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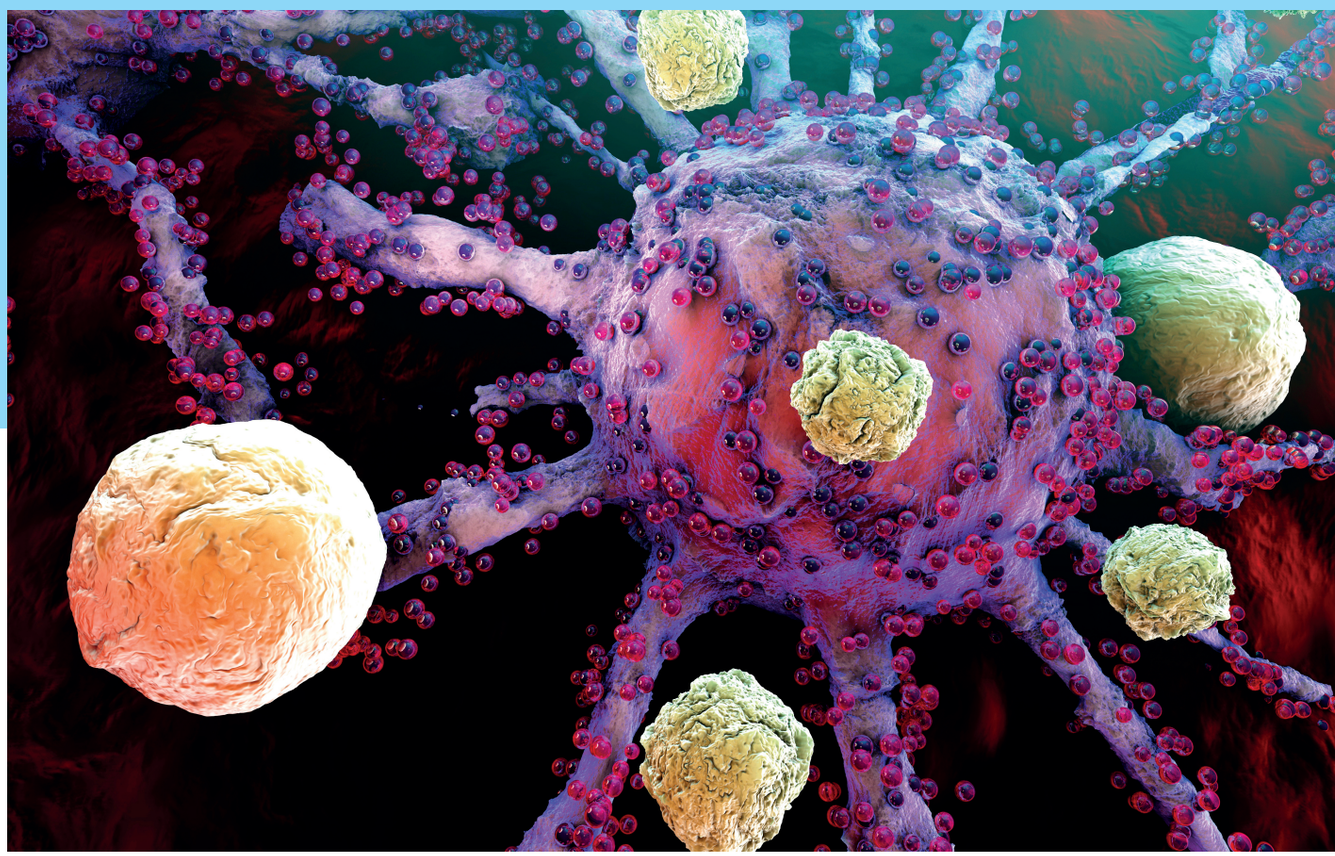

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Genetic Counselling, Testing, and Management of Hereditary Breast and Ovarian Cancer Syndrome in India: Updated Expert Consensus Recommendations from Indian Society of Medical and Pediatric Oncology

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Abstract

Introduction Hereditary breast and ovarian cancer (HBOC) is driven by mutations in BRCA1/2 and related genes. Their understanding is vital to appropriate management of such patients and at-risk families, including counselling and genetic testing. Several important recent advances have made it necessary to revise the previous recommendations we made for India in 2020.

Materials and Methods This consensus document was developed with the authors as key experts in the field. Published evidence, real-world data, and expert interpretation were used by a modified Delphi method to finalize these recommendations.

Results Detailed description and process for identifying patients at risk, doing their counselling, selecting the right molecular test, interpreting the results, and determining the optimal mode of action to attenuate risk of HOBC or its recurrence have been provided in a clear and lucid manner. Differences between germline and somatic mutations are described. Information from publicly available databases was used to fine-tune the guidelines—as more information had becomes available since the time of writing the first guidelines. Risk of various cancer types and corresponding risk reduction strategies have been explained.

Conclusion Community oncologists in India, SAARC region, and other low- and middle-income countries should use these guidelines in their clinical practice to optimize genetic counselling, molecular testing, and management of patients with HBOC.

Keywords

- hereditary cancer
- therapy
- driver mutations
- guidelines

Introduction

In India, breast cancer (BC) is still the commonest cancer in females as well as the leading cause of cancer-related death in women.[1] While the younger median age of onset reflects the population demographics (52% of Indians are below the age of 30 years), BC in Indian women has a high incidence-to-mortality ratio compared with the West.[2] It is therefore important to ascertain risk factors, high-risk biomarkers, and driver mutations to optimize management.

As the name suggests, hereditary breast and ovarian cancer (HBOC) syndrome leads to increased risk of early-onset BC and ovarian cancer (OC) in multiple family members. It usually follows an autosomal-dominant inheritance pattern.[3,4] HBOC syndrome results in higher lifetime risk of cancer in women—being 50 to 85% for BC, and 15 to 30% for OC.[5,6] Most common mutations responsible for HBOC include *BRCA1* and *BRCA2*.[7] Race and ethnicity have strong bearing in HBOC. For instance, specific mutations identified in a population sharing common ancestry (Founder mutations) first reported from the Caucasians were from Ashkenazi Jews, French Canadians, and Icelanders.[8] Similarly novel founder mutations in BRCA1, BRCA2, and MLH1 genes have been identified in India from several Maharashtrain, Gujarati, Jain, Bohri, Punjabi, Bengali, and Nepali communities[9,10] [personal communication Rajiv Sarin, 27 October 2023].

With the availability of drugs that have been proven to be useful in women with *BRCA1/2* mutations (e.g., poly (ADP-ribose) polymerase [PARP] inhibitors for both germline and somatic mutations; platinum-based chemotherapy in germline), their genetic testing is vital for optimal treatment decision making.[11] Identification of such mutations (and families at risk of HBOC) allows us to quantify their risk of future metachronous cancers as well as discuss pros and cons of appropriate surgical and/or nonsurgical prophylactic measures.[12] We can then enable families at risk to become informed previvors (a person who takes action to reduce or eliminate a genetic cancer before the cancer develops or is detected in his or her body).[13] We now know that "BRCA-ness" is also a result of mutations in several non-*BRCA* genes (e.g., *PALB2*, *CHEK2*, *ATM1, RAD51C*, and *RAD51D)* and similar preventive measures can also potentially benefit them also.[14]

The prevalence, nature, and frequency of germline mutations vary significantly—geographically and ethnically.[8,9] Their clinical significance and penetrance is also highly variable. BrCa Exchange collates data from across the world, including India, and makes them publicly available for real-time risk assessment for individual patient–related genetic mutations. In general, pathogenic genetic mutation occurs in about 10 to 15% of all patients with BC, and *BRCA1* and *BRCA2* account for almost half of the pathogenic/likely pathogenic mutations.[9,10,15,16]

While innumerable international guidelines have been published since 2010 for overall management of HBOC, almost all of them are based on the Caucasian population.[17] Our 2020 consensus document was the first to incorporate unique characteristics and needs of the Indian patients.[19]

In the intervening period, a lot of new data have been generated and our understanding of HBOC refined. It was therefore necessary to update our previous recommendations, making it more robust.

Materials and Methods

Our multidisciplinary expert group began the revision process by re-evaluating evidence from phase III randomized controlled studies, other prospective and retrospective studies, BrCa gene mutation publications, and real-world clinical experience. Google Scholar and PubMed databases were searched with the key words: "hereditary breast and ovarian cancer"; "HBOC"; "BRCA1/2 mutations"; "germline BRCA mutations"; "somatic BRCA mutations"; "brcaness"; "non-BRCA mutations"; and "genetic testing."

The first meeting involved extensive discussions moderated by the chairperson of the committee followed by preliminary voting using the modified Delphi process.[20] Thereafter, the Expert Committee continued to discuss via e-mail and WhatsApp to fine-tune the recommendation statements. All authors participated in the preparation of the draft, multiple rounds of voting, and the approval of the final manuscript.

Recommendations (Results)

1. Genetic Counseling in India: Importance and Awareness

While our understanding has improved considerably, genetic testing in HBOC in India lags behind for several reasons, most important ones being infrastructure and finances.[21] Lack of sufficient number of trained genetic counsellors also increases the burden of the treating physician and oncologist. Where feasible, individual or family members are helped appropriately using the following principles:

- Allow them to understand the medical facts in simple terms: diagnosis, probable future course, cancer risk, and management options.
- Understand role of heredity in the risk of cancer, its risk of recurrence in patients, and first-degree relatives who are carrying the mutation(s).
- Understand how to minimize the risk of recurrence.
- Select the course of action most suitable to their individual preference and family goals.
- Understand the advantages of positive lifestyle changes.[22–25]

Pretest genetic counselling is generally recommended globally. This is also endorsed by international oncology working groups.[4,26–28] Such counselling should be done by health care professionals who are adequately trained with respect to genetic and clinical aspects of HBOC, *BRCA1* and *BRCA2*.[29,30] In India, the burden often falls on the respective oncologists.[21,31] In view of the shortage of trained and qualified staff across India, use of tele-genetic counselling is recommended. This should be carried out in compliance with the Telemedicine Practice Guidelines as published by Government of India.

Components of HBOC Genetic Counselling

(A) ***Pretest counselling***: the patient/family members should be made aware of the following key factors:

- Medical history and available pedigree evaluation of up to three generations (►**Fig. 1**).
- Objective risk estimation using mathematical risk assessment models where available and qualitative criteria as an alternate (e.g., National Comprehensive Cancer Network [NCCN] tools).
- Genetic testing methodology recommendations.
- Interpreting test reports—possible test outcomes, e.g., pathogenic variant detected, variant of uncertain significance (VUS).
- Risks, benefits, and psychosocial implications.

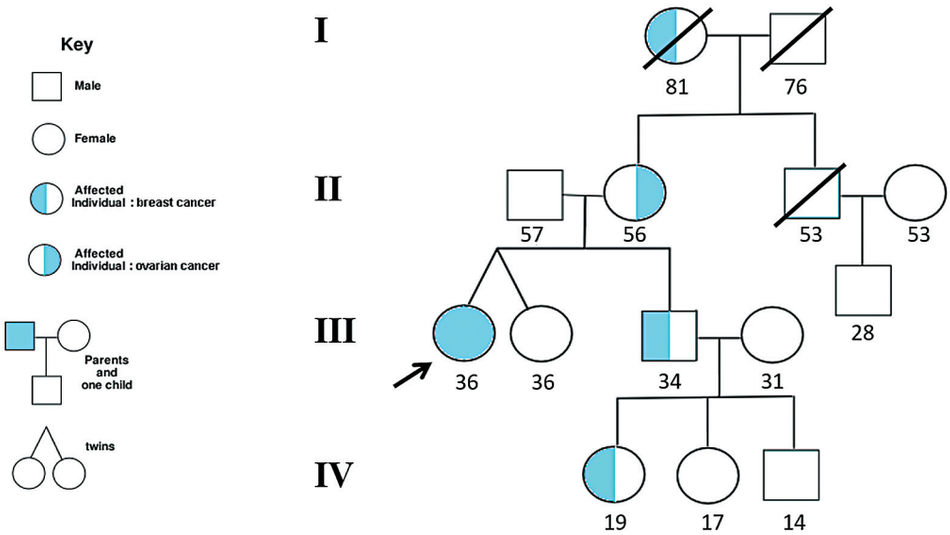

Fig. 1 Representative pedigree chart of HBOC family. HBOC, hereditary breast ovarian cancer.

- Implications of test results to other family members.
- Discussion of need to maintain confidentiality to prevent genetic discrimination.[22,24,25]

Once the pretest genetic counselling is documented, if the patient/subject allows to be subject to biological sample collection, consent is automatically implied.

Assessment of family history: while real-world limitations are well known in countries like India, attempts should be made to collect family history of three generations to prepare pedigree analysis. This should ideally include first- and second-degree relatives on both the maternal and paternal sides of the family. Details recommended are age, gender, pregnancies (if applicable), age at onset of cancer (if applicable), age at death (if applicable), cause of death (for deceased relatives), ethnic background of grandparents (maternal and paternal, if necessary), and history of consanguinity. Using this information to generate a pedigree chart gives a good visual understanding for all stake holders.[32–35]

(B)*Post-test counselling*: the key objective of this session is risk communication. Once the molecular testing report becomes available and the health care team has calculated the risk of cancers/recurrence, the same is communicated to the patient/family member as appropriate. Appropriate attention should also be given to emotional counselling. Pretest genetic counselling improves the family's journey through this process. Group discussions and use of information booklets, where available, are encouraged. This can minimize anxiety of the patients and their family members.[33–36] It is desired that all questions and doubts are answered to the satisfaction of the patient/family member during this session.[37]

2. Germline *BRCA* Testing

Assessment of risk and identifying patients: germline mutations in *BRCA1/2* genes are regarded as high penetrance—a cancer relative risk of greater than 5—and have been characterized in several populations globally. Mutations in other non-*BRCA* genes, such as *PALB2*, *TP53*, *PTEN*, *CDH1*, *STK11*, *CHEK2*, *RAD51C*, *RAD51D*, and *ATM*, are also known to confer risk of BC and/or OC, albeit with lower frequency and penetrance[26,31,35] (►**Tables 1** and **2**).

The lifetime risk of breast and ovarian malignancies is variable, with pathogenic mutations in *BRCA1* (BC: 46–87%; OC: 39–63%) and *BRCA2* (BC: 38–84%; OC: 17–27%). Other

Table 1 Genes associated with HBOC and their penetrance risk

Gene/locus	Syndrome	Risk	Mutation/minor allele frequency
High penetrance			
BRCA1 (17q21)	HBOC	60–85 × for BC	1/400
		15–40 × for OC	
BRCA2 (13a12.3)	HBOC	60–85 × BC	1/400
		13–23 × OC	
TP53 (17p13.1)	Li–Fraumeni	50–89 × by age 50 BC	<1/10,000
		90 × in Li–Fraumeni survivors	
PTEN (10q23.3)	Cowden	25–50 × BC	<1/10,000
CDH1 (16q22.1)	Familial diffuse gastric cancer	RR: 6.6	<1/10,000
STK11/LKB1 (19p13.3)	Peutz–Jeghers	30–50 × by age 70	<1/10,000
PALB2 (16p12)	BC, prostate Ca, pancreatic Ca	30% BC risk by age 70	<1/1,000
Moderate penetrance			
CHEK2 (22q12.1)	Li–Fraumeni 2	OR: 2.6	1/100–1/200 in some populations
BRIP1 (17q22)	BC	RR: 2.0	<1/1,000
ATM (11q22.3)	Ataxia telangiectasia	RR: 2.37	1/33–1/333
Low penetrance			
FGFR2 (10q26)	BC	OR: 1.26	0.38
TOX3 (16q12.1)	BC	OR: 1.14	0.46
LSP1 (11p15.5)	BC	OR: 1.06	0.3
TGFB1 (19q13.1)	BC	OR: 1.07	0.68
MAP3K1 (5q11.2)	BC	OR: 1.13	0.28

Abbreviations: BC, breast cancer; HBOC, hereditary breast ovarian cancer; OC, ovarian cancer; OR, odds ratio; RR, relative risk.

Table 2 Cancer risks associated with BRCA1 and BRCA2

Mutation	Lifetime risk of BC, %	Lifetime risk of OC, %
BRCA 1	50–85	35–46
BRCA 2	50–85	13–23
Cancer type	**Risk in carriers to age 70 years**	**Lifetime risk in general population, %**
Breast	BRCA1: 55–70	
	BRCA2: 45–70	
Contralateral breast	Up to 63 at 25 years post-diagnosis, but highly age-dependent	7 at 25 years post-diagnosis
Ovarian	BRCA1: ~40	~1
	BRCA2: ~15	
Colon	Unclear	~5
Prostate	Elevated; absolute risk not well defined	White: ~14
		African American: ~19
Male breast	BRCA1: 1	0.1
	BRCA2: 8	
Pancreatic	BRCA1: unclear	1.5
	BRCA2: 5	
Other sites	To be determined	Varied

Abbreviations: BC, breast cancer; OC, ovarian cancer.
Source: Adapted from Malhotra et al[19].

cancers associated with germline *BRCA1/2* mutations include male BC (1–9%), prostate cancer (9–20%), pancreatic cancer (1–7%), and melanoma.[38] The largest analysis of 1,010 high-risk families across India revealed *BRCA* mutations in 85% and non-*BRCA* mutations in 15% of families.[39] Additional analysis based on age and family history showed a high prevalence of germline variants (75%) in younger patients age younger than 40 years with a first-degree family member affected with BC/OC.[40–42] A methodical review investigating the prevalence of germline variants in high-risk HBOC susceptibility genes in 1,028 patients of Indian descent with familial/early-onset/triple-negative BC (TNBC) or OC identified 18 *BRCA1* and 16 *BRCA2* variants that were not reported in the Breast Cancer Information Core or ClinVar databases.[39] The putative Ashkenazi founder mutation *BRCA1* 185delAG was detected in a low proportion of patients (4.2%), the majority of whom were from South India or who were Malaysians of Indian origin.[40–42] In the last 5 years, several studies have been published from Indian researchers investigating the frequency of P/LP mutations in the Indian population. ►**Table 3** provides a summary of the important Indian-specific studies published till date. Most of the studies have a strong selection bias in patient enrollment and hence have reported higher frequencies.[39,43–48] However, two studies have been performed in unselected patients with BC and OC[39,43] In a predominantly North Indian population, Mittal et al have reported a frequency of 18.6% for P/LP variants, with their multigene next-generation sequencing (NGS) panel.[44] Using the NCCN 2019 criteria would have missed 11% of these mutations. This frequency is higher than those reported on unselected cases from Europe, the United States, and Africa. In their multicentric study, Gupta et al tested 239 unselected patients with OC for only BRCA1/2. They reported a prevalence of 21.4% P/LP mutations.[43] It is also important to keep in mind that NGS testing has the risk of leading to false positivity. In one study, out of all 41 samples analyzed for *BRCA1* and *BRCA2*, 5 were found with 950_951 insA (Asn319fs) at Chr13:32906565 position and 1 sample with 1032_1033 insA (Asn346fs) at Chr13:32906647, both being frame-shift mutations in *BRCA2* gene. The 950_951 insA (Asn319fs) mutation is reported as a pathogenic allele in NCBI dbSNP. On examination of IGV for all these samples, it was found that both mutations had "A" nucleotide insertion at 950 and 1032 positions in exon 10 of *BRCA2* gene. However, Sanger sequencing did not confirm these insertions.[49–53]

Clinical practice has been focused to test patients who fulfil NCCN criteria for testing (Box 1); however, recent publications have emphasized that using NCCN guidelines misses many patients with both *BRCA* and non-*BRCA* mutations.[54] At present, it is therefore uncertain whether testing for hereditary mutations is warranted beyond the standard criteria. As a rule of thumb, it is recommended to assess women with a personal or family history of BC, OC, tubal, or peritoneal cancer or those who are part of a family having known *BRCA1/2* gene mutations. Where appropriate, a familial risk assessment tool should guide whether further genetic counselling and/or genetic testing is warranted.[55]

Table 3 Pathogenic BRCA1/2 mutations identified in Indian patients

Study	Region	Tumor types	Testing method	Remarks
Valarmathi et al, 2003[118]	New Delhi, North India	Breast cancer	Direct sequencing	BRCA 1 (E1250X in exon 11; E1754X in exon 20)
Rajkumar et al, 2003[79]	Chennai, South India	Breast and ovarian cancer	Heteroduplex analysis/dHPLC	BRCA1 (Ex12 1386 delCTCTC Stop 1389, Ex13 CGA → TGA Arginine 1443 Stop), BRCA2 (Ex11O 1235delCTTAA stop 1237)
Saxena et al, 2006[119]	New Delhi, North India	Breast cancer	Heteroduplex analysis of PCR amplicons using exon-specific primers	BRCA1 (185delAG in exon 2; 4184del4; 3596del4 in exon 11), BRCA1 (4184del4 in exon 11)
Syamala et al, 2007[120]	Kerala, South India	Breast and ovarian cancer	Direct sequencing	BRCA2 (c.4642delAA, c.4926insGACC)
Thirthagiri et al, 2008[42]	Malaysia, Indian ethnicity	Breast cancer	dHPLC and DNA sequencing	BRCA1 (180 delA, 185 delAG, 5370 C.T), BRCA2 (9097 C. T)
Soumittra et al, 2009[121]	Chennai, South India	Breast and ovarian cancer	PCR-dHPLC	BRCA1 (c.4158_4162delCTCTC; p.Ser1369SerfsX2, c.4327C.T; p.R1443X, c.1148_1149delAT; p.Asn383Arg fsX6, c.4399C.T; p.Gln1467X, c.4705_4706insTGGAATC; p.Ile1567fsx5, c.5024_ 5025insT; p. Thr1675Thr fsX4, c.68_69delAG; p. Glu23Val fsX16, c.66_67delAG; p. Leu22Leu fsX18, c.5118_5120delAAT; p.del1707Ile); BRCA2 (c.6214_6218delCTTAA; p.Ser2072Ser fsX4, c.5130_5133delTGTA; p.Tyr1693X, c.2621_ 2627delAACTGTC; p. Ile873Ile fsX19)
Vaidyanathan et al, 2009[55]	South India	Breast and ovarian cancer	Heteroduplex analysis using CSGE and direct sequencing	BRCA1 (185delAG)
Kang et al, 2014	Malaysia, Indian ethnicity	Breast cancer	PCR and Sanger sequencing	BRCA1 (185delAG)
Mittal et al, 2022[44]	North India	Breast cancer	NGS, multigene panel with reflex MLPA	236 unselected, consecutive patients, 18.64% P/LP, 34% non-BRCA genes, 1 AJ founder mutation
Pramanik et al, 2022[45]	North India	Ovarian cancer	NGS, multigene panel with reflex MLPA	72 patients, selectively referred for genetic testing. 44% P/LP, 85% BRCA, and 15% non-BRCA
Gupta et al, 2021[43]	Pan India	Ovarian cancer	NGS, BRCA1/2	239 unselected patients, 21.4% P/LP
Kadri et al, 2021[46]	Western India	Breast and ovarian cancer	NGS, multigene panel	144 patients, selectively referred, 28% P/LP, 12% non-BRCA.
Singh et al, 2018[39]	Pan India	Breast and ovarian cancer	NGS, multigene panel	1,010 selectively referred, 30.1% P/LP, BRCA1/2 85%
Chheda et al, 2020[47]	Western India	Breast and ovarian cancer	NGS, BRCA1/2	160 women, selectively referred, 31.9% P/LP
Mehta et al, 2018[48]	North India	Breast and ovarian cancer	NGS, BRCA1/2	206 women, selectively referred, 30.6% P/LP

Abbreviations: MLPA, multiplex ligation-dependent probe amplification; NGS, next generation sequencing.

Whom to Test First?

Any woman affected by early-onset BC and/or OC should be tested first. A suspected family member who is below the age of 18 years should have testing differed till they become adults. Once an index case (proband) has been identified, testing of first-degree adult relatives is the next step. If a pathogenic variant/mutation has already been identified, single-site gene testing of the specific gene mutation is sufficient. This is called cascade testing (screening of at-risk biologic relatives of the individual who harbors a pathogenic variant).[56] Its objective is to identify asymptomatic at-risk relatives, calculate their risk, and discuss steps to reduce future morbidity and mortality from cancer (risk-reducing strategies include enhancing surveillance diagnostics, medical therapeutics, and/or surgical interventions).

Testing Methods

First-generation automated Sanger sequencing has generally been replaced by the more efficient NGS technology. It is less expensive and comprehensive genomic profiling or multigene panel testing becomes rapid. Where cost is not a significant consideration, multigene panels are being used as a first-line test for any patient suspected to have an inherited cancer syndrome.[57]

This approach is robust for detecting single-nucleotide variants (SNVs) and small insertion/deletion (indels), with high accuracy. However, this is not the case for larger genomic rearrangements (insertions/deletions) and/or copy number variants. Multiplex ligation-dependent probe amplification (MLPA) or array comparative genomic hybridization (aCGH) would have to be used to detect the same.[58] Several in silico tools make the bioinformatics part less labor-intensive and reduce turnaround time.

Based on genetic testing and interpretation, patients can be divided into three risk groups—high risk, moderate risk, and low or unknown risk (►**Table 1**).

Methods of Germline BRCA Detection

Sample for germline genetic testing could be blood, saliva, or cheek swab. As mentioned above, multigene panels using NGS enables high-throughput genetic testing with good accuracy. However such testing can be performed as follows:

- Single site.
- Founder/recurrent mutation associated with specific neoethnicity.
- Multigene/targeted panel.
- MPLA/aCGH for large genomic rearrangements/copy number variations.
- Whole exome sequencing.
- Whole genome sequencing.

While selecting a NGS workflow, the following criteria should be considered to suit the genetic testing[59–61] (Box 2):

- Design of multigene panel based on clinical utility and demand.
- Wet lab processes for enrichment of targets (hybrid capture or amplicons based capture) without compromising on sensitivity and specificity of the detection of pathogenic variants.
- Bioinformatics expertise.
- Qualified and trained personnel to interpret results.
- Reasonable turnaround time (approximately 4 weeks).

Having a reasonable turnaround time (approximately 4 weeks) helps in reducing anxiety and testing fatigue amongst patients, families, and providers.

At least three studies from India have reported the use of multigene panel testing by NGS for germline mutations in HBOC patients.[39,43–49] The majority of BRCA1/2 mutations identified are single base substitutions (missense or nonsense mutations); small insertions or deletions (result in prematurely truncated nonfunctional protein); and splice junction alterations (exon skipping or intronic inclusion, also resulting in a nonfunctional protein). In 5% of cases, there could be large genomic rearrangements which will be missed on conventional NGS testing. As mentioned above, MLPA technique or aCGH can be used in patients/families who are shown negative by NGS, but have a strong clinical suspicion of HBOC.[48,49] It is important to remember that besides BRCA1 and BRCA2, genes that need to be tested in suspected cases of HBOC syndrome includes ATM, BRIP1, CHEK2, RAD50, RAD51D, RAD51C, PALB2, BAARD1, P53, STK11, CDH1, MSH2, MSH6, MLH1, EPCAM, PMS2, ATM, PTEN, FGFR2, TOX3, LSP1, and MAP3K1.

Interpretation of Sequencing Results

International working groups have provided guidelines for the interpretation of germline sequence variants. The DNA sequence alterations are categorized qualitatively based on several factors—including functional evidence, family history, allele frequency data, computational and in silico predictions (►**Table 4**).

Failure to detect a deleterious germline mutation in a proband could be due to one or more of the following[49,62]:

- Patient has a pathogenic variant in another gene not included in the multigene panel.
- Tested gene has a sequence variant that cannot be easily detected by sequence analysis (e.g., large deletion; limitation of test methodology).
- Sequence variant in a region such as an intron or regulatory region of a gene (area not covered by the test).
- Involvement of genes not associated with known underlying phenotype.

Interpretation is facilitated by interrogating updated publicly available literature/databases (ClinVar, OMIM, BrCa Exchange, GWAS, HGMD, and SwissVar), correlation with population data, and in silico predictions of variant effect. Nonsynonymous variants' effects can be calculated using multiple algorithms such as PolyPhen-2, SIFT, Mutation Taster2, Mutation Assessor, and LRT. Only nonsynonymous and splice site variants found in the hereditary cancer gene panel should be used for clinical interpretation. Fortunately,

Table 4 Classification of mutation variants according to clinical significance (adapted from nomenclature of three international working groups)

International Agency for Research in Cancer		Clinical Molecular Genetics Society		American College of Medical Genetics	
Class	Description	Class	Description	Category	Description
5	Definitely pathogenic	1	Certainly nonpathogenic	1	Previously reported and recognized cause of the disorder
4	Likely pathogenic	2	Unlikely to be pathogenic	2	Previously unreported and is of the type that is expected to cause the disorder
3	Uncertain	3	Likely to be pathogenic	3	Previously unreported and is of the type that may or may not be causative of the disorder
2	Likely not pathogenic	4	Certainly pathogenic	4	Previously unreported and is probably not causative of disease
1	Not pathogenic			5	Previously reported and is a recognized neutral variant
				6	Previously not known or expect to be causative of disease, but is found to be associated with a clinical presentation

Source: Adapted from Malhotra et al[19].

germline BRCA testing is robust and the variants are well-curated.

Genetic Test Report

Molecular labs should transcribe their information into a report that describes the test results in a uniform manner, without using jargon, and facilitates explaining its significance to the proband and first-degree relatives. DNA change as a variant should be reported using the standard Human Genome Variation Society (HGVS) nomenclature, describing the mRNA reference sequence that was used, the nucleotide change in the cDNA as a c. and the consequent change in the amino acid and protein as a p.[63,64]

Validation of Test Result

Molecular testing laboratories should follow the joint consensus from the Association for Molecular Pathology and College of American Pathologists. They should validate every detected SNV or indel in the coding region that results in deleterious mutations and documenting it in terms of positive percentage agreement and positive predictive value.[64,65] Documenting concordance between results from a newly developed assay and a gold standard method such as Sanger sequencing is also advised. Participating in internal quality control as well as external quality assurance programs is desirable. If using the NGS for genetic testing, then validating NGS results by Sanger may not be necessary if all bioinformatic and quality parameters are controlled.[43,44,49,66]

How to Manage Variants of Uncertain Significance

VUSs are genetic alterations whose clinical significant is not yet established. They are usually single nucleotide polymorphisms—may be in the promoter regions, exons, and introns. They could also be small in frame insertions and deletions or synonymous substitutions.[64] More than 20,000 unique variants have been identified in the BRCA genes.[65] These constitute less than 10% of all mutations in BRCA1/2 (the remaining 90% have already been classified either as pathogenic or benign). The clinical implication is that once further data become available, up to 30% of current VUSs might turn out to be pathogenic.[67,68] In countries where data are still maturing, like India, up to half (30–50%) of mutations identified in BRCA1/2 genes could be VUS.[9] Data sharing initiatives like BrCa Exchange, BRCA Challenge, and Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) help in resolving the significance of VUS.[69] Such initiatives have already resulted in a 13% fall in the rate of mutations being called VUS between 2002 and 2013.[70] If the mutation is still reported as VUS, it should not be taken into consideration while arriving at risk assessment in HBOC. Unfortunately, clinicians often want to err on the side of abundant caution and factor in VUS as potentially pathogenic. Caution is advised against this because it may lead to unnecessary prophylactic medication/surgery and/or patient anxiety.[71–73]

Quality of Genetic Testing: The Backbone of Characterizing BRCA1/2 Mutations

All aspects of genetic testing need a robust quality. This includes sample collection and transport; wet lab processing; and bioinformatics. Following established standard operating procedures in genetic testing is extremely important to maintain test quality so as to avoid both false-positive and false-negative reporting (which is true for every aspect of health care management). Following guidelines developed by the American Association of Pathologists' Assistants and the College of American Pathologists for NGS bioinformatics pipelines has been shown to significantly reduce error rates.[66,74,75] National accreditation programs and quality assessment programs (e.g., EMQN [European Molecular Genetics Quality Network]) could help meet the goal of optimizing quality.

Somatic or Tumor BRCA Testing

DNA extracted from tumor tissue (freshly frozen or from the formalin fixed paraffin embedded [FFPE] blocks) is used to detect somatic mutations. The percentage of tumor cells in the sample processed for mutation analysis can determine the accuracy of the results. If the quality of DNA is compromised, that would also interfere with the test. Further, tissue preservation using formalin induces a chemical crosslinking reaction with nucleotides that results in artefactual sequence alterations and deamination of cytosine nucleotides. These features are of concern when FFPE specimens are used.[76] Use of shorter amplicons, de- crosslinking steps, and treatment with uracil-DNA glycosylase (DNA repair enzyme) are methods that can help reduce the number of sequence artefacts. Their use can improve the quality of extracted DNA.[77] Somatic NGS testing is generally recommended at 500X coverage to avoid false-negative assessment. The bioinformatic pipeline should also be set to take into consideration the percentage of tumor cells in the processed sample.

The ideal somatic mutation testing report should describe[78]:

- Suitability of tumor sample for tumor content and specific testing method.
- Number and names of genes tested (if using a multigene panel).
- Depth of coverage for each gene.
- Details of mutation (if detected) with HGVS nomenclature.
- Reference sequence of gene.
- Interpretation of results with reference to therapy.

When in doubt, somatic mutations identified using NGS may need to be confirmed by Sanger sequencing.

Significance of Somatic (Tumor) BRCA Mutations

In OCs, association with loss of heterozygosity suggests genomic scaring and instability.[73,74] Sporadic somatic BRCA1/ 2 mutations are seen in up to 33% of BRCA mutations in OC and 4 to 15% of unselected TNBC.[79–82] Amongst patients with high-grade serous OC (HGSOC), BRCA1/2 germline as well as somatic mutations are frequent (17–25%). In fact, somatic mutations have been reported in 18 to 30% of all patients with BRCA1/2 mutations. It is also interesting to note that 9% of patients with OC showed presence of somatic mutations in homologous recombinant genes (BRCA1/2, BRIP1, CHEK2, and RAD51C).[81]

Clinically, it is important to identify these because presence of somatic mutations is predictive of primary platinum sensitivity and improved overall survival.[83] Somatic BRCA1/2 pathogenic mutations and loss of heterozygosity are also predictive biomarkers for clinical response to PARP inhibitor.[83–86] Platinum-sensitive relapsed patients with serous OC and positive BRCA mutations have been shown to have the highest chance of benefiting from olaparib (median progression-free survival: 11.2 months in BRCA mutation-positive vs. 7.4 months in wild-type BRCA patients; hazard ratio: 0.54 [95% confidence interval: 0.34–0.85]; $p = 0.0075$).[87] Based on data from PAOLA-1 study, homologous recombination deficiency (HRD) testing (a composite genomic scar score) has also become an important predictive biomarker for response to PARPi.[88] Some tests developed by other laboratories that can be considered include Myriad MyChoice,[89] loss of heterozygosity, and telomeric allelic imbalance. A word of caution in interpreting their results, since different proprietary algorithms have been incorporated in these tests.[87] Thus, to ensure all eligible patients receive the benefit of PARPi therapy, it is necessary to evaluate all (somatic as well as germline) their BRCA1/2 pathogenic mutations.[90]

Somatic or Germline Testing First in Ovarian Cancer?

Somatic mutations testing is not interchangeable with germline testing. Because the bioinformatics pipeline is different, somatic testing is less sensitive. If it is used solely, a significant number (10%) of germline P/LP mutations can be missed.[91] For this reason, most guidelines, including American Society of Clinical Oncology (ASCO), recommend the use of germline testing first, and then if required, somatic testing.[92]

On the other hand, if only germline testing is carried out (on DNA extracted from blood sample), an estimated 5 to 7% of HGSOC cases who may have acquired (somatic) mutations in BRCA1/BRCA2 genes in their tumor will be missed.[50]

So another approach would be to first do tumor testing (to triage cancer genetics referrals) and then offer germline testing to the subset of HGSOC patients in whom a deleterious mutation was detected. For example, somatic P/LP variants seen in tumor specimens are common in some genes with germline implications (e.g., TP53, STK11, PTEN) and may not indicate the need for germline testing unless the clinical/family history is consistent with a P/LP variant in the germline.[51]

Since both approaches have their limitations, current recommendation is to consider parallel testing of somatic and germline panel. This can reduce turnaround time, reduce false negativity but at the cost of double the cost. This is also recommended especially for HGSOC.[52]

European Society for Medical Oncology has recommended either approach, based on physician's choice.[53] We recommend germline testing as the first approach, followed by HRD for those with gBRCA negative.

Management of HBOC

Risk Management for the Previvor (Unaffected Carrier of Mutation)

- *Lifestyle modifications recommended are:*
  - Regular exercise and maintaining a healthy body weight.
  - Limiting alcohol consumption.
  - Avoid hormone-replacement therapy.
  - Encourage breast feeding.
- *Risk reduction surgery:* NCCN recommends that BRCA carriers be offered prophylactic bilateral mastectomy.92–94 In both retrospective and prospective

observational studies, risk-reducing or prophylactic bilateral mastectomy decreases the incidence of BC by 90% or more in patients who are at risk for hereditary BC, with most studies focusing on BRCA mutation carriers. For BRCA1 carriers, risk-reducing bilateral salpingo-oophorectomy (rrBSO) is recommended for women who have completed child-bearing and should be performed by age 35 to 40 years or individualized on the basis of age of onset of OC in the family.95–97 In BRCA2 carriers, this procedure can be delayed until age 40 to 45 years. rrBSO not only decreases the risk of OC in BRCA mutation carriers, but also decreases the risk of mortality. NCCN does not routinely recommend hysterectomy at the time of rrBSO and indicates that salpingectomy alone is not the standard of care, discouraging it outside a clinical trial.[99]

- *Cancer surveillance: for female BRCA carriers who do not wish to pursue (or would r*ather delay) surgical risk reduction, BC surveillance should be offered, and OC screening may be performed.[100]

BC screening: the following strategy is recommended by expert groups for women with BRCA pathogenic variants who have not undergone risk-reducing surgery and should be individualized as needed[100,101]:

- Breast awareness from 18 years of age.
- Clinical breast examination every 6 to 12 months is recommended from the age of 25 or 10 years before the youngest BC.
- Annual screening using magnetic resonance imaging (MRI; days 7 to 15 of the menstrual cycle) should be commenced from age 25 years with the addition of annual mammography with or without tomosynthesis from age 30 years.[78]
- Age 30 to 75 years: annual mammogram and contrast-enhanced MRI of breast (alternating every 6 months).
- >75 years: management should be considered on an individual basis.
- Male BC: breast self-examination training and education starting at 35 years. Clinical breast exam every 12 months starting at 35 years.

OC screening: before rrBSO, 6 monthly transvaginal ultrasound and measure of serum CA-125 may be considered from age 30 years; however, the limited value of these tools as effective screening measures should be communicated to individuals.

- *Pharmacoprevention*: use of tamoxifen may be considered; however, the level of evidence is weak.[103] It should be used only for BRCA2 tumors or if the first cancer was estrogen receptor–positive. Its benefit is offset by risk of side effects. The dose (1–20 mg per day) and duration (2–5 years) are also not clear. If raloxifene is used instead of tamoxifen, both efficacy and toxicity are reduced.
- *Prevention of other BRCA-related cancers*: no evidence-based data exist. BRCA2 carriers may consider annual skin and eye examination as screening for melanoma, and annual screening for pancreatic cancer with endoscopic ultrasound or MRI/magnetic resonance cholangiopancreatography. There is no consensus when screening should commence; however, age 50 years or 10 years before the earliest diagnosed case in the family would be reasonable (►**Table 5**).

Table 5 Management recommendations for other genes implicated in hereditary breast cancers

Gene	Breast cancer risk management	Ovarian cancer risk management	Other cancers
ATM	• Absolute risk 20–40% • Consider CEMRI breast from 30–35	• Potentially increased risk • RRSO, insufficient evidence	• Counsel for autosomal-recessive condition in offspring
BARD1	• Potentially increased risk • RRM insufficient evidence	• Unknown	
BRIP1	• Unknown	• Increased risk of ovarian cancer • Consider RRSO at age 45–50	
CDH1	• Increased risk of lobular cancer • Annual mammography from age of 30 years • Discuss option of RRM	• No increase	• Gastric cancer: prophylactic total gastrectomy at 20 years or 5 years earlier than the earliest case of HDGC in the family.
CHEK2	• Increased risk • Annual mammogram from age 40 years	• No increase	• Colon
NF1	• Increased risk • Annual mammogram from 40 years age • RRM insufficient evidence	• No increase	• MPNST, GIST

Table 5 (*Continued*) Management recommendations for other genes implicated in hereditary breast cancers

Gene	Breast cancer risk management	Ovarian cancer risk management	Other cancers
NBN	• Increased risk • Annual mammogram from 40 years age • RRM insufficient evidence	• Unknown	
Lynch syndrome genes (MSH2/MSH6/MLH1/PMS2/EPCAM)	• Unknown or insufficient evidence	• Except for PMS2, the association is strong. RRSO should be discussed with the patient as evidence is limited.	• Colon, uterus
PALB2	• Absolute risk: 41–60% • Annual mammogram and breast MRI with contrast at 30 yc, • Risk reduction: discuss option of RRM • Strength of evidence of association with cancer: strong	• Absolute risk: 3–5% • Management: • Risk reduction: consider RRSO at age >45 years • Strength of evidence of association with cancer: strong	• Pancreatic cancer • Absolute risk: 5–10% • Management: screen P/LP variant carriers with a family history of pancreatic cancer
PTEN	• Breast awareness from 18 years age • CBE every 6–12 months from age of 25 years • MRI/mammography from age 30 years or 5–10 years before earlier case in family		• Endometrial cancer, education, and hysterectomy • Annual thyroid USG • Colonoscopy every 5 years from age of 35 years
RAD51C	• Absolute risk: 20–40% • Management: annual mammogram and consider breast MRI with contrast starting at age 40 years • Strength of evidence of association with cancer: strong	• Absolute risk: 10–15% • Management: risk reduction: recommend RRSO at 45–50 years • Strength of evidence of association with cancer: strong	
RAD51D	• Absolute risk: 20–40% • Management: annual mammogram and consider breast MRI with contrast starting at age 40 years • Strength of evidence of association with cancer: strong	• Absolute risk: 10–20% 10-12,63,64 • Management: risk reduction: recommend RRSO at 45–50 years • Strength of evidence of association with cancer: strong	
STK11	• Absolute risk: 32–54% • Management: screening: annual mammogram and breast MRI with contrast starting at age 30 years	• No established association	• Pancreatic cancer • Nonepithelial ovarian cancers
TP53	• Breast awareness from age of 18 years • CBE every 6–12 months from age of 25 years • Annual MRI with contrast ages 20–29 • Annual MRI + mammography ages 30–75 • RRM to be discussed		• Annual whole-body MRI • Annual brain MRI • Colonoscopy and UGIE every 2–5 years from age of 25 years • Comprehensive physical and neurological exam \follow Toronto protocol

Abbreviation: MRI, magnetic resonance imaging.

- *Reproductive counselling*: pathogenic variants in many BC genes, including BRCA, are inherited in an autosomal-dominant pattern, meaning that there is a 50% chance that children of BRCA carriers will have inherited the cancer predisposition variant (assuming that the pathogenic mutation is harbored on only one of the chromosomes of one of the parents). Reproductive counselling of BRCA carriers includes education about prenatal diagnosis and assisted reproduction.[20] One option is preimplantation genetic diagnosis, which is used to analyze embryos, obtained by in vitro fertilization, genetically before their transfer into the uterus.

