 2010;89(10):1106–1110
- 14 Ekambaram M, Itthagarun A, King NM. Comparison of the remineralizing potential of child formula dentifrices. *Int J Paediatr Dent* 2011;21(2):132–140
- 15 Thaveesangpanich P, Itthagarun A, King NM, Wefel JS. The effects of child formula toothpastes on enamel caries using two in vitro pH-cycling models. *Int Dent J* 2005;55(4):217–223
- 16 dos Santos AP, Nadanovsky P, de Oliveira BH. A systematic review and meta-analysis of the effects of fluoride toothpastes on the prevention of dental caries in the primary dentition of preschool children. *Community Dent Oral Epidemiol* 2013;41(1):1–12
- 17 Rasines G. Fluoride toothpaste prevents caries in children and adolescents at fluoride concentrations of 1000 ppm and above. *Evid Based Dent* 2010;11(1):6–7
- 18 Ammari AB, Bloch-Zupan A, Ashley PF. Systematic review of studies comparing the anti-caries efficacy of children's toothpaste containing 600 ppm of fluoride or less with high fluoride toothpastes of 1,000 ppm or above. *Caries Res* 2003;37(2):85–92

- 19 Freire IR, Pessan JP, Amaral JG, Martinhon CC, Cunha RF, Delbem AC. Anticaries effect of low-fluoride dentifrices with phosphates in children: A randomized, controlled trial. *J Dent* 2016;50:37–42
- 20 Dos Santos APP, de Oliveira BH, Nadanovsky P. A systematic review of the effects of supervised toothbrushing on caries incidence in children and adolescents. *Int J Paediatr Dent* 2018;28(1):3–11
- 22 Whitford GM. Fluoride toxicology and health effects. In Fejerskov O, Ekstrand J, Burt BA, eds. *Fluorides in Dentistry*. Hanover: International Society of Fluoride Research 1996;167–184
- 23 Wright JT, Hanson N, Ristic H, Whall CW, Estrich CG, Zentz RR. Fluoride toothpaste efficacy and safety in children younger than 6 years: a systematic review. *J Am Dent Assoc* 2014;145(2):182–189
- 24 American Academy of Pediatric Dentistry. Fluoride therapy. *Pediatric Dentistry. Reference Manual* 2018;40(6):250–253
- 25 European Academy of Paediatric Dentistry. Guidelines on the use of fluoride in children: an EAPD policy document. *Eur Arch Paediatr Dent* 2009;10(3):129–135
- 26 Lima CV, Pierote JJ, de Santana Neta HA, de Deus Moura de Lima M, de Deus Moura LdeF, de Moura MS. Caries, toothbrushing habits and fluoride intake from toothpaste by Brazilian Children according to socioeconomic status. *Pediatr Dent* 2016;38(4):305–310

Jasbir Singh Bajaj (1936–2019)

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Professor Jasbir Singh Bajaj was born on September 26, 1936 in Lahore as the younger of the two children of Sardar Makhan Singh Bajaj, a homeopath of repute. He grew up in an environment conducive to scientific pursuits. Post partition, his family was relocated to Delhi, where he excelled academically, earning honors and accolades throughout his schooling and education in college. During his undergraduate medical studies at Amritsar Medical College, he secured first position in Medicine and was awarded the Dr. H.B.N Swift Medal for securing highest marks in pharmacology and setting a new record in Punjab University in the subject. After his MD (Medicine) degree, he proceeded for his postdoctoral research in endocrinology and diabetes as a Commonwealth Scholar at the Royal Postgraduate Medical School, London, and at the University of Newcastle upon Tyne in England.

From his early student years, he was a polymath who read voraciously to expand his intellectual horizons. On the shelves of his personal library, one would find Bertrand Russell's "Theory of Knowledge" beside treatises on immunology; the leather-bound volumes of the Encyclopedia Britannica resided alongside his research papers on insulin kinetics.

He joined the faculty of AIIMS in 1967 where he served as a professor and later Head of the Department of Medicine since 1979, retiring in 1996. At AIIMS, he shaped the careers of generations of students with his famous teaching rounds. He derived his zeal as a teacher from the legendary Sir William Osler who was responsible for producing fine physicians who emphasized humility and humanity. Thus he set out on his ward rounds spending hours painstakingly examining the patient, instilling not only medical adeptness but also the ideals of discipline and patience in his students.