Risk Management for Patients

- *Decision for breast-conserving surgery (BCS) versus B/L mastectomy*: for a patient with BC, who is technically eligible for BCS, a BRCA mutation is not a contraindication for BCS. Both BCS and mastectomy are equally recommended by ASCO guidelines.[102] Surgical management of the index malignancy (BCT vs. ipsilateral therapeutic mastectomy and contralateral risk-reducing mastectomy [CRRM]) in BRCA1/2 mutation carriers should be discussed, considering the increased risk of contralateral BC (CBC) and possible increased risk of an ipsilateral new primary BC compared with noncarriers. The risks and benefits of both approaches and reconstruction options should be weightage to her age, expectations, wishes, risk acceptance attitude, and body image preferences. For women who have a mutation in a moderate-penetrance BC susceptibility gene, mutation status alone should not determine local therapy decisions for the index tumor or CRRM. BCT should be offered to those for whom BCT is an appropriate treatment option.
- *Risk of OC*: rrBSO. Recommendations are the same as those for previvors. There are conflicting data whether rrBSO reduces the risk of BC, with many recent studies not showing any association between rrBSO and BC risk.[96,97]
- *Risk of CBC*[103,104]: for women with BC who have a BRCA1/2 mutation and who have been treated or are being treated with unilateral mastectomy, CRRM should be offered. Nipple sparing mastectomy is a reasonable option. CRRM is associated with a decreased risk of CBC; there is insufficient evidence for improved survival. The following factors should be considered for assessing risk of CBC and role of risk-reducing mastectomy in BRCA1/2 mutation carriers: age at diagnosis (the strongest predictor of future CBC), family history of BC, overall prognosis from this or other cancers (e.g., ovarian), ability of patient to undergo appropriate breast surveillance (MRI), co-morbidities, and life expectancy.
  BRCA1/2 mutation carriers who do not have bilateral mastectomy should undergo high-risk breast screening of remaining breast tissue with annual mammogram and MRI. Risk-reduction mastectomy and CRRM are not advised routinely to patients with a personal history of advanced OCs, because of the increased chances of recurrence of the index ovarian malignancy.
- *Fertility preservation*: in a large analysis including 250 BRCA carriers and 578 controls, it was reported that female BRCA1 carriers had an approximately 33 percent lower anti-mullerian hormone levels relative to controls, although levels in BRCA2 carriers were not diminished. This finding may represent decreased ovarian reserve, and therefore fertility counselling may be appropriate for BRCA1 carriers, if decisions regarding chemotherapy or delayed child-bearing are being considered.[105]

Medical Implications of P/LP BRCA Mutations in BC

For patients harboring pathogenic or likely pathogenic BRCA1/2 mutation, PARPi have a role in the management of HER2-negative metastatic stages as well as select high-risk early-stage BCs (►**Table 6**).

- *Metastatic/advanced stage setting*: olaparib is recommended for patients with germline BRCA mutations and human epidermal growth factor receptor 2–negative BC previously treated with chemotherapy in the neoadjuvant, adjuvant, or metastatic disease setting (based on the Olympiad trial).[107]

Talazoparib is recommended for patients with germline BRCA mutations and human epidermal growth factor receptor 2–negative locally advanced or metastatic BC (based on the EMBRACA trial).[108]

- *Neoadjuvant setting*: neoadjuvant platinum chemotherapy is recommended for BRCA-positive patients (based on the GeparSixto and CALGB 40603 trials).[109,110]
- *Adjuvant setting*: one year of adjuvant olaparib is indicated after the completion of (neo)adjuvant chemotherapy and local treatment, including radiation, for patients with high-risk BC (based on the OlympiA trial).[111]

The high-risk patients are[112]:

Post-op TNBC with node-positive disease or an invasive tumor size of at least 2 cm.

Post-op TNBC who have also received neoadjuvant therapy with residual invasive cancer on histopathology (not in pathological complete response).

Hormone-positive BC with at least four involved axillary nodes.

Hormone-positive BC post-neoadjuvant therapy with residual disease and a CPS + EG score of at least 3 (CPS = clinical and pathological stage; EG = estrogen-receptor status and histologic grade).

Medical Implications of BRCA in OC

PARPi (olaparib, rucaparib, and niraparib) have been approved in OC for various indications. The broad applications are as below[88,113–116]:

- *Frontline setting*: as maintenance therapy for advanced epithelial OCs who experienced a response to frontline, platinum-based chemotherapy.
- *Relapsed/recurrent setting*: as maintenance therapy for platinum-sensitive relapsed epithelial OCs, irrespective of BRCA status, who has responded to platinum-based therapy rechallenge and who did not receive PARPi maintenance in frontline setting.

Discussion

These revised/updated consensus guidelines are recommended to be used by health care professionals in their real-world practice (changes in this version of the recommendation guidelines are highlighted in ►**Table 7** for convenience of the readers). In the absence of prospective randomized data from India, we have not made any attempt to grade or distinguish regarding the strength of the recommendations. Box 3 shows the summary of the expert group's recommendations, following which is expected to

Table 6 Results from selected PARPi studies in breast and ovarian cancer plus BRCA1/2 mutations

Study treatment (trial)	Indication	Efficacy findings
Breast		
Olaparib monotherapy (300 mg twice per day) vs. standard single agent therapy[93,106] (OLYMPIAD)	Metastatic breast cancer and gBRCA1/2m	ORR: 60% (olaparib) vs. 29% (standard therapy) Median PFS: 7.0 months (olaparib) vs. 4.2 months (standard therapy; HR: 0.58; 95% CI: 0.43–0.80; $p < 0.001$)
Talazoparib monotherapy (1 mg every day) vs. standard single agent therapy[90] (EMBRACA)	Metastatic breast cancer and gBRCA1/2m	ORR: 62.6% (talazoparib) vs. 27.2% (standard therapy) Median PFS: 8.6 months (talazoparib) vs. 5.6 months (standard therapy; HR: 0.54; 95% CI: 0.41–0.71; $p < 0.001$)
Olaparib (300 mg twice per day) vs. placebo[90] (OLYMPIA)	High-risk early breast cancer (see the text) and gBRCA1/2m	iDFS at 3 years: 86% (olaparib) vs. 77% (placebo; HR: 0.58; 95% CI: 0.41–0.82; $p < 0.001$) OS at 4 years: 89.8% (olaparib) vs. 86.4% (placebo; HR: 0.68; 98.5% CI: 0.47–0.97; $p = 0.009$)
Ovary		
Olaparib monotherapy (300 mg twice per day) vs. placebo[97] (SOLO 1)	High-grade serous or endometrioid OC, primary peritoneal cancer, or fallopian tube cancer and g/s BRCA1/2m (FRONTLINE)	PFS at 3 years: 60% (olaparib) vs. 27% (placebo; HR: 0.30; 95% CI: 0.23–0.41; $p < 0.001$)
Olaparib monotherapy (300 mg twice per day) vs. placebo[94] (SOLO 2)	Platinum-sensitive, relapsed high-grade serous or endometrioid OC, primary peritoneal cancer, or fallopian tube cancerandgBRCA1/ 2m	Median PFS: 19.1 months (olaparib) vs. 5.5 months (placebo; HR: 0.30; 95% CI: 0.22–0.41; $p < 0.0001$)
Niraparib monotherapy (300 mg once daily) vs. placebo[95] (NOVA)	Platinum-sensitive relapsed high-grade ovarian cancer gBRCAm cohort	Median PFS: 21 months (niraparib) vs. 5.5 months (placebo; HR: 0.27; 95% CI: 0.17–0.41; $p < 0.0001$)
Rucaparib monotherapy (600 mg twice per day)[96] (ARIEL2)	Relapsed high-grade ovarian carcinoma and a g/sBRCA1/2m	ORR: 53.8%; CR: 8.5%; PR: 45.3%; DOR: 9.2 months (95% CI: 6.6–11.6 months)
Olaparib + bevacizumab vs. placebo + bevacizumab (PAOLA-1)	1L maintenance of advanced HGSOC/endometrioid carcinoma patients who have responded to chemotherapy and bevacizumab	mPFS = 22.1 m vs. 16.6 m [PFS (HR): 0.59 (0.49–0.72)] HRD-positive and BRCA +: PFS (HR) = 0.33 (0.25–0.45) HRD-positive and BRCA −: PFS (HR) = 0.43(0.28–0.66)

Abbreviations: CI, confidence interval; HR, hazard ratio.
Note: Reference [95] to be changed - Mirza MR, Monk BJ, Herrstedt J, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med. 2016;375(22):2154–2164.

Table 7 Key highlights of the major revisions in the current HBOC article as compared to our previous publication (JGO 2020) are as follows:

1. List of non-BRCA genes updated (RAD51C and 1D)
2. Pretest counselling section added
3. Informed consent importance outlined
4. Pedigree chats with sample of the same with commonly used symbols added
5. Implications of germline BRCA and risks of germline BRCA1/2 mutations (U.S. Preventive Services Task Force recommendation added) as well as implications for management of HBOC
6. Details of how to avoid common mistakes made by labs while testing for hereditary cancer panel testing
7. Medical Implications of BRCA in BC section including implications with respect to use of PARPi

(*Continued*)

Table 7 (*Continued*) Key highlights of the major revisions in the current HBOC article as compared to our previous publication (JGO 2020) are as follows:

8. New, recent Indian references added and updated in ►**Table 3**
9. Several new genes added and updated in ►**Table 5**
10. New indications and approval for use of PARPi added in ►**Table 6**
11. Appropriately, several references updated in the whole manuscript, especially in sections on Somatic/Tumor BRCA testing, BRCA, and PARPi (earlier manuscript had 98 references and the updated one has 121 references)

personalize management plan for each patient and ultimately optimize outcome. In the absence of any nationwide genetic testing policy document, our guidelines will serve as its substitute.

Conclusion

HBOC is a complex syndrome with additional insights constantly being generated from real-world experiences combined with data science developments in global publicly available databases.[117] The community oncologists in India, SAARC region, and other low- and middle-income countries are in the need of guidelines that can be impropriated easily into their clinical practice to optimize genetic counselling, molecular testing, and management of patients with HBOC. This is because the implications are far-reaching in our socioeconomic milieu. This consensus statement provides a clear picture of why to test, whom to test, how to test, when to test, which test to use, how to interpret the results, and how to discuss options with the patient and families at risk.[118–121]

HBOC ISMPO Guidelines (2023)

Box 1 Guidelines for gBRCA risk assessment (adapted from NCCN Criteria v.3 2023)

1. Personal history of breast cancer with specific features:
   - ≤50 years
   - Any age:
     - ◊ Treatment indications
       - – To aid in systemic treatment decisions using PARP inhibitors for breast cancer in the metastatic setting
       - – To aid in adjuvant treatment decisions with olaparib for high-risk, HER2-negative breast cancer
     - ◊ Pathology/histology
       - – Triple-negative breast cancer
       - – Multiple primary breast cancers (synchronous or metachronous)
       - – Lobular breast cancer with personal or family history of diffuse gastric cancer
     - ◊ Male breast cancer
     - ◊ Ancestry: Ashkenazi Jewish ancestry
     - ◊ Family history
       - – ≥1 close blood relative with ANY:
         - ▪ breast cancer at age ≤50
         - ▪ male breast cancer
         - ▪ ovarian cancer
         - ▪ pancreatic cancer
         - ▪ prostate cancer
       - – ≥3 total diagnoses of breast cancer in patient and/or close blood relatives
       - – ≥2 close blood relatives with either breast or prostate cancer (any grade)

2. Personal history of epithelial ovarian cancer (including fallopian tube cancer or peritoneal cancer) at any age

3. General criteria:
   - Individuals with any blood relative with a known P/LP variant in a cancer susceptibility gene
   - Individuals meeting the criteria below but who tested negative with previous limited testing
   - A P/LP variant identified on tumor genomic testing that has clinical implications if also identified in the germline
   - To aid in systemic therapy and surgical decision-making
   - Individual who meets Li–Fraumeni syndrome (LFS) testing criteria or Cowden syndrome/PTEN hamartoma tumor syndrome (PHTS) testing criteria or Lynch syndrome

- Family history of cancer only:
- An affected individual (not meeting testing criteria listed above) or unaffected individual with a first- or second-degree blood relative meeting any of the criteria listed above (except for systemic therapy decision-making).
- If the affected relative has pancreatic cancer or prostate cancer only, first-degree relatives should be offered testing unless indicated based on additional family history.
- An affected or unaffected individual who otherwise does not meet the criteria above but has a probability >5% of a BRCA1/2 pathogenic variant based on prior probability models (e.g., Tyrer–Cuzick, BRCAPro, CanRisk)

Box 2 Points to be considered while selecting the right lab for genetic testing:

1. Certification (e.g., NABL)
2. Participation in external quality assurance program
3. Documentation of internal quality control SOPs
4. Design of appropriate multigene panel
5. Wet lab processes for enrichment of targets (hybrid capture or amplicon-based capture) without compromising on sensitivity and specificity of the detection of pathogenic variants
6. Bioinformatic expertise
7. Qualified and trained personnel to interpret results
8. Reasonable turnaround time (approximately 4 weeks)

Box 3 Summary of the ISMPO consensus recommendation statements for HBOC (with voting by authors)

Question	Recommendation (description)	Recommendation (strength)
1. Who should undergo genetic counseling?	All clinicians should assess: • Women with a personal or family history of breast, ovarian, tubal, or peritoneal cancer. • An ancestry associated BRCA1/2 gene mutations. and advise genetic counselling and, if indicated, genetic testing	100%
2. Who should undergo genetic testing?	• Any breast cancer (BC) diagnosed at age <50 years. • Any triple-negative BC • Any male BC • BC at any age and ≥1 close relative (first/second/third degree relative on same side of family) diagnosed with BC, ovarian cancer (OC), prostate, or pancreatic cancer. • Any woman with OC	96%
3. Who can perform genetic counselling?	Genetic counsellors and other medical professionals (medical/surgical/radiation oncologists/breast surgeons) knowledgeable in genetic testing can provide patient education and counselling and make recommendations regarding genetic testing and arrange testing	**100%**
4. What points should be included in pretest counselling?	• Rapport building, • Elicitation of need and comprehension levels • Medical history and pedigree evaluation • Decide the best test candidate to test first • Genetic testing recommendations • Implications of genetic testing: benefits/harms • Variants of unknown significance • Financial considerations • Risk reduction options	**100%**
5. What genetic test should be offered?	• Single-site mutation testing in families with a known mutation • For unknown mutation: – Essential: BRCA1/2 sequencing by next-generation sequencing plus multiplex ligation probe amplification (MLPA; BRCA1/2) for large genomic rearrangements (LGRs) – Desirable: multigene panel testing (a representative model panel should include BRCA1, BRCA2, p53, PTEN, CDH1, PALB2, CHEK2, ATM, RAD51C, STK11, RAD51D, BRIP1, MLH1, MSH2, MSH6, and PMS2) + MLPA (BRCA1/2) for LGRs	**100%**
6. What risk-reduction approaches should be offered to affected individuals?	• Risk management for future cancers: – Contralateral prophylactic mastectomy: ➢ Risk-reduction mastectomy should be offered to patients with a previous history of BC who carry a germline genetic mutation in BRCA1/2 ➢ Risk-reducing bilateral salpingo-oophorectomy (rrBSO): for BRCA1 carriers, rrBSO is recommended for women who have completed childbearing, and should be performed by age 35 to 40 years. In BRCA2 carriers, one can consider delaying this procedure until age 40 to 45 • Advanced OC with BRCA mutation: prophylactic bilateral mastectomy is not considered in these cases as the risk of death	**100%**

(*Continued*)

Box 3 (*Continued*) Summary of the ISMPO consensus recommendation statements for HBOC (with voting by authors)

Question	Recommendation (description)	Recommendation (strength)
	from the primary malignancy is high over the next 5 years. In these cases, nonsurgical measures and surveillance only are used for any new primary malignancy in breasts	
7. What risk-reduction approaches should be offered to unaffected mutation carriers?	BRCA1/2: • Lifestyle modifications: regular exercise, maintaining healthy body weight, limiting alcohol consumption. • Avoid hormone replacement therapy, encourage breast feeding. • Breast cancer: BRCA carriers should be offered prophylactic bilateral mastectomy; however, the final decision is based on personal preference, given that effective screening is available. • Bilateral salpingo-oophorectomy: for BRCA carriers, risk-reducing bilateral salpingo-oophorectomy is recommended for women who have completed childbearing, and should be performed by age 35 to 40 years. In BRCA2 carriers, one can consider delaying this procedure until age 40 to 45 years. • Cancer surveillance: for female BRCA carriers who do not wish to pursue (or would rather delay) surgical risk reduction, BC surveillance should be offered, and OC screening may be performed • Breast cancer screening: ➢ Breast awareness from age 18 years IIA ➢ Clinical breast examination (CBE) every 6–12 months is recommended from the age of 25 years or 10 years before the youngest BC VC ➢ Annual screening MRI (days 7–15 of menstrual cycle) should be commenced from age 25 years with the addition of annual mammography from age 30 years • OC screening: ➢ Concurrent transvaginal ultrasound (preferably days 1–10 of menstrual cycle) and CA-125 (best performed after day 5 of menstrual cycle) every 6 months beginning at age 30 years ➢ Before risk-reducing bilateral salpingo-oophorectomy, 6 monthly transvaginal ultrasound and measures of serum CA-125 may be considered from age 30 years; however, the limited value of these tools as an effective screening measure should be communicated to individuals ➢ Chemoprevention: use of tamoxifen may be considered; however, the level of evidence is weak; use tamoxifen only for BRCA2 tumors or if the first cancer was estrogen receptor-positive ➢ Surveillance in male previvors: There are no proven risk-reducing surgical options for men – Monthly breast self-examination starting at age 35 years – Clinical breast examination every 12 months starting at age 35 years – Prostate cancer screening starting at age 45 years for BRCA2 carriers and consideration of prostate screening for BRCA1 carriers also at age 45 years	100%
8. When should poly (ADP-ribose) polymerase inhibitors be used?	➢ Olaparib, niraparib, and rucaparib are indicated for maintenance treatment in adults with recurrent epithelial OC who are in complete response (CR) or partial response (PR) after platinum-based chemotherapy (irrespective of BRCA status) ➢ In g/s P/LP BRCA-mutated OC, olaparib should be used as maintenance after a CR/PR to first-line chemotherapy and cytoreductive surgery. Niraparib is indicated in this setting irrespective of BRCA status. ➢ 1L maintenance treatment for advanced ovarian cancer in combination with bevacizumab after CR/PR to 1L platinum-based chemotherapy which is HRD+ ➢ Talazoparib is indicated for adults with deleterious or suspected gBRCA-mutated, human epidermal growth factor receptor 2–negative locally advanced or metastatic BC	96%

Box 3 (*Continued*) Summary of the ISMPO consensus recommendation statements for HBOC (with voting by authors)

Question	Recommendation (description)	Recommendation (strength)
	➢ Olaparib is indicated for adjuvant treatment of P/LP gBRCA HER2-negative high-risk early breast cancer in adults previously treated with NACT or adjuvant CT ➢ Olaparib is indicated for P/LP gBRCA HER2-negative metastatic breast cancer patients who have received chemotherapy in neoadjuvant/adjuvant/metastatic setting. Hormone-positive breast cancer should have been treated with prior endocrine therapy or be considered inappropriate for endocrine therapy.	

Abbreviation: CT, computed tomography.

Patient Consent
Patient consent not required.

Conflict of Interest
None declared.

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Deep Learning-Based Pulmonary Nodule Screening: A Narrative Review

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Abstract

Given its capacity to generate three-dimensional pictures, computed tomography is the most effective means of detecting lung nodules with more excellent resolution of detected nodules. Small lung nodules can easily be overlooked on chest X-rays, making interpretation difficult. Artificial intelligence algorithms have recently demonstrated remarkable progress in medical imaging, especially with deep learning techniques such as convolutional neural networks (CNNs). CNN produces excellent results in natural image recognition and classification using abundant available data and the computational abilities of modern computers. It further reduces false-positive pulmonary nodules in medical image processing. This review article provides a detailed and inclusive review of recent advances, challenges, performance comparisons, and possible future directions for the problem of pulmonary nodule screening using deep learning methods.

Keywords
- deep learning
- lung neoplasms
- screening
- AI
- diagnosis
- treatment outcome
- prognosis

Introduction

Lung cancer is one of the most prevalent cancers and a major reason for cancer-related deaths across the world. Patients' survival and prognosis largely depend on age, morphology, and stage at detection time.[1] The number of fatal cases can be significantly decreased by early diagnosis as early detection of lung cancer improves the survival rate. Different diagnostic procedures available for the early diagnosis of lung cancer include chest radiographs, computed tomography (CT) scans, positron emission tomography (PET), and biopsy.

The best method for investigating lung pathologies is CT imaging.[2] Due to its capacity to provide three-dimensional (3D) pictures with the superior resolution of identified nodules, CT is the most efficient tool for locating lung nodules. However, CT scans have a high rate of false-positive (FP) findings with an additional burden of potential adverse effects of radiation. For screening purposes, low-dose CT has shown promising results in decreasing mortality. While a regular chest CT has an effective dose of 4 to 18 mSv, low-dose CT has an effective dose of only ~1.5 mSv. Low-dose CT can depict the structure of the lung without superimposition while causing less radiation exposure than conventional CT.[3] Hence, it has been used widely given its efficiency and ease of performance.

Besides CT, a chest X-ray is a potential option for screening nodules. Interpretation of chest X-rays is usually difficult, as small lung nodules can be missed. For effective lung cancer

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screening, it is highly necessary to maintain a low false-negative rate. Cancer-related mortality in the study population was dramatically decreased in those who underwent low-dose CT scans compared with chest radiographs, as seen in the National Lung Screening Trial database.[4,5]

Artificial intelligence (AI) algorithms have recently demonstrated remarkable progress in medical imaging, especially with deep learning techniques such as convolutional neural networks (CNNs). CNN produces excellent results in natural image recognition and classification using abundant available data and the computational power of modern computers. Efforts in AI are advancing to reduce FP pulmonary nodules in automated methods of medical image processing.[6–8]

This review article will provide a comprehensive review of recent advances, challenges, performance comparisons, and possible future directions for the problem of pulmonary nodule screening using deep learning methods. We will also discuss the following:

- Available public datasets with a brief overview of deep learning.
- Recent advances in deep learning for pulmonary nodule screening.
- The clinical application of deep learning models.

Finally, this study summarizes the current state of AI for pulmonary nodule screening and shares future research directions in pulmonary nodule detection.

Current Scenario

The sensitivity for detection of pulmonary nodules relies on the number of CT slices, more for thinner slices and with better image registration techniques. One scan can generate up to 500 sections or slices, depending on the slice thickness.[9] It takes ~2 to 3.5 minutes for an experienced radiologist to observe a single slice.[10] There is a significant increase in the radiologist's workload to screen a CT scan for the presence of a lung nodule. In addition to the section thickness of the CT slices, detection sensitivity also depends on multiple different features of the nodule, such as size, location, shape, adjacent structures, edges, and density. The following important task is to assess whether the nodules detected are benign or malignant. Benign and malignant nodules have considerable feature overlaps. Estimating the probability of malignancy is challenging but essential for follow-up and further management. Morphological examination using thin-section CT is crucial in addition to the clinical context and metabolic assessment. The most critical elements in determining a nodule's malignant potential are its size and growth. The chance of malignancy is directly proportional to nodule diameter: as the diameter grows, the risk of malignancy increases proportionately. Even though benign and malignant nodules share many characteristics, morphology is still essential and should not be undervalued.[8–10]

A perifissural position, a triangular morphology, interior fat, and benign calcifications are characteristics of benignity. Nodules with spiculation, lobulation, pleural indentation, vascular convergence sign, related cystic airspace, bubble-like lucencies, irregular air bronchograms, and subsolid morphology are suspicious for malignancy. Nodules frequently display a variety of characteristics, and the combined information is more useful.[11] However, radiologists may still overlook pulmonary nodules for multiple reasons. Besides the individual performance of observers, nodule characteristics such as small size, inconspicuity, ill-defined margins, and location very close to adjacent vessels may result in missed lung cancers on CT.[12] Large sets of images, now available due to multidetector low-dose CT, can result in visual and mental fatigue for radiologists, leading to errors in interpretation during routine practice.[13] Also, radiologists who evaluate CT screening images face challenges such as the mechanical and tedious nature of work, small nodules being easy to miss, and the lack of consistent criteria. Missing a lung nodule/cancer in a radiological investigation is very concerning and is among the common causes of malpractice claims against radiologists.[14,15]

Computer-Aided Detection

As mentioned earlier, extensive research is ongoing with computer-aided diagnosis to overcome the challenge. Research in computer-aided detection (CAD) has been ongoing for more than 20 years to improve the accuracy and efficiency of detecting small lung nodules. Typical traditional CAD processes included image acquisition, lung field segmentation, candidate nodule detection, and FP mitigation (►**Fig. 1**).

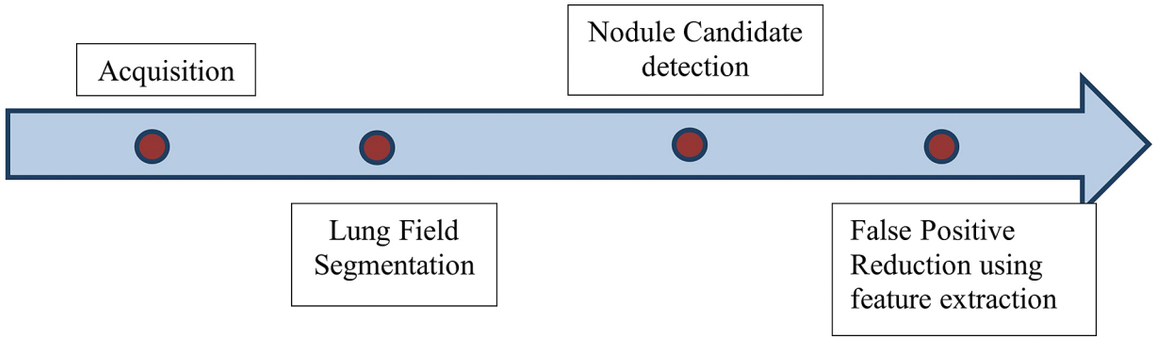

Fig. 1 Typical computer-aided detection processes: image acquisition, lung field segmentation, candidate nodule detection, and false-positive mitigation.

However, advanced detection algorithms and high-speed calculations have allowed the development of new CAD systems to be more effective and given them the ability to assist radiologists in detecting and characterizing lung nodules. Presently, using CAD alone is not generally acceptable in clinical practice.[16–18]

The CAD, as mentioned earlier, can be designed in multiple ways. (1) *Heuristic-based systems* use expert knowledge and traditional software. (2) *Data-driven systems* that learn the patterns in the data to meet specific objectives. Due to the complexity of the problem, the performance of heuristic-based systems is limited, and they are not robust to dynamic data. In recent years, data-oriented systems have surpassed the performance of heuristic-based systems. Earlier data-oriented systems were designed with manual handcrafted features such as histogram of oriented gradients), local binary patterns, and scale-invariant feature transform. These systems showed much promise; however, designing manual handcrafted features is challenging and domain-specific. Current state-of-the-art systems for AI are designed using deep learning methods that do not require feature engineering. These systems have surpassed heuristic-based systems and manual handcrafted features in terms of performance. Due to their adaptability and cutting-edge performance, these systems are rapidly being used to solve most computer vision challenges.[16–18]

Convolutional Neural Networks

CNNs, a particular type of deep learning model, are utilized for image interpretation. CNNs are effectively employed in various imaging detection and assessment procedures, including the identification of strokes, breast cancer lymph node metastases, skin cancer, diabetic retinopathy, colonic polyps, brain tumors, and lung cancers.[19–25]

AI detection of lung nodules has been awaited as a practical assistant in daily practice, especially for low-dose CT lung nodule screening. So far, multiple new deep neural network-based systems have shown potential for use in assisting radiologists to increase the accuracy and efficiency of nodule detection while being cost-effectiveness.

Open Datasets

Most of these systems have been taught using CT scans from the Lung Image Database Consortium/Image Database Resource Initiative, the Lung Nodule Analysis 2016 database, and the Automatic Nodule Detection 2009 database.[6,26–34]

Deep Learning

This section introduces deep learning, primarily deep convolutional neural networks (DCNNs). In general, deep neural networks process the input data layer by layer, feeding and output of one layer into the following. The output of the first layer is passed through a nonlinear activation function before passing it to the next layer. This design allows deep neural networks to learn very complex functions of data. Deep neural networks are also hierarchical because of their layered structure. Initial layers of the networks learn simple patterns, and later layers learn higher-level features. DCNNs are a specific type of neural network where shared weights of the neurons are designed with inductive bias that allows them to be shift/space invariant. The shared weights are used to perform an operation known as convolution.[6,26–30]

DCNNs are compelling models to work with image understanding problems. They were successfully used in LeNet for handwritten digit recognition. In 2012, Krizhevsky et al[35] used a DCNN named AlexNet to win the image challenge. With minimal preprocessing and no handcrafted features, the AlexNet-based solution won ILSVRC 2012 challenge by a large margin. This led to the adoption of DCNNs in various computer vision tasks. In the following years, new and improved architectures named VGG, ResNet, and DenseNet[36] have been proposed to improve the performance, ease of training, and scaling issues. Due to their effectiveness in computer vision, DCNNs have been the default first choice for medical imaging applications.

Deep Learning for Pulmonary Nodule

Pulmonary nodule-related AI applications can be divided into three settings: detection, segmentation, and classification. In detection, the objective of AI is to predict the position of the nodules inside the lung. Segmentation tries to predict the pixels or voxels of the nodule. Classification setting tried to predict the type of nodule, that is, benign or malignant.[6,26–36]

We will present these three applications of deep learning methods in this section.[6,26–36]

Pulmonary Nodule Segmentation

In this setting, AI aims to predict pixels/voxels of the nodule. The predictions can be used to do quantitative analysis of various clinical parameters of nodules, such as shape, volume, and distribution of pixel values (►**Fig. 2**).

Pulmonary Nodule Detection

The nodule detection setting locates the nodules by predicting 3D cubes around the nodule (►**Fig. 3**). This setting makes nodule AI explainable, which can assist radiologists in scan interpretations. The predictions can be used to analyze the size and location of the nodule.

Pulmonary Nodule Classification

Nodule classification AI uses predictions from nodule segmentation, nodule detection, or both modules. After extracting features using outputs from previous steps, AI can be trained on the features to classify the nodule into benign or malignant.

Major Challenges

As highlighted in the section on Deep learning for Pulmonary Nodule, many advances in AI have been using deep learning in recent years. Performance shows that, when deployed, these technologies can assist radiologists or can even operate

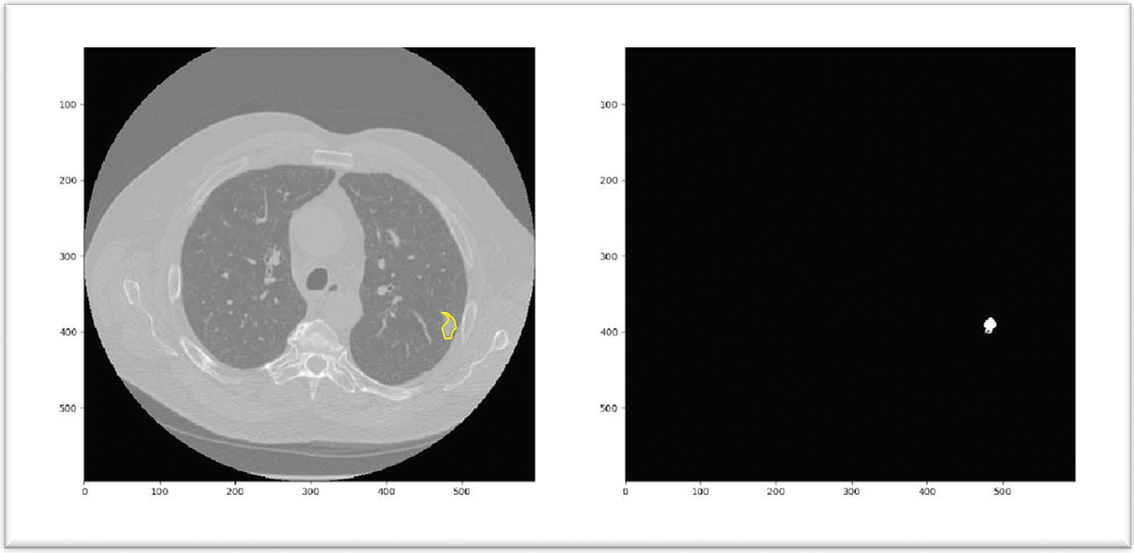

Fig. 2 Pulmonary nodule segmentation: an irregular solid nodule is segmented in the left lung highlighted in yellow.

independently. It will reduce the load on radiologists and also reduce diagnosis duration. However, there are challenges as we develop these systems for real-world deployments.[6,26–36] We highlight these challenges in this section.

Deep Learning Requires Large Datasets

Deep learning systems require large amounts of data to learn patterns in the data that are generalized well. Otherwise, they tend to overfit the data, and predictions on new patients will not be good. There have been significant strides in this direction with the release of many datasets in recent years, as described in the section on deep learning. However, more data will further help improve these systems, especially for different demographics of patients. Collaboration between AI research laboratories and hospitals might lead to more such datasets. It will also lead to a detailed analysis of the weaknesses of these systems.[6,26–36]

Need for Transfer Learning

Even though these systems perform well on large-scale open-source datasets, making them work on datasets specific to a demographic based on country or gender requires retraining for a particular dataset. Transfer learning resolves this by taking a pretrained network on a sizeable open-source dataset and retraining the last few layers of the network for a specific dataset. This is required because there are variations in scans such as size, resolution, and quality. Most of the time, this dataset also needs to be large enough for the AI to get the

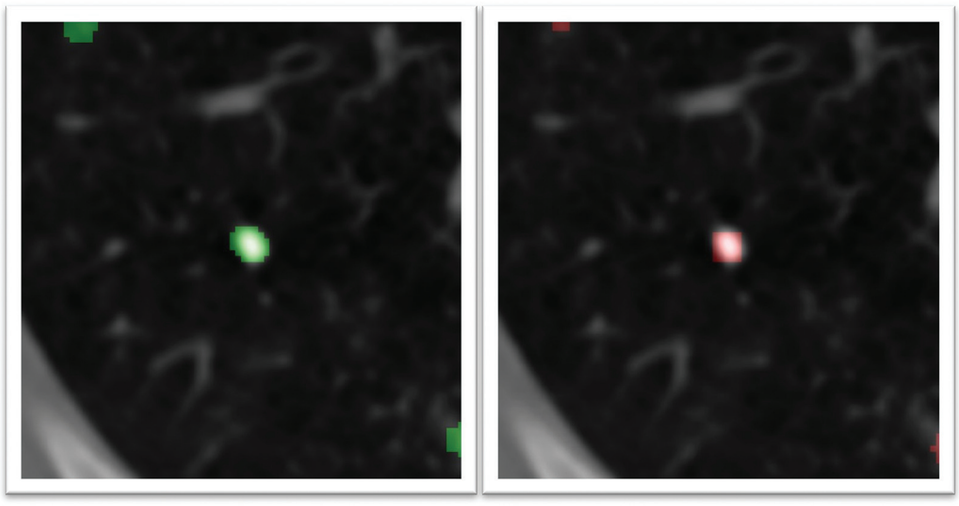

Fig. 3 Pulmonary Nodule deep learning model detected a nodule in the right lower lobe (highlighted in green) which corresponds to the nodule marked by the radiologist (highlighted in red).

expected performance. Further reducing this data requirement will improve the deployment of these systems.[6,26–36]

Interpretability, Explainability, and Bias

For health care systems such as pulmonary nodule AI, the system must be interpretable, explainable, and unbiased. Due to the complex nature of deep neural networks, making them interpretable and explainable is challenging. However, improving interoperability and explainability will give radiologists more confidence, and AI. as assistive technology will benefit tremendously.[6,26–36]

There is also inherent bias in deep learning systems if the systems are not trained on carefully curated datasets and evaluated before deployment. This also makes it necessary to test these systems against bias on carefully curated data before deployment.[6,26–36]

Conclusion

The conclusion infers that AI detection modules with/without CAD can be an effective tool to help future radiologists with adequate global and local standards for early diagnosis of lung malignancies and lowering mortality.

Note

The article is not under consideration for publication elsewhere. Each author participated sufficiently for the work to be submitted. The publication is approved by all authors.

Authors' Contributions

A.M. and K.S.S.B. contributed to the conceptualization, and design of the work. A.M., U.A., R.A., A.V., S.S., K.S.S.B., M.L.V.A., V.P., and V.P. contributed to writing—original draft, and writing—review and editing.

Ethical Approval

Not applicable.

Patient Consent

Patient consent not required.

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Conflict of Interest

None declared.

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Retrospective Study of B Lymphoblastic Leukemia to Assess the Prevalence of *TEL/AML1* in South India: A Study of 214 Cases and Review of Literature

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Abstract

Introduction Translocation t(12;21)(p13;q22), a recurrent and an invisible chromosomal abnormality, resulting in *TEL/AML1* gene fusion, associated with good prognosis, has been described to be a common abnormality, in children with B-acute lymphoblastic leukemia (B-ALL).

Objectives The initial observation of very few TEL/AML1 positive patients at this center on testing by fluorescence in situ hybridization (FISH) led to study the prevalence of the abnormality, compare with the global distribution, and evaluate clinical, pathological, molecular, and cytogenetic features in *TEL/AML1* positive patients.

Materials and Methods A retrospective study of all B-ALL patients tested for *TEL/AML1* gene fusion during the period January 2009 to November 2020 was undertaken. Clinicopathological, molecular, cytogenetic, treatment, and follow-up details were collected. All publications dealing with *TEL/AML1* gene rearrangement were reviewed post Google and PubMed search.

Results *TEL/AML1*gene rearrangement was assessed by FISH in 178 patients and by reverse transcription polymerase chain reaction in 36 patients and detected as the sole abnormality in 8.4% patients with additional genetic abnormalities noted on FISH evaluation. Normal karyotype was noted in 14/18 (77.7%) of these patients and 2 had complex karyotype. Complete blood count revealed hemoglobin to range from 35 to 116 g/L (median: 74 g/L), white blood count: 1.01–110$\times 10^9$/L (median: 7.8$\times 10^9$/L), platelet counts: 10–115$\times 10^9$/L (median: 42$\times 10^9$/L), blast count in peripheral smear: 0–98% (median: 41%). Immunophenotyping demonstrated 94.4% were CD34 positive, common acute lymphoblastic leukemia associated antigen (CALLA) positive with aberrant expression of CD13, CD33, CD56, singly or in combination in 58.8%.

Keywords
- TEL/AML1
- t(12;21) fluorescence in situ hybridization
- acute lymphoblastic leukemia (ALL)
- RT-PCR

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Conclusion TEL/AML1 fusion is rare in Indian patients with B-ALL and appears to be much rarer in our region. The detection of relevant specific abnormalities is of fundamental importance in B-ALL patients and these geographic variations can be used in defining management policies.

Introduction

Detection of recurrent genetic abnormalities in hematological malignancies by fluorescence in situ hybridization (FISH) is part of routine standard of care. Recurrent cryptic translocation t(12;21)(p13;q22) in pediatric B-acute lymphoblastic leukemia (B-ALL) is associated with specific clinicopathological characteristics and favorable prognosis.[1–4] Assessment for *TEL/AML1(ETV6/RUNX1)* fusion enables proper treatment strategies in a given population. Review of online hospital-based reports, international projects, multicenter studies, and review articles[3–5] demonstrated a wide variation in global incidence, with incidence in west being ~25%[4] and lesser in India (►**Table S1** and **S2-supplementary material**, available in the online version).[5–30] However, the initial observation of very less number of prognostically favorable TEL/AML1 rearranged B-ALL patients from our tertiary care cancer center in south India prompted focus on evaluating the prevalence of this abnormality. The aim and objectives of the study were to see the prevalence of *TEL/AML1* gene fusion in newly diagnosed B-ALL patients, compare with the global distribution of the fusion gene, and evaluate the clinical, pathological, molecular, and cytogenetic features where available in *TEL/AML1* positive patients.

Materials and Methods

A retrospective study of all cases, previously diagnosed as B-ALL during the period January 2009 to November 2020, at a tertiary care cancer center from South India, was undertaken. Data on demographic, clinical, hematological, interphase FISH, cytogenetics, reverse transcription polymerase chain reaction (RT-PCR) findings, treatment protocol, and follow-up details were collected from the case files in a format. All the newly diagnosed cases for which ALL prognostic panel (*BCR-ABL, TEL-AML1, MLL)* was performed and TEL AML result was available were included in the study. All cases where adequate blasts counts were not evident and also relapsed ALL cases were excluded from the study. Primary outcome provides information on the prevalence of TEL/AML1 gene fusion positivity in patients diagnosed with B-ALL from this region and for understanding how the prevalence compares with the global incidence for managing the patients. Secondary outcome includes defining the clinicopathlogic features, cytogenetics and FISH findings, baseline lab values and overall survival in the TEL AML1 positive patients that were analyzed.

FISH: Interphase FISH studies were performed till November 2019, on heparinized bone marrow/peripheral blood fixed samples in 178 patients. LSI ETV6(TEL)/RUNX1(AML1) extra signal dual color translocation probe set and ZytoLight*ETV6/RUNX1(TEL/AML1)* dual color dual fusion probe were used for testing in 81and 97 samples, respectively. The probe details, typical signal patterns and interpretation are provided in ►**Table S3** (**Supplementary material**, available in the online version). The denaturation and hybridization procedures were performed according to the manufacturer's instructions and counterstained with 4,6-diamino-2-phenyl-indole stain. Fluorescent signals were visualized at 1250 × magnification. The cutoff for TEL/AML1 positivity was 5%. For each case, 200 interphase cells were evaluated by two analysts.

The status of *BCR ABL* fusion and *MLL* gene rearrangement was also evaluated using appropriate probes (►**Table S3**, available in the online version).

RT-PCR: *TEL/AML1* mutation was assessed by RT-PCR in 36 patient samples from December 2019 onward. RNA was extracted from bone marrow/peripheral blood samples in ethylenediaminetetraacetic acid, using Qiagen RNA blood mini kit and, c-DNA synthesis and real time PCR was performed using TRU-PCR ALL Panel Kit Version 2.1 (3B Black Biotech India Ltd, Bhopal, India).

Data on karyotype was available in 92 ALL cases. Twenty-four or forty-eight-hour unstimulated heparinized bone marrow/peripheral blood cultures had been set up as per standard protocol. Twenty metaphases were evaluated, and karyotype reported as per International System for Human Cytogenomic Nomenclature (2016).[31] Correlation with cytogenetics was done where available.

Statistical Analysis

Since the number of *TEL/AML1* positive cases were few, simple statistical measure, percentage was used to express the prevalence and other data.

Ethical Approval

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964, as revised in 2013. Patient consent was taken prior to the enrolment of participants in the study. Approval of the study was obtained from Basavatarakam Indo American cancer Hospital & Research Institute's ethical committee. (EC Reference study code: 96, dated 27 May 2021).

Results

The patient cohort consisted of 214 B-ALL patients, at diagnosis, both adults and children. Data on demographic, clinical hematological, FISH, and karyotype findings are

Table 1 Clinical, pathological data, FISH, RT-PCR, and karyotype findings in B-ALL patients positive for *TEL/AML1* gene rearrangement.

Case no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Age(y) and gender M/F	5 M	3 M	4 M	4 M	2 M	4 M	4 F	18 M	5 M	2 M	8 M	4 M	13 M	5 M	3 M	8 M	6 M	7 M
Clinical presentation and history	Fever and weakness—2 months	Fever and weakness—1 week	Fever—2 weeks and multiple petechial rashes	Fever—1 week and multiple joint pains	Fever—2 weeks and joint pain for 1 week	Fever—1 month, bilateral cervical LN, S+.	Fever—1 month, cough cold —vomiting petechial rash—1 day.H+S+	Fever and AD	Fever and weakness—1 week.	Fever—2 weeks and multiple petechial rashes	Fever—1 month, cough cold, vomiting, petechial rash—1 day. H+S+	Fever—1 week and multiple joint pains	Fever—2 weeks, joint pain—1 week, bilateral cervical LN, S+	Fever—1 month bilateral cervical LN,S+	Fever and pallor	Fever—1 week vomitting	Fever—2weeks Cough cold	Fever >1 month
HB g/L:	73	62	89	35	69	48	97	85	74	91	116	89	77	74	69	88	76	7
WBCX10(12)/L:	9.7	110	3.2	36.7	5.92	4.5	6.8	17.95	4.59	1.91	6.12	3.66	8.9	1.01	50.31	17.5	11.22	23.62
Platelet x10(9)/L:	100	10	45	12	86	17	65	89	22	74	115	24	15	62	18	50	39	45
PS smear blasts (%)	25	95	10	90	18	6	50	46	0	30	36	65	4		98	70	56	80
BM blasts (%)			85	90	77	88	95	60	95	58	80	88	40	69	77		3	55
PAS	+	+	NC	ND	+	+		+			N		G +					
B-ALL CD10 +/−	+					+	+		+		−	+	+		+	+		
Aberrant markers	CD13 & CD33					CD56	CD13 & CD33				CD13 & CD33	CD13			CD13			
TEL AML1	*P	*P	P	P	P	P	P	P	P	P	P	P	P	P	P	P#	P#	P#
Signal pattern	AT	AT	AT	AT	AT	AT	AT	AT	AT	T	AT	AT	AT	AT	AT			
FISH signal pattern	1G 2O3F	2O1F	1G1O1F	3G1O1F	2G2O1F. 2G 1O 1F	2G1O1F	2G1O1F	2G2O2F	1G1O1F,	1G1O2F,	2G2O2F,	2G1O1F,	1G1O1F,	1G1O1F	2G2O1F, 3G3O			
Interpretation	1	2	3	3 + 4*(1-2)*	6(1)−3+ 4 (1) +5(1) 6(2)−3+ 4 (1)	3 + 4 (1)	3+ 4 (1)	4(1) + 5(1)	3		4(1) + 5(1)	3 +4(1)	3	3	6(1)- P 3+ 4 (2) +5(1) 6(2)- N, 4 (1) +5(1)			
Clone no and size (%)	39	61	10	89	38 20	47	42	83	95	64	91	11	8.9	5	P 16 N			
BCR-ABL, MLL, E2A-PBX	N N	N N	N N	N N	ND,N	ND, N	N, N, N	ND, N	ND, N loss of MLL −91%	ND,N	ND,N	#N , N	ND, MLL-ve	# N, N	ND,N	ND	ND	ND
Karyotype	NK	NK	NK	NK	IM	NK	NK	NK	NK	NK	NK	* 46	NK	NK	NK	AK	NM	AK

Abbreviations: #, TEL AML1/BCR-ABL(p210,p190)-RT-PCR; +/G +, block/granular positive; AD, abdominal distension; B-ALL, B-acute lymphoblastic leukemia; FISH, fluorescence in situ hybridization; $H^{+}S+$, hepatosplenomegaly; LN, lymphadenopathy; N, negative; NC, noncontributory; ND, not done; P, positive; RT-PCR, reverse transcription polymerase chain reaction; S +, splenomegaly
*Cases 1 & 2 (Vysis Probe) &, 3–15 (Zytovision Probe). Signal: green: G, orange: O, fusion: F, typical signal pattern:(T), normal nuclei—2G2O, abnormal nuclei with fusion—1G2O1F (Vysis)/ IGIO2F (Zytovision). Atypical signal pattern: AT, Interpretation: **1**—extra fusion genes *TEL/ AML1 on der 21q*, **2**—Del of untranslocated TEL, **3**—single fusion loss of AML1/TEL fusion on der 12p, **4**—extra copies of *AML 1*(number), **5**—extra copies of *TEL* (number), additional genetic abnormality: +, **6**—clones (Number), clone size (positivity rate). Normal/abnormal karyotype: NK/AK, inadequate/no metaphases: IM/NM, *Ploidy analysis—chromosome number.
Case16: 46,XY,dup(17)(q21q22),t(?;17)(?;q25)[3]/47,XY, + 11,dup(17)(q21q22)t(?;17)(?;q25)[4]/46,XY,del(11)(q23),dup(17)(q21q22)t(?;17)(?;q25)[3]/46,XY,dup(17)(q21q22)t(?;17)(?;q25),t(?;18)(?;p11.2)[3]/46,XY[7].
Case18: 46,XY,inv(11)(p13q13.1)[1]/46,XY,inv(11)(p13q13.1),del(17)(p11.2)[5]/46,XY,inv(11)(p13q13.1),del(12)(p13)[4]/ 46,XY,inv(11)(p13q13.1),del(12)(p13),del(17)(p11.2)[8]/46,XY[2].

Table 2 Data of demographic and *TEL/AML1* results in 214 B-ALL patients

Parameters	Group 1: 1–18 years	Group 2: 19–39 years		Group 3: >40 years
Overall, no of patients (%)	125(58.41)	63(29.43)		26(12.14)
Median age of presentation (y)	5	26		46.5
No of males No of females Gender of baby not disclosed	77 47 1	45 18		15 11
Male female ratio	1.6:1	2.5:1.0		1.3:1
TEL AML1 fusion positive cases	Total:18/214 (8.41%), FISH:15/178(8.6%) / RT-PCR:3/36(8.3%)			
TEL AML1 fusion positive cases as per age group by both technologies: No (%)	17/125(13.6%)	1/63(1.58%)		–
TEL AML1 negative cases:196/214(91.58%)		*TELAML1*negative with additional genetic findings:39/163(23.9%)		
Types of additional abnormalities in negative group No of patients (%) &% cells with abnormal findings in different patients.				
Additional copies of *AML1*—21(12.8%) 24–92% cells 2 patients:1 copy 4patients;2 copies 1 patient;1–2 copies 2 patients:3 copies 3 patients:3–4 copies 4 patients:4 copies 5patients:5 copies	Additional copies of *TEL*gene—2 patients (1.2%) 43–63% cells—3 copies in each patient	Additional copies of *TEL and AML1*genes—8 (4.9%) Patient:2 clones seen: Clone 1–44% cells had 4 copies of only *AML1*. Another clone—34% cells had 3copies of *Tel*& 4 copies of *AML1* Patient: 2 clones seen. Clone 1: 42.3%cells had 3–4 copies of *AML1*only. Clone 2: 28.46% cells had 3 copies of *Tel*& 3–4 copies of *AML1* genes Patient: 3–4 copies *AML1*&*TEL* genes seen in 81%cells Patient: Clone1–50% cells had4 copies of *AML1*&3 copies of *TEL* gene 2ndclone—34% had 3 copies of *TEL* gene Patient: 83% cells had 3 copies *TEL* gene & 77% cells had 3–7 copies of *AML1* Patient: 65% cells had 5 copies *TEL*& 4–5 copies *AML1* gene Patient:40% cells had (2 copies) *AML1* gene & 1 copy of *TEL* gene Patient:18% cells had 1 copy of *TEL* gene and 2 copies of *AML1* gene	Deletion of *TEL* gene and amplification of *AML1*2(1.2%) Patient: Loss of *TEL* gene in all cells and 40% cells—3 copies of *AML1* Patient: Loss of *TEL* gene and 4 copies AML*1*—59% cells	Del of *TEL* gene6(3.7%) 14.5–86.8% cells

Abbreviations: B-ALL, B-acute lymphoblastic leukemia; FISH, fluorescence in situ hybridization; RT-PCR, reverse transcription polymerase chain reaction.

Table 3 Correlation of FISH and cytogenetic results of 6 cases lacking *TEL/AML1* gene rearrangement

Case no	Cytogenetic findings	FISH findings	Correlation of karyotype and FISH findings
1	47,XX,+8[14],46XX[2]*	*TEL/AML1* negative, with amplification of the *AML1* gene—2 copies in 45% cells	Cryptic amplification of *AML1* gene
2	50–57,XX/XXX,+X[11],+4[10],+5[6],+6[13],+8[6],+9[7],+10[6],+11[8],+12[3],t(?;12)(?;q24.1)[5],+14[4],+15[2],+16[3],+17[12],+18[6],−9[11],+20[3],+21[10][cp16]/46,XX[4]	*TEL/AML1* negative, amplification of *AML1* gene (1 copy)	Karyotype demonstrating a+21 corroborating with FISH amplification.
3	46,XY,dup(9)(q34.1q34.1)[4]/47,XY,+mar[2]/46,XY[9]	*TEL/AML1* negative, amplification of *AML1* gene1–2 copies -33%	Cryptic amplification of *AML1* seen.
4	46,XY,del(8)(p22)[8]/46,XY[6]	*TEL/AML1* negative, Amplification of Tel and AML1 genes	Cryptic amplification of *TEL* and *AML1* genes
5	50–53,XXY/XY,+X,+4,+6,+8,+10,+14,+17,+18, +21,+22[cp15]/46,XY[5]	*TEL/AML1* negative, amplification of *AML1* gene—2 copies	Karyotype shows gain of chromosome 21 (only 1 copy) and the other copy of *AML 1* is cryptic.
6	54–55,XY,+4[4],+6[2],+8[3],+10[3],+16[1],+17 [3],+17×2[1],+18[3],+21[2],+21×2[2],+22[3][cp4]/46,XY[16]13	*TEL/AML1* negative, amplification of *AML1* gene—2 copies—58%	Karyotype and FISH demonstrate gain of 2 copies of chromosome 21 and 2 copies of *AML1* gene

Abbreviation: FISH, fluorescence in situ hybridization.

presented in ►**Tables 1**, **2**, and **3**. A total of 8.4% (18/214) patients harbored the *TEL/AML1* gene rearrangement. The fusion was detected by FISH in 15/178(8.4%) patients and by RT-PCR in 3/36 (8.3%) patients. The clone size or positivity rate varied from 8.9 to 95%. The fusion was frequent in 17/125 (13.6%) children, aged between 2 and 13 years (median: 4 years), and an 18-year-old young adult. Fusion was noted in 94.4% males (►**Table 1**).

Additional genetic abnormalities characterized 14/15 (93.3%) patients, reflected as atypical signal patterns on FISH suggestive of various abnormalities (►**Table 1**) (►**Fig. 1**).

TEL/AML1 fusion was absent in 91.58% cases. Additional genetic abnormalities were also noted in 23.9% of these patients, with amplification of *AML1*, seen more often (►**Table 2**).

Cytogenetic analysis in 92 B-ALL patients revealed the karyotype was normal in 57 (61.95%) and abnormal in 35 /92 (38.04%) patients. It is important to note that 14 patients with normal karyotype and cases 16 and 18 with abnormal complex cytogenetics had *TEL/AML1* rearrangement/ mutation. Case 18 showed evidence of del(12)(p13)in 60% metaphases (►**Table 1**-footnote).

Correlation of FISH results and cytogenetics demonstrated *TEL/AML1* rearrangement to be the sole abnormality and mutually exclusive as BCR ABL and MLL were negative for rearrangement in all cases wherever done. Additional genetic abnormalities noted in the positive group, occurred when the chromosome complement was 46 with two intact homologous chromosomes 12 and 21 suggesting that the abnormalities were submicroscopic/cryptic level.

Among the 39 cases negative for TEL/AML1 rearrangement but with additional abnormalities, karyotype was normal in 8 and abnormal in 6 cases. Correlation studies demonstrated that the loss or gain of the genes was cryptic in the former. However, in six cases having an abnormal karyotype, *AML1* amplification was cryptic in 50% and corroborated by additional chromosome 21 (►**Table 3**).

Complete blood count (CBC) in patients positive for *TEL/AML1*fusion revealed low hemoglobin in all patients (range: 35–116 g/L, median: 74 g/L), high white blood cell (WBC) counts in 8/18 (44.4%) and 7/17(41.2%) pediatric patients (range: 11.2–110 $\times 10^9$/L) and low counts in 4/18 (22.2%) and 4/17 pediatric patients (23.52%) (range: 1.01–3.6). Platelet counts were low in all patients (range: 10–115 $\times 10^9$/L, median: 42 $\times 10^9$/L). The blasts ranged from 0 to 98% (median: 41%) in peripheral smear and 0 to 95% (median: 80%) in the bone marrow (►**Fig. 2**). Immunophenotyping revealed patients had CD34 positive phenotype, common acute lymphoblstic leukemia associated antigen (CALLA) positive in 17/18 (94.4%) and aberrant expression of CD13, CD33, CD56, singly or in combination in 10/17(58.8%) patients. Coexpression of CD13 and CD33 occurred in 70% (►**Fig. 3**), only CD3 in 20%, and only CD56 in 10%.

Treatment and follow-up details: All the 18 patients were treated with ALL-Berlin-Frankfurt-Munster (BFM 95) protocol. One child died after 1 month due to thrombotic complication. Three patients completed induction and consolidation and lost to follow-up. Fourteen patients who completed the whole treatment are alive and disease free. The median survival among those who completed treatment is 35.5 months (range: 19–44 months).

Discussion

ALL is the most frequent malignancy accounting for 85% of all childhood leukaemia.[32–36] B-ALL constituted 76% of all ALL that

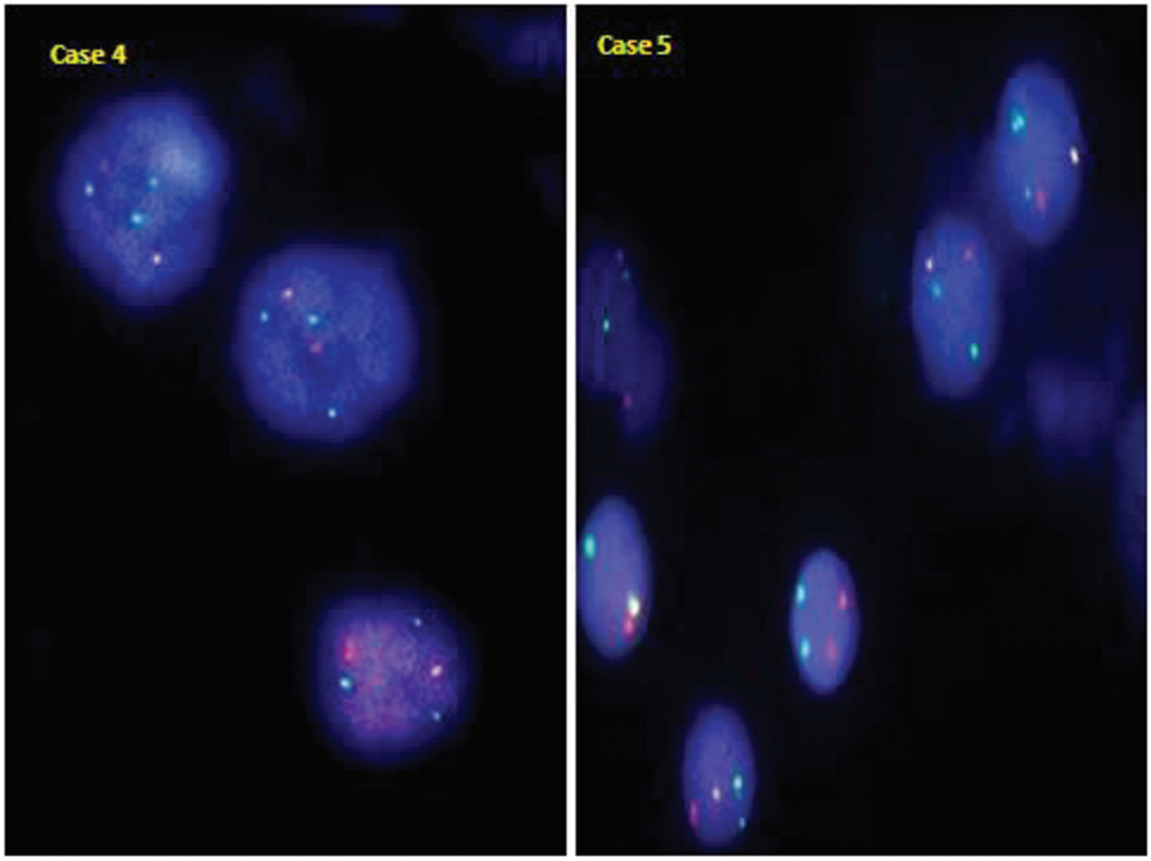

Fig. 1 TEL/AML1 positive case with an atypical signal pattern showing 1 fusion with additional copies of *AML1* (green) and *TEL* (orange) gene in case 4 and an additional copy of only *AML1* gene (green) in case 5 (clone 2).

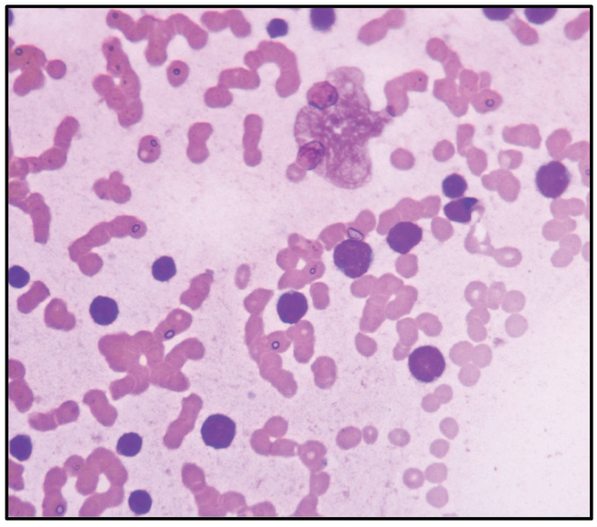

Fig. 2 Blasts showing high N/C ratio coarse chromatin and scanty cytoplasm (Leishman stain,400x).

comprised of predominantly CALLA positive ALL in 73%, early precursor pro B-ALL in 3%.[33] The World Health Organization classification of hematopoietic and lymphoid tissue (2017) defines B lymphoblastic leukemia/lymphoma into various subtypes including "B lymphoblastic leukemia/lymphoma with 7 recurrent genetic abnormalities."[1] Conventional cytogenetics, interphase FISH, RT-PCR find application in the detection of recurrent genetic abnormalities as they play a pivotal role in prognostication, selection of therapy, and response to treatment.[1,13,31–37]

Translocation t(12;21)(p13;q22), a somatically acquired early lesion, results in the critical *TEL/AML1* fusion on chromosome 21, and *AML1/ TEL* fusion on derivative 12p that can be lost. TEL allele is often deleted the fusion protein acts in a dominant negative fashion inhibiting the normal function of transcription factor *AML1*.[4] The abnormality can occur as a sole abnormality or within the context of a complex karyotype characterized by three or more chromosomal abnormalities. Conventional cytogenetics is the gold standard to detect both known and unknown genomic abnormalities in the genome; however, poor chromosome morphology, few malignant metaphases encountered in ALL, and inherent cytogenetic invisibility of the abnormality limit the detection of t(12;21) to 0.05% of patients. Cryptic and subtle abnormalities, below 7 to 8 MB resolution, can be missed on karyotyping. FISH and RT-PCR enable detection of *TEL/AML1* in 20 to 25% of precursor B ALL.[2] Translocation t(12;21) has a favorable outcome and useful for the prediction of outcome in childhood ALL. Coexistence of the same,

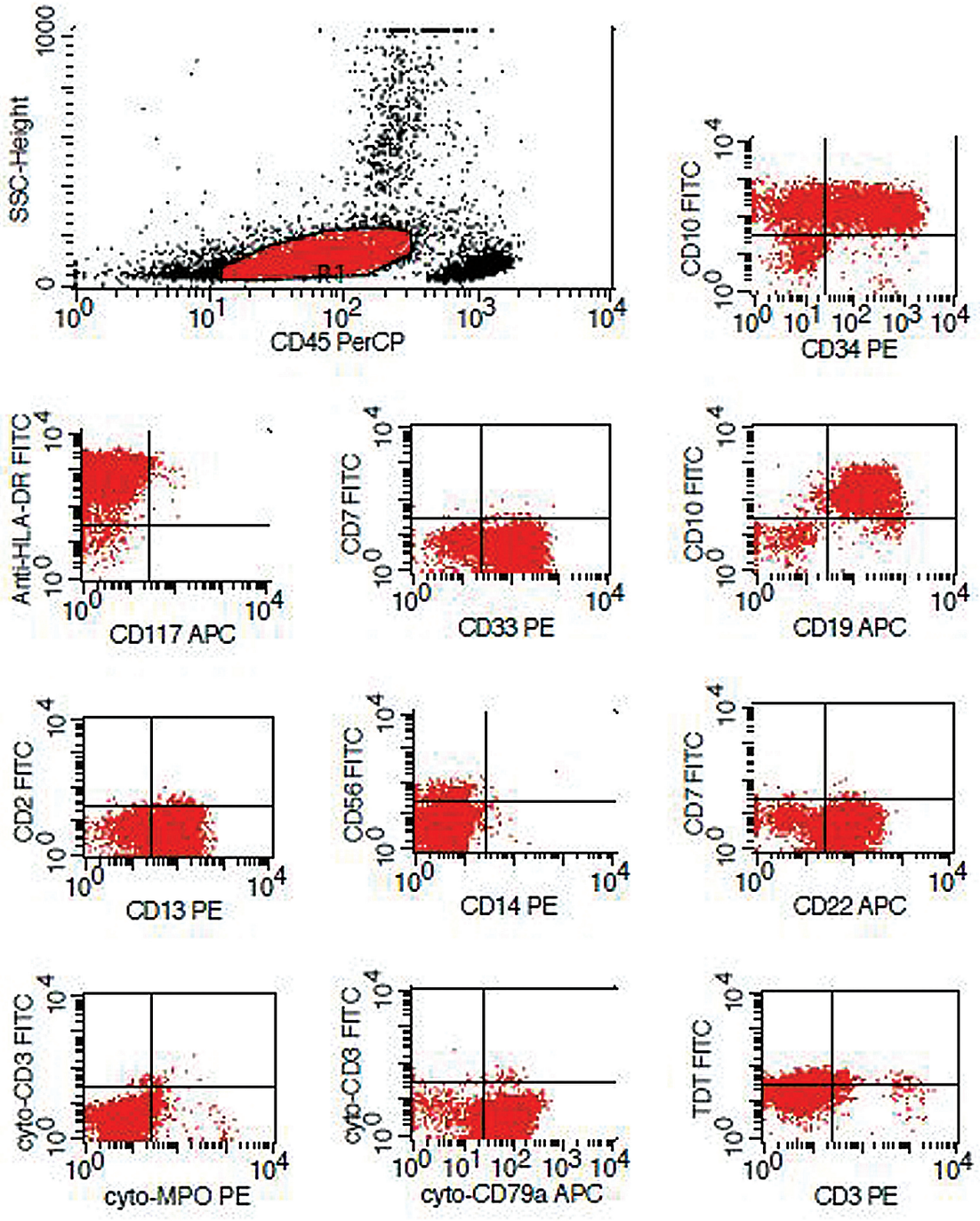

Fig. 3 Immunophenotyping in a prototype case showing aberrant expression of CD13 and CD33.

with other gene rearrangements and in complex setting, favors poorer clinical outcome.

B-ALL patients with t(12;2l) abnormality are younger, ~3–5 years, blasts have B precursor phenotype, and are CD19, CD10 and CD34 positive with expression of myeloid antigens CD13, sensitive to chemotherapy and associated with favorable prognosis and late relapse.[1–3,33]*TEL/AML1* fusion is seen rarely in adults (1–4%);[2,3] however, increased numbers of adults diagnosed with B ALL, prompted assessment for the same. In the present study, under-representation of the cryptic *TEL/AML1* rearrangement overall (8.4%) and in children (13.6%) is low contrary to the expected high prevalence of 25% in western countries.[4] Hospital-based reports,[5–7,9,12–30] international projects,[1,10,24] multicenter

studies,[11,24] and review articles[8,15] indicated existence of geographical differences in distribution of *TEL/AML1* worldwide, including India (►**Table S1** and **S2**). Global studies indicated the incidence of *TEL/AML1* positive B ALL was high ranging from 17% in Spain,[6]18.9% in Brazil,[17]28% in Tunisia,[7] 31% in United States,[5] 37.5% in Egypt,[14] to 40% in Israel.[18] Exception was Turkey (8.5%).[16] The incidence was low in Asian countries, 14.1 to 17.9% in China and Korea,[13–15] 5 to 10.6% in Pakistan,[10–12] and 0 to 19.8% in north and western India,[19–26] 8.3% in Chennai,[27]4.3% in Karnataka,[28]4.8% in Kerala,[29] and 13.64% in Hyderabad.[30] The low prevalence of *TEL/AML1* positivity is 13.6% children with B-ALL, which resonates with previous studies from this region.[27,30] Variation in the incidence of *TEL/AML1* positivity may be dependent on the technology used. When conventional cytogenetics and FISH were used for testing, the global incidence varied from 8.3 to 19.8%[13,17,25–27] however, when only FISH was used it varied from 8.5 to 40%,[5–7,9,14,16,18,28,30] and 4.3 to 13.64% in south India.[28,30] RT-PCR enabled detection of patients with the abnormality in 7.4 to 10.8% in developing countries like Pakistan[10–12] and in 0 to 16.2% in India.[19–22,24,29] Preselection of precursor B ALL enabled increased detection of the *TEL/AML1* rearranged cases. Affordability for the test is an important factor. In the present study, both technologies enabled detection of the fusion gene at the same rate of 8.3 to 8.4%. The population studied, sample size, patient selection, technology(s) used for testing affect the detection rate. If FISH is the modality, the probes used in house/commercial, company, design of the probe, size, validation, cutoff value set, and interpretation are important for defining the status of the abnormality. For RT-PCR, the primers were used.

It is well known that reciprocal translocation t(12;21) (p13;q22) results in the pathogenic *TEL/AML1* fusion on chromosome 21, and *AML1/TEL* fusion on derivative 12p. The latter may be lost sometimes. Deletion of *TEL* allele on the normal chromosome as a consequence of del(12)(p12) and duplication of der(21)t(12;21) that looks like a +21 hyperdiploid karyotype with <5 *AML1* and rarely gene amplification with cluster of >5 or intra-chromosomal amplification of *AML1* is also encountered. Interphase FISH enabled the detection of not only *TEL/AML1* fusion but also associated additional genetic abnormalities in 93% of these cases and in the negative group too. The reported loss of the untranslocated *TEL*,[5,7,14,16,25,27] duplication of *TEL/AML1* fusion (der 21t(12;21)) were seen infrequently in our study as compared with 35 and 17%, respectively, reported previously.[25] An unusual finding was the occurrence of cryptic loss of the reciprocal *AML1-TEL* fusion on der12p in 66.5% of our patients. This appears to be rare[8] and has not been reported earlier from this region. Loss of derivative 12p was also associated with extra copy(s) of *AML1* in 40%, with amplification of *TEL* gene (33.3%). These findings were consistent with the previous reports.[5–8,13,14,16–18] *TEL/AML1* rearrangement with one or more copies of der 21t(12;21) has been reported in 42 to 50% with additional abnormalities,[5,6,8,13,17,18,38] including additional *AML1* in 6 to 23.8% patients,[5–7,14,16,25] and clones with loss of *TEL* gene and an extra *AML1* in 9.5%.[6] Duplication of the fusion gene is rare and may occur in the form of duplication of der 21t (12;21) or ider 21(q10)t(12;21). *TEL* gene functions as a tumor suppressor gene, and its function may be inhibited when in combination with *TEL/AML1* fusion in a dominant negative fashion. Alternatively, *TEL* gene may inactivate the fusion protein necessitating the deletion of the *TEL* gene for leukemogenesis. Submicroscopic deletions and point mutations of *TEL* homologue may contribute to inactivation of *TEL*.[25] Additional genetic abnormalities in relation to the cryptic t(12;21) translocation are envisaged to be secondary events critical to leukemogenesis and not disease progression. The significance of additional genetic changes with respect to clinical features and outcome between presence or absence is debatable.[14]

The observation of *TEL/AML1* rearrangement and additional genetic abnormalities detected by FISH in patients having normal cytogenetics in 14/18 (77%) patients, complex karyotype in 2 (11.11%), one with 12p deletion in 80% metaphases connoting loss of *AML1/TEL* fusion, and the other case with nonrandom chromosomal abnormalities indicate (1) *TEL/AML1* rearrangement is the sole, cryptic abnormality; (2) additional mutational events were submicroscopic too, and (3) the normal karyotype is actually a pseudodiploid karyotype.

Evaluation of clinical and pathological features demonstrated occurrence of *TEL/AML1* positivity mainly in children (94.4%), aged 2 to 13 years (median: 4 years) predominantly among males (94.1%). The fusion was absent in all adults tested. The patients had classic CD34 and CD10 positive phenotype (CALLA positive B-ALL) with aberrant expression of cross lineage antigens, CD13 and CD33.[2,33] WBC counts were high in 44.4% patients, low in 22.2%, and rest within normal range. Another study demonstrated significant association of *TEL/AML1* positive group with lower age group >1 to 5 years (median: 5 years), WBC counts of 10–20 $\times 10^9$/L (median: WBC count of 11.2 $\times 10^9$/L), CD34 negative phenotype, and absence of CD13/33 myeloid marker expression and low relapse rate.[25]

Our hospital is a regional tertiary care cancer center for cancer treatment in a metropolitan city. The possibility of geographic variation in presentation of the disease cannot be ruled out. India is genetically and ethnically a diverse country. Genetic propensity for *TEL/AML1* fusion in childhood ALL has been described.[37] Regional, population based, biological differences, gene–environment interactions, are critical in leukemogenesis and may differentially contribute to the prevalence of *TEL/AML1* fusion occurrence.

This study revealed additional genetic abnormalities in 23.9% *TEL/AML1* negative patients lacking the *BCR-ABL* and *MLL* gene rearrangement. These include extra AML1 copies, extra *AML1*, alone or in combination with *TEL* aberrations (amplification or deletion) and abnormalities of TEL gene (►**Table 2**). Extra *AML1* was cryptic or corroborated by cytogenetics (►**Table 2**). One study reported no loss of *TEL* homologue, extra copies of *AML1* in 37%. The authors cited that *AML1* may be the current target of genetic abnormalities in ALL and acute myeloid leukemia, which probably induces

the transcriptional defect in *AML1* in the process of leukemogenesis, and showed abnormalities. These occur as a consequence of "tri/tetra/polysomy 21, high degree of amplification, and jumping translocations/insertions." The biological significance of the observed anomalies is not known. Although we have not analyzed these cases for clincopathological correlations, *TEL/AML1* negative cases with extra *AML1*/trisomy 21 were reported to be associated with CD13/33-negative and CD34-positive phenotype, low LDH value, low WBC counts, age ranging from 1 to 5 years, high hyperploidy.[25] B-ALL with iAMP21[1] has been defined as the presence of 3 or more *RUNX1* signals on one marker chromosome or a total of five or more *RUNX1* signals per cell. Metaphase FISH with Vysis *TEL/AML1* dual color dual fusion probes display one signal connoting a normal chromosome 21, and several red signals along an abnormal chromosome 21 as tandem duplication, while interphase FISH displays a cluster of red signals (*AML1*) and one separate red signal representing the normal chromosome 21.[1,38,39] To the best of our knowledge, interphase FISH showed distinct well separated signals of *AML1*, and no localized clusters in all with multiple copies of *AML1*. However as per the definition, three patients with five or more *AML1* signals per cell can be construed as having an iAMP amplification. It is important to identify patients with iAMP who have a poor prognosis.

Each technology has its advantages and limitations. Prevalence of good prognostic *TEL/AML1* positive patients was found to be low at this center. Multicenter studies should be undertaken to understand this genetic subtype of B-ALL better in larger sample volume and prognostic implications.

The recurrent genetic abnormalities tested in B-ALL patients have revealed large number of patients lacking these abnormalities and need further interrogation. Comprehensive emerging technologies have identified several genetic determinants. Next-generation sequencing and other evolving technologies will address these genetic abnormalities. Hence, use of orthogonal testing and algorithmic approach will propel identification of new entities in our population.

Conclusion

This is the first hospital-based large study from south India demonstrating low prevalence of cryptic *TEL/AML1 in* 13.6% children with BALL, lower than western data but comparable with south Indian studies. FISH was useful in identifying unique additional genetic abnormalities in *TEL/AML1* positive and negative cases. Clinicopathologic associations were classic. The study adds to the Indian data. The detection of relevant specific abnormalities in B-ALL patients is of fundamental importance.

Conflict of Interest

None declared.

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Germ Cell Tumors in Children: A Retrospective Review of a 04-Year Single-Center Experience

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Abstract

Introduction Pediatric germ cell tumors (GCTs) are rare neoplasms that can be benign or malignant and occur in children and adolescents. They can arise from germ cells in the extragonadal or gonadal sites and have numerous histologic subtypes. The International Germ Cell Cancer Cooperative Group classification is used to guide treatment, with most children experiencing excellent overall survival (OS). Chemotherapy is historically recommended for all malignant GCTs. Nevertheless, surgery and observation alone may be sufficient for stage I gonadal GCTs. Using chemotherapy can lead to successful salvage of relapses..

Objectives Our institute has conducted a study with the objective of evaluating a group of 97 patients diagnosed with GCTs, who received treatment within the past 4 years.

Materials and Methods From January 2018 to April 2022, a total of 97 pediatric patients diagnosed with GCTs underwent surgical treatment at Indus Hospital & Health Network. The diagnosis was established by considering clinical features, tumor marker levels, imaging, and histology. Treatment was determined based on the risk stratification utilizing the United Kingdom Children's Cancer Study Group GC 2005–04 protocol. Patients classified as LR (low-risk) received chemotherapy only if a recurrence occurred after the initial surgery. On the other hand, IR (intermediate-risk) and HR (high-risk) patients received four and six cycles of JEB (chemotherapy regimen), respectively, followed by surgery. Recurrence was closely monitored through suspicion, tumor marker levels, or imaging. Patients experiencing a recurrence after JEB chemotherapy were treated with TIP (paclitaxel, ifosfamide, and cisplatin) chemotherapy and subsequently underwent surgery.

Results In this retrospective study, a group of 97 patients diagnosed with GCTs was analyzed. The cohort included 59 gonadal tumors and 38 extragonadal tumors. The most common histopathological types observed were yolk sac tumor and dysgerminoma. Out of the patients, 33 (34%) were classified as HR, 35 (36.1%) as IR, and 29 (29.9%) as LR. Among the patients, 16 experienced recurrence, while the remaining 90 patients (92.8%) were alive at the time of the analysis. The study determined the 5-year

Keywords
- germ cell tumors (GCT)
- children
- gonadal
- yolk sac tumor
- overall survival
- event-free survival

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event-free survival (EFS) rate as 83.5% and the OS rate as 92.8%. The presence of residual disease was found to be the only significant factor that influenced EFS.

Conclusion To manage GCTs in children, a multidisciplinary approach involving surgeons, oncologists, and radiation therapists is required. Surgery and chemotherapy have improved outcomes for children with GCTs, but personalized treatment planning is crucial. With advancements in pediatric oncology care, the prognosis for children with GCT in Pakistan has improved, providing hope for better outcomes in the future.

Introduction

Pediatric germ cell tumors (GCTs) are rare tumors. GCTs originate from aberrant differentiation of germ cells and encompass a heterogeneous collection of neoplasms, exhibiting notable variations in histological characteristics and site of occurrence.[1,2] Children and adolescents can exhibit both benign and malignant GCTs, and the incidence rates of these tumors vary depending on their age.[3]

The overall incidence of GCTs in children up to 15 years of age can be estimated at 0.9 per 100,000 children.[1,3] In infants, majority are benign GCTs, with the most common site being sacrococcygeal. GCT accounts for 2 to 3% of all pediatric malignancies.[3] Out of all pediatric GCTs, approximately 60% originate from extragonadal sites, such as the mediastinum, pineal and sacrococcygeal regions, and retroperitoneum. On the other hand, the remaining 40% of cases are attributed to GCTs arising from the gonadal sites, specifically the ovary and testis.[4,5] GCTs are the most frequently occurring tumors in the gonads among children and adolescents.[6] While GCTs may exhibit notable variations depending on their anatomical location, they possess a significant degree of similarity and are generally regarded as a cohesive group.[1] GCTs shows a wide range of histologic subtypes. The histologic characteristics of each subtype are not influenced by the clinical presentation. However, the behavior of the tumor and its biological attributes vary based on factors such as the site of origin, stage of the tumor, and age of the patient.[7]

The International Germ Cell Cancer Cooperative Group (IGCCCG) classification is globally employed to identify patients at high risk (HR) and provide guidance for first-line treatment.[8] As per this classification, patients diagnosed with non-germinomatous GCTs or those with elevated levels of tumor markers are categorized as HR individuals.

The majority of children diagnosed with GCTs achieve remarkable overall survival (OS) rates, which has consequently resulted in a reduction in the extent of chemotherapy administered.[9] In the past, chemotherapy was typically advised for all cases of malignant GCTs. Nevertheless, research has demonstrated that stage I gonadal GCTs can be effectively managed with surgical intervention and close monitoring, without the need for additional therapy in many patients. Even in cases of relapse, chemotherapy has proven highly successful in salvaging the patients.[10,11]

To gain deeper insights into the clinical features and treatment approaches for pediatric GCTs, we conducted a comprehensive analysis of a cohort consisting of 97 patients with GCTs who were treated at our institution over the past 4 years.

Materials and Methods

During the period spanning from January 2018 to April 2022, a group of 97 pediatric patients (16 years old or younger) diagnosed with GCTs received surgical treatment at the Indus Hospital & Health Network. The hospital's electronic database was utilized to gather comprehensive information including clinical records, radiological data, laboratory results, and pathological findings.

The diagnosis of GCTs was established by considering various factors, including clinical manifestations, elevated levels of tumor markers (such as serum α-fetoprotein [AFP] and β human chorionic gonadotropin [β-HCG]), imaging studies, and histological analysis. Each patient underwent contrast-enhanced computed tomography scans of the chest, abdomen, and pelvis. For pelvic tumors, magnetic resonance imaging of the pelvis with contrast was performed. Additionally, bone scintigraphy was conducted for staging purposes.

The United Kingdom Children's Cancer Study Group GC 2005–04 protocol was employed for both staging and treatment purposes. Patients were categorized into three risk groups: low risk (LR), intermediate risk (IR), and HR, based on their stage and prognostic factors. Patients classified as HR included those with AFP levels greater than 10,000 ng/mL, stage IV disease (excluding testis $<$ 5 years and all germinomas), or stage II to IV mediastinal tumors. For stage I tumors (LR), chemotherapy was administered only if there was disease recurrence following surgery. IR and HR patients received first-line chemotherapy consisting of four and six cycles of JEB (carboplatin 600 mg/m^2 on day 2, etoposide 120 mg/m^2 for 3 days, and bleomycin 15 mg/m^2 on day 3), respectively, followed by surgical intervention. Tumor markers were monitored to assess treatment response. After completing the initial treatment, all patients underwent long-term surveillance to detect any recurrence of the disease. In cases where patients experienced a recurrence after JEB chemotherapy, they received four cycles of second-line chemotherapy known as TIP (paclitaxel 175 mg/m^2 on day 1,

ifosfamide 1500 mg/m^2 from day 2 to 6 , cisplatin 20 mg/m^2 from day 2 to 6) followed by surgery.

The patients were closely monitored to assess their event-free survival (EFS) and OS rates. An "event" was defined as disease relapse/progression or death from any cause. EFS was determined by calculating the time from the start of treatment to the occurrence of the event, while OS was calculated from the start of treatment to the date of the last follow-up or date of death. In survival analysis, all patients were considered until the date of the last follow-up or until April 30, 2022, whichever came earlier.

Primary Outcome

In this study, EFS and OS were the primary outcomes.

Secondary Outcome

Most of the tumor recurrences occurred in the HR group of GCT and was found as an additional information.

Inclusion Criteria

1. Patients with biopsy-proven extracranial GCTs within the age range of 0 to 16 years.
2. Patients who received surgical treatment outside of our hospital were also included.

Exclusion Criteria

1. Patients older than 16 years were excluded.
2. Patients with intracranial GCTs.
3. Patients with refractory or progressive extracranial GCT treated outside of our institution.

Statistical Analysis

The data analysis was performed using the Statistical Package for Social Sciences (SPSS; IBM, version 24.0, Armonk, New York, United States). For age, AFP, HCG, and lactate dehydrogenase (LDH) variables, the median (interquartile range) was calculated as the data did not follow a normal distribution. Frequencies and percentages were calculated for sex, histology, stage of the disease, presence of metastatic disease, initial treatment characteristics, recurrence, and outcomes. The frequency distributions along with corresponding percentages were also calculated for the categories of age, AFP, β-HCG, and LDH. A chi-square test/Fisher's exact test was run to check the association of outcome and recurrence of disease with demographic, laboratory, and clinical parameters. The Mann–Whitney test was employed to assess the median difference in AFP, β-HCG, and LDH values. The Kaplan–Meier method was utilized to estimate the OS and EFS rates. Univariate and multivariate analyses were conducted using Cox's proportional hazard model. The significant parameters identified in the univariate analyses were subjected to multivariate analysis, and their association with recurrence/outcome was expressed as hazard ratios (HRs) with a 95% confidence interval. A significance level of < 0.05 was set to determine a significant association between recurrence/outcome and the factors under investigation.

Ethical Approval

This analysis was conducted under an ethical exemption granted by the institutional ethical review board with the reference IHHN_IRB_2022_07_020 on August 3, 2022. All procedures performed in the study involving human participants adhered to the ethical principles outlined by the institutional and/or national research committee. The study also complied with the guidelines set forth in the 1964 Helsinki Declaration and its subsequent amendments or equivalent ethical standards.

Results

This retrospective study included a cohort of 97 patients, with a median age of 4 years (1.9–10) at the time of diagnosis. The male-to-female ratio was approximately 1:1.7, with males having a median age of 2 years (1.5–3.5) and females having a median age of 8.4 years (2.5–11).

According to the histopathology, the majority were the yolk sac tumor (YST) (50.5%), followed by dysgerminoma (17.5%). The study included a total of 97 patients, with 33 (34%) classified as HR, 35 (36.1%) classified as IR, and 29 (29.9%) classified as LR. The disease was metastasized in 28 (29%) patients, 17 (60.7%) had pulmonary metastatic diseases while 11 (39.3%) had nonpulmonary metastatic diseases at the time of diagnosis. The surgical excision was done in 84 (86.6%) patients, in which 55 (65%) had residual disease treated with adjuvant first-line chemotherapy and 29 (35%) were LR with complete surgical excision. The tumor was nonresectable in 13 (13.4%) patients, and neoadjuvant chemotherapy was given.

The first-line chemotherapy (JEB) was given to 68 (70%) patients. The recurrence has occurred in 16 patients in which 6 (38%) were from the LR group (chemo-naive) patients, 1 (6%) patient from IR, and 9 (56%) were from the HR group. The IR and HR patients were offered the second-line chemotherapy (TIP).

Out of the total patient population, 90 (92.8%) were reported as alive, while 7 patients (7.2%) had unfortunately passed away; the nonsignificant association of recurrence and outcomes with demographic, clinical, and laboratory parameters are depicted in ►**Table 1**.

There were 59 (60.8%) gonadal and 38 (39.2%) extragonadal GCTs. The anatomical distribution is shown in ►**Fig. 1A**, in which the common site of extragonadal was the abdomen, 14 (36.8%), followed by the sacrococcygeal, 13 (34.2%), while other 11 (29%) were the mediastinum and retroperitoneum sites. In the 13 sacrococcygeal region, 5 (38%) showed mature teratoma (MT), the age of these 5 patients were: 1 (20%) was < 1 year, 3 (60%) were < 5 years, and 1 (20%) patient was of 11 years.

In gonadal GCT, in testis, the most common was YST, 21 (80.8%), followed by mixed GCT (MGCT) 3 (11.5%) and each of one case of mature and immature teratoma (IMT). In ovarian tumor, dysgerminoma, 14 (42.4%), was the most common, followed by YST 8 (24.2%), MGCT 6 (18.2%), 1 (3%) MT, and two of each case of embryonal carcinoma (EC) and IMT.

Table 1 Demographic, clinical, and laboratory parameters in study patients

Characteristics	Recurrence		*p*-Value	Outcomes		*p*-Value
	Yes ($n=16$)	No ($n=81$)		Alive ($n=90$)	Expired ($n=7$)	
Age (y) (*n*) (%)						
<10 >10	13 (81.3) 03 (18.8)	56 (69.1) 25 (30.9)	0.385[a]	63 (70) 27 (30)	06 (85.7) 01 (14.3)	0.669[a]
Sex (*n*) (%)						
Male Female	04 (25) 12 (75)	31 (38.3) 50 (61.7)	0.312[b]	32 (35.6) 58 (64.4)	03 (42.9) 04 (57.1)	1.000[a]
Histology (*n*) (%)						
Mature teratoma	01 (6.3)	10 (12.3)	0.685[a]	11 (12.2)	0	1.000[a]
Immature components (teratoma)	01 (6.3)	04 (4.9)	1.000[a]	05 (5.6)	0	1.000[a]
Dysgerminoma	02 (12.5)	15 (18.5)	0.730[a]	16 (17.8)	01 (14.3)	1.000[a]
Mixed GCT	03 (18.8)	09 (11.1)	0.412[a]	11 (12.2)	01 (14.3)	1.000[a]
Yolk sac tumor	09 (56.3)	40 (49.4)	0.616[b]	44 (48.9)	05 (71.4)	0.436[a]
Embryonal carcinoma	0	03 (3.7)	1.000[a]	03 (3.3)	0	1.000[a]
Risk (*n*) (%)						
Low risk Intermediate risk High risk	06 (37.5) 01 (6.3) 09 (56.3)	23 (28.4) 34 (42) 24 (29.6)	0.019[b]	29 (32.2) 31 (34.4) 30 (33.3)	0 04 (57.1) 03 (42.9)	0.191[a]
Metastatic disease (*n*) (%)						
Yes No	05 (31.3) 11 (68.8)	23 (28.4) 58 (71.6)	0.772[a]	27 (30) 63 (70)	01 (14.3) 06 (85.7)	0.669[a]
AFP (ng/mL) (*n*) (%)						
$<10{,}000$ $>10{,}000$ Normal	03 (18.8) 10 (62.5) 03 (18.8)	28 (34.6) 30 (37) 23 (28.4)	0.164[b]	29 (32.2) 35 (38.9) 26 (28.9)	02 (28.6) 05 (71.4) 0	0.169[a]
HCG (IU/L) (*n*) (%)						
$<5{,}000$ $>5{,}000$	14 (87.5) 02 (12.5)	73 (94.8) 04 (5.2)	0.274[a]	80 (92.9) 06 (7.1)	07 (100) 0	1.000[a]

Abbreviations: AFP, α-fetoprotein; GCT, germ cell tumor; HCG, human chorionic gonadotropin.
*Significant value.
[a]Fisher's exact test.
[b]Pearson's chi-square test.