Recognized as a physician and diabetologist of exceptional merit, he was appointed as the Honorary Physician to the President of India from 1977 to 1982 and again from 1987 to 1992. He also served the Prime Minister of India as Chief Physician from 1991 to 1996.

He was appointed as Member, Planning Commission, in 1991, in the rank of Minister of State, Government of India, in which capacity he served till 1998. His uncanny ability

to assimilate information hitherto unfamiliar to him was such that, upon being the first ever biomedical scientist to be nominated to the Planning Commission, in no more than a fortnight, he had mastered modern economic theory and was able to participate in financial policy drafting.

In 2007, he was appointed Vice-Chairman of the Punjab State Planning Board.

Prof. Bajaj was recipient of many distinctions, awards, and honors at national and international levels. He was a fellow of the Royal College of Physicians of London and Edinburgh, and the National Academy of Medical Sciences. He was a founder fellow of the Indian College of Physicians.

In recognition of his highly distinguished services to the country, he was decorated with Padma Shri by the President of India in 1981, and with the coveted Padma Bhushan in 1982 and Padma Vibhushan in 2009, being the first diabetologist and endocrinologist of India to be so honored.

His interest in diabetes and endocrinology spanned over nearly four decades. His most outstanding research included the original work on entero-hypothalamo-insular axis, insulin dynamics in health and disease, hormonal contraception in males, and endocrinal and metabolic profile of protein calorie malnutrition. His proposed classification of a separate category of malnutrition-related diabetes mellitus, with its two subtypes, was accepted and incorporated in the new international classification of diabetes (ICD 10), recommended by the World Health Organization (WHO) Expert Committee; the eponym of *Bajaj's syndrome* was assigned to this entity by international bioscientists.

He published five books on insulin and metabolism, diabetes mellitus and glucagons, and contributed chapters in several national and international textbooks, with more than 240 publications and research communications to his credit.

He was honored as Doctorate in Medicine (*honoris causa*) by the Karolinska Institute, Stockholm, during their 175th anniversary celebration in 1985. The citation



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presented on the occasion recognized him as “a leading research investigator in diabetes” and made a special mention of “his most outstanding work concerning the role of central nervous system in insulin secretion, insulin dynamics in health and disease, and endocrinal and metabolic profile of malnutrition.” The citation also recognized “Prof. Bajaj as a leading exponent in the field of health manpower development for diabetes health care and an advocate for the incorporation of diabetes in the newly emerging models of primary health care.”

Prof. Bajaj was the first scientist from outside Europe and the United States to have been elected as the President of the International Diabetes Federation (IDF) in which capacity he served from 1985 to 1988. He had the singular distinction of having been unanimously elected as the Honorary President (for life) of the Federation in 1988.

He had been deeply involved in medical education and health planning since 1967. He was responsible for the planning, organizing, and implementing the Rehbar-e-Sehat health care delivery model in Jammu and Kashmir. As the Chairman of Expert Committee on Health Manpower Development and the Chairman of Consultative Group on National Education Policy in Health Sciences (1991), he steered major initiatives for reorientation of medical and paraprofessional

education and facilitated the development of health-related vocational courses in secondary education.

He was the first sub-Dean of AIIMS in 1972. He was a Member of the Governing Body, and Chairman, Academic Committee, AIIMS from 1994 to 1999.

He was the President of National Academy of Medical Sciences from 1992 to 1994 and National Board of Examinations from 1994 to 1997, and served on the Executive Board of the World Federation for Medical Education from 1988 to 2003, and participated in developing policy initiatives and implementation of strategies to enhance quality and relevance of medical education at regional, national, and international levels.

For his distinguished achievements as an Educationist, the Management Committee of Dr. B.C. Roy National Award Fund selected him for the Senior Award under the category of “Eminent Medical Man, for the year 1992” which was presented by the President of India.

Prof. Bajaj passed away on January 8, 2019. This medical luminary, being a Padma Awardee, was honored with a State funeral as a mark of deep respect and in recognition of his outstanding services to the nation and contribution to health policy planning, medical education, and applied research in endocrinology and metabolic medicine.

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