Total 49 cases of YST were reported in our study, the AFP level was high $\geq$ 10,000 (ng/mL) in 34 (69.4%), $<$10,000 (ng/mL) were 13 (26.5%), and 2 (4.1%) were in normal range.

The histopathological distribution is shown in ►**Fig. 1B**, YST and dysgerminoma were commonly found in 49 and 17 patients, respectively. MT and IMT were in 11 and 5 patients, respectively, however, 3 were EC.

MGCT was found in 12 patients; we have reported 3 patients in each MGCT with EC + YST and IMT + EC + YST. Two patients in each MGCT with YST + dysgerminoma, YST + IMT, and YST + MT. One in each MGCT with IMT + dysgerminoma (►**Fig. 1B**).

Survival Analysis

The study followed all patients for a median duration of 17.2 months (8.2–25.3). Seven patients died, 03 were related to progressive disease, 01 had secondary malignancy (brain tumor), 02 had bacterial and fungal infections, and 01 patient had a recurrence of the disease.

Recurrence has occurred in 16 patients in which 10 (62.5%) were off treatment after first-line chemotherapy and 06 (37.5%) were LR chemo-naive patients. In these 6 patients, 1 had lymphovascular invasion and 2 patients had mixed histology, while 3 had dysgerminoma and 1 YST.

For the entire patient cohort ($n=97$), the 5-year EFS rate was determined to be 83.5%, while the 5-year OS rate was calculated as 92.8% (►**Fig. 2A**).

►**Fig. 2B** shows the 5-year EFS rates among different risk groups (LR, IR, and HR), demonstrating a statistically significant difference. Owing to female predominance and primary nononcological surgeries outside the tertiary care center, we found lower EFS in the LR group as compared with the IR group.

Furthermore, ►**Fig. 2C** illustrates the EFS rates for children without metastases (84.1%) and those with metastases

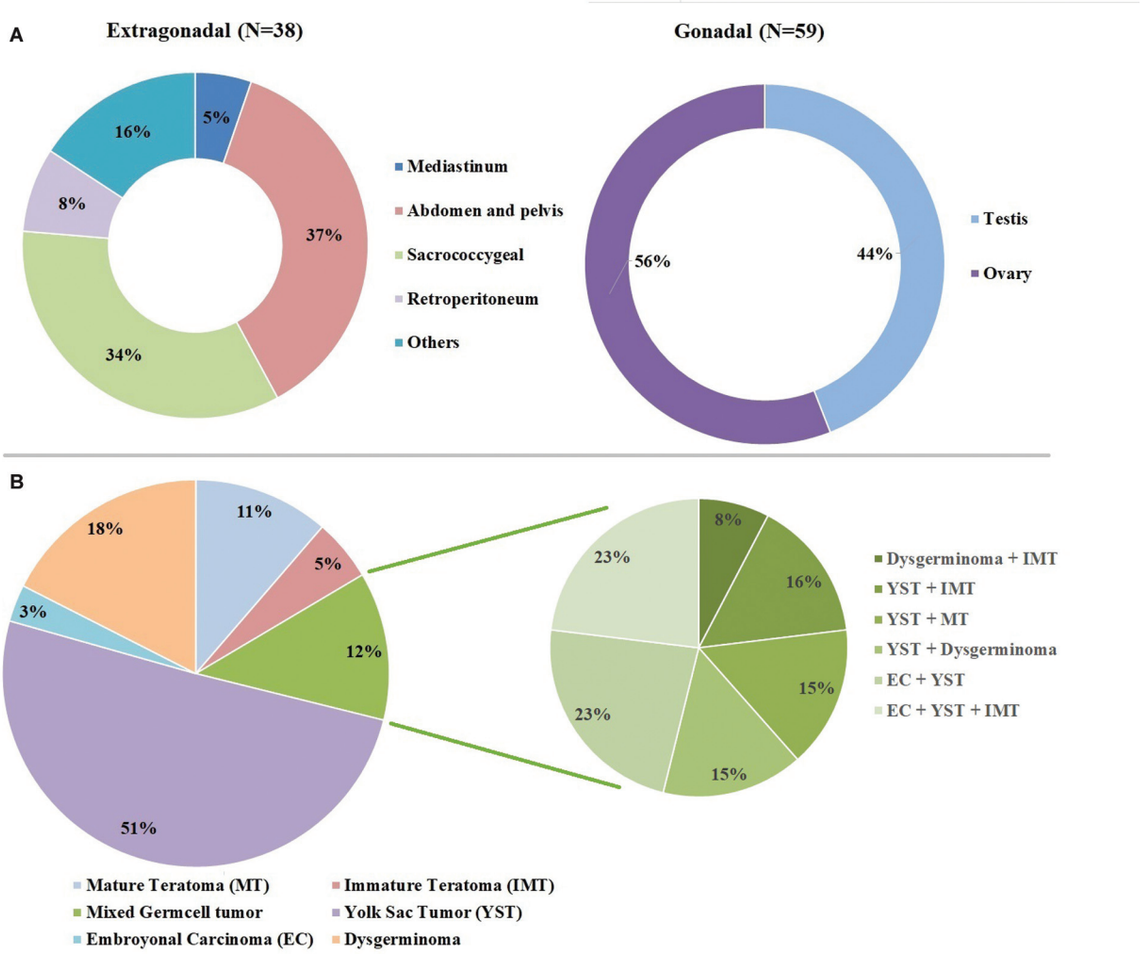

Fig. 1 (A) Anatomical distribution of gonadal and extragonadal germ cell tumors. (B) Distribution according to histopathology.

(82.1%), showing no statistically significant difference. In ►**Fig. 2D**, patients with AFP < 10,000 ng/mL exhibited a higher EFS compared with those with elevated AFP ≥ 10,000 ng/mL, although the difference was not statistically significant. The EFS of patients after the first-line treatment with the small residual disease was 42.1% as compared with patients with complete remission, 93.6%, which was statistically significant (►**Fig. 2E**). Through multivariable analysis, it was determined that the presence of residual disease emerged as the sole independent prognostic factor, with a HRs of 7.42. The residual disease was small in size and was not amenable for surgery without mutilation. We did not encounter condition like growing teratoma syndrome. Also, patients in the HR group and AFP level > 10,000 ng/mL were found to have 2.4 and 1.3 times higher HRs, respectively, which was statistically nonsignificant. There were 36% higher chances of patients with metastatic disease to have disease recurrence, but it was statistically nonsignificant (►**Table 2**).

Discussion

GCTs are uncommon neoplasms characterized by diverse histological subtypes, encompassing both benign and malignant forms. Due to their rarity, a retrospective study was conducted to evaluate the treatment outcomes of pediatric GCTs at our medical facility. Consistent with established international guidelines, all patients received chemotherapy as part of their treatment regimen.[12] In our study, girls were more frequently affected than boys, which is consistent with the literature.[13,14]

Malignant testicular tumors are less frequently reported compared with extragonadal GCTs and mature teratomas.[15] In our study, the majority of cases (60.8%) exhibited gonadal involvement, while 39.2% showed extragonadal involvement. Among the histological subtypes, YST was the most frequently observed, which aligns with findings from previous studies.[14,15] However, our study revealed that ovarian GCTs accounted for a larger proportion (56%) of the cases.

In our study, we also identified MGCTs, with the most prevalent combination being YST and EC. This finding is consistent with previous studies that have reported similar results, indicating the recurrent occurrence of this specific combination in MGCTs.[16] Tumor markers play a crucial role in the diagnosis, detection of relapse, and follow-up of GCTs. In our study, we found that serum

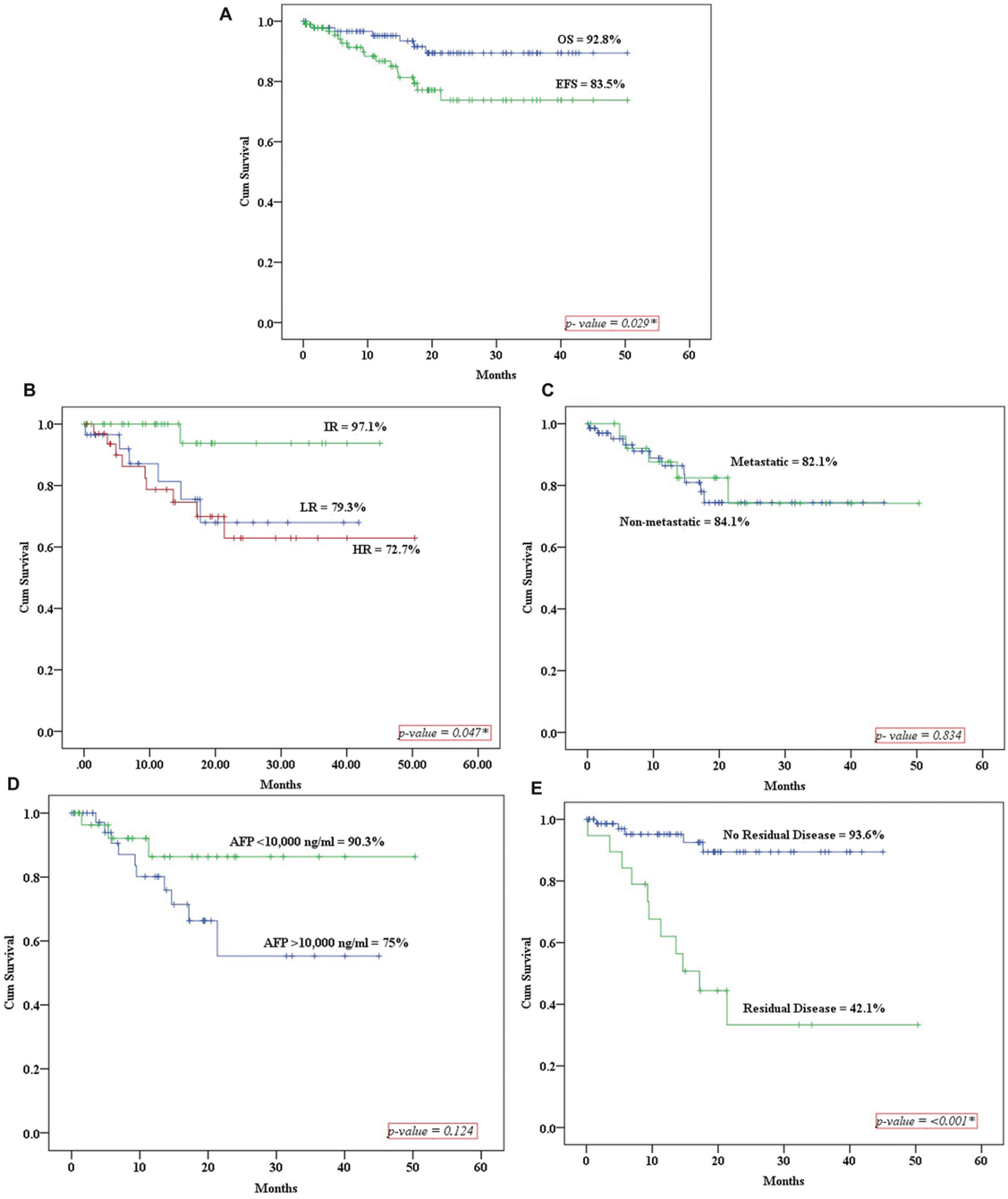

Fig. 2 (**A**) The 5-year event-free survival (EFS) and overall survival (OS). (**B**–**E**) The 5-year EFS according to the risk factors (IR = intermediate risk; LR = low-risk; HR = high-risk; AFP = α-fetoprotein.).

AFP elevation, which is commonly associated with the presence of a YST component, was observed in 87.5% of patients with GCTs. These findings align with the results reported by Kumar et al, indicating a high concurrence in the incidence of elevated AFP levels among patients with GCTs.[16] Among the cases of YST in our study, 35 individuals (71.4%) exhibited an AFP level exceeding 10,000 ng/mL. Interestingly, our study did not identify elevated AFP as a significant prognostic factor, which aligns with the findings of a study conducted by Marina et al. This suggests that AFP levels may not independently predict the prognosis of YSTs in pediatric patients, consistent with the previous research.[17]

In our study, patients with β-HCG levels below 5,000 IU/L experienced a recurrence rate of 87.5%, with 92.9% of these patients still alive. In contrast, those with β-HCG levels above 5,000 IU/L had a recurrence rate of 12.5%, with only 7.1% alive. These findings are consistent with previous research that

Table 2 Multivariate analysis for hazard ratio

	HRs	95% CI	*p*-Value
High-risk group	2.416	0.656–8.895	0.185
Metastatic disease	0.361	0.098–1.331	0.126
AFP > 10,000 (ng/mL)	1.370	0.424–4.422	0.598
Residual diseases	7.426	2.455–22.46	< 0.001[a]

Abbreviations: AFP, α-fetoprotein; CI, confidence intervals; GCT, germ cell tumor; HR, hazard ratio.
[a]Significant value < 0.05.

identifies β-HCG as a significant prognostic marker in GCTs. For example, Lorch et al[18] demonstrated that elevated β-HCG levels in patients with metastatic GCTs were associated with poorer survival outcomes and a higher likelihood of recurrence.

The management approach for GCTs is influenced by various factors such as tumor location, disease extent, patient age, and coexisting medical conditions. Typically, the management strategy involves surgical resection for localized disease, chemotherapy for cases with residual or metastatic disease, and a combination of neoadjuvant chemotherapy and delayed surgical excision for unresectable lesions. These treatment modalities are implemented based on individual patient considerations to optimize outcomes in GCT management.[19,20] Localized GCTs are primarily treated through surgical resection. The primary objective of the surgical procedure is to achieve complete removal of the tumor while ensuring the preservation of normal tissue and organ function. The surgical approach aims to maximize tumor removal while minimizing potential damage to surrounding healthy tissues and organs. In some cases, a radical orchiectomy (removal of the testicle) may be necessary for testicular GCTs. In ovarian GCTs, surgical resection may involve removal of the affected ovary or ovaries.[19,20] Chemotherapy is the primary treatment for residual or metastatic disease. Platinum-based chemotherapy, which includes drugs such as cisplatin, carboplatin, and oxaliplatin, has been shown to be highly effective in treating GCTs. Chemotherapy is typically given in cycles, with a period of rest between each cycle to allow the body to recover.[19,20] Unresectable lesions in GCTs may be managed through a combination of neoadjuvant chemotherapy and delayed surgical excision. Neoadjuvant chemotherapy refers to the administration of chemotherapy before surgery, with the goal of reducing the size of the tumor and increasing the feasibility of surgical removal. This approach allows for tumor shrinkage, facilitating subsequent surgical intervention by improving the chances of achieving complete resection while preserving normal tissue function. Neoadjuvant chemotherapy followed by delayed surgical excision is employed to optimize the management of GCTs in cases where immediate surgical resection is not feasible. Delayed surgical excision involves waiting until the tumor has responded to chemotherapy before removing it surgically.

The introduction of platinum-based chemotherapy has led to a remarkable improvement in the chances of survival for children diagnosed with GCTs. Currently, approximately 90% of children with GCTs can expect to survive for at least 5 years, a substantial increase compared with the less than 50% survival rate in the period before platinum-based treatments were available. Nevertheless, the outlook for each child is influenced by various factors such as the specific type and location of the tumor, the stage of the disease at diagnosis, as well as the age and overall health of the patient.[19,20]

Our study revealed consistent findings with regard to the cure rates of ovarian and testicular GCTs at different stages. Specifically, stage I GCTs exhibited a 100% cure rate, indicating successful treatment for all patients in this stage. For stage III GCTs originating from any primary site, the cure rate was determined to be 82%, while for stage IV GCTs from any primary site, the cure rate stood at 75%. Furthermore, we observed that the histology, or tumor type, played a significant role in prognosticating immature malignant teratoma ovarian tumors. Among these ovarian tumors, those of the neural type with grades II and III demonstrated the least favorable prognosis.[21,22] Two cases of ovarian tumors with progressive disease were identified in our report. Unfortunately, these patients were lost to follow-up after undergoing second-line chemotherapy.

A study conducted by Akyüz et al[23] from Turkey reported the OS and EFS rates in ovarian tumors as 68 and 57%, respectively, but in our study, we reported 97% OS and 84.8% EFS in ovarian tumors. We have 96.6% OS for gonadal tumors and 86.8% for extragonadal tumors; however, in a study conducted by Feltbower et al,[24] the 5-year survival rates for gonadal GCT were reported to be 93 to 95%, while the rates for extragonadal GCT were 70 to 75%. Mann et al[25] also reported similar findings, with 5-year survival rates of 90.9 and 87.8%, which aligned with our study. Furthermore, a more recent extensive population-based study from Germany indicated a 5-year survival rate of 92% for both gonadal and extragonadal GCTs.[26]

Risk of recurrence is more in patients with HR disease as we reported in our study, 27.3% in HR. Actual recurrence occurred more in patients with residual disease after first-line chemotherapy, that is, 55.6%, rather than patients with no residual.

Pediatric and adult GCTs have many differences in terms of histology, different staging systems, and survival. In adult gonadal GCT, testicular GCT is staged as per the American Joint Committee on Cancer and ovarian as per the International Federation of Gynecology and Obstetrics, while pediatric GCT as per the IGCCCG.[27] MGCTs are more common in adolescent

and young adults. Children with testicular non-seminomatous GCT have improved survival compared with adults.[28]

The study provides confirmation that positive outcomes can be attained for children diagnosed with pediatric GCTs. It highlights the significant impact of a multidisciplinary approach to pediatric oncology care, which has substantially enhanced survival rates for children with GCTs in Pakistan. The recent advancements in health care services within the country have played a crucial role in raising life expectancy and achieving better outcomes for children with cancer.

Study Limitations

We have presented in this study the in-depth analysis of GCTs but our data has some limitations. We were not able to do follow-up of this large cohort for short duration, so far. We aim to follow this cohort with longer duration and add assessment of long-term toxicities of chemotherapy.

Conclusion

In conclusion, a comprehensive approach involving collaboration among surgeons, histopathologists, oncologists, and radiation therapists is crucial for effectively managing GCTs in children. The combination of surgical interventions and chemotherapy has played a vital role in enhancing the overall outcomes for pediatric GCT patients. However, it is essential to develop personalized treatment plans to maximize the effectiveness of interventions. With the continuous advancements in pediatric oncology care, there has been a notable improvement in the prognosis for children with GCTs in Pakistan, instilling optimism for even better outcomes in the future.

Patient Consent

Informed patient consent was obtained for this study.

Funding

None.

Conflict of Interest

None declared.

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Epidemiology of Cancer Incidence Estimates and Statistics 2000–2025: Analysis from National Cancer Registry Programme in India

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Abstract

Introduction Several hospital-based and population-based cancer registries (PBCRs) have been collecting cancer data systematically since 1982, according to the National Cancer Registry Programme-National Centre for Disease Informatics and Research, an initiative of the Indian Council of Medical Research.

Objective Planning, observing, and assessing cancer control efforts require knowledge of current cancer statistics. This article's goal is to provide an update on cancer incidence projections for India by age groups, sex, and anatomical sites for the period 2020 to 2025.

Materials and Methods The cancer incidence, patterns, trends, projections, and mortality from 28 PBCRs were analyzed in this study, along with the kind of therapy and stage of presentation of cancer patients from 58 HBCRs ($N = 667{,}666$) from the pooled analysis for the composite period 2012 to 2016. Data regarding the population at risk were obtained from the Indian Census (2001 and 2011) to estimate the age- and sex-stratified population. To gain a better understanding of the epidemiology of cancer, the states and areas of the nation were divided into PBCR groups.

Results For both males and females, the districts with the highest age-adjusted incidence rates were Aizawl (269.4) and Papum Pare (219.8). It is anticipated that there will be 1392,179 cancer patients in India by 2020 and 1569,793 by 2025. In Delhi, the northern region of India, the incidence rates of tobacco-related malignancies were high (62.1% for men and 18.5% for women). High incidence rates were seen in the southern districts of Kollam (males: 52.9) and Bangalore (20.1), respectively. Age-adjusted rates (AARs) for males and females in Kolkata, East, were 42.3 and 13.7, respectively. Western cities with high AARs were Mumbai (18.2) and Ahmedabad Urban (54.3) for men and women, respectively. For lung cancer, in terms of male and female incidence rates, Aizawl district ranked highest at 38.8 and 37.9 per 100,000, respectively.

Conclusion This study offers a methodology for evaluating cancer trends and status in India. To meet the national targets for noncommunicable diseases and the sustainable development goals, it will direct adequate support for action to boost efforts to promote cancer prevention and control.

Keywords
- cancer incidence
- lung cancer
- breast cancer
- tobacco-related cancer
- relative proportion

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Introduction

Nowadays, one of the main causes of sickness and death worldwide is cancer, which is also a health concern. An estimated 70% increase in cancer incidence is predicted in the next 20 years, despite continuous global efforts to prevent cancer.[1] Noncommunicable diseases (NCDs) accounted for 71% of all fatalities worldwide.[2] According to estimates, NCDs caused 63% of deaths in India, with cancer being among the top causes (9%). It is acknowledged that cancer registries are essential parts of national initiatives to manage cancer.[3] Current data on cancer incidence, trends, and projections can be found in publications from both industrialized and developing nations. Many National Cancer Registry Programme (NCRP) cancer reports from various Indian registries have been made public.[4–6] Nearly half of the world's cancer cases and over half of cancer-related deaths occur in Asian nations, according to the GLOBOCAN 2018 database.[7] Research from both domestic and international sources indicates that the Indian subcontinent has seen a marked rise in the incidence, morbidity, and death related to cancer.[8–12]

In a population that is characterized by geography, population-based cancer registries (PBCRs) offer data on the incidence and prognosis of cancer. In addition, they offer the structure for evaluating community cancer control. The main applications of HBCRs are performance reviews of clinical trials and hospital cancer programs, which deal with the documentation of data on cancer patients seen in a specific hospital. The PBCR now serves a purpose besides only providing data on the prevalence of cancer in a certain catchment region.[13] The Indian Council of Medical Research (ICMR) program report 2020 predicts that, given present trends, there will be 13.9 lakh cancer cases in the country in 2020 and that number is likely to increase to 15.7 lakh by 2025. These approximations are derived from cancer-related data gathered from 28 PBCRs. Additionally, information on cancer was provided by 58 hospital-based cancer registries (HBCRs). There will be 3.7 lakh (27.1%) tobacco-related cancer cases worldwide in 2020, according to estimates. Among women, breast cancer accounts for an estimated 2.0 lakh cases (14.8%), whereas cervical cancer accounts for 0.75 lakh occurrences (5.4%). Gastrointestinal tract malignancies account for an estimated 2.7 lakhs (19.7%) of all cancer cases, affecting both men and women. The incidence of cancer per 100,000 people in the male population varies from 269.4 in Aizawl district (the highest in India) to 39.5 in Osmanabad and Beed district. Comparably, the incidence of cancer in female populations varies from 219.8 cases per 100,000 people in the Papum Pare district to 49.4 cases in the Osmanabad and Beed areas. Roughly 94,000 new cases of cervical cancer are expected to occur annually in India alone, where it accounts for 7.92% of all malignancies diagnosed in women globally.[14,15] Breast cancer cases in Indian women have been discovered to be 10 years younger than in Western women, indicating that breast cancer develops earlier in premenopausal life in India.[16–19]

Materials and Methods

Materials

Under the ICMR-NCDIR-NCRP (ICMR-National Centre for Disease Informatics and Research-NCRP), there are now 236 HBCRs and 36 PBCRs registered. However, this article contains data from 58 HBCRs and 28 PBCRs that have at least a year's worth of high-quality data. Both during and after data submission, the registration data are subjected to multiple quality checks. These include range, consistency, and family checks in accordance with the guidelines set forth by the International Association of Cancer Registries. Both the web-based PBCR data entry application and PBCRDM 2.1 include all the checks. After the changes are received, the registry database is updated with the list of instances that may have been incorrectly sent to the relevant registries for verification using the original medical records. The NCDIR-NCRP greatly facilitates cancer registration with innovative software applications. The desktop and web-based PBCR software performs quality checks (including consistency, range, improbable, and family), matching, and duplicate checks to ensure that the data are accurate and legitimate. Furthermore, duplicate names that sound similar but have different spellings are filtered out using a phonetics program. The software is used to identify fluctuations in the number of cancer cases over time from each source of registration so that the appropriate action can be taken. When mortality data and incidence are matched, the unmatched mortality cases are classified as either death certification only or death certificate notification. After obtaining clarification from each registry at each stage, the data are finalized and ready for further analysis.

Methods

Study Design

Secondary data from HBCRs and PBCRs were used in the study.

Primary and Secondary Outcomes

This study's primary outcome is to estimate India's cancer incidence utilizing HBCRs and PBCRs. The estimation of cancer cases projected for 2025 using age-specific incidence rate is the secondary outcome.

Statistical Analysis

The following formulas are used to compute the crude incidence rate, age-specific rate (ASpR), age-standardized rate, cumulative risk, and truncated age-adjusted incidence rate for the obtained data. The statistical software Joinpoint Regression Program, Version 4.7.0, is used to analyze trends using Joinpoint models, which are regression lines that are connected at many locations. In this article, the population estimates for the years 2012 and 2016 were computed using the difference distribution method (which estimates populations by five annual age groups) using data from the censuses of 2001 and 2011. The number of cancer cases in India for 2024 was then calculated by applying the age-standardized incidence rate (ASIR) of each distinct anatomical site for the

years 2012 to 2016 to the predicted population. The total estimate of all site cancer was obtained by adding the cancer estimates for each individual anatomical site (International Classification of Diseases, Tenth Revision [ICD-10]: C00–C97). Utilizing the population-weighted average of the rates from the PBCRs, the pooled age, sex, and site-specific incidence rates were calculated. The 28 PBCRs were assumed to be representative of the nation with a steady incidence rate throughout time for the purpose of estimating the incidence of cancer.

Ethical Approval

This study received ethical approval from the Institutional Ethics Committee (IEC) of the National Centre for Disease Informatics and Research (NCDIR) under reference number NCDIR/IEC/3020/2020. Participating Population-Based and Hospital-Based Cancer Registries also obtained approval from their respective Ethics Committees and secured consent from local authorities, citizen groups, and community representatives.

Crude Incidence Rate

Crude rate (CR) is the rate found by dividing the total number of cancer cases by the corresponding mid-year population estimate and then multiplying the result by 100,000.

$$\mathrm{CR} = \frac{\text{New cases of cancer of a particular year}}{\text{Estimated population of the same year}} * 100{,}000.$$

Age-Specific Rate

ASpR is the rate determined by dividing the total number of cancer cases by the estimated population in that age group, gender, site, geographic area, and time, then multiplying the result by 100,000.

$$\mathrm{ASpR} = \frac{\text{New cases of cancer of a particular year in the given age group}}{\text{Estimated population of the same year for the given age group}} * 100{,}000$$

Age-Standardized Rate

Cancer incidence increases as age increases. Accordingly, the number of cancer cases increases with the percentage of the population that is older. The proportion of older people is higher in most developed and Western countries. Hence, age-adjusted rate (AAR) or age-standardized rate are calculated using a world standard population that accounts for this to make cancer rates comparable between nations. By collecting the ASpRs and applying them to the standard population in that age group, this is estimated using the direct technique[20] (Boyle and Parkin, 1991).

$$\mathrm{AAR} = \frac{\sum(\mathrm{ASpR}) \times (\text{No.of persons in Std.world population in that 5 yearage group})}{100{,}000}$$

Cumulative Risk

The probability that a person would acquire a specific cancer at a given age, barring any other cause of death, is known as cumulative risk. An approximation of the cumulative risk is the cumulative rate (CuR). It is calculated by multiplying the yearly age-specific incidence rates by 5 (the 5-year age interval), times 100/100,000. This process is repeated for each 5-year age interval, up to 64 or 74 years of age, or for whatever age group is to be used to compute the cumulative risk.

$$\mathrm{CuR} = \frac{5*\sum(\mathrm{ASpR})*100}{100{,}000}$$

Truncated Age-Adjusted Incidence Rate

This is similar to the age-adjusted rate, with the exception that it is based on a reduced age range of 35 to 64.

Results

Calculation of Cancer Incidence

The number of men and women covered by PBCRs is provided using data from 32 geographic locations. There are 865 girls for every 1,000 males in Mumbai PBCR, which has the lowest sex ratio in the data. In comparison to other PBCRs, the percentage of rural residents reporting was higher in the northeastern (NE) PBCRs. There are 12 pure urban PBCRs, 1 pure rural PBCR, and 15 PBCRs that cover the populations of both urban and rural areas in varying percentages. ►**Table 1** shows that the top five PBCRs with the highest number of cases registered were Thiruvananthapuram district (27,833), Bangalore (29,049), Chennai (31,271), Delhi (60,097), and Mumbai (53,714). With the exception of Manipur, Imphal West district, and Papum Pare district in Arunachal Pradesh, the majority of registries in the NE region of the country showed a greater percentage of cancer cases in men. Except for Delhi, the Kollam district, Kolkata, and Ahmedabad urban, there were more female cancer cases reported in other places.

Crude Rate

According to ►**Table 2**, Aizawl district (206.2) has the highest CR per 100,000 men, followed by Mizoram state (146.1), Kollam district (159.4), Kamrup urban (190.5), and Thiruvananthapuram district (170.4). Similarly, the district with the highest CR for females is Aizawl (174.6), followed by Kollam (139.1), Chennai (141.4), Kamrup urban (150.8), and Thiruvananthapuram (164.8). Greater crude incidence rates in both males and females have been reported by the registries covering the country's NE and southwest coastal regions.

Age-Adjusted Rates

Osmanabad and Beed in Maharashtra have an AAR of 369.5, whereas Aizawl in the state of Mizoram has an AAR of 269.4. East Khasi Hills in Meghalaya has an AAR of 227.9. Under the West Arunachal PBCR, the female population varied from 49.4 in the Osmanabad and Beed districts to 219.8 in the Papum Pare district, with Aizawl district coming in second at 214.1.

Truncated Rates

Between Osmanabad and Beed district (71.5) and East Khasi Hills district (494.5), there was a variation in the truncated rate (TR) per 100,000 population for men; Aizawl district came in second with 485.5. The range for females was

Table 1 Total number of cancer cases registered in 28 PBCRs under NCRP

S. No	Registry	Males		Females		Total
NORTH						
		n	%	*n*	%	*N*
1	Delhi (2012–14)	31,032	51.6	29,065	48.4	60,097
2	Patiala (2012–16)	5,394	47.0	6,077	53	11,471
SOUTH						
3	Hyderabad (2012–14)	5,143	44.4	6,453	55.6	11,596
4	Kollam (2012–16)	9,930	50.4	9,780	49.6	19,710
5	Thiruvananthapuram (2012–16)	13,506	48.5	14,327	51.5	27,833
6	Bangalore (2012–14)	13,221	45.5	15,828	54.5	29,049
7	Chennai (2012–14)	14,468	46.3	16,803	53.7	31,271
EAST						
8	Kolkata (2012–2015)	10,186	52.7	9,151	47.3	19,337
WEST						
9	Ahmedabad urban (2012–2016)	14,579	56.9	11,025	43.1	25,604
10	Aurangabad (2012–2016)	1,923	49.0	2,001	51.0	3,924
11	Osmanabad and Beed (2012–2015)	3,635	44.9	4,467	55.1	8,102
12	Barshi rural (2012–2016)	726	47.2	813	52.8	1,539
13	Mumbai (2012–2015)	26,256	48.9	27,458	51.1	53,714
14	Pune (2012–2016)	9,687	47.2	10,818	52.8	20,505
CENTRAL						
15	Wardha district (2012–2016)	2,389	48.5	2,537	51.5	4,926
16	Bhopal (2012–2015)	3,567	49.8	3,589	50.2	7,156
17	Nagpur (2012–2016)	5,952	49.6	6,047	50.4	11,999
NORTHEAST						
18	Manipur state (2012–2016)	3,702	45.1	4,500	54.9	8,202
19	Mizoram state (2012–2016)	4,323	53.6	3,736	46.4	8,059
20	Sikkim state (2012–2016)	1,172	50.9	1,131	49.1	2,303
21	Tripura state (2012–2016)	6,559	57.2	4,914	42.8	11,473
22	West Arunachal (2012–2016)	1,222	51.1	1,171	48.9	2,393
23	Meghalaya (2012–2016)	4,688	62.3	2,832	37.7	7,520
24	Nagaland (2012–2016)	1,403	58.6	992	41.4	2,395
25	Pasighat (2012–2016)	321	51.4	303	48.6	624
26	Cachar district (2012–2016)	4,663	54.2	3,943	45.8	8,606
27	Dibrugarh district (2012–2016	2,535	53.1	2,238	46.9	4,773
28	Kamrup urban (2012–2016)	6,223	56.5	4,790	43.5	11,013

Abbreviations: *n*, number of cancer cases, males or females; *N*, total number of cancer cases; NCRP, National Cancer Registry Programme; PBCR, population-based cancer registry.

likewise 108.2 in the Osmanabad and Beed districts to 499.0 in the Papum Pare, Arunachal Pradesh area.

The estimated top sites of cancer cases by time periods (2015, 2020, and 2025) are shown in ►**Table 3**. All-site cancers in females are expected to rise to 806,218 in 2025 from 627,202 in 2015, whereas in males they are expected to rise to 763,575 in 2025 from 601,737 in 2015. As of 2025, the number of males and females with anticipated leading cases of lung and breast cancer, respectively, would be 81,219 and 232,832 accordingly. Between 2015 and 2025, there is projected to be a 27.7% increase in cancer cases across all sites combined.

The top five cancer sites in men expected in the year 2024 are the mouth (8.9%), tongue (6.3%), stomach (5.2%), prostate (6.5%), and lung (11%). They are also shown in ►**Figs. 1** and **2**. Breast (30.1%), cervix (11.2%), ovary (6.6%), corpus uteri (4.1%), and lung (4%) were the estimated top five sites of cancer in women. Males were more likely to have liver cancer

Table 2 Incidence rates: CR, AAR, and TR (35–64 years) per 100,000 population for all sites of cancer in 28 PBCRs under NCRP

S. No	Registry	Males			Females		
		CR	ARR	TR	CR	ARR	TR
NORTH							
1	Delhi (2012–2014)	112.3	147.0	232.2	119.6	141.0	279.0
2	Patiala district (2012–2016)	101.6	108.2	196.4	127.7	124.6	271.4
SOUTH							
3	Hyderabad district (2014–2016)	84.2	101.6	172.2	109.8	136.0	278.3
4	Kollam district (2012–2016)	159.4	127.7	198.0	139.1	107.1	205.7
5	Thiruvananthapuram district (2012–2016)	170.4	137.8	211.5	164.8	127.3	242.8
6	Bangalore (2012–2014)	96.8	122.1	181.7	125.1	146.8	283.6
7	Chennai (2012–2016)	121.8	119.9	185.2	141.4	132.8	260.5
EAST							
8	Kolkata (2012–2015)	109.9	91.2	145.2	105.9	89.2	175.9
WEST							
9	Ahmedabad urban (2012–2016)	89.1	98.3	183.2	74.7	76.7	158.0
10	Aurangabad (2012–2016)	56.6	70.9	121.6	62.9	75.1	158.5
11	Osmanabad and Beed (2012–2015)	39.3	39.5	71.5	52.8	49.4	108.2
12	Barshi rural (2012–2016)	53.9	50.6	80.5	67.2	61.0	126.5
13	Mumbai (2012–2015)	97.3	108.4	155.1	117.6	116.2	207.6
14	Pune (2012–2016)	67.5	83.0	120.0	83.3	94.0	177.7
CENTRAL							
15	Wardha district (2012–2016)	70.4	64.5	109.7	78.7	69.9	148.9
16	Bhopal (2012–2015)	83.3	101.0	180.0	90.4	106.9	223.3
17	Nagpur (2012–2016)	89.0	91.1	158.6	93.1	89.8	188.2
NORTHEAST							
18	Manipur state (2012–2016)	47.0	62.8	91.0	57.8	71.1	129.6
19	Mizoram state (2012–2016)	146.1	207.0	357.7	127.5	172.3	313.2
20	Sikkim state (2012–2016)	69.9	88.7	131.5	75.3	97.0	175.2
21	Tripura state (2012–2016)	67.0	80.9	145.9	52.0	58.3	127.3
22	West Arunachal (2012–2016)	56.6	101.1	199.9	56.3	96.3	215.7
23	Meghalaya (2012–2016)	92.6	176.8	386.0	55.7	96.5	201.1
24	Nagaland (2012–2016)	74.5	124.5	223.8	56.3	88.2	193.6
25	Pasighat (2012–2016)	90.7	120.4	207.6	88.1	116.2	260.3
26	Cachar district (2012–2016)	99.2	129.0	233.4	87.0	104.8	234.2
27	Dibrugarh district (2012–2016)	72.5	91.9	155.9	66.0	76.8	170.7
28	Kamrup urban (2012–2016)	190.5	213.0	339.7	150.8	169.6	320.8

Abbreviations: ARR, age-adjusted rate; CR, crude rate; NCRP, National Cancer Registry Programme; PBCR, population-based cancer registry; TR, truncated rate.

(4.2%) among the top 10 cancers than females, whereas females were more likely to have thyroid (3.8%) and gallbladder (3%) cancers.

According to age group (0–14, 15–39, 40–64, and 65+), ►**Table 4** presents the predicted top five sites of cancer (%) in India by gender for the year 2024. Lymphoid leukemia is the most common site in the childhood (0–14 years) group for both boys (29.06%) and girls (24.07%). Boys' brain nerve systems (NSs) come in second with 12.6% and girls' 14.17%, respectively. For males, in the 15- to 39-year-old age group, the most common sites are the mouth (12.6%), tongue (9.53%), brain NS (7.4%), myeloid leukemia (6.72%), and non-Hodgkin lymphoma (6.23%); for females, the most common sites are the breast (27.05%), thyroid (13.07), ovary (7.82%), cervix (6.69%), and myeloid leukemia (3.56%). The mouth (11.08%), tongue (7.52%), and lung (11.0%) were the most common sites among males in the 40 to 64 age group. The most common sites in females were the breast (32.3%),

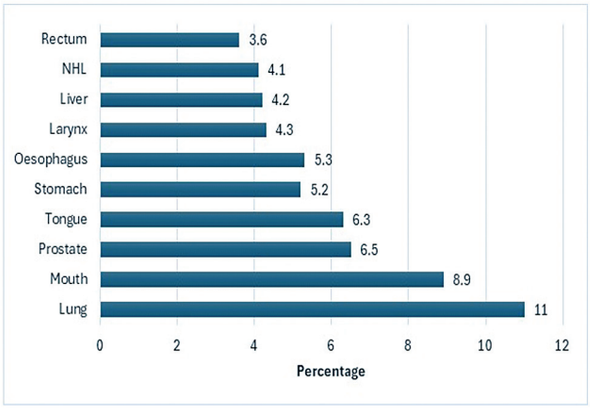

Fig. 1 Estimated proportion of top 10 leading sites of cancer in India by sex, males 2024.

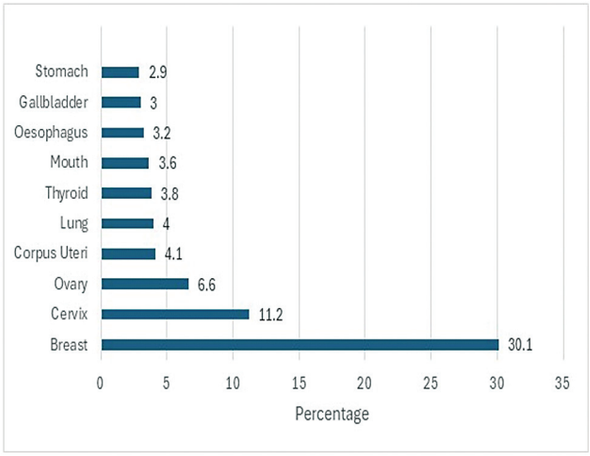

Fig. 2 Estimated proportion of top 10 leading sites of cancer in India by sex, females 2024.

cervix (12.2%), and ovary (6.70%); there were high incidence of cases in this age range in both males (350,141) and females (439,960). In men over 65 years, the prostate (11.9%) was the second most common site after the lung (12.90%). Lung cancer was the most prevalent cancer in men over 40 years, while breast cancer was the most common in women.

►**Supplementary Fig. S1** (online only) shows India's projected ASIR and number of cancer patients by gender and age group over a 5-year period in 2024. In both males and females, the ASIR for all cancer sites began to rise at age 25. The ASIR was higher in females than in males between the ages of 25 and 59 years. The ASIR for males was higher than that of females above the age of 60, peaking at 710.8 per 100,000 at 75 years or older. In contrast, the ASIR fell to 452.7 per 100,000 in females aged 75 year and above. The age group of 60 to 64 years old had the greatest number of cancer cases (105,561 female cases and 106,542 male cases).

The Relative Proportion of Cancer Cases Across All Sites in Hospital-Based Cancer Registries

A total of 58 HBCR centers that have successfully finished data transmission and quality checks for 1 or more years between 2012 and 2016 were chosen for inclusion in the study out of the 236 HBCR centers registered with NCRP. Several of these hospitals' data (42 out of 58) are included in the NCDIR-NCRP network for the first time. The analysis excludes the remaining HBCRs due to insufficient data. According to pooled data from 58 HBCRs, the number and relative proportion of patients by treatment types, educational attainment, and clinical severity of disease at the time of diagnosis are shown for a few selected locations. The reporting institute (RI) has exclusively treated cases; instances that were previously processed outside of RI have not been analyzed. In relation to all instances of cancer with microscopically diagnosed tumors, the major histologic type (according to the World Health Organization categorization of tumors) and its relative proportion have been described for the 58 HBCRs in this study. Males made up 52.9% and females made up 47.1% of the 667,666 cases that were registered. Male and female cancer patients at Tata Memorial Hospital in Mumbai accounted for the greatest number of newly reported cases across all cancer sites. The relative proportion and new cases reported for all sites of cancer in 58 HBCRs is provided in ►**Supplementary Table S1** (online only).

Table 3 Estimated trends in number of cancers for the leading sites (ICD-10 codes) in India (2015, 2020, and 2025)

Males				Females			
Cancer site (ICD 10)	**2015**	**2020**	**2025**	**Cancer site (ICD 10)**	**2015**	**2020**	**2025**
Lung (C33–34)	63,087	71,788	81,219	Breast (C50)	180,252	205,424	232,832
Mouth (C03–C06)	50,779	57,380	64,519	Cervix (C53)	65,978	75,209	85,241
Prostate (C61)	36,419	41,532	47,068	Ovary (C56)	38,607	43,886	49,644
Tongue (C01–C02)	35,336	39,902	44,861	Corpus uteri (C54)	23,175	26,514	30,121
Stomach (C16)	28,815	32,713	36,938	Lung (C33–34)	23,163	26,490	30,109
Others	387,301	436,106	488,970	Others	296,027	335,235	378,271
All sites	**601,737**	**679,421**	**763,575**	**All sites**	**627,202**	**712,758**	**806,218**

Abbreviation: ICD-10, International Classification of Diseases, Tenth Revision.

Table 4 The estimated top five leading sites of cancer (number and proportion) in India by age group (0–14, 15–39, 40–64, and 65+ age groups) and sex for the year 2024

Males			Females
Cancer site	***n* (%)**	**Cancer site**	***n* (%)**
0–14 years			
Lymphoid leukemia (C91)	6,365 (29.06)	Lymphoid leukemia (C91)	3,568 (24.07)
Brain and NS (C70–C72)	2,740 (12.6)	Brain and NS (C70–C72)	2,100 (14.17)
NHL (C82–86, C96)	1,720 (7.6)	Bone (C40–C41)	1,226 (8.27)
Hodgkin's disease (C81)	1,624 (7.6)	Myeloid leukemia (C92–C94)	1,270 (8.56)
Myeloid leukemia (C92–C94)	1,601 (7.31)	NHL (C82–86, C96)	953 (6.42)
Other sites	7,848 (36)	Other sites	5,705 (38.5)
All sites	**21,898 (100)**	**All sites**	**14,822 (100)**
15–39 years			
Mouth (C03–C06)	10,122 (12.60)	Breast (C50)	27,562 (27.05)
Tongue (C01–C02)	7,652 (9.53)	Thyroid (C73)	13,324 (13.07)
Brain and NS (C70–C72)	5,923 (7.4)	Ovary (C56)	7,966 (7.82)
Myeloid leukemia (C92–C94)	5,400 (6.72)	Cervix (C53)	6,821 (6.69)
NHL (C82–86, C96)	5,012 (6.23)	Myeloid leukemia (C92–C94)	3,625 (3.56)
Other sites	46,216 (57.5)	Other sites	42,568 (41.8)
All sites	**80,325 (100)**	**All sites**	**101,866 (100)**
40–64 years			
Lung (C33–34)	38,537 (11.0)	Breast (C50)	142,124 (32.3)
Mouth (C03–C06)	38,800 (11.08)	Cervix (C53)	53,687 (12.20)
Tongue (C01–C02)	26,354 (7.52)	Ovary (C56)	29,514 (6.70)
Esophagus (C15)	19,964 (5.7)	Corpus uteri (C54)	20,142 (4.6)
Stomach (C16)	19,231 (5.5)	Lung (C33–34)	15,841 (3.60)
Other sites	207,254 (59.19)	Other sites	178,652 (40.6)
All sites	**350,141 (100)**	**All sites**	**439,960 (100)**
65+ years			
Lung (C33–34)	37,600 (12.90)	Breast (C50)	51,123 (22.7)
Prostate (C61)	34,562 (11.9)	Cervix (C53)	21,134 (9.4)
Esophagus (C15)	15,963 (5.5)	Lung (C33–34)	13,258 (5.9)
Stomach (C16)	15,623 (5.3)	Ovary (C56)	13,245 (5.87)
Mouth (C03–C06)	15,784 (5.40)	Mouth (C03–C06)	10,246 (4.54)
Other sites	172,231 (59.0)	Other sites	116,258 (51.6)
All sites	**291,763 (100)**	**All sites**	**225,264 (100)**

Abbreviations: NHL, non-Hodgkin lymphoma; NS, nervous system.

Cancer Associated with Tobacco in Males and Females

It is established that tobacco use is linked to malignancies in several anatomical locations. The World Health Organization's monographs on general assessments of carcinogenicity, published by the International Agency for Research on Cancer (IARC) in 1987, have been the basis for the NCRP's classification system. More anatomical sites discussing the connection between tobacco use and cancer have been added to the most current IARC monographs. The following regions are used to group the data from all 58 HBCRs: North, South, East, West, Central, and North East. This is performed irrespective of the patient's residency status.

With a cancer incidence rate of 70.4% in men and 46.5% in women, the East Khasi Hills district of Meghalaya has the greatest relative proportion of malignancies linked to tobacco smoking. Thiruvananthapuram district had the lowest number of cancer sites associated with tobacco use (10.1%) among females, while West Arunachal had the lowest percentage among males (24.5%). The NE states of India had the highest percentage of female smokers with cancer, followed

by the central and western regions of India with the highest number of registries. The relative proportion of specific sites of cancer associated with the use of tobacco by region is mentioned in ►**Supplementary Table S2** (online only).

Lung Cancer

The incidence rates of lung cancer in both males and females in the PBCRs of Bangalore, Delhi, Chennai, and Kamrup urban have significantly increased. In contrast to the 11 PBCRs involving females, 5 PBCRs involving males showed a statistically significant rise in incidence rates. In terms of male and female incidence rates, Aizawl district ranked highest at 38.8 and 37.9 per 100,000, respectively. Data on the overall number of lung cancer cases that have been reported, together with the disease's relative proportion and incidence rates, are included in ►**Supplementary Tables S3** and **S4** (online only).

Lung cancer rates among nonsmokers are increasing, and significant contributing factors are radon gas, indoor smoke from solid fuels, outdoor air pollution, and hazardous dust exposure. Other notable concerns include genetic predisposition and passive smoking. Preventive measures such as promoting smoke-free environments, improving indoor ventilation, and reducing air pollution can play a vital role in mitigating these risks.

Discussion

Cancer is not homogeneous in India. For both males and females, the incidence rates in Aizawl district were found to be seven times and four times higher, respectively, than those in Osmanabad and Beed district PBCRs. Compared with other parts of the nation, the NE region had the highest cancer incidence rate (six PBCRs for males and four PBCRs for females). Cancer was most common in the NE region in the following areas: stomach, liver, gallbladder, esophagus, nasopharynx, hypopharynx, cervix uteri, lung, and breast. The low 5-year survival rates of head and neck, breast, and cervical cancer in comparison to the rest of India indicate that the NE region lacks the necessary infrastructure in terms of specialist treatment facilities and human resources. A significant percentage of cancer patients from the NE region go outside of the region for their care. As shown in Thailand, the variety in cancer incidence pattern and variances in India may have been influenced by local cultural variables and lifestyle choices.

The most prevalent malignancies in men were those of the lung (9 PBCRs), mouth (9 PBCRs), esophagus (5 PBCRs), stomach (4 PBCRs), and nasopharynx (1 PBCR). The most common cancer in urban areas and the south was lung cancer, whereas the most common cancer in the west and central regions was mouth cancer. The most prevalent malignancies among men in the Indian subcontinent were lung and mouth cancers. Most cases in the NE part of India were stomach, nasopharyngeal, and esophageal cancers. Compared with the rest of India, this region has a different cancer incidence pattern. The cancer incidence pattern is comparable to that of Southeast Asia. All things considered, these cancer pattern findings aligned with previously released data under NCRP.

The hospital database was employed for this type of study since it is challenging to extract data from PBCRs regarding the clinical severity of the illness and its course of treatment. Most cases of cervix uteri and breast cancer were discovered after they were already locally progressed. The standard treatment for cervical cancer in locally advanced or metastatic stages is a combination of chemotherapy and radiation. Additionally, vascular endothelial growth factor inhibitors and immunotherapy have enhanced treatment options, providing a more comprehensive approach to managing the disease. In India, a multi-institutional study on cervix cancer revealed that, in the locally advanced stage, chemotherapy and radiation proved to be substantially more effective in survival than radiation therapy alone. Concurrent chemoradiation for locally advanced cervical cancer produced the best disease-free survival, according to a study from Chennai. When it came to head and neck malignancies from HBCRs, two-thirds of the patients had a locoregional cancer diagnosis. The data from Uttarakhand reveal a concerning trend in the diagnosis of head and neck cancer, with most patients (88.1%) presenting at advanced stages. This suggests that early detection methods are insufficient, highlighting the need for more effective screening programs and public awareness initiatives. The fact that only 8.5% of patients are diagnosed in the early stages points to a significant gap in health care practices, which could lead to worse outcomes for patients. To address this issue, focused efforts are required to raise awareness, enhance detection capabilities, and implement region-specific strategies for early diagnosis and intervention. This is particularly crucial in areas with high incidence rates of head and neck cancers, where timely treatment can significantly improve survival rates. According to an estimate from a multi-institutional study, poor survival occurred in 65% of newly diagnosed head and neck malignancies with locally advanced disease because they did not receive the best possible care.

The lack of notification for cancer makes it difficult to register cases in India and makes data collection more difficult.[21–24] The anticipated cancer burden in India for 2001 was derived from the data obtained from the three PBCRs.[25] Updates to cancer estimates were released based on PBCRs that were available as a result of their expansion.[26–29] Recently, GLOBOCAN estimated the cancer incidence in India for 2020 using data from 27 PBCRs from 2012 to 2014 and the ASIR, which estimates cancer incidence across five continents.[30,31] Based on the assumption that the ASIR in 2020 would remain unchanged, China predicted the incidence for the year 2022.[32] There are various flaws in the mortality registration system, one of which is the erroneous and incomplete certification of the reason for death.[33,34] A framework for evaluating India's cancer trends and status is provided by this research. As a result, the national NCD targets and the sustainable development goals will be met, and work to promote cancer prevention and control will receive the proper support.[8,35]

Breast cancer is the leading cancer in women, with family history being a key risk factor. Women with close relatives who had breast cancer, especially at a young age, are at higher risk. Genetic testing can identify mutations in genes like BRCA1 and BRCA2, which significantly increase the risk of breast and ovarian cancers. Other genetic mutations, such as in PALB2 and CHEK2, can also raise risk. Genetic testing helps guide preventive measures and informed health decisions, though not all cases are hereditary. Family history and genetic testing are valuable for assessing risk and prevention.

Adolescent human papillomavirus (HPV) vaccination helps prevent cervical cancer by focusing on the virus that causes the disease. Early screening for women, such as Pap smears and HPV tests, can detect precancerous alterations in the cervix. Early treatment is possible with the timely discovery of these lesions, which lowers the risk of malignancy. Implementing screening and immunization initiatives can significantly reduce the incidence of cervical cancer. These preventive approaches decrease the burden of cervical cancer and improve overall health outcomes.

In conclusion, India's cancer incidence burden is still rising. Breast cancer ranked highest among the five most common cancers in women, followed by cancers of the cervix, ovary, and corpus uteri. Three sites were restricted to tobacco-related malignancies in males: the tongue, mouth, and lungs. To lessen the burden of cancer in the future, a preventative action must be performed. The updated estimates are beneficial for early diagnosis, risk reduction, and management initiatives related to cancer prevention and control in India. To go further into the causes of the cancer burden and offer practical remedies, however, appropriate research is required.

Limitations of the Study

There are several challenges to be addressed while registering a case in India to gather data, as cancer is not a disease that needs to be reported. Because of limits in the number of deaths from cancer, mortality data were incomplete due to inadequate coverage of the Civil Registration System. Accurate death statistics have proven difficult to obtain, and the quality of the data differs throughout PBCRs. As a result, no attempt was made to estimate incidence from death or survival. Statistical modeling techniques like age–period–cohort could not be included because most of the PBCRs did not have longer data periods available. In low- and middle-income nations, launching a PBCR presented several difficulties. For the goal of cancer control, a sample of regional PBCRs or a group of regional PBCRs with 10% of coverage would be very beneficial. This is the finest cancer data available in the nation for estimation under these circumstances. The burden estimates for India and a few of the neighboring nations in South Asia have been derived from GLOBOCAN and IARC using the identical PBCRs. In India, cancer incidence might be better covered with fewer resources if health care providers registered through passive notification. For the smooth enhancement of cancer statistics, cancer registries must be connected to many national and local databases (Ayushman Bharat, other insurance programs, mortality databases, Health Management Information System, etc.).

Conclusion

The NE region of India had the highest cancer incidence recorded. The most prevalent malignancies in men were those of the mouth, throat, stomach, and esophagus. The most prevalent malignancies in women were cervix uteri and breast cancer. Oropharyngeal, nasopharyngeal, hypopharyngeal, esophageal, stomach, liver, gall bladder, larynx, lung, and cervix uteri cancers had the highest cancer burden in the NE. For stomach and lung cancer, systemic therapy was the most often used form of treatment. In the world, people over 65 years account for half of all cancer cases, while in India, the proportion is one-third. In India, however, the 40- to 64-year-old age group accounts for half of the estimated cancer burden. According to estimates, the incidence of cancer cases will rise from 2.8% in 2020 to 12.8% in 2025. By 2025, 29.8 million disability-adjusted life years are expected to be caused by cancer in India, according to a recent NCRP study. The burden of cancer in the future must be decreased by taking a preventative action. When it comes to early detection, risk reduction, and management initiatives aimed at controlling cancer in India, the updated estimations are beneficial. But to address the causes of the cancer burden and offer practical remedies, adequate research is required.

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Conflict of Interest
None declared.

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Biallelic Mismatch Repair Deficiency in Children and Adolescents: A Review of Published and Unpublished Data from India—Need for an Indian Consortium

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Abstract

Introduction Biallelic mismatch repair deficiency or constitutional mismatch repair deficiency (CMMRD) is a rare and aggressive pediatric cancer predisposition syndrome that occurs as a result of homozygous (biallelic) pathogenic variants in mismatch repair genes. The primary malignancies that occur in CMMRD are mainly hematological and brain malignancies. Most published data are from the western populations and the Middle East. Data from India are limited to case reports. We performed an analysis to determine the prevalence of CMMRD in the Indian population.

Materials and Methods All children aged less than 18 years with a diagnosis of CMMRD from various centers in India were included. CMMRD confirmed using genetic, molecular, and clinical criteria by an international consensus was included in the analysis. Literature search and data submitted by individual centers were reviewed.

Results The analysis revealed that 22 children had genetically confirmed CMMRD. The median age of the cohort was 6.5 years, with a male predominance (male:female, 2:1). The classical phenotype of café-au-lait macules was observed in 72.7 % of subjects. The most common pathological variant was found in the *PMS2* gene, which accounted for 77.3 % of children. Hematological malignancy (T cell acute lymphoblastic leukemia) was the most common primary malignancy in our study that occurred at a median age of 5 years (interquartile range 4–6 years) followed by brain tumors. The age at initial presentation for CMMRD with mutations in *MSH2*, *MSH6*, and *PMS2* was 5.4, 4, and 7.5 years, respectively.

Keywords
- CMMRD
- India
- childhood and adolescence
- genetic testing

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Conclusion The diagnosis of CMMRD requires a high index of suspicion for the early diagnosis, management, surveillance, counseling, and testing of family members. The awareness about CMMRD in clinicians is important so that diagnosis is made early, and a second malignancy is detected and treated early. The need for an Indian consortium to determine the actual burden of the disease, genetic characteristics, and course of illness in our country has been emphasized.

Introduction

Biallelic mismatch repair deficiency (BMMRD) or constitutional mismatch repair deficiency (CMMRD) is a rare childhood cancer predisposition syndrome with an autosomal recessive inheritance.[1] While Lynch syndrome (LS) is associated with heterozygous (monoallelic) germline pathogenic variant in one of the mismatch repair (MMR) genes, CMMRD occurs because of homozygous (biallelic) pathogenic variant in these genes.[2] Primary malignancies that occur in LS are usually of colorectal and endometrial origin. In addition to hematological and central nervous system (CNS) malignancies, colorectal malignancies are well-known and frequent in CMMRD.[3]

Most of the published data are from the western population, as well as from the Middle East. In 2024, the findings of the study with a large cohort of more than 200 patients with CMMRD, led by the International Replication Repair Deficiency Consortium (IRRDC), was published by Ercan et al.[1] Data from India were limited to case reports. We performed a literature search to determine the prevalence of CMMRD in the Indian population.

Materials and Methods

An online literature search was done to obtain published data on pediatric CMMRD cases from India. Various centers across India were contacted for data on unpublished and confirmed CMMRD cases. Only pathogenic variant-proven CMMRD cases in children from India were included in this study.

Study Design

Retrospective study: Sample size – 22 children with CMMRD.

Primary and Secondary Outcome

The primary objective was to find the clinical presentation, type of cancer, and age of onset of primary and secondary malignancy and progression of the disease. The most common malignancy was hematological malignancy with a median age of 6.5 years (interquartile range [IQR] 4–9years) at presentation and the second most common malignancy was brain tumors at a median age of 11.5 years (IQR 8–15 years) with parental consanguinity a vital pointer toward diagnosis.

The secondary objective was to find the severity of illness and survival associated with each MMR gene and its pathological variant. Children with pathogenic variants in *MSH2* and *MSH6* tend to have an earlier onset of malignancy. *PMS2* pathogenic variants were the most common and children with *MSH2* or *MLH1* had severe disease. The incidence of pathogenic variants in *MLH1* and *MSH2* were much less than the incidence of pathogenic variants of *PMS2* and *MSH6*.

Inclusion Criteria

All children less than 18 years with a diagnosis of CMMRD from various centers in India were included. CMMRD confirmed using clinical, genetic, and molecular criteria by an international consensus was included in the analysis. Data collected from published as well as unpublished data provided by centers treating children with malignancies were considered the full study cohort. The initial literature search was executed by searching the PubMed, Cochrane Library, and Web of Science databases for studies in the English language. The search words were "CMMRD, BMMRD" restricted to "India" and then limited to "childhood" and "adolescence."

Exclusion Criteria

Duplicate publications and cases with no confirmatory genetic study were excluded.

Data Abstraction

Two investigators reviewed all the studies that were obtained and confirmed that they fulfill the inclusion criteria. Duplicate publications were excluded from analysis. Patients who had no confirmatory diagnosis of CMMRD based on molecular studies were excluded. The studies selected for data collection are included in ►**Annexure 1** (available in the online version).

All subject data submitted by individual centers were also reviewed for eligibility for entry into the analysis. An overview of this study is given in ►**Fig. 1**.

Analysis

The median values, IQR, and percentages were calculated from the data.

Various clinical parameters such as age at diagnosis, gender, types of first and second malignancies, consanguinity and other affected family members, and their molecular profiles were analyzed. All patients were also given a score as per the scoring system to determine germline testing eligibility for CMMRD (►**Supp. Table S1**, available in the online version).

Ethical Approval

The Institutional Ethics Committee had granted approval for this retrospective study (Institutional Ethics Committee, MVR Cancer Centre & Research Institute, Kerala, India).

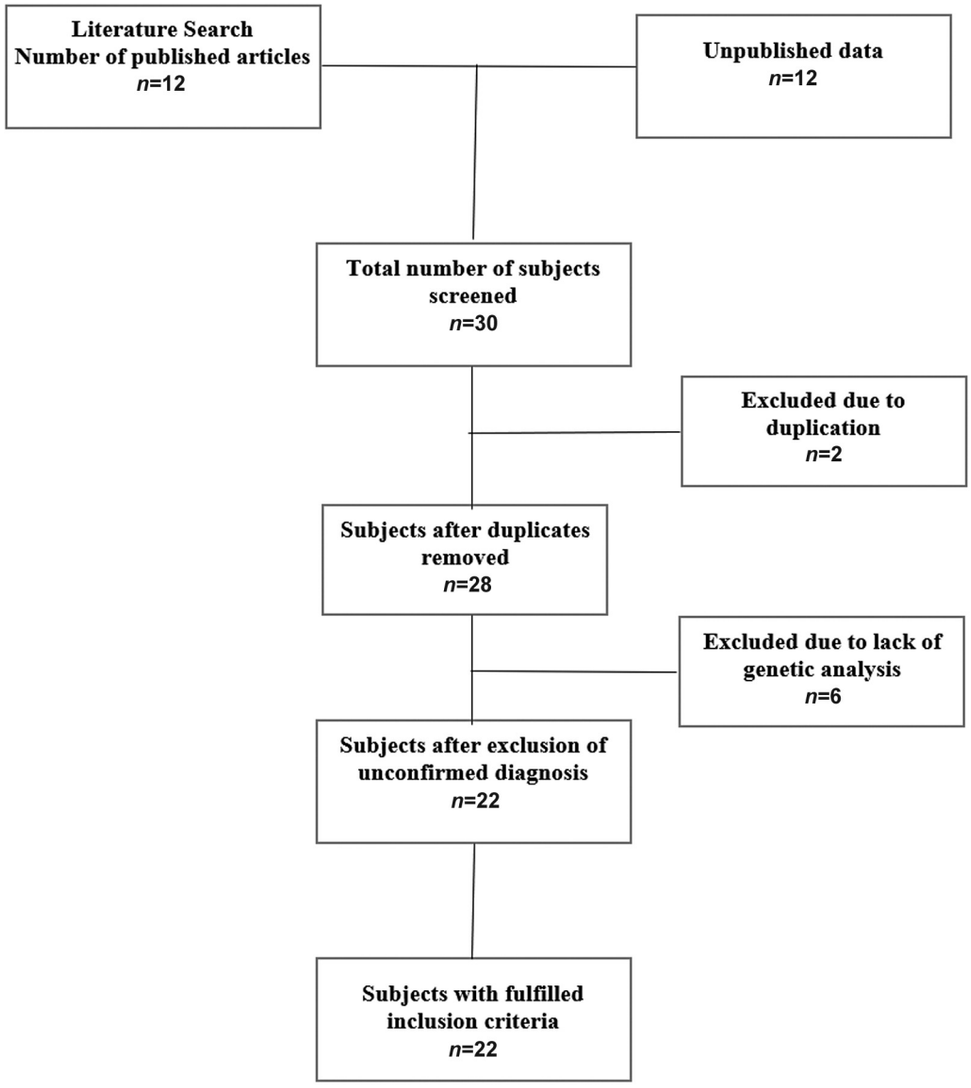

Fig. 1 Study overview of constitutional mismatch repair deficiency subjects included in the analysis.

Approval No.: EC Ref No.: IEC2023/III/02, dated: 08/12/2023.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Results

We analyzed the data of 22 children with genetically confirmed CMMRD from different centers across India. Of the 22 subjects analyzed, 15 were males and 7 were females. The male:female ratio was 2:1. The median age at diagnosis of the first malignancy was 6.5 years (IQR 4–9 years). The median age at diagnosis was 7 years (IQR 4–11 years) and 6 years (IQR 4.4–9 years) for males and females, respectively. In this study, a high incidence of consanguinity was observed. Fourteen of 22 (63.6%) children were born out of a third-degree consanguineous marriage which is a high rate of consanguinity. The parents were unaffected and did not have features of LS.

Sixteen (72.7%) participants had siblings with malignancies. Upon analyzing the clinical parameters, all children had normal development and intelligence (95%), except for one child who had delayed speech development. Skin involvement in the form of café-au-lait macules (CALM) was observed in 16 children (72.7%). One child each had a hypopigmented macule, pilomatricoma, and nevus spilus, in addition to CALMs. All children had strong evidence of CMMRD according to the CMMRD diagnostic criteria as per updated international diagnostic criteria for CMMRD published by Aronson et al in 2022.

All patients were given a score according to the scoring system to determine germline testing eligibility for CMMRD, and the median score in our study was 5.5. A score of ≥ 3 requires testing for MMR gene pathogenic variant. The indications for CMMRD testing are listed in ►**Supp. Table S1** (available in the online version).

Table 1 Clinical parameters of children diagnosed as CMMRD

Parameter	Number (percentage)
Median age at first malignancy (range)	6.5 y (IQR 4–9 y)
Median age in males	7 y (IQR 4–11 y)
Median age in females	6 y (IQR 4.4–9 y)
Sex	
Male	15 (68.2)
Female	7 (21.8)
Consanguineous marriage	14 (63.6)
Family history	16 (72.7)
Skin findings	16 (72.7)
Normal development and intelligence	21 (95
Median age at second malignancy, $N = 8$	12 y (IQR 10–15 y
CMMRD score, mean	6
Relapse	5 (24)
Pathologic variants, genes affected	
PMS2	17 (77.2)
MSH2	4 (18.1)
MSH6	1 (4.5)
MLH1	0 (0)
Additional pathologic variants	*PMS2*
	POLE
	TP53

Abbreviations: CMMRD, constitutional mismatch repair deficiency; IQR, interquartile range.

Of all the pathogenic variants analyzed, the most common was in *PMS2*, accounting for 77.3%. *MSH2* and *MSH6* constituted 18.2 and 4.5% of the total, respectively. There were no children with *MLH1* pathogenic variant in our study. Additional pathogenic variants were detected in *PMS2* (additional second pathogenic variant), *POLE*, and *TP53* genes (►**Table 1**).

The spectrum of primary and secondary malignancies diagnosed, with the median age at diagnosis in our series, is shown in ►**Table 2**. The most common first malignancy diagnosed in our children was a hematological malignancy, accounting for 52.4% (T-acute lymphoblastic leukemia was the most common). The median age at diagnosis was 5 years (IQR 4–6 years). The second most common malignancy was brain tumor, accounting for 38.1%, and the median age at diagnosis was 10 years (IQR 7–12 years). Colorectal cancers were observed in two children, with a median age at diagnosis of 9.5 years (IQR 9–10 years). In our series, a second malignancy was seen in eight children (36.3%), and the median age of occurrence was 12 years (IQR 10–15 years). The malignancies noted were colorectal cancer, non-Hodgkin's lymphoma (NHL), astrocytoma, and sarcoma (osteosarcoma and alveolar soft part sarcoma) (►**Table 3**).

Children who developed brain tumors had a worse outcome than children who developed hematological or colorectal malignancy. Only 3 of the children with brain tumor survived to develop a second malignancy.

The ages at initial presentation for CMMRD with pathological variants in *MSH2*, *MSH6*, and *PMS2* were 5.4, 4, and 7.5 years, respectively. Children with pathological variants in *MSH2* and *MSH6* tend to have an earlier onset of malignancy. Of the 17 children with *PMS2* pathological variants, 8 (47%) developed a second malignancy at a median age of 12 years (IQR 10–15 years). None of the children with *MSH2* or *MLH6* pathological variants lived long enough to develop a second malignancy. There were no children affected with *MLH1* pathological variants in our study.

The detailed genetic characteristics of the entire cohort with detected malignancies are shown in ►**Table 4**.

Discussion

The role of MMR genes in the pathogenesis of malignancy is well known to the scientific community. LS occurs due to the heterozygous (monoallelic) germline pathological variants in the MMR genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*, and are autosomal dominantly inherited.[4] The majority of CMMRD were probably misreported as LS until the case report by Ricciardone et al, which was first published in 1999.[5] The authors identified a family with hereditary nonpolyposis

Table 2 Genes affected in the CMMRD cohort and age at presentation

Genes affected	Number of patients *N* (%)	Median age in years at first malignancy (IQR)	Median age at second malignancy in years (IQR)	Skin findings	Siblings affected
PMS2	17 (77.2)	8 (4–5)	($N = 8$) 12 (10–15)	11 (64%)	8
MSH2	4 (18.1)	10 (1–11)		4 (100%)	4
MSH6	1 (4.5)	4		1 (100%)	1
MLH1	0	0			
Total	22 (100)			15	12

Abbreviations: CMMRD, constitutional mismatch repair deficiency; IQR, interquartile range.

Table 3 Types of malignancies and median age of onset in each type

Type of malignancy	First malignancy *N* (%)	Median age at first malignancy in years (IQR)	Second malignancy *N* (%)	Median age at second malignancy (y)
Hematological				
T-ALL	6 (27.4)	6 (4–8)		
B-ALL	3 (13.6)	4 (3.5–4.4)		
NHL	2 (9)	4	2 (25)	13
Brain tumors				
GBM/Astrocytoma	4 (18.2)	9 (5–11)	2 (25)	10.5
Medulloblastoma	3 (13,6)	11		
High-grade glioma	1 (4.6)	15		
Lynch syndrome associated				
Colorectal cancer	2 (9)	9.5	2 (25)	16
Other cancers				
ASPS			1 (12.5)	8
Osteosarcoma			1 (12.5)	12
No malignancy	1 (4.6)			
Total	22 (100)		8 (100)	

Abbreviations: ALL, acute lymphoblastic leukemia; ASPS, alveolar soft part sarcoma; GBM, glioblastoma multiforme; IQR, interquartile range; NHL, non-Hodgkin lymphoma.

Table 4 Genetic characteristic of the entire cohort with malignancies detected

Sl. no.	*PMS2*	Exon	*MSH2*	Exon	*MSH6*	Exon	Homozygous	First and second malignancies (age at diagnosis in years)
1	delC	11					Yes	T-ALL (8); GBM (12)
2	delC	11					Yes	T-ALL (6); ASPS (8)
3	double heterozygous (p. Ser815Leu and p.Gln275Gln)						Yes	CRC (9); GBM (9)
4	c.1500delC at codon 501	11					Yes	GBM (11); CRC (15)
5	c.1500delC at codon 501	11					Yes	Medulloblastoma (9); NHL (11)
6	c.2402C > T	14					Yes	GBM (9)
7	c.2402C > T	14					Yes	CRC (10)
8	c.325dupG	4					Yes	T-ALL (4); Relapse (8)
9	c.325dupG	4					Yes	T-ALL (4); NHL (15)
10	c.325dupG	4					Yes	NHL (7)
11	c.478C > T	5					Yes	GBM (4)
12			(c.221_231del)	2			Yes	GBM (5)
13			(c.221_231del)	2			Yes	High-grade glioma (15)
14	(c.2404C > T)	14					Yes	

Table 4 (*Continued*) Genetic characteristic of the entire cohort with malignancies detected

Sl. no.	*PMS2*	Exon	*MSH2*	Exon	*MSH6*	Exon	Homozygous	First and second malignancies (age at diagnosis in years)
15	(c.2404C > T)	14					Yes	B-ALL (4)
16					c.1670G > A	4	Yes	B-ALL (4) Relapse
17	c.128_130del	2					Yes	T-ALL (6); Osteosarcoma (12)
18	c.525_534del	5					Yes	B-ALL (3); Relapse (4)
19	c.778del	7					Yes	Medulloblastoma (12); CRC (17)
20	c.778del	7					Yes	Medulloblastoma (12)
21			c.1165C > T	7			Yes	T-ALL (11 mo)
22			c.1165C > T	7			Yes	NHL (1 y)

Abbreviations: ALL, acute lymphoblastic leukemia; ASPS, alveolar soft part sarcoma; CRC, colorectal cancer; GBM, glioblastoma multiforme; NHL, non-Hodgkin lymphoma.

colorectal cancer, with three children who had hematological malignancy at a very young age and had a neurofibromatosis phenotype. Deoxyribonucleic acid analysis revealed the presence of a homozygous *MLH1* pathological variant. Since then, biallelic germline pathologic variants involving MMR genes have been described in approximately 200 patients and have been recognized as distinct cancer predisposition syndromes: constitutional or biallelic MMR deficiency (CMMRD/BMMRD) syndrome (OMIM #276300).[6]

The IRRDC was established in 2007, and since have identified more than 100 patients from different countries across the world.[3]

A large cohort of patients with CMMRD was reported by Wimmer and Etzler in 2008, with 78 cases detected in 46 families.[7] In 2013, a European consortium was formed —"Care for CMMRD" (C4CMMRD)—which identified 146 patients from 91 families.[8,9] In 2022, latest recommendations and guidelines from the international consensus working group (IRRDC, C4CMMRD, and experts dedicated to CMMRD) for diagnosis and surveillance for individuals with CMMRD was published by Aronson et al. They established six diagnostic criteria (four criteria with strong evidence and two criteria with moderate evidence) and also outlined the surveillance and ancillary tests needed in each group (►**Supp. Table S2**). A scoring system to identify the eligibility for genetic testing was also published.[3]

In 2024, Ercan et al published a study involving more than 200 patients; the largest study led by the IRRDC.[1] The Middle East Network on Hereditary Colorectal Cancer was also established with the aim of obtaining more information on the epidemiology of hereditary colorectal cancer and CMMRD in the Middle East.[10] A position paper in 2020 highlighted the challenges of CMMRD diagnosis in low-resource settings and the need for more data given the high levels of consanguinity in this particular population.[11] In our Indian study, we identified 22 cases from 11 families.

The most common malignancies associated with CMMRD in the study by Ercan et al were CNS tumors (51%), followed by gastrointestinal (GI) malignancy (22%).[1] In our study, the most common malignancy was hematological malignancies accounting for 52.4% of all cases followed by CNS tumors (38.1%) and GI malignancies (9%). This disparity could be primarily due to the reason that the study was conducted in children less than 18 years of age, where hematological malignancy is the most common malignancy. A large population-based study is required to establish age and the type of malignancies that occur in our population.

The median age of onset of malignancies was younger in our study (6.5 years) than in other studies (Ercan et al, 8.9 years).[1] The typical age of onset is the first decade for hematological and brain malignancies, and the second or third decade for LS-associated malignancies. According to data from C4MMRD, NHL was the most common hematological malignancy, mainly the T-lymphoblastic type, followed by acute lymphoblastic leukemia (ALL) and acute myeloid leukemia.[9] The high incidence of T-cell lymphoma suggests an inefficient immunoglobulin class switch and subclinical immune deficiency.[5] Among malignant brain tumors, glioblastoma and high-grade astrocytic tumors constitute the majority, followed by medulloblastoma and supratentorial primitive neuroectodermal tumors.[3,9] Among the LS-associated tumors, GI malignancies are the most common; endometrial and urinary tract/bladder malignancies being less frequently reported.[3] Childhood-onset colorectal cancer with a mean age of 16.4 years at diagnosis is the most common GI malignancy, followed by that of the small intestine.[12] Other embryonal tumors such as neuroblastoma, Wilms tumor, and rhabdomyosarcoma have also been reported.[3] In our analysis, although ALL was the most common malignancy, the mean age at diagnosis was younger than that in other studies described in the literature.[1,9] A similar trend was

observed with the mean age of patients with colorectal malignancy also.

Children with CMMRD have a phenotype characteristic of this condition. The most common clinical finding is the presence of CALM. These hyperpigmented macules have more diffuse irregular borders and have hypopigmented areas within, when compared to the CALMs observed in patients with neurofibromatosis 1 (NF1).[13,14] Lisch nodules, axillary freckling, and plexiform neurofibroma seen more commonly in NF1 are rarely observed in CMMRD.[1,3], Developmental abnormalities of the brain, such as agenesis of the corpus callosum and venous and vascular anomalies, are among the described clinical findings.[3] In our analysis, all children with *MSH6* and *MLH2* pathogenic variants had skin findings in the form of CALMs. In children with *PMS2* pathogenic variants, 64% had CALMs. Pilomatricoma and nevus spilus were also seen in 2 children with *PMS2* pathogenic variants. The other benign tumors described are polyps of the stomach, small and large intestines, and hepatic adenoma.[9]

The overall incidence of CALMs was 72.7% as against 89% in the study by Ercan et al.[1] Almost 10 to 25% of children with CMMRD can present without skin involvement. A high index of suspicion is required in children who present without CALMs, for early diagnosis especially in a setting of consanguinity and occurrence of second malignancy.

The most common gene affected was *PMS2*, accounting for 77.3% (as against 60% in Aronson et al[3] and 65% in Ercan et al)[1]. *MSH2* and *MSH6* constitutes 18.2% (as against 10–20% by Aronson et al and 5% by Ercan et al) and 4.5% (as against 20–30% by Aronson et al and 26% by Ercan et al) of the total, respectively.[1,3]

A history of parental consanguinity is the most important clue that might allow the physician to suspect CMMRD, especially in a child presenting with a malignant brain tumor, hematological malignancies, or childhood-onset GI cancer.[13] Results from communities with high rates of consanguinity suggest a high incidence of CMMRD.[13,15] Our study also found high rates of consanguinity (63% as against 54% in study by Ercan et al and 39–45% by Aronson et al).[1,3]

National Family Health Survey from India suggests that the prevalence of consanguinity is high in certain communities, especially in South India, and only a nationwide study on CMMRD can provide a clearer picture of the situation in India.[15] Most parents of the affected children are asymptomatic and do not have a history of malignancy, even though they are heterozygous carriers of the disease. This is probably because malignancies associated with LS usually develop during the fourth decade of life. In addition, owing to the low penetrance of *PMS2* (the most common MMR pathogenic variant), clinical evidence of LS in family may be absent.[1]

Once a pathogenic variant is identified, the pathogenic nature of the variant has to be determined from the available genetic databases. Biallelic pathogenic variants in the MMR genes *PMS2*, *MLH1*, *MSH6*, and *MSH2* are responsible for the development of CMMRD, with *PMS2* and *MSH6* being the most common. In the IRRDC cohort of 201 patients, 65% carried the *PMS2* pathogenic variants and the rest carried the *MSH6* and *MLH1*/*MSH2* bialleles.[1] This observation is in contrast to LS, in which the majority of patients carry heterozygous *MLH1* or *MSH2* mutations.

In our analysis, *PMS2* was the most common mutation identified. No child was found to have pathogenic variants in *MLH1*. This could be either due to, less testing for CMMRD or because *MLH1* mutations are rare in India. Or it could be that the disease was so severe that the affected proband did not survive long enough to undergo genetic testing. None of the children in our study had a synchronous malignancy. All the children who developed a second malignancy ($n = 8$) had it only beyond 6 months from first diagnosis (metachronous).

CMMRD is the most penetrant and aggressive pediatric cancer predisposition syndromes and patients can develop another malignancy every 2 years and *MLH1* and *MSH2* genes were found to be more aggressive.[1]

In the literature, it has been observed that primary hematological malignancies were infrequent or absent in the *MLH1* or *MSH2* pathological variant group compared to GI and brain tumors that were seen with all the MMR genes.[1] In our cohort of four children who had *MSH 2* mutation, two children who presented early (11 months and 1 year; exon 2 mutation) had hematological malignancy and two children who had late presentation had brain tumors (glioblastoma multiforme at 5 years and glioma at 15 years; exon 7 mutation). None of these four children survived the first malignancy. In the study by Ercan et al, hematological malignancy was absent in MLH1 or MSH2 pathogenic variants; and no survivors were found in cohort with MSH2 pathogenic variants.[1] Only one child had an *MSH6* mutation and developed B-cell ALL at 4 years of age. In our cohort, only children with *PMS2* mutations survived the first malignancy and developed a second malignancy. The presence of additional mutations in genes such as *POLE1* was observed in our analysis, which has also been described previously.[1]

Though genetic testing is the gold standard for diagnosing CMMRD, testing challenges can arise due to pseudogenes and frequent gene conversion in *PMS2*; the most frequently affected gene with the highest incidence of variants of uncertain significance. These challenges for interpretation of the results can be overcome by specialized assays and also by following the scoring system to know the eligibility for genetic testing and the diagnostic criteria for CMMRD.

The C4CMMRD consortium has devised criteria for indications for genetic testing among suspected patients using a point-based system.[3] The main criteria used were type of malignancy and age at presentation, such as LS spectrum tumors or multiple bowel adenomas, grade III or IV glioma, and NHL of T-cell lineage or primitive neuroendocrine tumor, in both children and young adults. The additional features considered were NF1 signs, developmental brain abnormalities, and the family history in siblings or first-degree relatives (►**Table 1**). Aronson et al in the year 2022 defined two newer hallmark malignanciesin CMMRD: (1) glioma or CNS embryonal tumors < 18 years and (2) GI adenocarcinoma < 18 years. Only 11 to 15.6% cases presented beyond 18 years of age. The first consensus for diagnostic criteria for CMMRD (members of the IRRDC and the C4CMMRD consortia) laid down six diagnostic criteria

and recommendations for surveillance and genetic counseling of the family.[3]

CMMRD diagnostic criteria are presented in ►**Supp. Table S2**.[3]

Definitive genetic diagnosis has an important role in tumor surveillance, family testing, and further treatment. In addition to genetic testing, use of ancillary testing like microsatellite instability (MSI) in tissue and MMR immunohistochemistry (IHC) showing loss of MMR protein expression can help diagnose CMMRD. IHC from normal tissue was found to have more than 90% sensitivity and specificity in diagnosing CMMRD. Another assay named ex vivo MSI (ev MSI) MSI is considered to be 100% sensitive and 100% specific. Similarly, next-generation sequencing-based MSI is also highly sensitive and specific for CMMRD. These ancillary tests can help when facing atypical cases with diagnostic challenges.[3]

Low-pass genomic instability characterization assay for CMMRD was found to be more sensitive tool than MSI, IHC, and tumor mutational burden and it was able to distinguish CMMRD from other cancer predisposition syndromes. It is useful for diagnosis as well as surveillance of individuals with CMMRD.[16]

Cascade testing (genetic counseling and testing) of family members, especially siblings, is the most important goal, as it helps for early detection and treatment of disease at an early stage.[17]

In the IRRDC study it was found that patients who underwent surveillance had better outcome and surveillance was the single most important confounding factor in the MLH1/MSH2 group (40% survival against 0% survival). The first consensus for diagnostic criteria for CMMRD (members of the IRRDC and the C4CMMRD consortia) has laid down recommendations for surveillance and genetic counseling of the family (►**Table 5**).[1,17,18]

Apart from the conventional treatment modalities for childhood malignancies, multiple studies have found that treatment with immune checkpoint inhibitors like nivolumab and ipilimumab greatly improved survival in advanced metastatic and recurrent cancers, especially in brain tumors (especially high-grade glioma). Combined modality of reirradiation and synergistic immune agents have helped in hypermutant high-grade glioma even after progression of disease.[18–22]

The limitation of our study was that the data were a combined analysis of published and unpublished data from individual centers. This might not be helpful in determining the nationwide prevalence of CMMRD. A more structured analysis with clinical diagnostic criteria, ancillary testing, and genetic testing of all children and family members who satisfy the CMMRD criteria might be ideal. The financial implications of the same are the most important restrictive factors in a low-resource country such as ours. In our analysis, despite the patient having a high CMMRD score, a substantial number of patients did not undergo genetic testing. Genetic analysis was not performed in a centralized laboratory as in IRRDC cohort, but at multiple centers across India.

Conclusion

CMMRD is an aggressive pediatric cancer predisposition disease that can have rapid fatal outcome if not diagnosed early and treated. It needs a high index of suspicion for early diagnosis, more so in a setting of consanguinity. The awareness regarding surveillance and cascade testing for early detection of malignancies in the affected child and siblings at the earliest to ascertain the gene involved is to be emphasized. The probability and risk of a child developing second malignancy in a life time is overwhelming in terms of social, psychological, and financial aspects. The biggest limitation is financial burden as we live in low- to middle-income country where testing and treatment are costly. The genes commonly affected are different in our study cohort and disease characteristics too are different. We need a large population-based study to ascertain if the findings represent the genetic characteristics of Indian population. The authors would like to reemphasize the need for a national policy for management and an Indian consortium on CMMRD.

Table 5 Surveillance protocol for patients with CMMRD

Examination	Start age	Frequency	Tumors	Comment
MRI brain	At diagnosis	Q 6 months	Brain tumors	Should not be replaced with WBMRI
WBMRI	6 years	Once a year	All tumors	Should not replace dedicated CNS imaging
CBC	1 year	Q 6 months	Leukemia	May be considered
Abdominal ultrasound	1 year	Q 6 months	Lymphoma	May be considered
Upper gastrointestinal endoscopy; video capsule endoscopy, ileocolonoscopy	4 to 6 years	Once a year	Gastrointestinal tumors	Upper and lower endoscopy, to increase once polyps are found
Gynecological exam, transvaginal ultrasound, pipelle curettage, urine cytology, dipstick	20 years	Once a year	Genitourinary cancers	As per Lynch syndrome guidelines

Abbreviations: CBC, complete blood count; CMMRD, constitutional mismatch repair deficiency; CNS, central nervous system; MRI, magnetic resonance imaging; WBMRI, whole body MRI scan.

Patients' Consent

The written consent of caregivers for publication has been obtained.

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Conflict of Interest

None declared.

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Effect of Smart Pill Box on Improving Adherence to 6-Mercaptopurine Maintenance Therapy in Pediatric ALL

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Abstract

Introduction 6-Mercaptopurine (6-MP) forms the backbone of maintenance chemotherapy for acute lymphoblastic leukemia (ALL). A Children's Oncology Group study found 3.9-fold increased risk of relapse in children with 6-MP adherence less than 90%.

Objective This article estimates the impact of smart pill box in improving adherence to 6-MP during maintenance phase chemotherapy in children with ALL.

Materials and Methods It is a prospective interventional study done at pediatric oncology clinic of a tertiary care hospital. Participants being 40 newly diagnosed children with ALL. Baseline adherence was assessed and impact of smart pill box was estimated after using it for 60 days. Subjective and objective assessment of baseline adherence and adherence after intervention was done by subjecting the parents of the children to Morisky Medication Adherence Score 8 (MMAS-8) and measurement of patient's red blood cells (RBC) 6-MP metabolites (6-thioguanine [TGN] and 6-methylmercaptopurine [MMP]) levels, respectively, pre- and postintervention.

Results The mean age was 7.39 ± 4.29 years. NUDT15*3 polymorphism was present in 10.26%, and none had *TPMT* polymorphism. Baseline assessment of adherence to 6-MP by MMAS-8 revealed low, medium, and high adherence in 7.5, 35, and 57.5%, respectively. Baseline 6-TGN and 6-MMP levels by cluster analysis revealed poor adherence in 10%. Following intervention, mean MMAS-8 improved from 7.34 ± 0.78 to 7.66 ± 0.55 (p-value < 0.015) and the median 6-TGN level improved from 150 to 253 pmol/8×10^8 RBCs (p-value < 0.001).

Conclusion Nonadherence to 6-MP is widely prevalent in Indian children. Simple measures like smart pill box can improve adherence.

Keywords
- ALL
- maintenance chemotherapy
- adherence
- smart pill box
- 6MP metabolites

Introduction

Acute lymphoblastic leukemia (ALL) is among the most common malignancies in children. Cure rates vary between the risk groups and in developed countries cure rates are up to 90% in low-risk group.[1–4] Maintenance chemotherapy of 2 to 2.5 years of duration is an essential component of the treatment to achieve long-term disease-free remission in ALL.[5–7] Daily oral 6-mercaptopurine (6-MP) and weekly methotrexate administration form the backbone of

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maintenance chemotherapy.[6] Similar to other conditions requiring chronic oral intake of medications, nonadherence to 6-MP is frequently reported in children with ALL.[8,9] Moreover, children are asymptomatic during maintenance phase which may further increase chances of nonadherence. Nonadherence to 6-MP puts them at increased risk of relapse. Indeed, a Children's Oncology Group (COG) study by Bhatia et.al found 3.9-fold increased risk of relapse in children with less than 90% adherence.[8] This increased relapse risk has greater significance in the context of resource-limited setting as observed in most low-and-middle income countries (LMICs), as children with relapsed ALL have very poor access to intensive and novel therapy required for the cure.[10] Lack of adherence to 6-MP has been studied in children with ALL from developed countries.[9] Yet, little is known about its prevalence in LMICs.

Adherence to 6-MP can be measured by various methods including pill count, smart monitoring system, parent and/or child self-report, or therapeutic drug monitoring.[11] 6-MP has short half-life of 1.5 hours.[8] Hence, 6-MP level is not useful for therapeutic monitoring. However, 6-MP metabolites 6-thioguanine (6-TGN) and 6-methylmercaptopurine (6-MMP) accumulate in red blood cells (RBCs) over 1 to 3 weeks and monitoring 6-MP metabolite level is a good objective marker of long-term adherence.[11] Using a single cutoff value of active metabolite 6-TGN may not be helpful as there is a lot of variability in its level even among children with wild-type TPMT and NUDT15 genotype.[12] Hierarchical cluster analysis of RBC 6-MP metabolite level has been shown as good method for picking up children with poor adherence.[13] It is recommended that both objective and subjective methods should be used to better assess adherence to 6-MP.[8,9] In this first study from India, we aimed to estimate prevalence of nonadherence to 6-MP in children with ALL by using both subjective and objective methods. We also aimed to study the effect of simple intervention like use of smart pill box in improving adherence to 6-MP during maintenance phase chemotherapy.

Materials and Methods

This was a prospective interventional study conducted in the pediatric oncology clinic of a tertiary care hospital, over a duration of 1 year, after obtaining ethical clearance from the institutional review board.

Participants

A total of 40 newly diagnosed children with ALL between 0 and 18 years of age, who had completed at least 1 month of maintenance chemotherapy according to the Indian Collaborative Childhood Leukemia protocol were enrolled in the study, after taking informed consent from their parents and assent from children more than 12 years. The children who had received blood transfusion in the 2 months prior to the enrollment, or those in whom 6-MP was withheld for more than 5 days due to 6-MP-related toxicities, were excluded from the study. Clinical, demographic, and laboratory data, which included complete blood count and liver function test of the recruited children, were noted and *TPMT* and *NUDT15* mutation analysis was done by gene sequencing for mutant alleles as per standard guidelines.[14]

Assessment of Adherence to 6-MP

Adherence was assessed first by the subjective method by Morisky Medication Adherence Scale (MMAS-8).[15] Parents of the subjects were interviewed as per the questionnaire in MMAS-8 (►**Annuexure 2, 3** and **5**, available in the online version). The total score range on the MMAS-8 was 0 to 8. Children with scores of more than 7.2, between 6 and 7.2 and less than 6 were classified as having high, medium, and low adherence, respectively.[8] Following this, objective assessment of adherence was done by cluster analysis of RBC 6-MP metabolites, 6-TGN, and 6 MMP.[16,17] Briefly, 4 mL blood was collected from each patient and RBCs were cryopreserved at –80°C until analysis of 6-TGN and 6-MMP by mass spectroscopy. Cryopreserved packed RBCs were suspended in 500 µL in peripheral blood smear, and 250 µL of the solution was dispensed into a 1.5-mL microfuge tube. The hydrolysis and extraction process were performed as follows: diluted RBC solution (250 µL) was mixed with 20 µL of isotonic saline, 20 µL of 1.1 M dithiothreitol, and 50 µL of distilled water, vortexed for 30 seconds, and spun down. Then, 34 µL of 70% perchloric acid was added, vortexed for 30 seconds, and centrifuged at 3,000 × *g* for 15 minutes at room temperature. The supernatant (220 µL) was transferred to another polypropylene tube and hydrolyzed at 100°C for 1 hour. After cooling at room temperature, the acidic solution was neutralized with 220 µL of sodium hydroxide. Then, 50 µL of this solution was transferred to 1.5 mL of microfuge tube and dried using Speed Vacuum (Thermofisher Scientific, Cat. No. SPD1030-230) at low energy for 30 to 35 minutes. Samples pellets were then resuspended using 50 µL methanol:water (1:1, water:methanol) mixture for injection. Ultra-high performance liquid chromatography-mass spectrometry analysis was performed using a Dionex Ultimate 3000. Ultra-high performance liquid chromatography chromatographic system combined with a Q Exactive mass spectrometer fitted with a heated electrospray source operated in the positive ion mode. The software interface was Xcalibur 4.2, SII 1.3, and MSTune 2.8 SP1 (Thermo Fisher Scientific, Breda, The Netherlands). The levels of 6-TGN and 6-MMP in the samples were calculated on the basis of comparison of peak intensities with that of the internal standards.[18]

Intervention

Parents and children more than 10 years of age were educated regarding adherence to 6-MP and they were provided with I-store medicine storage box (smart pill box) with inbuilt alarm for a duration of 60 days. The smart pill box had a display for time with a facility to schedule three alarm timings and four compartments to keep the medications. This alarm box acted as a reminder for the patients to take the medication at the scheduled time. For assessment of the impact of intervention, the patients were again subjected to subjective and objective measurement of adherence by MMAS-8 and 6-MP metabolite level measurement as described before.

Primary Outcomes

To estimate the prevalence of nonadherence to 6-MP in maintenance phase chemotherapy and assess the impact of smart pill box as simple cost-effective intervention for improving adherence to 6-MP in maintenance phase chemotherapy in pediatric ALL.

Secondary Outcomes

1. To assess effectiveness of MMAS-8 as a subjective method for assessing adherence and its correlation with the objective method of estimating drug metabolite levels.
2. To look for NUDT and TPMT polymorphism in children with ALL and there correlation with the metabolite levels.
3. To study the factors affecting adherence in children with ALL.

Inclusion Criteria

Newly diagnosed children with ALL who had completed at least 1 month of maintenance chemotherapy.

Exclusion Criteria

1. Patients in last 3 months of maintenance phase.
2. Patients for whom 6-MP was withheld for more than 5 days by treating physician due to any reason during previous 1 month.
3. Patients who received RBC transfusion in the last 90 days.
4. Children with relapsed ALL.
5. Patients not consenting for the study.

Statistical Analysis

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables. Categorical outcomes were compared between study groups using the chi-square test and/or Fisher's exact test. Categorical variables at different time periods of follow-up were compared using the McNamar test; reported frequencies and proportions along with *p*-values. For normally distributed quantitative parameters, the mean values were compared between study groups using independent sample *t*-test (two groups). The change in the quantitative parameters, before and after the intervention was assessed by paired *t*-test. For normally distributed quantitative parameters, the mean values were compared between study groups using analysis of variance. For nonnormally distributed quantitative parameters, medians and interquartile range (IQR) were compared between study groups using the Mann–Whitney *U* test. The change in the quantitative parameters, before and after the intervention was assessed by Wilcoxon signed-rank test. For nonnormally distributed quantitative parameters, medians and IQR were compared between study groups using the Kruskal–Wallis test. Two numerical parameters (6-TGN and 6-MMP levels) were used to perform cluster analysis by hierarchical agglomerative method. Cluster 1 were characterized by very low levels (above 20th percentile of cutoff point) of 6-TGN levels and 6-MMP levels and these were considered to be nonadherent and other three clusters were considered adherent. A *p*-value of < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis. The mean score of MMAS-8 were calculated and the pre- and postintervention mean scores were compared by using the chi-square test. The hierarchical cluster analysis of drug metabolite concentrations was done and the pre- and postintervention levels were compared. Correlation between MMAS-8 and metabolite levels was done and *p*-value was calculated.

Ethical Approval

IEC, JNMC, Belgaum; No. MDC/DOME/161 dated January 25, 2021. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All authors approved the final manuscript.

Results

A total of 40 children with ALL in complete remission 1 (CR1), who had completed at least 1 month of maintenance phase of chemotherapy were enrolled in the study. The mean age was 7.39 ± 4.29 years and male-to-female ratio was 1.5:1. B-cell ALL was the most common immunophenotype of ALL accounting for 87.50% of the cases. As per the National Cancer Institute risk stratification, 55% belonged to standard risk and 45% to high risk. As far as distribution of patients in different maintenance cycle was concerned, 32.50, 20.00, 5.00, 10.00, 15.00, 7.50, and 10% belonged to M1, M2, M3, M4, M5, M6, and M7 number of maintenance cycles, respectively. Evaluation of *NUDT15* and *TPMT* polymorphisms revealed presence of NUDT15*3 polymorphism in heterozygous state in 4 out of 39 (10.26%) patients, and none had *TPMT* polymorphism (►**Table 5**).

The baseline subjective assessment of adherence to 6-MP by MMAS-8 revealed that 3 children (7.5%) had low adherence, 17 (42.5%) had medium adherence, and 20 (50%) had high adherence (►**Table 1**). Objective assessment of baseline adherence was done by quantifying 6-TGN and 6MMP levels and subjecting them to cluster analysis. Cluster 1 consisted of those with very low 6-MMP and very low 6-TGN levels, cluster 2 included those with low 6-MMP and low 6-TGN, cluster 3 consisted of those with medium 6-MMP and low 6-TGN, and cluster 4 included those with low 6-MMP and high 6-TGN levels (►**Fig. 1**). Cluster analysis revealed that 4 (10%) had very low 6-TGN and 6-MMP levels reflecting poor

Table 1 Descriptive analysis of baseline MMAS-8 in the study population ($N = 40$)

MMAS-8	Frequency	Percentage
High adherence (> 7.2)	20	50
Medium adherence (6–7.2)	17	42.50
Low adherence (< 6)	3	7.50

Abbreviation: MMAS-8, Morisky Medication Adherence Scale 8.

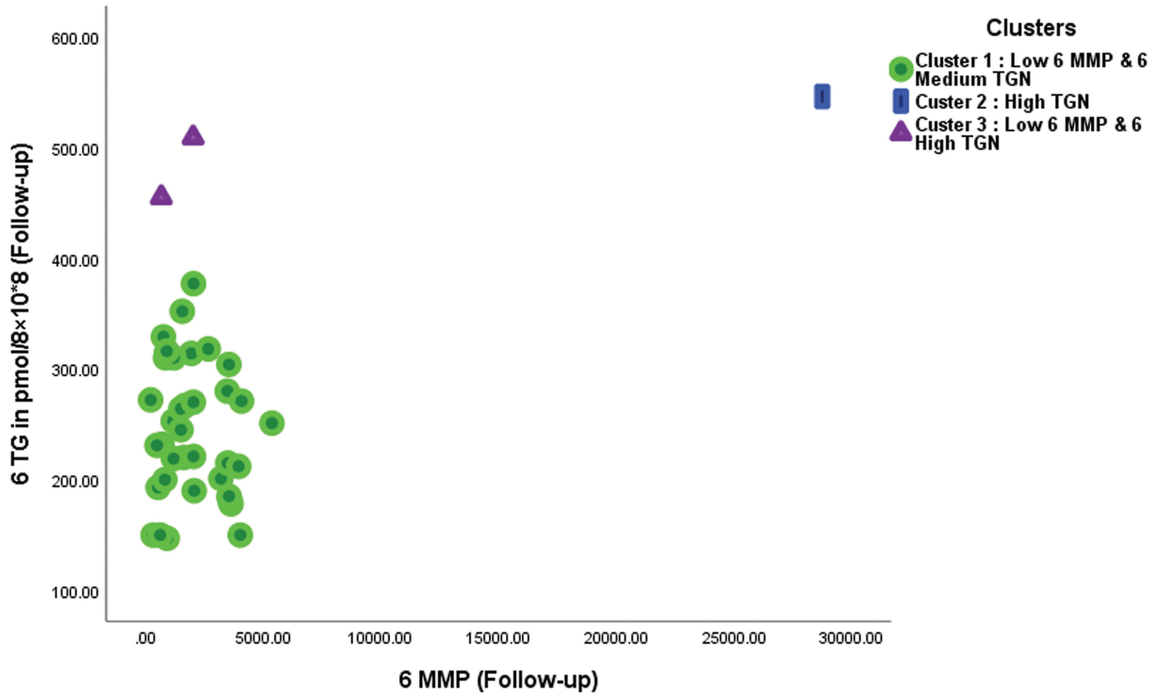

Fig. 1 Scatter plot showing the clusters for 6-methylmercaptopurine (6-MMP) and 6-thioguanine (6-TGN) follow-up levels.

adherence (►**Fig. 2**). The baseline mean MMAS-8 score was 7.34 ± 0.78 and the baseline median 6-TGN and 6-MMP levels were 150 (100, 221) and 879 (270, 1528) pmol/8×10^8 RBCs, respectively. There was no statistically significant association of baseline adherence with patient- or parent-related factors (►**Table 2**).

The assessment of adherence by MMAS-8 and RBC 6-TGN and 6-MMP levels after the intervention with smart pill box showed statistically significant impact of intervention on improving the adherence. Following the intervention, MMAS-8 results illustrated that none had low adherence, 11 (27.5%) had medium adherence, and 29 (72.5%) had high

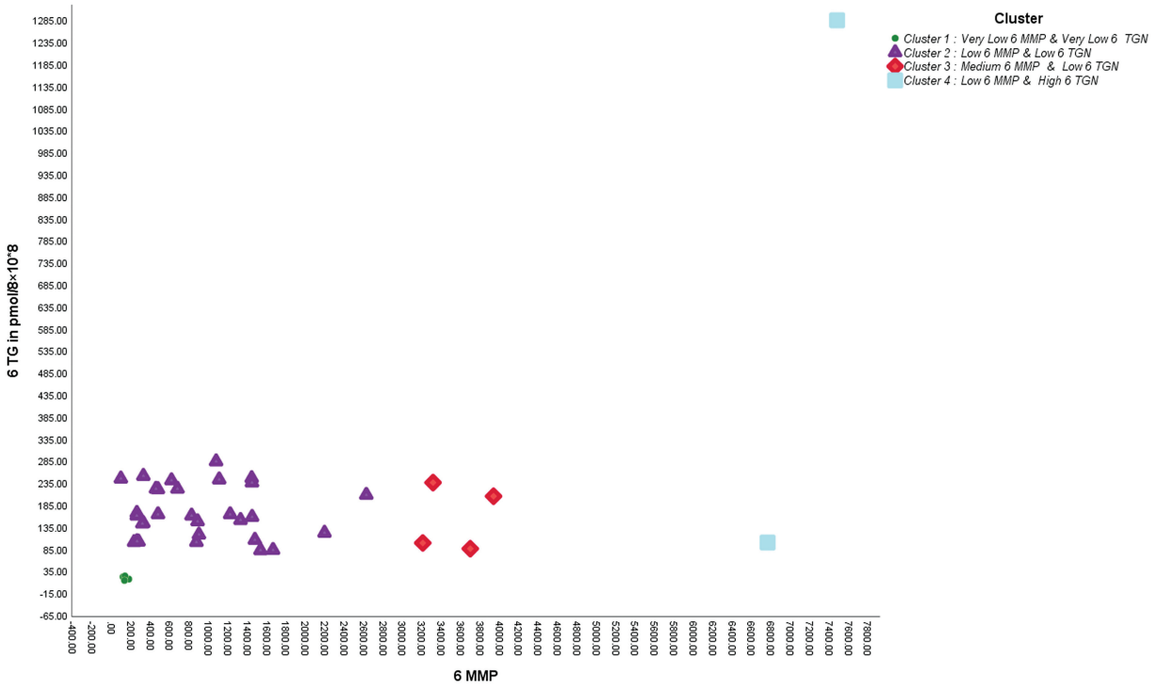

Fig. 2 Scatter plot showing the clusters for 6-methylmercaptopurine (6-MMP) and 6-thioguanine (6-TGN) baseline levels.

Table 2 Comparison between gender, number of siblings, and socioeconomic status, type of family, and education (mother and father) with Morisky scale (parent) baseline in the study population ($N = 40$)

Parameter	MMAS-8 baseline			Chi-square value	*p*-Value
	High adherence ($N = 20$)	Medium adherence ($N = 17$)	Low adherence ($N = 3$)		
Gender					
Male	11 (55.00%)	10 (58.82%)	3 (100.00%)	–	*
Female	9 (45.00%)	7 (41.18%)	0 (0.00%)		
Number of siblings					
Zero	0 (0.00%)	2 (11.76%)	0 (0.00%)	–	*
One	11 (55.00%)	9 (52.94%)	0 (0.00%)		
Two	6 (30.00%)	5 (29.41%)	3 (100.00%)		
Three	3 (15.00%)	1 (5.88%)	0 (0.00%)		
Socioeconomic status (as per modified B.G. Prasad classification[30])					
I	3 (15.00%)	1 (5.88%)	0 (0.00%)	–	*
II	2 (10.00%)	3 (17.65%)	0 (0.00%)		
III	4 (20.00%)	3 (17.65%)	1 (33.33%)		
IV	2 (10.00%)	7 (41.18%)	2 (66.67%)		
V	9 (45.00%)	3 (17.65%)	0 (0.00%)		
Type of family					
Joint	9 (45.00%)	6 (35.29%)	2 (66.67%)	1.13	0.5686
Nuclear	11 (55.00%)	11 (64.71%)	1 (33.33%)		
Education (mother)					
No formal education	3 (15.00%)	0 (0.00%)	0 (0.00%)		
Primary education	2 (10.00%)	6 (35.29%)	2 (66.67%)	–	*
Secondary education	11 (55.00%)	9 (52.94%)	1 (33.33%)		
Graduate	4 (20.00%)	2 (11.76%)	0 (0.00%)		
Postgraduate	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Education (father)					
No formal education	3 (15.00%)	1 (6.25%)	0 (0.00%)	–	*
Primary education	3 (15.00%)	3 (18.75%)	1 (33.33%)		
Secondary education	7 (35.00%)	9 (56.25%)	1 (33.33%)		
Graduate	7 (35.00%)	2 (12.50%)	1 (33.33%)		
Postgraduate	0 (0.00%)	1 (6.25%)	0 (0.00%)		

Abbreviation: MMAS-8, Morisky Medication Adherence Scale 8.
*No test applicable due to the nature of the data.

adherence (►**Table 3**). The mean MMAS-8 significantly improved to 7.66 ± 0.55 (p-value < 0.015) after the intervention and the median 6-TGN level improved to 253 pmol/8×10^8 RBCs (p-value < 0.001) as shown in ►**Table 4**. Similarly, on cluster analysis, there was no cluster of patients with very low 6-TGN and 6-MMP levels (►**Fig. 1**). The correlation between the 6-MMP levels and the liver enzymes was not statistically significant.

Table 3 Descriptive analysis of follow-up MMAS-8 in the study population ($N = 40$)

MMAS-8 (follow-up)	Frequency	Percentage
High adherence	26	65.00
Medium adherence	14	35.00

Abbreviation: MMAS-8, Morisky Medication Adherence Scale 8.

Discussion

The subject of nonadherence to 6-MP in pediatric ALL has been well studied in developed countries; nonadherence leads to significant increase in relapse risk.[8,11,19,20] In the first Indian study exploring adherence to 6-MP in children with ALL, we utilized both subjective and objective methods, as only subjective method of parent or child interview is

Table 4 Comparison of MMAS-8, 6-TGN, and 6-MMP levels at baseline and 2 months after intervention

Parameter	Mean ± SD	Mean difference	*p*-Value
Morisky scale parent score ($N = 40$)			
Baseline	7.34 ± 0.78	−0.32	0.015
Follow-up	7.66 ± 0.55		
6-TGN in pmol/8 × 10^8 RBC	**Median (interquartile range)**	**Wilcoxon's sign rank test statistics**	***p*-Value**
Baseline	150 (121)	−4.80	< 0.001
Follow-up	253 (114)		
6-MMP in pmol/8 × 10^8 RBC			
Baseline	879 (1258)	−3.82	< 0.001
Follow-up	1678 (2593)		

Abbreviations: 6-MMP, 6-methylmercaptopurine; 6-TGN, 6-thioguanine; MMAS-8, Morisky Medication Adherence Scale 8; RBC, red blood cell; SD, standard deviation.

Table 5 Comparison of mean of 6-TGN in pmol/8 × 10*8 (baseline) and 6-MMP (baseline) between NUDT mutation ($N = 39$)

Parameter	NUDT mutation (mean ± SD)		*p*-Value
	Positive ($N = 4$)	Negative ($N = 35$)	
6-TGN in pmol/8 × 10*8 (baseline)	177.25 ± 42.95	220.21 ± 252.87	0.739
6-MMP (baseline)	499.75 ± 262.27	1670.64 ± 1719.99	0.187

Abbreviations: 6-MMP, 6-methylmercaptopurine; 6-TGN, 6-thioguanine; SD, standard deviation.

likely to overestimate adherence.[21,22] Indeed, only 3 out of 40 (7.5%) children were found to have low adherence (< 75%) by MMAS-8 at baseline, while RBC metabolites cluster analysis identified one more child (4 out of 40) with poor adherence to 6-MP. Our finding of 17 out of 40 children having medium level of adherence (75–90%) is of particular concern. Overall, 40% of children were found to have adherence below 90% by MMAS-8. We could not identify separate RBC metabolite cluster for children with medium adherence measured by MMAS-8, probably because they were part of large cluster 2 consisting of 30 patients with low 6-TGN and 6-MMP levels. It is possible that all these children in cluster 2 were at increased risk of nonadherence as suggested by Bhatia et al.[8] Alternatively, low RBC metabolite levels could be explained by ethnicity and pharmacogenomics.[23,24] We need larger study to establish RBC metabolite levels, during 6-MP maintenance therapy in Indian children with ALL. In our cohort, we did not find any patient with *TPMT* polymorphism; however, 4 out of 39 patients tested positive for *NUDT15**3 polymorphism in heterozygous state. This is in line with similar prevalence in other Indian studies.[12,25] None of the factors studied influenced adherence in our cohort. This is in contrast with larger COG study which found ethnicity and socioeconomic factors as significantly associated with adherence.[8] Our finding of lack of association with these factors could be because of smaller sample size and most of the patients belonging to similar ethnic background. Various interventions have been studied to improve adherence to medications which are used chronically with conflicting results.[26,27] In particular, interventions like mobile text reminders, alarms, and parental education have failed to improve adherence in children on 6-MP maintenance therapy.[26] However, our simple, affordable, smart pill box with inbuilt alarm significantly improved adherence in our cohort as measured by both methods. Notably, none of the patient showed poor (< 75%) adherence after the intervention. Both the MMAS-8 and median 6-TGN level significantly increased after the use of smart pill box for 2 months. This positive effect on adherence could be in part due to higher number of patients with low and medium adherence at baseline. This finding is of particular relevance to children from LMIC as it may help in reducing relapse risk and overall survival, as very few children with relapse undergo curative treatment like transplant or novel therapies like immunotherapy due to inadequate access and financial constraints.[28,29] In this study, we found high prevalence of NUDT polymorphism, which can contribute to severe toxicity due to 6-MP. Hence, NUDT and TPMT studies can be done upfront, so that the 6-MP dose can be adjusted during consolidation itself, thus leading to reduced morbidities and mortalities due to 6-MP toxicity. Also, dose adjustment depending on NUDT and TPMT polymorphism would prevent interruption of chemotherapy, eventually improving the outcome.

Limitations

Our study is limited by small sample size and lack of measurement of adherence at multiple time points during maintenance treatment.

Conclusion

This study also highlights the fact that nonadherence to 6-MP is widely prevalent in Indian children during maintenance treatment for ALL and simple measures like smart pill box can improve adherence and more such approaches should be studied in LMIC setting. We did not find any factor that had significantly affected the adherence of the children.

What is Already Known?

The issue of nonadherence to 6-MP and the consequent increased risk of relapse is well studied in western countries.

What this Study Adds?

1. This is the first Indian study to assess nonadherence by subjective and objective methods.
2. This study emphasizes the fact that simple intervention by smart pill box can significantly reduce nonadherence.

Patient Consent
Informed patient consent was obtained for this study.

Funding
None.

Conflict of Interest
None declared.

Acknowledgments
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Periodontitis and Its Role in Oral Cancer Susceptibility: A Case-Control Study

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Abstract

Introduction Oral cancer and periodontitis are complex, multifactorial diseases, influenced by common risk factors such as genetic predisposition, lifestyle choices, and oral health practices. While certain studies indicate a positive correlation between periodontitis and oral cancer, the precise mechanisms and causation remain unclear.

Objective This study aims to determine if individuals with periodontitis have a heightened risk of developing oral cancer compared with those with healthy periodontal conditions.

Materials and Methods One hundred and twenty-six participants, 63 with oral cancer and 63 without oral cancer, were enrolled. A structured questionnaire was developed to gather data on demographics, socioeconomic status, lifestyle risk factors, dietary habits, periodontal condition, oral hygiene practices, and complete oral health status. Statistical analysis used chi-squared and Mann–Whitney *U* tests and logistic regression to understand potential influences on oral cancer development.

Results Notable associations were identified between oral cancer occurrence and specific socioeconomic factors and lifestyle behaviors, including gender, age, education level, and tobacco and alcohol usage. Average Silness and Loe plaque index values, probing pocket depth, and clinical attachment loss values were significantly higher in cases than controls. Patients with periodontitis exhibited a higher incidence of oral cancer (63.9%) compared with those without periodontitis (32.4%).

A substantial majority of oral cancer patients (72.9%) exhibited stage 4 periodontitis, contrasting with controls (30.6%).

Conclusion Periodontitis emerges as a significant individual risk factor influencing oral cancer development. Rigorous monitoring is recommended for individuals with compromised periodontal health, particularly with severe periodontitis and concurrent risk factors. Prioritizing preservation of periodontal health in high-risk individuals holds promise for mitigating oral cancer–associated risks.

Keywords
- periodontitis
- oral cancer
- risk factors
- chronic inflammation
- tobacco

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Introduction

Periodontal diseases constitute a prevalent oral health issue affecting over 50% of the Indian population. While these conditions can impact individuals of all ages, their likelihood increases with advancing age.[1] The widespread occurrence of periodontal diseases poses an important public health challenge due to their adverse effects on oral health, including tooth loss, disability, aesthetic concerns, and masticatory issues. Moreover, these conditions can have systemic consequences and may contribute to undernourishment. The psychosocial and economic implications of periodontal diseases are substantial.[2] Many research studies have emphasized the connection between periodontitis and a range of systemic illnesses, such as diabetes, cardiovascular conditions, and adverse pregnancy outcomes. Furthermore, emerging evidence suggests a connection between periodontal disease and oral cancer (OC).[3]

Both periodontitis and OC share established risk factors, including smoking, tobacco use, alcohol consumption, poor oral hygiene (OH), unhealthy diet, age, systemic conditions like diabetes, autoimmune diseases, certain medications, bacterial infections, and genetic predisposition. Despite the shared risk factors contributing to both diseases, the specific mechanisms and their influence can vary.[4] Persistent inflammation in the mouth, frequently linked to periodontal conditions, is regarded as a separate risk factor for OC, as it can lead to deoxyribonucleic acid (DNA) damage, cell proliferation, and creation of a microenvironment conducive to the growth and survival of cancer cells. Furthermore, distinct risk factors and condition-specific elements play crucial roles in the onset and progression of each oral health issue. Recognizing these distinctions is crucial for the prevention, early detection, and effective management of gum disease and OC.[5] In this study, a reliable radiographic index (RI) assessing interproximal alveolar bone loss (iABL) in conjunction with clinical screening tools is included, providing a comprehensive approach to understanding the oral health status of individuals. The study aims to assess the association between periodontal disease and the potential risk of OC development within the population of Bengaluru city, India.

Materials and Methods

Study Design

This study is a case-control study conducted in our in our faculty involving oral squamous cell carcinoma (OSCC) patients recruited from the department database between January 2020 and January 2024, based on histological confirmation.

Inclusion Criteria

The study enrolled 126 participants (63 with OC and 63 controls) ranging in age from 18 to 90 years. Among them, 63 patients were in the case group, diagnosed with OSCC based on histological confirmation, and were recruited from the department database between January 2020 and January 2024. The control group consisted of 63 age- and sex-matched individuals without a history of OC, recruited from the outpatient section.

Exclusion Criteria

Patients with cancers other than OSCC and patients with a history of jaw resection as a part of cancer therapy were excluded from the study.

Questionnaire

A comprehensive questionnaire was designed to collect data on demographic information, socioeconomic status, risk factors, detailed dental and medical history, site of OC, and various parameters related to potential risk factors and confounding variables.

Assessment of Dental and Oral Health

The decayed, missing, and filled teeth (DMFT) index was employed to evaluate dental health and the extent of dental caries. The Silness–Löe plaque index (SLPI) was utilized to assess OH status. Periodontal condition was evaluated through measurements of probing pocket depth (PPD) and clinical attachment loss (CAL) at six locations on each tooth. The average data for bleeding on probing (BOP) were subsequently computed. Periodontitis severity was categorized according to the criteria set forth by the World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions in 2018. Apart from the clinical evaluations, an RI was employed to assess the extent and severity of iABL relative to the lengths of individual roots. This approach utilized available panoramic radiographs to conduct a thorough assessment. Patients were briefed about the study's objectives, and written consent was obtained from all participants. The primary cancer-related treatment for the patients was managed appropriately. Referrals were made for any required conservative dental or periodontal treatment.

Primary Outcome

The primary outcome of this study was to determine the association between periodontitis and OC susceptibility.

Secondary Outcome

The primary outcome of this study was to assess the periodontal status and other oral health parameters (PPD, CAL, BOP percentage, SLPI, and DMFT index, radiographic assessment of alveolar bone loss) among individuals with OC compared with controls.

Statistical Analysis

Statistical analysis was conducted using SPSS Statistics 18 software (IBM Corporation), utilizing the chi-squared and Mann–Whitney *U* tests and logistic regression. Statistical significance was determined at a *p*-value of less than 0.05.

Ethical Approval

Ethics committee approval was obtained from the institutional ethics committee, dated January 4, 2020, with reference number EC 2020/PG/081. The procedures followed were

in accordance with the ethical standards of the responsible committee on human experimentation and with the Declaration of Helsinki 1964, as revised in 2013. Waiver of informed patient consent was obtained from the ethics committee.

Results

Patients with periodontitis exhibited a higher incidence of OC (63.9%) compared with those without periodontitis (32.4%). A substantial majority of OC patients (72.9%) exhibited stage 4 periodontitis, contrasting with controls (30.6%). Significant associations were identified among age groups, education, tobacco use, alcohol consumption, and diet about case and control groups. The incidence of OSCC was higher among individuals older than 45 years compared with those younger than 45 years. The occurrence of OSCC was more among the patients/participants with lower levels of formal education as compared with patients/participants with higher education and employment. However, occupation and marital status did not prove to be significant factors. The calculated *p*-value of approximately 0.000102 for tobacco use strongly suggests a substantial association, indicating a connection between tobacco use and variations in the distribution of cases and controls. A total of 77.7% were current tobacco users among cases as compared with 38.5% among controls. Those who consumed more than 20 cigarettes or sachets of smokeless tobacco (SLT) per day exhibited elevated rates of OC. No significant correlation was found between passive smoking and the occurrence of OC. Percentage of daily alcohol consumption among cases was 56.79% as compared with 23.85% in controls. A notable correlation was observed between the incidence of OSCC and the quantity of alcohol consumption ($p = 0.027$; ►**Table 1**). In our study, excessive alcohol consumption emerged as an independent risk factor for the onset of OC.

Finally, diet was recognized as a likely factor influencing the distribution of cases and controls. The majority of OCs were found on the buccal mucosa (54%), followed by gingiva/gums (18%), tongue (11%), floor of the mouth (8%), labial mucosa (6%), and palate (3%). Analysis of dental status revealed that the case group had a higher proportion of

Table 1 Comparison of demographic characteristics, socioeconomic risk factors, and lifestyle habits between the cases and control groups

Variables		Cases	Controls	*p*-value
Age (y)	>45	45	20	< 0.001
	< 45	18	43	
Gender	Male	36	36	> 0.05
	Female	27	27	
Education	Elementary school	42	7	< 0.05
	High school	14	20	
	Degree	7	36	
Occupation	Employed	18	27	> 0.05
	Unemployed	12	9	
	Home maker	19	14	
	Student	1	1	
	Retired	13	12	
Marital status	Single	16	11	> 0.05
	Married	30	39	
	Widowed/divorced	17	13	
Tobacco	Smoking	18	10	< 0.05
	Smokeless	27	18	
	Both	4	0	
	Never	11	33	
Alcohol consumption	Daily	32	15	0.027
	Weekly	20	19	
	Monthly	4	9	
	Never	7	20	
Diet	Vegetarian	29	45	0.72
	Nonvegetarian	34	18	

Table 2 Comparison of periodontal staging, CAL, PPD, BOP, RI (iABL), SLPI, DMFT INDEX between case and control groups

Periodontitis stage	Cases	Controls	*p* value
I	2	1	<0.05
II	4	0	<0.05
III	27	18	0.02
IV	27	5	<0.05
CAL (mm)	6.2 ± 1.3	2.8 ± 1.1	<0.05
PPD (mm)	5.6 ± 1.3	2.5 ± 1.1	<0.05
RI (iABL)	Codes 3 and 4	Codes 2 and 3	<0.05
BOP (%)	45.9 ± 27	27.9 ± 18.9	<0.05
SLPI	2.7 ± 0.9	1.3 ± 0.9	<0.05
DMFT index	24.7 ± 9	13.2 ± 8.01	<0.05
Completely edentulous	6	3	>0.05

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment loss; DMFT, decayed, missing, and filled teeth; iABL, interproximal alveolar bone loss; PPD, probing pocket depth; RI, radiographic index; SLPI, Silness–Löe plaque index.

completely edentulous patients compared with the control group. The control group exhibited a significantly higher rate of filled teeth (F), whereas the case group had a higher rate of missing teeth (M). There was no significant difference in the number of decayed teeth (D) between the two groups. The mean DMFT value was 21.65 ± 8.46 in the case group and 14.18 ± 8.26 in the control group (►**Table 2**). A significant correlation was found between the occurrence of OC and periodontitis. The occurrence of OC was 57.1% among patients with periodontitis, whereas those without periodontal disease had a lower incidence of 26.6%. A significant correlation was identified, indicating that as the severity of periodontitis increased, there was a corresponding rise in the risk of OC development (►**Figs. 1** and **2**). The majority of OC patients, constituting 72.1% of the case group, were diagnosed with stage 4 periodontitis. In contrast, in the control group, most individuals had stage 2 periodontitis, accounting for 51.6%. Significant disparities were observed in the

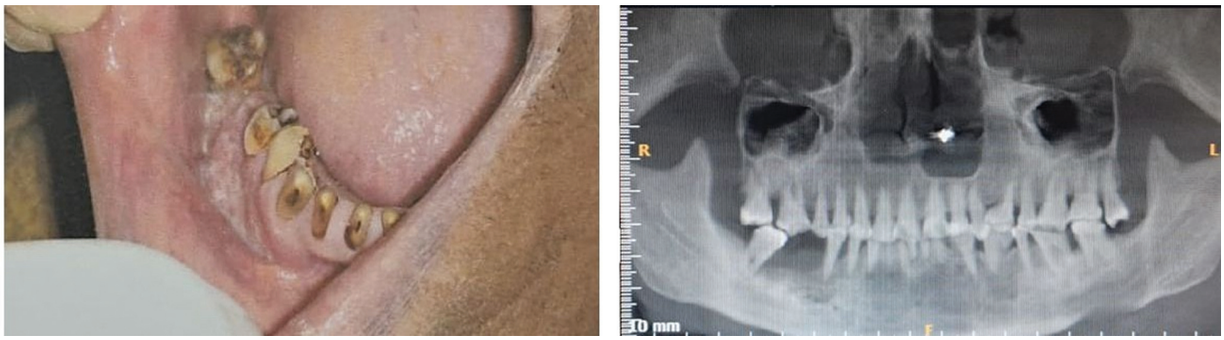

Fig. 1 Clinical and radiographic image of a 54-year-old female patient with cancer of the lower right alveolus with no known risk factors apart from periodontitis.

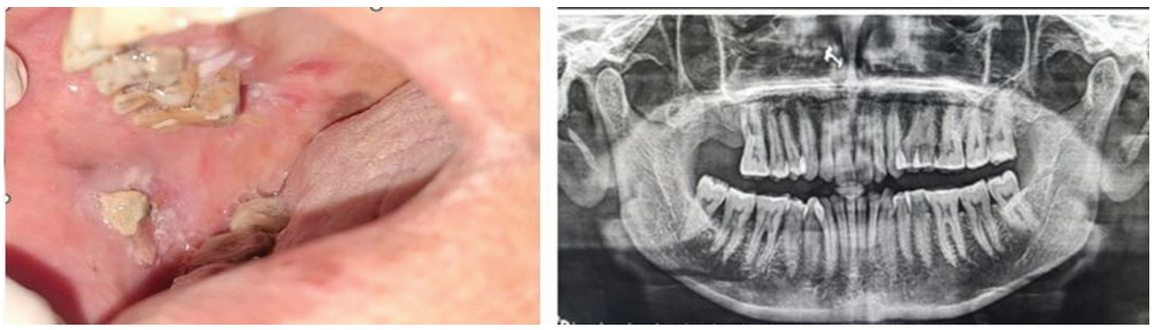

Fig. 2 Clinical and radiographic images of a 36-year-old female patient with cancer of the upper right alveolus with no known risk factors other than periodontitis.

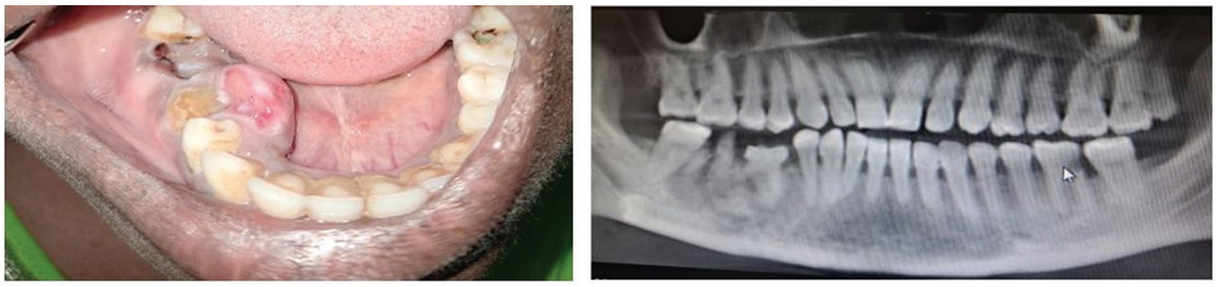

Fig. 3 Clinical and radiographic images of a 48-year-old female patient with cancer of the lower right alveolus and the floor of the mouth with no known risk factors other than periodontitis.

mean values of CAL and PPD between the two groups. The case group demonstrated substantially higher values, with CAL at 6.2 ± 1.3, compared with 2.8 ± 1.1 in the control group. Similarly, the mean values of PPD and bleeding on probing percentage (BOP%) were higher among cases compared with controls. The RI (iABL) index scoring codes was 2 and 3 (moderate to severe alveolar bone loss) among controls (►**Figs. 3** and **4**) and 3 and 4 (severe to very severe alveolar bone loss) among cases (►**Figs. 1** and **2**). In the case group, there were more completely edentulous patients ($n = 6$) compared with the control group ($n = 3$). Among the completely edentulous patients, 11.3% were diagnosed with OC. Logistic regression analysis confirmed that the prevalence and severity of periodontitis were statistically significant factors.

Discussion

India has the highest number of OC cases globally and is recognized as the global epicenter of this disease. Besides tobacco use, risk factors include the consumption of areca nut, alcohol, diet, human papillomavirus (HPV) infection, advancing age, male gender, and socioeconomic factors. Notably, periodontitis is also widespread in India, and both OC and periodontitis share common established risk factors like tobacco use, poor OH, etc.[6] Periodontitis is a persistent inflammatory condition that affects the supporting structures of the teeth, resulting in the deterioration of periodontal tissues leading to tooth mobility and tooth loss. It is known that the impact of periodontitis extends beyond the confines of the oral cavity, potentially giving rise to systemic consequences like diabetes, cardiovascular diseases, and cancer.[3] Although complex, evidence suggests a connection between periodontal disease and OC due to the considerable role of inflammation in both conditions.

In the present study, patients with periodontitis had a higher rate of OC (63.9%) compared with those without periodontitis (32.4%), and a large proportion of OC patients (72.9%) were diagnosed with stage 4 periodontitis, in contrast to just 30.6% of the control group. Five (7.9%) of the OC cases had severe periodontitis (stages III and IV) as their only identified risk factor. This finding underscores the potential unique contribution of periodontitis to OC susceptibility. The chronic inflammation associated with periodontitis may create an environment conducive to carcinogenesis, even in the absence of other major risk factors such as tobacco use, significant alcohol consumption, poor diet, or genetic predisposition.[7] Although 7.9% is a relatively small percentage, it is substantial in identifying periodontitis as a potential independent risk factor for OC. The present study confirms a significant correlation between OC and periodontitis, aligning with the findings of Javed et al's 2016 systematic review.[8] This highlights the importance of monitoring and managing periodontal health to potentially reduce the risk of OC in susceptible individuals.

The study also revealed that a notable number of individuals diagnosed with OC exhibited advanced periodontitis (stages III and IV), in stark contrast to the control group, which showed a lower prevalence of severe periodontitis. CAL was markedly elevated in cases of OC, as demonstrated by a substantially higher mean CAL of 5.7 mm, contrasting sharply with controls in whom the mean CAL was 2.7 mm. Likewise, PPD displays notable distinctions between cases (5 mm) and controls (2.2 mm). This considerable variance

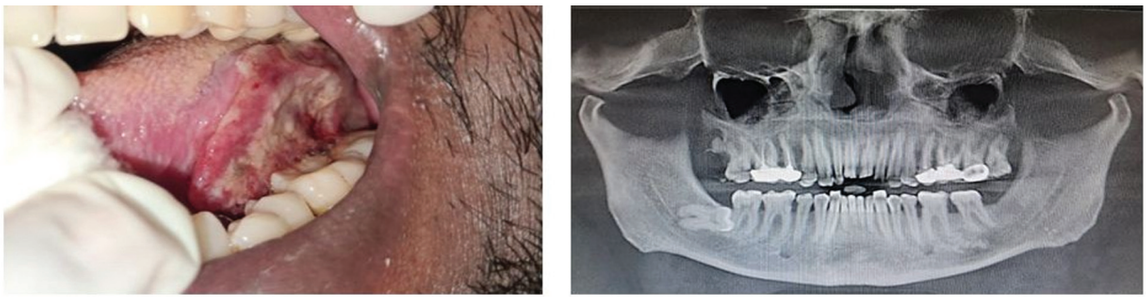

Fig. 4 Clinical and radiographic images of a 40-year-old male patient with cancer of the left lateral border of the tongue and the floor of the mouth with no known risk factors other than periodontitis.

underscores the existence of more profound periodontal pockets among individuals with OC, highlighting the potential gravity of periodontal involvement in this cohort. The study demonstrates a distinct positive correlation between the severity of periodontitis and the prevalence of OC, consistent with the results of Komlós et al.[9] Severe periodontitis and OC may be linked through several mechanisms. Chronic inflammation caused by severe periodontitis can facilitate cancer development by leading to DNA damage and mutations. The development of a malignant lesion is often linked to inflammation, particularly due to oxidative damage to the cell's DNA.[10,11] Periodontitis is distinguished by increased levels of proinflammatory cytokines, acute phase proteins, and proteinases in the bloodstream.[12] Moreover, inflammatory mediators such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) found in periodontal lesions are linked to carcinogenesis.[13] Recent research indicates a direct connection between pathogens associated with periodontal disease and the onset of OC. Additionally, certain bacteria involved in periodontitis, such as *Porphyromonas gingivalis*, have been detected in higher levels in OC tissues, potentially contributing to cancer development.[14] Periodontal pockets could serve as reservoirs for cytomegalovirus, HPV, and Epstein–Barr virus, all of which are agents linked to OC.[15] This lends support to the hypothesis proposed by Sahingur et al, indicating that the severity of periodontitis and changes in the oral microbiome play a role in creating a favorable environment for the onset of OC.[9]

Shared risk factors, like tobacco and alcohol use, further complicate the relationship, as these can exacerbate both conditions. Poor OH associated with periodontitis may also delay OC detection as it may go unnoticed.[16] Thus, managing severe periodontitis and maintaining good oral health are crucial for reducing the risk of OC. Advanced periodontitis has the potential to compromise the local immune response, impacting the body's ability to regulate abnormal cell growth and potentially contributing to OC development. The proposition that periodontitis and OC may follow a sequential progression in certain cases is plausible, where severe periodontitis could serve as an early indicator or a contributing factor in the sequence of events leading to the development of OC.[17]

Radiographs, especially panoramic radiographs, facilitate precise assessment of the interproximal alveolar bone, aiding in the localization and measurement of bone loss. Radiographic indices like RI(iABL) facilitate the quantification of iABL.[18] The codes likely correspond to specific degrees of bone loss, allowing for a standardized and measurable assessment of severity. The RI (iABL) distribution shows a marked difference between cases and controls, with a higher prevalence of advanced iABL in OC patients, suggesting a potential link between OC and more severe bone loss. BOP is a common clinical indicator of periodontal inflammation, and its elevated levels among cases implies the need for attention to periodontal health in this population. The higher mean SLPI in cases, compared with controls, points to an increased level of dental plaque among OC patients. Elevated plaque levels not only impact the condition of the teeth and gums but also have systemic implications.[19] Consideration should be given to interventions aimed at improving OH and reducing plaque accumulation in OC patients to maintain overall oral health and potentially mitigate associated risks.

The observation that cases have a significantly higher mean DMFT index compared with controls indicates a higher prevalence of dental caries and tooth loss among individuals diagnosed with OC. Several factors could lead to the higher DMFT index observed in OC cases, including the effects of cancer treatments on oral health, potential alterations in salivary flow, and the presence of risk factors like tobacco use and inadequate OH. Additionally, there was a greater number of completely edentulous individuals among cases compared with controls, indicating a higher prevalence of complete tooth loss primarily associated with periodontitis among OC patients.

The findings of the study suggest that OC patients (cases) exhibit higher prevalence of periodontal conditions, including advanced periodontitis stages, higher CAL and PPD values, greater iABL, increased BOP, elevated SLPI, a higher DMFT index, and a higher rate of complete edentulism compared with the control group. These findings highlight the potential interplay between periodontal health and OC, emphasizing the relevance of comprehensive oral health assessments in patients.[20] It is important to note that while there is evidence suggesting a potential association, not all individuals with periodontitis develop OC, and the relationship is likely influenced by a combination of genetic, environmental, and lifestyle factors.[21,22]

This study offers novel insights into the association between periodontitis and OC, particularly within the Indian population, which experiences high rates of both conditions. It quantitatively establishes that individuals with periodontitis, especially in advanced stages, face a significantly higher risk of developing OC. The study integrates both clinical and radiographic evaluations to show that severe periodontitis is linked to increased OC risk, influenced by socioeconomic, lifestyle, and OH factors. It suggests that periodontitis may be an independent risk factor for OC, regardless of tobacco and alcohol use, and offers region-specific data to guide preventive strategies and further research.

Conclusion

This study highlights the complex link between OC and periodontal health, showing that poor OH and plaque buildup create chronic inflammation that may promote OC development. The findings of this study support the notion that periodontitis is an independent risk factor for OC, with the risk increasing as periodontal disease progresses. Preventive measures for periodontal disease include regular dental visits and maintaining optimal OH. Dentists can contribute to reducing the risk of OC by evaluating and addressing patients' lifestyle and habits and monitoring compromised periodontal health. Further research and longitudinal studies are crucial to deepening our understanding of the intricate interplay between OC and periodontitis, laying the groundwork for more precise and effective preventive strategies in clinical practice.

Funding
None.

Conflict of Interest
None declared.

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Pattern of Expression of PML-RARα Transcripts in Acute Promyelocytic Leukemia Patients and Its Correlation with Overall Survival: A Retrospective Study from Western India

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Abstract

Introduction Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia with specific molecular pathogenesis, clinical features, and treatment. It is cytogenetically characterized by translocation t (15;17) (q24;q21). The location of breakpoints within the PML gene determines the formation of distinct promyelocytic leukemia-retinoic acid receptor α (PML-RARα) transcript subtypes which have prognostic significance. Breakpoints in intron 6 result in the long (L or bcr-1) subtype, those in exon 6 produce the variant (V or bcr-2) subtype, and breakpoints in intron 3 lead to the short (S or bcr-3) subtype.

Objectives The aim of our study was to determine the frequencies of the different PML-RARα transcripts in patients with APL at baseline and to investigate the impact of bcr-3 transcript as a prognostic factor on survival in newly diagnosed patients with APL.

Materials and Methods A retrospective study was conducted that included 54 newly diagnosed patients with APL. Clinicopathological parameters were evaluated in all cases for prognostic significance with overall survival. Real-time quantitative polymerase chain reaction was used for the quantification of PML-RARα transcripts.

Results Out of 54 patients, 53 (98.1%) patients expressed bcr-3 transcript either alone or in combination with either bcr-1 or bcr-2 transcripts. Twenty-one patients (38.9%) in our study showed expression of bcr-3 transcript only. Twenty eight (51.9%) patients in our study expressed all the three transcripts. Out of the 54 patients, 20 (37%) patients died of disease, out of which 19 patients died before completion of induction therapy. Complete remission was obtained in 34 patients (63%) after induction therapy. The survival rate for patients expressing the bcr-3 transcript alone was 66.66% as compared with 60.71% for patients expressing bcr-3 transcript in combination with other two transcripts. The survival rate for patients receiving all-*trans* retinoic acid (ATRA) in combination with arsenic trioxide was far better than patients receiving ATRA alone.

Keywords
- acute promyelocytic leukemia
- PML-RARα transcripts
- bcr-3 transcript

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Conclusion The total leucocyte count is an independent prognostic factor in patients with APL as it was statistically significant with overall survival in our study. The bcr transcript either alone or in combination with bcr-1 and/or bcr-2 transcripts was the most frequent pattern observed.

Introduction

Acute promyelocytic leukemia (APL) is a distinctive subtype of acute myeloid leukemia characterized by specific molecular pathology, clinical features, and treatment approaches. Cytogenetically, it is characterized by translocation t (15;17) (q24; q21), which is a balanced translocation. This translocation results in the fusion of retinoic acid receptor α (RARα) gene on chromosome 17 with promyelocytic leukemia (PML) gene on chromosome 15.[1] RARα breakpoints are seen in intron 2. Breakpoints within the PML gene which are located at intron 6, exon 6, and intron 3 result in the formation of three different PML-RARα transcripts which are referred to as long (L or bcr-1), variant (V or bcr-2), and short (S or bcr-3), respectively.[2]

The most common breakpoint subtype of PML is bcr-1 (breakpoint cluster region) or long form, which occurs at intron 6, and is identified in 45 to 55% of cases of APL. The second most common subtype is the short form, or bcr-3, at intron 3 of PML, and is seen in 35 to 45% of cases. The least common subtype is the variable form, or bcr-2, occurring at exon 6 of PML, and accounts for 5 to 10% of cases.[3]

Reverse transcriptase polymerase chain reaction (RT-PCR) remains the gold standard method for genetic confirmation of APL, as it allows the identification of the specific PML/RARα transcript subtype.[4] Conventional karyotype and fluorescence in situ hybridization are the other techniques that can be used for confirmation of diagnosis. Isoform-specific quantitative real-time PCR allows for the evaluation of response during therapy, minimal residual disease monitoring, and detection of molecular relapse during the follow-up period.[5,6] Posttreatment leukemic relapse is indicated by re-appearance of bcr transcripts.

APL patients are divided into low-, intermediate-, and high-risk categories based on the total leucocyte count (TLC) at presentation, which is also an important prognostic factor. At diagnosis, a TLC count greater than 10,000/μL places patients into a high-risk subset, whereas those with TLC count less than or equal to 10,000/μL are further classified into low- and intermediate-risk categories based on platelet count cut-offs of $>$40,000 and $\leq$40,000/μL.[7]

Combination of chemotherapy with arsenic trioxide (ATO) and all-*trans* retinoic acid (ATRA) has shown excellent outcomes with approximately more than 90% complete remission rates. In spite of these results, the incidence of death rate continues to remain high with this leukemia, which is particularly more frequent in patients who belong to the high-risk category. High-risk features in APL are defined by TLC count $>10 \times 10^9$/L at presentation, CD34 expression by flow cytometry, bcr-3 transcript expression on PCR, and FLT3-internal tandem duplication (ITD) mutations.[8]

Thus, we investigated the frequencies of the PML-RARα transcripts in 54 APL patients at baseline and the impact of bcr-3 transcript as a prognostic factor on survival in newly diagnosed patients with APL.

Need of the study: our present study evaluates the impact of bcr-3 transcripts on newly diagnosed APL patients as well as certain pathological and clinical risk factors such as TLC and platelet count. In APL, intensification of therapy in addition to conventional ATRA is based on these factors.

Materials and Methods

Sample Size

Newly diagnosed patients with APL during the period from January 2021 to October 2023.

Inclusion Criteria

All newly diagnosed patients with APL in which quantification of PML-RARα transcripts was done were included. Patients were divided into low- and high-risk groups on the basis of TLC at the time of diagnosis.

The treatment protocol was decided on the basis of risk stratification. Low-risk patients received combination of ATRA and ATO, while high-risk patients were treated with chemotherapy and ATRA. Treatment decision was not based on the pattern of transcripts.

Exclusion Criteria

Patients without PML-RARα transcripts at baseline were excluded.

Fifty-four newly diagnosed patients with APL were studied using bone marrow examination, flow cytometry, and cytogenetic analysis of t(15;17). Real-time quantitative PCR was used for the quantification of all the three different PML-RARα transcripts.

Peripheral blood was used for RNA extraction using an RNA extraction kit (Qiagen, Hilden, Germany). A complementary DNA (cDNA) synthesis kit (Thermo Scientific) was used for converting RNA to cDNA. Quantification was done on the Aria Mx instrument using Qiagen Ipsogen PML-RARα kits.

Statistical Analysis

Statistical analysis was done using SPSS software version 22 (IBM Company, Armonk, New York, United States). The difference between variables was analyzed by the χ^2-test. Overall survival (OS) was determined using the Kaplan–Meier analysis and was measured from the start of treatment to the either last follow-up date or to the date of death.

Ethical Approval

This study is approved by the Institutional Review Committee (IRC/2024/P-52, July 9, 2024).

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Results

Out of 54 patients, 31 (57.4%) were females and 23 (42.6%) were males. The median age was 34 years and the age range was 05 to 72 years. In total, 25 (46.3%) patients had TLC count $\leq$10,000 cells/mm^3 and were categorized as low risk and the remaining 29 (53.7%) had TLC count $>$10,000 cells/mm^3 and were categorized as high risk. Platelet count was $\leq$40,000 cells/mm^3 in 45 (83.3%) patients, whereas 9 (16.7%) patients had platelet count $>$40,000 cells/mm^3. Bone marrow morphological examination showed 52 (96.3%) patients having hypergranular variant of promyelocytes and only 2 (3.7%) had microgranular variant of promyelocytes. Cytogenetic analysis showed 49 (90.7%) patients were positive for t (15;17), whereas 5 (9.3%) patients showed variant positivity for t(15;17). Flow cytometry was also done in all the cases and 44 (81.5%) patients showed the classic immunophenotype of APL with 2 (3.7%) patients showing CD34 positivity, 5 (9.3%) with HLA-DR positivity, and 3 (5.5%) patients with HLA-DR and CD34 positivity.

Out of the 54 patients, 53 (98.1%) patients expressed bcr-3 transcript either alone or in combination with other transcripts. In our study, the bcr-3 transcript alone was detected in 21 (38.9%) patients. A total of 28 (51.9%) patients in our study expressed all the three transcripts (►**Table 1**).

Nine patients (16.6%) who were treated with only ATRA succumbed to disease and hence could not receive further treatment. Twenty five (46.3%) patients were treated with combination of ATRA and ATO. A total of 20 patients received chemotherapy, out of which, in 12 (22.2%) patients chemotherapy was given in combination with ATRA and in 8 (14.8%) patients it was given in combination with ATRA and ATO. Chemotherapy was given in 6 (21.4%) patients expressing combination of transcripts in contrast to 12 (57.1%) patients showing isolated bcr-3 transcript.

Out of the 54 patients, 20 (37%) patients died, of which 19 patients died before completion of induction therapy. Complete remission was obtained in 34 patients (63%) after induction therapy.

Table 1 Frequency distribution of different PML-RARα transcripts

PML-RARα transcripts	Frequency ($n = 54$)
bcr-1 + bcr-2 + bcr-3	28 (51.9%)
bcr-2 + bcr-3	02 (3.7%)
bcr-1 + bcr-3	02 (3.7%)
bcr-3	21 (38.9)
bcr-2	01 (1.8%)

Table 2 Clinicopathologic characteristics of bcr-3-positive cases alone and in combination with bcr-1 and bcr-2 transcripts

Clinicopathologic parameters	bcr-1 + bcr-2 + bcr-3 ($n = 28$)	bcr-3 ($n = 21$)
Total leukocyte count		
$\leq$10,000 cells/mm^3	16 (57.1%)	08 (38.1%)
$>$ 10,000 cells/mm^3	12 (42.9%)	13 (61.9%)
Platelet count		
$\leq$40,000 cells/mm^3	22 (78.6%)	20 (95.2%)
$>$ 40,000 cells/mm^3	06 (21.4%)	01 (4.8%)
Morphology		
Hypergranular	28 (100.0%)	19 (90.5%)
Microgranular	00 (0.0%)	02 (9.5%)
Cytogenetics		
Classic t(15;17)	26 (92.8%)	19 (90.5%)
Variant t(15;17)	02 (7.2%)	02 (9.5%)
Immunophenotyping		
Classic IPT	24 (85.7%)	15 (71.4%)
CD34 positive	01 (3.6%)	01 (4.8%)
HLA-DR positive	00 (0.0%)	05 (23.8%)
CD34 and HLA-DR positive	03 (10.7%)	00 (0.0%)
Treatment		
ATRA	05 (17.9%)	04 (19.1%)
ATRA + chemotherapy	03 (10.7%)	07 (33.3%)
ATRA + arsenic trioxide	17 (60.7%)	05 (23.8%)
ATRA + arsenic trioxide + chemotherapy	03 (10.7%)	05 (23.8%)

Abbreviation: ATRA, all-*trans* retinoic acid.

In our study, we also compared the clinicopathologic characteristics of bcr-3-positive cases alone and in combination (►**Table 2**).

Survival Analysis

In our study, we studied the effects of different variables on OS. OS was determined using the Kaplan-Meier analysis. Patients having TLC count $>$10,000 cells/mm^3 belonged to the high-risk category and had significant differences in OS compared with patients having TLC count $\leq$10,000 cells/mm^3 ($p = 0.007$; ►**Fig. 1**). Patients expressing bcr-3 transcript alone had a survival rate of 66.66% as compared with 60.71% for patients expressing bcr-3 transcript in combination with other two transcripts (►**Fig. 2**). However, the median OS in patients with combination of transcripts was 5 months as compared with 9 months for patients expressing bcr-3 transcripts alone. The survival rate for patients receiving ATRA in combination with ATO was far better than patients receiving ATRA alone (►**Fig. 3**). The

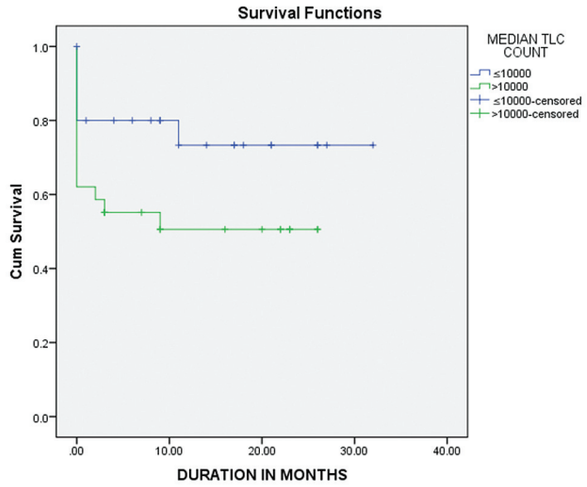

Fig. 1 Kaplan–Meier overall survival of APL patients according to TLC counts. APL, acute promyelocytic leukemia; TLC, total leukocyte count.

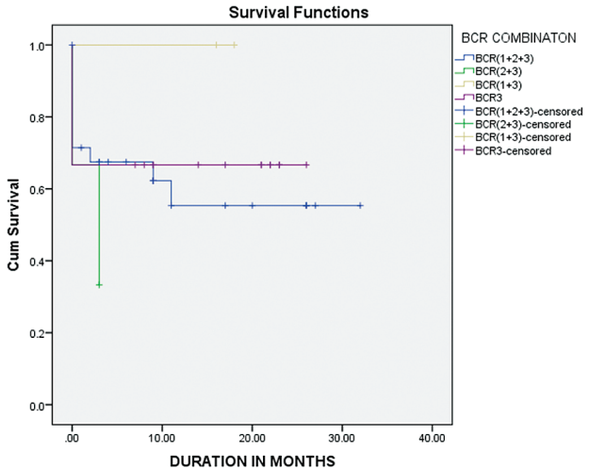

Fig. 2 Kaplan–Meier overall survival of APL patients according to PML-RARα fusion gene transcripts. APL, acute promyelocytic leukemia.

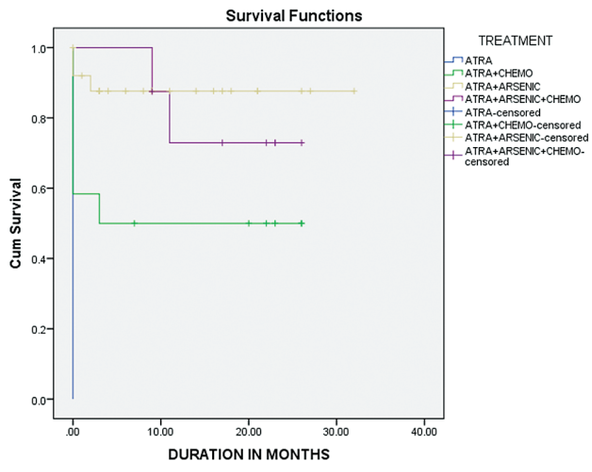

Fig. 3 Kaplan–Meier overall survival of APL patients according to treatment. APL, acute promyelocytic leukemia.

survival rate was also better for patients receiving ATRA and ATO in combination with chemotherapy (►**Table 3**).

Discussion

Depending on the location of breakpoints within the PML gene, APL shows three different transcripts (bcr-1, bcr-2, and bcr-3), which can be seen either singly or in combination. Monitoring of these transcripts is considered as an important part of treatment management in APL.

We aimed to study the pattern and distribution of these transcripts in APL and their effect on survival in our study.

The most frequent pattern of expression of transcripts in our study was a combination of all three transcripts (bcr-1 + bcr-2 + bcr-3) seen in 28 patients (51.9%), followed by bcr-3 alone in 21 patients (38.9%).

Another study conducted by Baba et al studied 45 cases of APL, of which bcr-1 expression was seen in 26 patients, followed by bcr-3 in 16 patients and bcr-2 in 3 patients. Combination patterns were not described in this study.[9]

Rasekh et al studied expression of bcr-3 and bcr-1 in 118 patients with APL, of which bcr-3 was seen in 31.4% and bcr-1 in 52.5%.[10] Similarly, Nath et al also analyzed bcr transcript expression in 29 patients with APL, of which 17 patients had bcr-1 and 12 had bcr-3 expression.[11] Expression of bcr-2 transcripts and combination patterns was not described as in our study.

Of hematological parameters, survival was better in low-risk patients with TLC count $\leq$10,000 cells/mm^3 (p-value = 0.007). Other parameters such as platelet count, age, gender, and immunophenotyping were not statistically significant with OS in our study.

Median survival of patients expressing bcr-1 + bcr-2 + bcr-3 transcripts was 5 months as compared with 9 months in patients only expressing bcr-3 transcripts. However, OS was not statistically significant with pattern of transcripts ($p = 0.445$).

Baba et al's study showed prognostically significant differences in OS with bcr transcripts, which was 100% with bcr-2 transcripts, 88.5% with bcr-1 transcripts, and 56.3% with bcr-3 transcripts.

Nath et al also highlighted higher induction death rates in patients expressing bcr-3 transcripts (34%) in comparison to bcr-1 transcripts (12%).

In our study of 54 patients with APL, bcr-3 was detected in 53 cases, either alone or in combination. Effect of bcr-3 alone was not found to be statistically significant with survival as compared with other studies, which could be explained as combination of all three transcripts was the most common pattern in our study. Hence true impact of bcr-3 alone could not be assessed with survival.

We also observed that in our study, 28 patients showed combination of all three transcripts in contrast to 21 patients showing isolated bcr-3 transcripts, which leads to a possible hypothesis that the prognostic impact of bcr-1 and bcr-2 transcripts cannot be ignored. However, larger cohorts of patients and longer follow-up periods are needed to test this hypothesis.

Table 3 Effect of different variables on overall survival in APL

Variables		Total	Death	Alive		*p*-Value
				N	%	
Overall		54	20	34	62.96	–
Median age	≤34	28	10	18	64.28	0.843
	>34	26	10	16	61.53	
Gender	Female	31	13	18	58.06	0.367
	Male	23	07	16	69.56	
TLC count	≤10,000 cells/mm^3	25	06	19	76.00	0.007
	>10,000 cells/mm^3	29	14	15	51.72	
Platelet count	≤40,000 cells/mm^3	45	15	30	66.70	0.206
	>40,000 cells/mm^3	09	05	04	44.40	
Morphology	Hypergranular	52	19	33	63.46	0.657
	Microgranular	02	01	01	50.00	
Cytogenetics	Classic t(15;17)	49	18	31	63.26	0.765
	Variant t(15;17)	05	02	03	60.00	
Immunophenotyping	Classic IPT	44	17	27	61.36	0.798
	CD34 positive	02	00	02	100.00	
	HLA-DR positive	05	02	03	60.00	
	CD34 and HLA-DR positive	03	01	02	66.66	
PML-RARα transcripts	bcr-1 + bcr-2 + bcr-3	28	11	17	60.71	0.445
	bcr-2 + bcr-3	02	01	01	50.00	
	bcr-1 + bcr-3	02	0	2	100.00	
	bcr-3	21	07	14	66.66	
	bcr-2	01	01	00	00.00	
Treatment	ATRA	09	09	00	00.00	<0.000
	ATRA + chemotherapy	12	06	06	50.00	
	ATRA + arsenic trioxide	25	03	22	88.00	
	ATRA + arsenic trioxide + chemotherapy	08	02	06	75.00	

Abbreviations: APL, acute promyelocytic leukemia; ATRA, All-trans retinoic acid; TLC, total leukocyte count.

This is, to our knowledge, as of today, the only study which has assessed the correlation of combination patterns of bcr transcripts with survival. Patients with combination of all three transcripts were doing worser than patients with bcr-3 transcripts alone, as highlighted by the Kaplan–Meier curve (►**Fig. 2**), even though *p*-value was not found to be statistically significant. Hence, we conclude that bcr-3 has major prognostic impact on OS because the median OS of patients with bcr-3 transcripts alone was also only 5 months.

Future Directions

Cytogenetic and molecular abnormalities such as FLT3-ITD along with PML-RARα transcripts can be studied in patients with APL to evaluate association with prognosis and hence, modification of therapy can be based on presence of poor prognostic molecular markers.

Conclusion

High TLC is a bad prognostic factor in APL as it was statistically significant with OS in our study. Bcr-3 transcript either alone or in combination with bcr-1 and/or bcr-2 transcripts was the most frequent pattern observed. No statistically significant association was observed between OS and pattern of transcripts in our study. Combination of all three transcripts together was noted in our study only till date. Although the results in our study were found to be statistically insignificant, further exploration of combination of patterns is needed to study their prognostic significance.

Study Limitation

Larger cohorts of patients need to be studied to establish prognostic significance of various transcripts on OS and management. Previous studies have not reported

combination of transcripts. Hence comparison with other studies was not possible. Comparisons between bcr transcripts and clinical features were not done in our study.

Conflict of Interest
None declared.

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When Less Is More: An Avenue for Academia–Industry Collaboration in Pediatric Cancer

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We read with interest the article by de Wilde et al that explores partnerships between academia and industry in conducting trials for pediatric cancer drug approval.[1] Cancers in the pediatric group have high cure rates and offer the possibility of significant gains in terms of years of life lived in the event of sustained remission. The pace of cancer drug development has traditionally been slower in children, with reported median lag periods of over 5 years for approvals in comparison to adult use.[2] Legislation mandating pediatric studies for drugs being tested in adults has helped accelerate this process over the last decade.[1] However, the global reach of such approvals is limited. Childhood cancer survival rates are reported to be as high as 80% in high-income countries (HICs), while low- and middle-income countries (LMICs) lag behind.[1,3] Treatment abandonment and social barriers to obtaining treatment are significant issues in LMICs, which may be further exacerbated by high costs of care.[4] Thus, drug approval is only an initial step in the process of translating drug development into survival benefits in childhood cancer. The role of academia–pharmaceutical collaborations in enhancing drug access remains unexplored.

Traditionally, the degree of academic involvement in drug trials, as opposed to pharmaceutical company involvement, is higher in pediatric cancers than in adult cancers. The article by de Wilde et al has emphasized the vital role of partnerships between academia and industry in conducting trials for pediatric cancer drug approval.[1] Clinicians can access large hospital databases that provide real-world patient data about drug use patterns, efficacy, and toxicity. They also have more opportunities to understand the patient's perspective holistically regarding reasons for satisfaction with treatment, nonadherence, treatment refusal, and abandonment. Thus, they may be better placed to identify routine clinical problems and design patient-friendly and cost-effective solutions. On the other hand, pharmaceutical companies could provide funding and rigor to trial conduct and may be better equipped to steer trial protocols in a direction that facilitates approval. In India and other LMICs, drug trials are predominantly pharmaceutical driven.[5] The lack of academic involvement in clinical trials may be due to financial, technical, and regulatory constraints. It has been seen that randomized trials of cancer drugs conducted in LMICs are more likely to identify larger effect sizes and identify effective therapies in comparison to those in HICs.[6] Thus, collaborative ventures in LMICs are likely to reap rewards for both academicians and clinicians. ►**Fig. 1** shows how such collaborations may enable better drug access and benefit the partners involved.

Tannock et al found in their review that drug doses used in adult clinical trials of new drugs are often higher than the effective doses identified in earlier phases.[7] Many investigator-initiated trials have explored the efficacy of lower doses of approved drugs (►**Table 1**).[8–12] While some of these trials may have been conducted with the aim of reducing drug-related toxicities with the support of government funding sources, they have the collateral benefit of reducing treatment-related costs and health care resource utilization.[10] In general, cost-reduction strategies are perceived as not being in line with the commercial interests of pharmaceutical companies. However, a few pharmaceutical-funded trials have also attempted to seek out pharmacoeconomic strategies. A pharmaceutical-funded phase III study of low-dose nivolumab in India demonstrated significant improvement in advanced head and neck carcinoma outcomes.[8] This criti-

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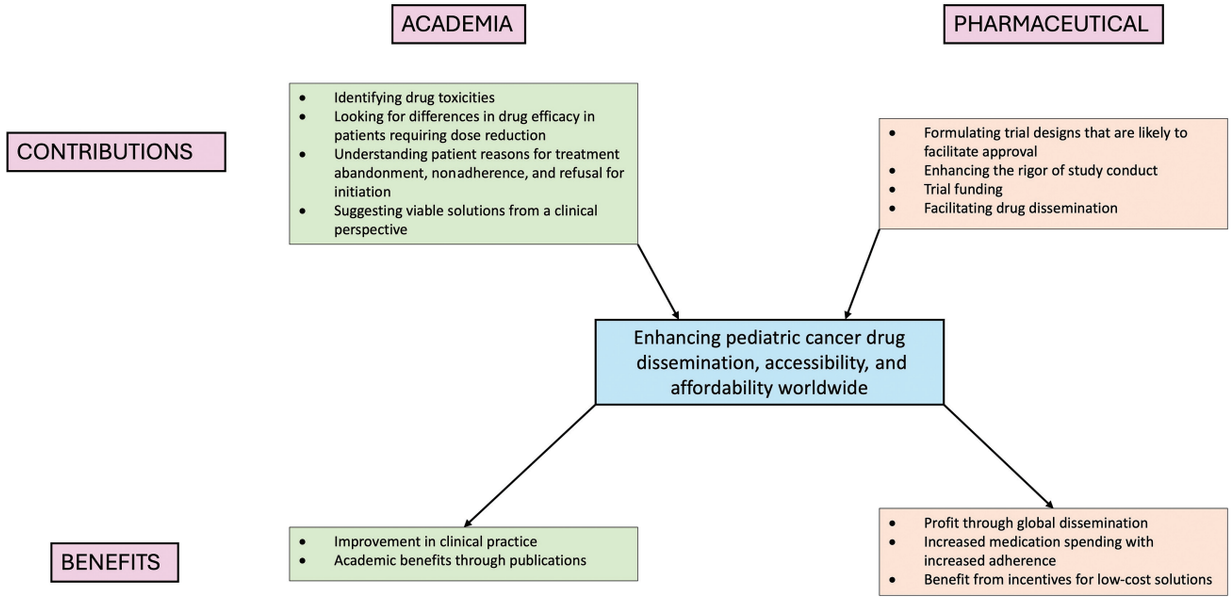

Fig. 1 Academia–drug manufacturer partnerships for enhancing cancer drug access: the contributions of and benefits to each partner.

cal finding has opened up the possibility of expanding the use of immunotherapy for cancer patients in resource-challenged settings.

Cost-effectiveness analyses play a vital role in decision-making for cancer therapeutics. For instance, a retrospective study conducted at a tertiary care center in India showed that oral metronomic therapy and pazopanib showed similar efficacy in the management of advanced soft tissue sarcoma; notably, the cost of oral metronomic therapy was only approximately 1/10th that of pazopanib.[13] However, a systematic review by Al-Badriyeh et al demonstrates that cost-effectiveness analyses sponsored by pharmaceutical companies tend to report results favorable to the sponsor, likely for furthering corporate interests.[14] Pharmaceutical companies could be incentivized by the state to develop innovative low-cost solutions. Drugs found to be cost-effective on independent review may gain the benefit of distribution through the public health care system, thus benefiting both patient care and the pharmaceutical industry.

Although drug manufacturers mention drug development costs as significant contributors to corporate expenditure, drug marketing costs may be greater in magnitude.[15] Drugs that fill a clinician-perceived lacuna in care may potentially have greater uptake among clinicians and patients. Financial toxicity is a significant cause of treatment nonadherence among young cancer patients. Increasing drug adherence

Table 1 Examples of trials exploring the use of lower-dose alternatives for established anticancer drugs in adult cancers

Sl. no.	Drug	Study	Trial description	Funding agency
Targeted therapeutics				
1	Ibrutinib	Chen et al[10]	Pilot study of ibrutinib dose reduction in chronic lymphocytic leukemia	Government
2	Trastuzumab	Earl et al[9]	Phase III trial 6 vs. 12 mo of adjuvant trastuzumab in HER2-positive breast cancer	Government
Immunotherapy				
1	Nivolumab	Patil et al[8]	Phase III randomized study evaluating the addition of low-dose nivolumab to palliative chemotherapy in head and neck carcinoma	Pharmaceutical company
Supportive care drugs				
1	Rasburicase	Vadhan-Raj et al[11]	Trial of single-dose rasburicase vs. daily dosing for 5 d in adult patients at risk of tumor lysis syndrome	Pharmaceutical company
2	Granulocyte colony-stimulating factor	Clemons et al[12]	Trial comparing 5- vs. 7- vs. 10-d schedules of filgrastim for primary prophylaxis of febrile neutropenia in early-stage breast cancer patients	Academic collaboration

could significantly increase medication expenses, thus potentially benefiting pharmaceutical companies. Cost-effectiveness strategies may allow for an increase in drug accessibility to more parts of the world and larger sections of society, which may ultimately allow for monetary benefits through increased consumption. Simultaneously, this may help drastically improve treatment outcomes for childhood cancers in underserved areas worldwide.

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Conflict of Interest
None declared.

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Outcomes of Allogeneic Stem Cell Transplant in Chronic Myeloid Leukemia-Blast Phase: A Single-Center Experience from South India

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Abstract

The blast phase (BP) is challenging to treat and leads to inferior survival in chronic myeloid leukemia (CML). Allogeneic hematopoietic stem cell transplant (AlloSCT) is the only curative option for CML-BP. We are sharing our experience of AlloSCT in seven patients with CML-BP who underwent transplants during the period from January 2017 to December 2019. Three patients each had myeloid-BP, lymphoid-BP, and one patient had mixed phenotypic BP. Donors were matched siblings in four, mismatched siblings in one, and haploidentical in two. All patients received peripheral blood stem cell grafts. The median CD34+ dose was 7.6 (range: 6.6–8.9) $\times 10^6$ cells/kg. Neutrophil engraftment was observed at a median of 15 (10–20) days and platelet engraftment at 19 days (10–22). At a median follow-up of 24 months, the 2-year leukemia-free survival (LFS) and overall survival (OS) were 43% and 57%, respectively. Transplant-related, non-relapse mortality was observed in three patients. AlloSCT results in promising survival for carefully selected patients of CML-BP, especially with a matched sibling donor.

Keywords

- chronic myeloid leukemia blast phase
- allogeneic hematopoietic stem cell transplant
- survival

The blast phase (BP) remains a significant challenge in the treatment of chronic myeloid leukemia (CML). The World Health Organization (WHO) defines BP as peripheral blood or bone marrow blasts of $\geq$ 20% or extramedullary proliferation of blasts.[1] BP can be initial presentation in less than 5% of CML patients.[2] In the era of tyrosine kinase inhibitors (TKI), 2 to 5% of patients progress to the advanced phase (accelerated phase or BP), compared with 10 to 50% in the pre-TKI era.[3–5] The management of BP depends on the type of BP (myeloid or lymphoid) and previous treatment course. The goal of therapy for CML BP is to revert to the chronic phase with the use of newer TKIs (based on prior TKI use and the presence of resistant mutations) with or without chemotherapy (based on the BP subtype), followed by allogeneic hematopoietic stem cell transplant (AlloSCT) preferably within a year of diagnosis.[6] Currently, AlloSCT remains the best chance of cure for BP with 3-year survival ranging from 35 to 40%.[7,8] Patients with BP treated with TKI alone without AlloSCT have poor outcomes with a 4-year survival of only 10%.[9] Similar results were reported in an Indian study with a median survival of 12 months with imatinib alone.[10] There is limited contemporary data of AlloSCT in CML BP from resource-limited settings. We hereby present our experience of AlloSCT in patients with CML BP from a growing transplant unit in a tertiary care cancer center.

The transplant program started at our institute in 2013. The first allogeneic transplant was done in 2014. A total of 48 cases of CML advanced phase were registered in the

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transplant clinic from 2015 to 2020. We have included all consecutive patients of CML-BP who underwent AlloSCT at our center in the given period. We collected data on the baseline patient and disease characteristics, pre-transplant response and donor type, peri-transplant features, post-transplant morbidity and mortality, post-transplant response, and survival from the medical records of the Department of Medical Oncology. Leukemia-free survival (LFS) was taken as the time from the AlloSCT to relapse or death due to any cause. Overall survival (OS) was defined as the time from AlloSCT to death due to any cause. Descriptive statistics were used to summarize baseline and peri-transplant data. The Kaplan–Meier method was used to estimate LFS and OS. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 19.0 (SPSS Inc., Chicago, IL, USA). The institute ethics committee approved the study, and a waiver of consent was granted (approval number JIP/IEC/2016/30/979).

Between October 2017 to December 2019, seven CML-BP (WHO criteria) patients underwent AlloSCT at our center. The median age was 40 years (9–52), and the male-to-female ratio was 5:2. Overall, four patients had de novo BP; the subtype of BP was lymphoid in three, myeloid in three, and mixed phenotypic (B/myeloid) in one. No patient had a detectable mutation on imatinib resistance mutation analysis (IRMA). Dasatinib was the most commonly used TKI before AlloSCT in five patients, Imatinib was used in two. In five patients, chemotherapy (vincristine and steroids for lymphoid BP) or hypomethylating agent decitabine (for myeloid BP) was used along with TKI to achieve remission. All patients reverted to the chronic phase before AlloSCT, i.e., complete cytogenetic response (CCyR) in five; $\geq$ major molecular response (MMR) in one; and complete hematologic response (CHR) in one. The median time from the diagnosis to AlloSCT was 6 months (range: 3–12 months). Pre-transplant disease features, donor characteristics, graft versus host disease (GVHD), veno-occlusive disease (VOD) prophylaxis, post-transplant course, and outcome for all patients are summarized in ►**Table 1**.

All patients received myeloablative conditioning (Flu-Bu in six and Cy-Bu in one, without any dose modifications), and stem cells were harvested from peripheral blood. Four patients received stem cells from a matched sibling donor (MSD), one had a mismatched sibling donor (MMSD, 9/10), and two from the haploidentical family donor. The median CD34+ dose was 7.6 (range: 6.6–8.9) $\times 10^6$ cells/kg. Neutrophil engraftment was observed at a median of 15 days (range: 10–20 days) and platelet engraftment at 19 days (range: 10–22 days). Peri-transplant toxicities in patients within the first 100 days included febrile neutropenia with septic shock in three, cytomegalovirus (CMV) reactivation in three, $\geq$ grade 3 mucositis in two, veno-occlusive disease (VOD) in two, primary graft failure in one, and poor graft function in one patient. Day 100 transplant-related mortality (TRM) was observed in two patients: one had moderate VOD (d22), and the other had primary graft failure (d24). The final event was disseminated infections as the cause of death for both patients. One patient with poor graft function received CD34+ stem cell boost on D + 102 but died because of grade 4 acute graft versus host disease GVHD on D + 174. Post-AlloSCT, all alive patients received dasatinib maintenance starting at a median of 50 days (range: 47–60 days) at a dose of 50 to 100 mg depending on blood counts and tolerance. Among alive patients ($n = 4$), one patient had mild chronic graft versus host disease on long-term follow-up. Another developed dasatinib-related pleural effusion 18 months post-transplant, which resolved with supportive treatment and change of TKI to imatinib. One patient developed CNS relapse at 16 months post-transplant. She was not willing for intensive systemic therapy and second AlloSCT. Hence, dasatinib was continued, and CNS-directed therapy was given with triple intrathecal therapeutics (methotrexate, cytarabine, and hydrocortisone) followed by craniospinal radiotherapy. She had a response in the CNS disease and continues to be on dasatinib dosage with a complete cytogenetic response on the last follow-up. The other three patients are in MMR and are continuing maintenance TKI. On the last follow-up on April 30, 2021, and with a median follow-up of 24 months, the 2-year LFS and OS were 43% and 57%, respectively.

From our small series of AlloSCT in patients with CML BP, we report a promising 2-year LFS and OS of 43% and 57%, respectively, especially with MSD. In the pre-TKI era, CML accounted for ~25% of cases of allogeneic transplants worldwide. In contrast, in recent times, only 2% of AlloSCT in hematological malignancies are done for CML.[11] Present indications for AlloSCT in CML include advanced disease (BP or refractory/progressive AP) and intolerance or resistance to multiple TKIs.[6]

Though there has been a remarkable improvement in outcomes of CML with long-term survival of more than 90% with current therapy for newly diagnosed CML, outcomes for patients in the developing countries are far removed from that in the west.[12] Several challenges are encountered in the day-to-day management of patients with CML in resource-limited settings, including but not limited to higher disease burden, late presentation, poor treatment compliance, lack of resources and expertise, irregular follow-up, monitoring of response, limited accessibility, and affordability to second or higher generation TKIs.[13,14] Thus, a higher proportion of patients present with or progress to advanced phase disease, thereby underscoring the need for AlloSCT in these patient subgroups. However, performing AlloSCT in these settings is even more challenging again due to the lack of centers with expertise and resources for transplant, socioeconomic constraints, the long waiting period for transplant, risk of disease progression, mortality during treatment of advanced disease, lack of general awareness for donor safety and transplant outcomes, and difficulties in donor availability.[15] Our record of transplant clinic registration shows that only 7 (15%) had undergone a transplant in the 48 cases of CML advanced phase registered over the past 5 years.

Despite all the challenges, we have observed an encouraging 2-year survival of 57% in our small patient cohort of CML-BP transplanted in the second chronic phase. Our

Table 1 Pre-transplant features and post-transplant course for all patients ($n = 7$)

Patient	Date of AlloSCT	Age/Sex	Time from diagnosis to transplant (Months)	Pre-Transplant Response	Donor (type/sex)	EBMT Score	HCT- CI Score	Conditioning	GVHD Prophylaxis	Graft failure	VOD[†]	Post-transplant Response (D-100)	Acute GVHD	Chronic GVHD	Relapse	Survival status at LFU
P1	Oct 2017	47Y/M	4	CHR	MSD, male	4	1	Cy-Bu	CSA + MTX	no	yes	CCyR (D30)	No	NA	No	Died (d44)
P2	Jan 2018	9Y/M	6	CCyR	HID,male	3	0	Flu-Bu	PTCY + Tac + MMF	No[*]	No	DMR	Yes[*] (Grade 4)	No	No	Died (d174)
P3	Jan 2019	49Y/M	12	CCyR	MSD, male	4	0	Flu-Bu	CSA + MTX	No	No	MMR	Yes (Grade 2)	Yes (Mild)	No	Alive (d842)
P4	Feb 2019	40Y/F	4	CCyR	MSD, male	4	1	Flu-Bu	CSA + MTX	No	No	DMR	No	No	Yes CNS (d494)	Alive (d801)
P5	May 2019	52Y/M	3	CCyR	MSD, male	4	1	Flu-Bu	CSA + MTX	No	No	DMR	No	No	No	Alive (d716)
P6	June 2019	26Y/F	7	MMR	MMSD, male	4	1	Flu-Bu	PTCY + Tac + MMF	No	Yes	DMR	No	No	No	Alive (d672)
P7	July 2019	31Y/M	11	CCyR	HID, male	4	0	Flu-Bu	PTCY + Tac + MMF	Yes[#] (d24)	No	Unknown (D30)	No	NA	No	Died (d65)

Abbreviations: CCyR, complete cytogenetic response; CHR, complete hematological response; CNS, central nervous system; CSA, cyclosporine; Cy-Bu, cyclophosphamide 60 mg/Kg on Day-7 to Day-6 and Busulphan 3.2 mg/kg/day D-5 to D-2; DMR, deep molecular response; EBMT, European Group for Blood and Marrow Transplantation; Flu-Bu, fludarabine 30 mg/m^2 Day-6 to Day-2 and Busulphan 3.2 mg/Kg/day Day-6 to Day-3; GVHD, graft versus host disease; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; HID, haploidentical donor; LFU, last follow-up; MMF, mycophenolate mofetil; MMR, major molecular response; MMSD, mismatched sibling donor; MSD, matched sibling donor; MTX, methotrexate; PTCY, posttransplant cyclophosphamide; Tac, tacrolimus; VOD, veno-occlusive disease.

[*]D84 had poor graft function, which was treated with stem cell (CD34) boost on d102–this was followed by acute grade 4 GVHD and death on d174.

[†]UDCA (ursodeoxycholic acid) was used for VOD prophylaxis in all until Day 100; patient 1 had moderate VOD (treated with diuretics and other supportive care, defibrotide was not available) followed by sepsis and death; patient 6 had mild VOD that resolved with supportive treatment (fluid restriction and diuretics).

For the two patients with acute GVHD and one with chronic GVHD, treatment was done with systemic corticosteroids (methylprednisolone in acute and prednisolone in chronic GVHD) in the first line.

[#]Patient had primary graft failure, died due to disseminated sepsis before a plan of second allogeneic transplant could be executed.

Table 2 Summary of studies evaluating allogeneic stem cell transplant outcomes in chronic myeloid leukemia advanced phase

Study	*N*	Indication for AlloSCT (*n*)	Phase before AlloSCT (*n*)	Donor (%)	TRM (y)	LFS (y)	OS (y)
CML-IV study 2010[19]	28	CML-Advanced phase (28)	CP2 (15) AP/BP (13)	Sibling (32%) Unrelated (68%)	18% (3)	Not available	59% (3)
CIBMTR 2012[7]	449	CML-advanced phase	CP2 (184) AP (185) BP (80)	MSD (27%) MRD (3%) MMRD/MUD (70%)	CP2: 39% (3) AP: 37% (3) BP: 54% (3)	CP2: 27% (3) AP: 37% (3) BP: 10% (3)	CP2: 36% (3) AP: 43% (3) BP: 14% (3)
Ma et al 2016[21]	90	CML-BP (90)	Not available	MSD (25%) HID (75%)	27% (3)	49% (3)	58% (3)
Swedish CML 2019[16]	118	CP (81) AP (11) BP (21) Unknown (5)	CP1 (56) $\geq$ CP2 (48) AP/BC (14)	MSD (27%) URD (72%) HID (1%)	CP: 11.6% (5) AP/BP: 23% (5)	CP1: 34% (5) CP2: 46% (5) AP/BP: 20% (5)	CP1: 96% (5) CP2: 70% (5) AP/BP: 37% (5)
EBMT data 2019[8]	170	CML-BP	CP2 (95) BP (75)	Related (46%) Unrelated (56%)	CP2: 20% (3) BP: 27% (3)	CP2: 34% (3) BP: 12% (3)	CP2: 51% (3) BP: 24% (3)
TMC abstract 2019[17]	40	CP (26) AP (8) BP (6)	Not available	MSD (83%) MUD (2%) HID (15%)	20% (5 y)	57% (5 y)	70% (5 y)
Neiderweiser et al., 2021[18]	147	BP (96) AP (51)	CP2 (70) AP (40) BP (37)	MSD (39.5%) MUD (24.5%) Unknown (36%)	28%	26% (15 y)	34% (15 y)
Current study	7	CML-BP (7)	CP2 (7)	MSD (57%) MMRD (14%) HID (29%)	43% (2 y)	43% (2 y)	57% (2 y)

Abbreviations: AlloSCT, Allogeneic stem cell transplant; AP, Accelerated phase; BP, Blast Phase; CIBMTR, Center for International Blood & Marrow Transplant Research; CML, Chronic Myeloid Leukemia; CP1, first chronic phase; CP2, second chronic phase; EBMT, European Society for Blood and Marrow Transplantation; HID, Haploidentical donor; LFS, Leukemia free survival; MMRD, Mismatched related donor; MSD, matched sibling donor; OS, Overall Survival; TMC, Tata Memorial Center; TRM, Transplant related mortality; URD, Unrelated donor.

survival outcomes are comparable to those reported in the literature for CML-BP post-transplant.[7,8,16–19] The largest series of transplant outcomes in CML-BP in the literature reports 3-year survival of around 40% in the Center for International Blood & Marrow Transplant Research (CIBMTR) Registry and European Society for Blood and Marrow Transplantation (EBMT) Registry.[7,8] Swedish CML population-based data for all phases of CML have reported 5-year survival of 70% post allogeneic transplant.[16] There are sparse data of transplants in CML advanced phase from developing countries. In one recent report from India, for patients with TKI resistant and advanced phase CML ($n = 40$; including six patients of CML-BP), 5-year OS was 70%.[17] In the most recent report by Neiderweiser et al, donor age >36 years, BP at AlloSCT, and low CD34+ count were shown to be risk factors for inferior OS.[18] ►**Table 2** summarizes the contemporary data on transplant outcomes in CML advanced phase. For patients with CML-BP, the data show definitively that AlloSCT represents the best chance of long-term remission or cure.

Some of the specific challenges we faced were:

(a) Early TRM in three patients, from disseminated infection and sepsis with underlying VOD, graft failure, and severe acute GVHD,
(b) poor graft function, and secondary graft failure for patients with haploidentical donor transplant.

Multidrug-resistant infection is a significant problem across all transplant centers in developing countries, contributing to early deaths and poorer outcomes.[20] Our study's adverse outcomes for the haploidentical transplant patients underscore the need for careful donor selection and reflect the initial learning curve. Studies reporting haploidentical transplant in CML advanced phase have shown that with improvement in post-transplant GVHD prophylaxis and supportive care, survival outcomes were similar to MSD in CML.[21] We suggest that transplant outcomes can be improved for these high-risk patients with thorough counseling of patients and their family caregivers to allay their fear of risk to the donor and general transplant outcomes, proper patient and donor selection, persistent efforts at careful monitoring of pre- and post-transplant conditions, rigorous infection control measures in the transplant unit, and initial mentorship from a high-volume center for both nurses and physicians, if feasible.

The role of post-AlloSCT TKI maintenance in CML is not well-defined. Although it is adopted as a common practice, large studies investigating this approach are lacking. In a recently published report by the CIBMTR study group, only 23% of the patients in their cohort ($n = 390$) received TKI maintenance.[22] A landmark analysis from D + 100, demonstrated no benefit in 5-year clinical outcomes (LFS, OS, relapse, TRM, or cGVHD) in the "TKI maintenance group" when compared with the "no maintenance" group. However, the authors conclude that there were several limitations in their retrospective analysis and choice to initiate TKIs after AlloSCT should be an individualized decision, based on patient, disease, and transplantation-related factors, which may benefit a subgroup of patients.[22] Another retrospective study has shown that TKI maintenance (mostly with dasatinib) might reduce the risk of relapse and improve post AlloSCT survival outcomes in CML and Ph+ acute lymphoblastic leukemia.[23] Maintenance with dasatinib is generally followed in lymphoid BP for neuroprophylaxis as it is known to cross the blood–brain barrier. Presently, with five available TKIs targeting BCR-ABL1, there is no clear choice for optimal TKI following AlloSCT. In our series, all surviving patients received dasatinib maintenance until unacceptable toxicities or medullary relapse. It was feasible to deliver TKI maintenance with minimal toxicities in our setting. Regular monitoring of BCR-ABL transcript levels with a sensitive assay is imperative after AlloSCT to identify early signs of relapse. Other post-SCT strategies such as donor lymphocyte infusion, change in TKI, or use of investigational agents may improve outcomes.

Despite its limitations of retrospective small case series with short follow-up, our experience suggests that AlloSCT can result in promising survival for carefully selected patients of CML-BP, especially with a matched sibling donor. Nevertheless, efforts should be made to improve compliance to first-line treatment for CML in general and enhance transplant resources for eligible patients with advanced phase disease.

Ethics Approval
Institute Ethics Committee approval was taken before the commencement of the study.

Patient Consent
Waiver of consent was granted for the retrospective data and analysis.

Author's Contributions
Study conceptualization and methodology: TN, AB, SK, BD, and PG. Data collection and analysis: TN, AB, NK, SD, DBT, and SK. Manuscript writing: TN, AB, SK, NK, DBT, and BD. Review and editing: SK, BD, SD, and PG. Final approval of manuscript: all authors.

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Conflict of Interest
None declared.

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Isolated Cerebral Metachronous Metastasis in Fibular Osteosarcoma: A Rare Case Report with Review of Literature

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Abstract

Isolated brain involvement is rarely reported as isolated metachronous metastasis from osteosarcoma. Herein, we report a case of fibular osteosarcoma in a young female who presented with solitary hemorrhagic metachronous cerebral metastasis after years of disease-free interval. Imaging showed a large mass lesion in the right posterior temporal lobe with internal areas of bleed not associated with calcification or ossification mimicking high-grade glioma. No other sites of distant metastases were found on the workup. Two-dimensional echocardiography was done to rule out any cardiac anomaly, including the shunt defect, but no abnormality was detected. She was operated for the cerebral lesion, and histopathology of the resected specimen showed osteosarcoma. The patient was started on chemotherapy and is doing well so far. This case presents a unique scenario of osteosarcoma with an isolated lesion in the brain without any other site of distant metastasis.

Keywords
- osteosarcoma
- brain metastasis
- high-grade glioma
- lung metastasis

Introduction

Osteosarcoma is the most common primary malignant bone tumor of the long bones, usually presenting in the second decade.[1] Sixty to seventy percent of localized osteosarcoma treated with surgery and intensive chemotherapy will have 5-year event-free survival.[2] A 3-monthly follow-up for at least 2 years is recommended postchemotherapy, subsequently 6 monthly for the next 3 to 5 years, 6 to 12 monthly for the next 5 to 10 years, and then every 6 to 24 months according to tumor grades and genetics.[3] Low-dose computed tomography (CT) protocol for the chest CT for detection of lung metastasis is recommended, as most of the patients with osteosarcoma are younger population and will have a risk of radiation-induced second malignancy.[3] Osteosarcoma recurring with isolated metastasis to the brain is relatively rare because the lungs are the most common site in approximately 90% of patients, followed by the skeleton in 50% of patients.[4] Bindal et al demonstrated that four patients with osteosarcoma had brain metastasis without lung involvement, but there was an inadequate examination of the lungs by CT scan.[5] Two cases of isolated brain metastasis were also seen in patients with osteosarcoma without having active lung metastasis.[6,7] Another case of osteosarcoma described presented with isolated brain metastasis without lung involvement due to patent foramen ovale.[8] This case raises pertinent questions regarding the management of similar

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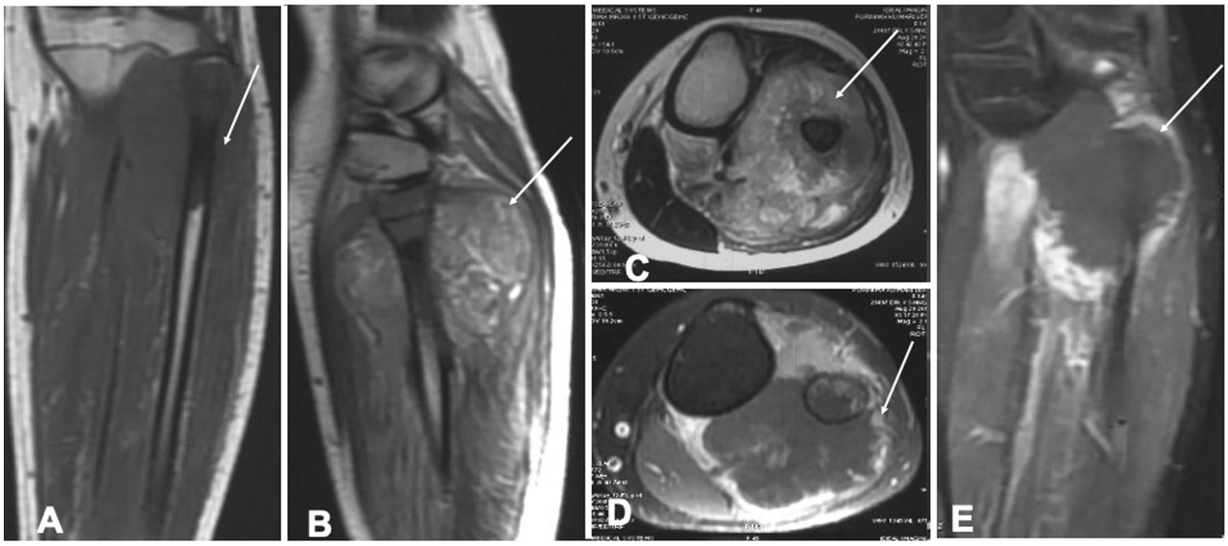

Fig. 1 Magnetic resonance imaging of the calf showing the fibular osteosarcoma. Sagittal T1 (**A**), sagittal T2 (**B**), and axial T2 (**C**) showed heterogeneous a heterogenous lesion involving the upper end of the fibula with adjacent soft tissue components (arrow). Axial phase-contrast (PC; **D**) and sagittal T1 PC (**E**) showed peripheral enhancement with a non-enhancing internal necrotic component (arrow).

patients because the optimal treatment in such patients is not yet well defined. We report a case of isolated brain metastasis in a patient with recurrent fibular osteosarcoma without any documented cardiac abnormality or another site of metastasis.

Case Report

A 17-year-old female initially presented with pain and swelling in the left calf for 1 month. Initial evaluation with magnetic resonance imaging (MRI) of the lower limb (►**Fig. 1A–E**) showed a heterogeneous lesion in both T1-weighted and T2-weighted images at the left upper fibula with surrounding peripheral enhancing soft tissue lesion ($70 \times 63 \times 63$mm) and medullary changes that suggested the radiological possibility of osteosarcoma, Ewing's sarcoma, or chronic osteomyelitis. Histopathology findings on biopsy showed the tumor cell had hyperchromatic pleomorphic nucleoli with a high nuclear/ cytoplasm ratio and a moderate amount of cytoplasm. The tumor showed overall areas of necrosis (80–90%) with few areas of chondroblastic differentiation with features consistent with conventional osteosarcoma. CT chest was done for metastatic screening and found to be normal. The patient was started on neoadjuvant ifosfamide, doxorubicin, cisplatin protocol followed by surgery and adjuvant chemotherapy within 8 months. She had developed ifosfamide encephalopathy during chemotherapy post the eighth cycle, following which further chemotherapy was withheld. She was on regular follow-up, doing fine for the next year and CT chest was normal. The patient started having dull aching, right temporal headache after 1 year of the last cycle of chemotherapy for which she was evaluated with CT scan in a local hospital, which showed a neoplastic mass in the right cerebral hemisphere causing a mass effect with midline shift to the left side (►**Fig. 2A**). MRI was performed after 20 days of symptom onset showed a right supratentorial mass lesion in the right posterior temporal lobe with internal bleeding and peripheral enhancement, mass effect with midline shift. The lesion was T1 hypointense with focal areas of hyperintensities, T2 hyperintense with fluid–fluid level, multiple areas of blooming on susceptibility-weighted imaging, and without restriction on diffusion-weighted imaging (►**Fig. 2B-E**). Elevated perfusion was seen within the lesion and irregular shaggy enhancement on the postcontrast study (►**Fig. 2F, G**). No soft ossification or calcification was seen on phase images. With these constellations of findings, an imaging diagnosis was made of high-grade glioma. The second possibility was osteosarcoma metastasis, as known primary was osteosarcoma of fibula but was confounded by the absence of other metastatic areas and no calcification within the tumor mass. She was admitted to the oncology department and evaluated with positron emission tomography-computed tomography, which showed remission in the primary carcinoma site, with no other metastatic lesion anywhere in the body. Neurosurgery opinion was taken and was they advised for surgery as the tumor was quite big with radiological features suggestive of a high-grade tumor. Right frontotemporal craniotomy and gross total excision of the tumor were done. Intraoperative findings showed an intra-axial firm to hard whitish solid cystic lesion arising from the right lateral ventricle and extending to the temporal base, invading the pial surface. The tumor was dissected circumferentially in a piecemeal fashion and excised with no gross residual disease. Histopathology of the resected lesion showed a tumor in the right lateral ventricle extending to the temporal lobe comprised of multiple firm-to-hard tissue pieces measuring $9 \times 8 \times 1$cm. Microscopy sections examined showed that the tumor was composed of spindle cells, mitosis, and areas of necrosis with lacy osteoid formation and a few chondroblastic areas

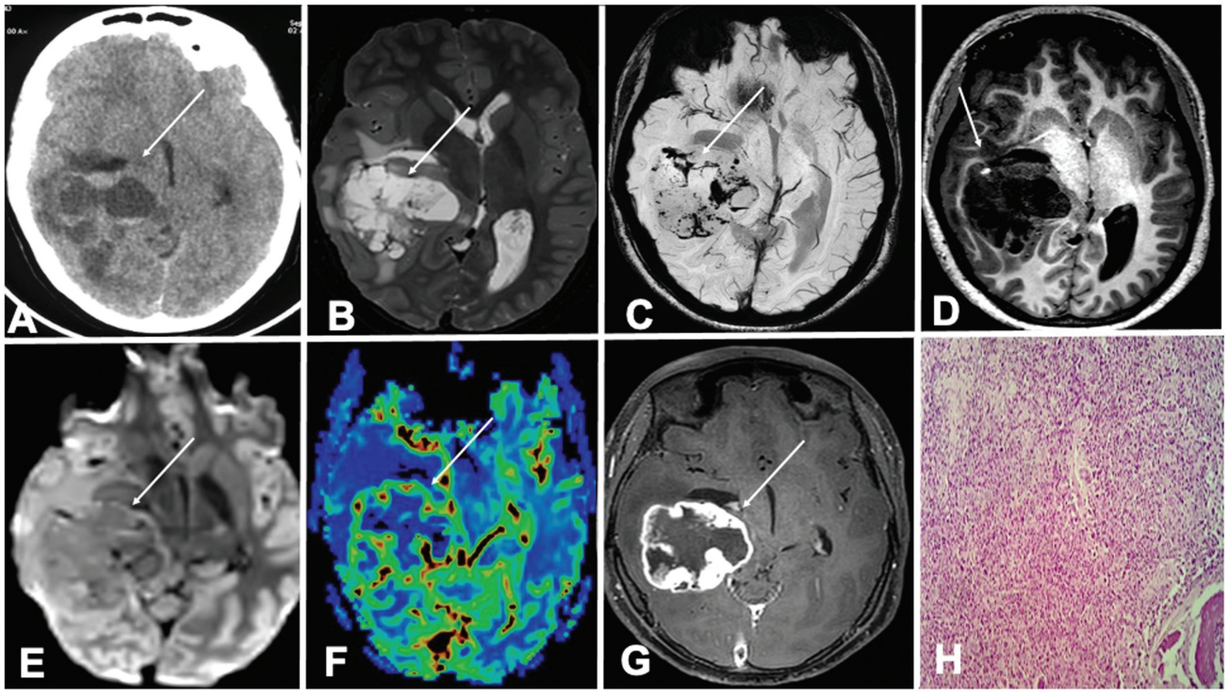

Fig. 2 Magnetic resonance imaging of the brain showing the metastatic lesion within the brain. Axial noncontrast computed tomography head (**A**) showed a large heterogeneous solid cystic lesion with internal areas of hemorrhage and without calcification in the right posterior temporal lobe (arrow). Axial T2-weighted (**B**), axial susceptibility-weighted imaging (**C**), axial T1 (**D**), and axial diffusion-weighted imaging (**E**) showed a large solid cystic multiloculated lesion (arrow) with the fluid–fluid level in the right posterior temporal lobe with internal blooming and without restriction. T2* perfusion (**F**), and axial T1 phase-contrast (**G**) showed elevated perfusion and irregular shaggy enhancement with nodularity (arrow). Photomicrograph (**H**) showed malignant mesenchymal tumor exhibiting pleomorphism and hyperchromasia with osteoid matrix production.

(►**Fig. 2H**). The above features were suggestive of metastatic osteosarcoma. The patient was symptomatically better after the procedure, and was further planned for methotrexate, ifosfamide, and etoposide.

Discussion

Osteosarcoma is the most common malignant bone tumor in children and adolescents, distinguished by an osteoid matrix with atypical cells.[1] Osteosarcoma commonly involves the metaphysis of the long tubular bones[9] and can also occur primarily in skull bones.[10] Different varieties of osteosarcoma in histopathology are osteoblastic, chondroblastic, and fibroblastic, according to predominant features of the cells, but the prognosis and clinical outcome remain the same in these groups.[11] The common mechanism for the spread of bone tumors is hematogenous, as the bones are devoid of lymphatics.[8] Brain metastases are relatively rare in osteosarcoma, with an incidence of 1.8 to 5.6%, and commonly seen in association with lung metastasis, with the postulated mechanism being tumor emboli from the lung migrating to the brain.[12] Mean duration of time interval from the initial diagnosis to brain metastasis in osteosarcoma is 20 months.[13] We report a case of fibular osteosarcoma in a young female with 2 years of disease-free interval, later presenting with solitary hemorrhagic metachronous cerebral metastases without the involvement of the lung. Metastasis in the brain is more common with approximately having ten times higher incidence than primary brain tumors.[14–16] Breast cancer, nonsmall cell lung cancer, and melanoma have their affinity to metastasize to the brain with melanoma as the most common tumor presenting as hemorrhagic brain metastasis.[14,15] Due to its higher vascularity, these lesions can mimic other tumors like glioblastoma[17] and higher association with intratumoral bleeding.[18] Brain metastasis in sarcoma is relatively rare and approximately only 3% of all brain metastases can develop from sarcomas according to the few studies.[16,19] Postulated mechanisms are hematogenous dissemination into the brain as the primary cause and contiguous extension from the skull bone to the brain as the secondary cause.[20] Similar to other metastatic lesions to the brain, the most common site in osteosarcoma metastasis is the gray-white matter junction in anterior circulation due to an abrupt change in caliber of the perforating vessels supplying the brain parenchyma.[21] A unique radiological finding in osteosarcoma metastasis brain is large calcifications within the mass lesion and typically described as "bone within the brain" appearance.[22,23] Our case showed lack of calcification which could be due to the chondroblastic nature of the tumor as evidenced in histopathology. Param et al observed 87 patients and showed that 45% of patients had lung involvement and only 13% of patients had brain metastasis with lung involvement on follow-up. In their study, of those patients who were having brain metastasis,[4] two had

hemorrhagic components, and one had osteoblastic component and unknown nature in two of the cases on histopathology.[4] Two of these cases of osteoblastic osteosarcoma were presented with brain metastasis simultaneously with or after lung involvement, for which they suggested periodic screening with disease recurrence.[24] Similar case reports were observed where fibroblastic osteosarcoma presented with brain metastasis after 15 months of pneumonectomy for lung metastasis[25] and pulmonary, isolated central nervous system relapse can occur in patients with fibroblastic osteosarcoma.[26,27] Osteosarcoma can present with hemorrhagic cerebellar metastasis, where the patient presented with acute neurologic deterioration[28] and multifocal osteosarcomas of the skull presenting with intracranial hemorrhage.[29] Isolated cerebral metastasis was also observed in a few cases, and most of the patients presented with raised intracranial hypertension.[17] Sarcoma with brain metastasis is relatively rare and provides a therapeutic challenge to the treating physicians because of resistance to both chemo and radiotherapy.[16] Our case was unique as there was brain metastasis without having lung metastasis and a cardiac defect. No such case report or study was previously available in the literature. We did echocardiography for any septal defect for the right to left shunting and found it to be normal. A possible explanation for our case was that tumor microemboli might escape through the pulmonary circulation to the systemic circulation and get deposited in the brain. One similar case report was available in the literature isolated brain metastasis without lung involvement in a 12 years male child due to patent foramen ovale resulting in the right to left shunt.[8] Due to the availability of the new chemotherapeutic and radiotherapeutic treatments for soft tissue and bony sarcomas, there was prolonged survival due to systemic disease control, but there was an increased incidence of brain metastases due to less effective intracranial control.[14,15,19] There was also a higher incidence of brain metastasis in patients with other site metastasis at diagnosis of the tumor and patients with recurrence at 1 year of interval period.[14] The average period from the initial diagnosis of sarcoma to brain metastases is approximately 20 months, according to this study.[13] Yonemoto et al suggested screening imaging for the brain at a periodic interval in those patients with pulmonary metastasis.[30] Contrary to the suggestion by Yonemoto et al, Marina et al stated that routine imaging outcomes are debatable in patients already who had metastasis at the time of diagnosis or recurrence within 12 months of interval period.[31] Multimodality treatment is often involved in the management of brain metastasis in osteosarcoma patients.[13] Treatment options for brain metastasis are surgical excision of the lesion with radio and neoadjuvant chemotherapy for the residual disease if any.[13] Although the long-term follow-up details were not available for solitary brain metastasis, Yonemoto et al in their report showed 6 years of disease-free interval after craniotomy and irradiation for solitary brain metastasis.[30]

Hemorrhagic brain metastasis without calcification from osteogenic sarcoma, lung involvement, and a septal heart defect, presenting with neurological complaints, is very rare. It has been postulated that prolonged survival in patients with osteogenic sarcoma due to advances in treatment, results in higher incidence of systemic metastasis.

Conclusion

This case highlights the unique pattern of intracranial metastasis in a case of fibular osteosarcoma that was rarely described in the literature. While following up on osteosarcoma patients, complaints of headache should be investigated for the possibility of brain metastasis.

Authors' Contributions

M.K.N. and S.R. designed and wrote the manuscript. A.N. provided the histopathology and images. S.L.J., G.T., S.R., A.N., and M.K.N. critically reviewed and revised the manuscript. Finally, all the authors have read and approved the manuscript. The requirements for authorship have been met, and that each author believes that the manuscript represents honest work.

Patient Consent

The authors certify that the appropriate consent forms were taken from the patient. In the consent, she had given the consent for the images and clinical information to be reported to this journal. The patient understands that her name and identity will not be disclosed but anonymity cannot be guaranteed.

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Conflict of Interest

None declared.

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Tuberculosis of Frontal Bone—A Rare Entity: Case Report and Review of Literature

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Abstract

Tuberculosis of the frontal bone is a rare entity, most commonly occurring in childhood. In situations of painless scalp swelling coupled with or without a discharging sinus that has not responded to antibiotics, a strong index of suspicion must be raised. Generally, conventional radiography and computed tomography are used for establishing the diagnosis along with microbiological confirmation. We present an interesting case of a young boy who presented with a 2-month-old bone lesion in the frontal scalp, accompanied by mediastinal lymph node involvement. Histopathology revealed tuberculous origin and successful antitubercular therapy resulted in significant improvement within 3 months. The calvarial bones can rarely be affected by tuberculosis, which can present with swelling, sinus discharge, pain, or in rare cases as blindness. Prompt diagnosis of this disease is needed as it is potentially curable and has an excellent prognosis. The cornerstone of treatment is antitubercular therapy, while surgical intervention may sometimes be required. Our case highlights the importance of keeping tuberculosis as a differential at the back of the mind when dealing with scalp swellings, particularly in children.

Keywords
- case report
- tuberculosis
- calvaria
- CT
- PET-CT

Introduction

Infection with mycobacterium tuberculosis (TB) is still widespread in developing nations. With the resurgence of immunocompromised states like human immunodeficiency virus infection, malignancies, etc. there is a rise in the incidence of TB in developed countries. In the last 10 years, there has been a dramatic transformation in the clinical pattern and presentation of TB. Disseminated TB may occur either due to activation of a latent focus of infection or secondary to progressive primary pulmonary TB. The tubercular bacilli are said to erode the epithelial layer of the alveoli, and enter the pulmonary vein. Following this, they enter the left side of the heart, and get disseminated into the systemic circulation thereby reaching bones, kidneys, brain, and other organs. Only 0.2 to 1.3% of skeletal TB cases involve calvarial bones, which is almost always secondary to disseminated TB. The first case was reported in Germany by Reid.[1] Strauss reported 220 cases in 1933.[2] Meng and Wu reported 40 cases.[3]

Case History

An 8-year-old boy, born of a nonconsanguineous marriage and with no family history of cancer, presented with a 2-month-old history of bulge in the frontal region of the scalp. It was initially peanut-sized, and showed subsequent increase in size. The swelling was associated with mild pain. Skin over the swelling, however, was normal. There were no

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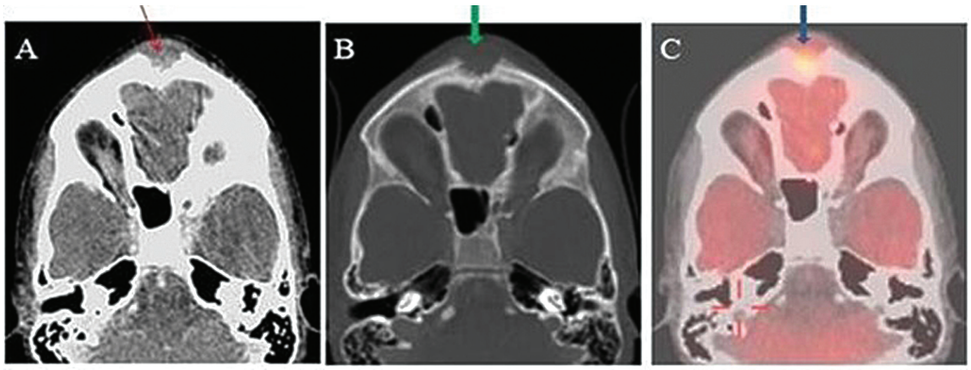

Fig. 1 (**A**) Axial computed tomography (CT) soft tissue window shows a lytic lesion in the frontal bone with associated soft tissue (red arrow). (**B**) Axial CT bone window shows soft tissue in the frontal bone associated with erosion of underlying bone. No periosteal reaction was seen. (**C**) The fused transverse image shows 18F-fluorodeoxyglucose avid lytic frontal bone lesion. Possibilities on imaging were Langhans cell histiocytosis, metastasis. Frontal bone lesion biopsy revealed necrotizing granulomatous inflammation of tuberculous etiology.

constitutional symptoms, no loss of weight, or loss of appetite. On examination, the vitals were stable. There was no sinus at the site of the swelling. No neurological deficits were present.

There was no pallor or organomegaly. Positron emission tomography-computed tomography was done outside our institute that revealed a hypermetabolic osteolytic lesion in the frontal bone in the midline as shown in ►**Fig. 1** and D10 vertebra body associated with soft tissue component. Pleural and parenchymal nodules with associated mediastinal lymph nodes (left hilar, left prevascular, aorta pulmonary, paratracheal, pretracheal and subcarinal nodes) were also seen as shown in ►**Fig. 2**. A frontal bone lesion biopsy was performed under ultrasonography guidance for further assessment, and the sample was tested for acid-fast bacilli staining and polymerase chain reaction (cartridge based nucleic-acid amplification) testing. Results revealed necrotizing granulomatous inflammation of TB etiology. The patient took antitubercular drugs including rifampicin (10 mg/kg), ethambutol (15 mg/kg), isoniazid (5 mg/kg), and pyrazinamide (25 mg/kg) for a period of 3 months followed by rifampicin (10 mg/kg) and isoniazid (5mg/kg) for the next 9 months. The swelling size reduced and complete resolution of the scalp lesion was noted 3 months post initiating the therapy.

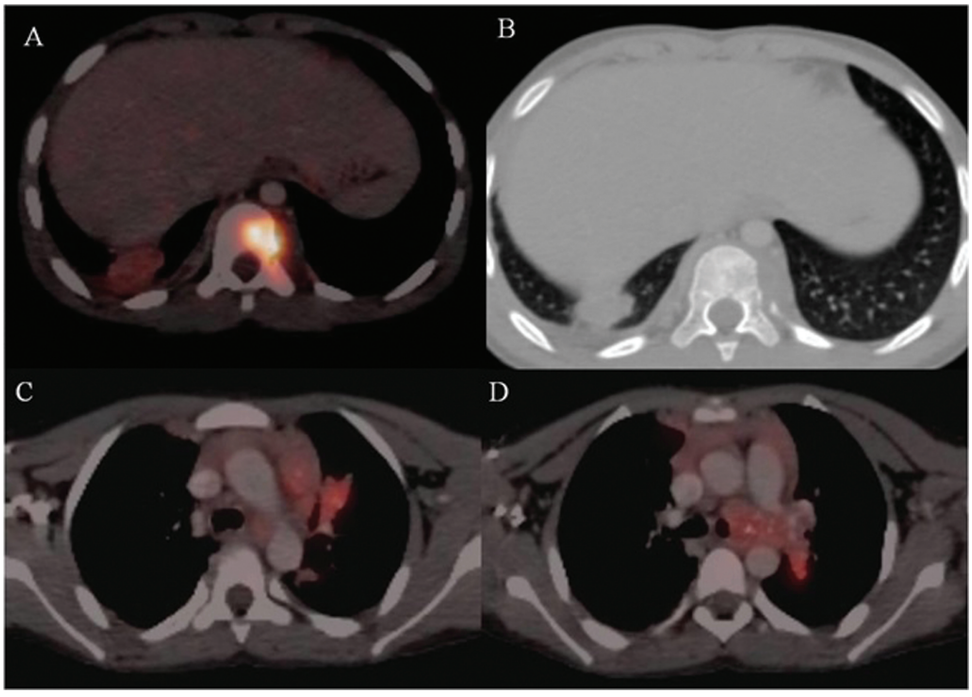

Fig. 2 (**A, B**) Axial positron emission tomography-computed tomography (PET-CT) shows a lytic lesion with associated soft tissue involving body of D10 vertebrae. (**C, D**) Axial PET-CT shows pleura and parenchymal nodules with associated mediastinal lymph nodes with foci of calcification within.

Table 1 Differential diagnosis of calvarial lesion

Pathology	Age	Radiograph	CT	MRI	PET	Additional features
Pyogenic osteomyelitis	Any age; however, more common in children and adolescents	ACUTE: Ill-defined lucent area with or without periosteal reaction, usually associated with sinusitis/mastoiditis CHRONIC: Osteolytic lesion with or without sequestrum and/or involucrum	An osteolytic lesion with or without dead necrotic bone / new bone formation	T1: Isointense to hypointense lesion Cortical destruction T2: Hyperintense lesion with surrounding high SI edema T1c+: Enhancement of periphery of associated collections and surrounding soft tissue	Hypermetabolic	Constitutional symptoms Raised WBC count Blood cultures are positive for bacterial/fungal organisms
Langhan's cell histiocytosis	Children	Geographic skull: Single (eosinophilic granuloma) or multiple osteolytic lesions without sclerotic rim Button sequestrum	An osteolytic lesion with a "bevelled edge" appearance (due to asymmetrical involvement of the inner and outer tables) ("hole within a hole" sign) Button sequestrum	T1: Isointense to hypointense T2: Heterogeneously hyperintense T1c+: Marked enhancement	Hypermetabolic	Asymptomatic or constitutional symptoms may be present depending on multisystem involvement. Histopathology shows "Langhans cells" and immunohistochemistry is positive for CD1a and/or CD207
Multiple myeloma	>40 years, With maximum cases between 50 and 70 years	Raindrop skull: Multiple, variable-sized well-defined punched out osteolytic lesions without sclerotic rim	Osteolytic lesions throughout the skull Axial and appendicular skeletal involvement in the form of diffuse osteopenia, osteolytic lesions with endosteal scalloping, and vertebral compression fractures	T1: Hypointense T2: Hyperintense T1C+: Post-contrast enhancement seen	Hypermetabolic	Monoclonal gammopathy (IgA and/or IgG) Reversal of albumin/globulin ratio Bence Jones protein proteinuria Hypercalcemia
Metastasis	Depend on primary cancer, however more common in the older age group. Commonly from neuroblastomas and Ewing's sarcoma in children	Commonly osteolytic, maybe be mixed/sclerotic depending upon the primary malignancy. May show spiculated ("hair-on-end") periosteal reaction	Commonly osteolytic, maybe be mixed/sclerotic.	T1: Isointense to hypointense T2: Hyperintense T1C+: Post-contrast enhancement seen	Hypermetabolic	
Dermoid/ epidermoid cyst	Any age, however common in 3rd to 4th decades	Well-defined osteolytic lesion with lobulated margins and sclerotic rim	Well-demarcated osteolytic lesion with smooth/lobulated margins, affecting the inner table more affected than the outer table. Internal fat density and/or calcification	T1: Both cysts appear hyperintense T2: Varying signal depending on content T1C+: Dermoid cysts may show peripheral enhancement Intradiploic epidermoid cysts do not enhance	Variable metabolic activity	Dermoid cysts develop along the midline Painless swellings with or without associated sinus tract Epidermoid cysts show restricted diffusion on DWI

Abbreviations: CT, computed tomography; DWI, diffusion-weighted imaging; IgA, immunoglobulin A; MRI, magnetic resonance imaging; PET, positron emission tomography; WBC, white blood cell.

Discussion

TB of the calvarium is almost always secondary to primary tubercular infection at various other sites such as lungs, lymph nodes, bones, gastrointestinal tract, and central nervous system. It is highly unusual to have isolated calvarial TB. There is a male preponderance, with the male to female ratio being 2:1. The maximum numbers of cases (75–80%) occur in less than 20 years of age.[3] It is extremely rare in infants, likely due to a paucity of cancellous bone. Due to the relatively higher proportion of cancellous bone, the frontal and parietal bones are most frequently damaged, followed by the occipital and sphenoid bones. Sphenoid bone involvement has been reported in a few cases.[4] The tubercular bacilli from the primary focus hematogenously seed the diploe to begin the pathogenesis of calvarial TB. The virulence depends on the patient's state of immunity. In immunodeficiency states, there is increased pathogenicity of the organism that causes the infection to spread to both the outer and inner table, thus destroying them, causing obliteration of capillaries, and replacement of bony trabeculae by granulation tissue. Microscopic examination shows necrotic areas, caseation, granulation tissue, epithelial cells, and Langhans giant cells. Involvement of the outer table leads to the formation of soft tissue swelling in the subgaleal region also referred to as Pott's puffy tumor and discharging sinuses. On the other hand, extradural granulation tissue forms when the inner table is involved and its extension continues. Since cranial sutures do not prevent granulation tissue from spreading, substantial bone destruction may take place before a sinus or swelling manifests. The majority of patients with frontal bone TB have painless scalp edema and discharge-producing sinuses. Initial presentation with seizures, focal neurological deficits, and meningeal signs is uncommon. Despite the dura being an effective barrier, subdural empyemas, meningitis, and intraparenchymal extension can also occur. Usually, skeletal TB occurs along with pulmonary involvement.[3] Sometimes, it may have local spread from adjacent mastoiditis. Trauma is also considered as one of the contributing factors due to increased vascularity and decreased resistance.[5] Radiological features are not always classical and diagnostic. The earliest finding is an area of rarefaction. This is followed by a punched-out lytic lesion with a central sequestrum. Sometimes, the defect may be surrounded by a sclerotic rim. There can be single or multiple lesions. Diffuse involvement or base of the skull is less commonly involved.[5] Since sometimes there may be multiple lytic lesions, multiple myeloma and metastases are considered differentials. Conventional radiography, computed tomography (CT), and magnetic resonance imaging (MRI) can be used for the diagnosis of frontal bone TB. The disintegration of one or both of the tables is visible on CT with associated soft tissue swelling.[6] On conventional radiography, three types of tubercular osteitis lesions are identified based on the type of calvarial destruction. The most common subtype are small, punched-out lesions with granulation tissue covering the inner and outer tables of the calvaria also known as "circumscribed lytic lesions." This term was used by Volkmann.[7] It is not associated with periosteal reaction.

Our case likely belonged to this subtype of tubercular osteitis, based on its radiological features as seen in ►**Fig. 1**.

"Spreading-type" is the term coined for lesions causing extensive extradural granulation tissue in addition to substantial damage of the inner skull table. The least common is the "circumscribed sclerotic variant."[8] Periosteal reaction is very rare but has been reported. CT is used to assess the degree of bone involvement, demonstration of the soft tissue component, sequestrum, and spread of disease to the extradural space meninges and brain parenchyma. A hypoattenuating lentiform or crescent-shaped collection describes the characteristic appearance of epidural granulation tissue on CT. The surrounding meninges show intense post-contrast enhancement on contrast-enhanced CT scan. MRI reveals a high signal intensity soft tissue mass within the bone defect projecting into the epidural space, with intense capsular enhancement on post-contrast sequences. MRI has higher sensitivity for the detection of meningeal enhancement, ventriculitis, and intraparenchymal granulomas or tuberculomas (rarely present along with calvarial TB). MRI was not required in our case as neurological symptoms suggestive of complications were absent.

Microbiological and histological confirmation is essential before starting Ziehl Neelsen staining. Biopsy in our case proved fruitful in clenching the diagnosis of TB as necrotizing granulomatous inflammation was detected on histopathological examination, which was further confirmed to be of tubercular etiology by Ziehl Neelsen staining and polymerase chain reaction testing. Other causes of necrotizing granulomas, which could be considered in cases of noninfective cases, include vasculitis such as Wegener's granulomatosis, rheumatoid nodules, and sarcoidosis.

Frontal bone or calvarial TB was primarily treated through surgery, before the advent of modern antitubercular therapy. Surgery is now reserved for patients with large epidural collections, extensive bone destruction, and sequestrum formation or in cases of mass effect on the brain or focal neurological deficits. The sinus tract is removed along with the complete excision of the damaged bone and debridement of the granulation tissue. However, the treatment of tubercular osteomyelitis of the skull is antitubercular treatment with appropriate antibiotic cover for secondary infections. Current guidelines advocate five drugs for at least 24 months. The important differentials to be considered in cases of TB of the frontal bone are pyogenic osteomyelitis; Langhans cell histiocytosis, multiple myeloma, epidermoid/dermoid cyst, and metastases. ►**Table 1** shows differential diagnosis of osteolytic calvarial lesions that can mimic calvarial TB.

Conclusion

The frontal and parietal bones are rarely affected by calvarial TB, which is typically a complication of pulmonary TB. With this case report, we aim to highlight the fact that in a patient

presenting with scalp swelling and a punched out lytic lesion, a suspicion of calvarial TB can be made over a metastatic lesion. Conclusive diagnosis requires imaging findings to be combined with histological and microbiological evidence of the disease. In countries endemic to TB, the differential of tubercular etiology should always be kept in the mind of the clinicians and radiologists to administer prompt and effective care to the patient. This case report hopes to raise awareness that despite isolated calvarial TB being rare, the inconvenience of going through a biopsy for the patient may be avoided if there is a high level of suspicion and clinic-radiological correlation is made. Calvarial TB must be treated surgically and pharmaceutically, and continuous follow-up is essential.

Patient Consent

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

Funding

None.

Conflict of Interest

None declared.

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Small-Cell Lung Carcinoma in an 8-Year-Old Boy: A Rare Case Report with Review of Literature

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Abstract

Lung cancer typically affects older adults, with occurrences in children being extremely rare. In adolescents, metastatic lung disease is more common than primary lung cancers. The pathological spectrum of lung cancer in children varies, with small-cell carcinoma being a rare type, accounting for around 4.5% of cases. An 8-year-old boy, previously healthy, presented with moderate-intensity, short-duration chest pain on the left side. Histomorphology, immunohistochemistry characteristics, and fluorescence in situ hybridization confirmed the diagnosis of extensive-stage small-cell lung carcinoma (SCLC). The patient responded positively to six cycles of chemotherapy with etoposide and carboplatin, resulting in the resolution of symptoms. This case underscores the rare instance of SCLC in the pediatric population, emphasizing the diagnostic challenges due to nonspecific symptoms and the necessity for aggressive treatment. It highlights the importance of adapting adult SCLC treatment protocols for pediatric patients to enhance prognosis, given the limited specific guidelines available.

Keywords
- small-cell lung carcinoma
- pediatric
- metastatic lung disease
- histomorphology
- chemotherapy

Introduction

Primary lung neoplasms predominantly occur in late adulthood and old age with occurrence in children being extremely rare.[1] The overall incidence rate is 1 in 2 million, or 0.2% of all pediatric malignancies.[2] Based on the classification by the World Health Organization (WHO), malignant pulmonary tumors of epithelial origin include non-small-cell lung cancer (NSCLC), which is divided into adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma. Additional lung carcinomas include small-cell lung cancer (SCLC) and neuroendocrine tumors.[3]

During childhood, lung and endobronchial cancers make up a very small percentage of all pediatric malignancies.[4] The different types of lung cancer found in children are quite varied, and one of the rarer forms is SCLC, which makes up approximately 4.5% of cases.[5] Only a small number of patients receive a diagnosis at an early stage, while the majority have an advanced-stage disease, which unfortunately has a very grim prognosis, with a survival rate of less than 5% after 2 years.[6–8] Here is the case summary of an 8-year-old boy who received extensive-stage SCLC diagnosis and underwent investigative workup and treatment at our center.

Case Presentation

An 8-year-old school boy from a suburb of Jaipur, Rajasthan, presented to our medical oncology department in July 2023, with complaints of left-sided chest pain of moderate severity and short duration. On admission, he did not exhibit symptoms such as cough, fever, hemoptysis, weight loss, fatigue, or dyspnea. He had no history of smoking or any exposure to passive smoke.

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Upon examination, pallor was noted, but there were no signs of icterus, edema, clubbing, cyanosis, or lymphadenopathy. Auscultation revealed decreased breath sounds in the left lower basal area without any other additional sounds, and the rest of the systemic examination was normal. The results of routine hematological and biochemical tests returned to normal. Chest X-ray revealed a mass on the left lower lung. Contrast-enhanced computed tomography (CECT) scan of the chest revealed a large, well-defined, lobulated, heterogeneously enhancing mass in the left lower lobe, adjacent to the descending aorta and the left posterior aspect of the pericardium, measuring $7 \times 8.5 \times 7.5$ cm. There was minimal left pleural effusion (►**Fig. 1**). Multiple enlarged, heterogeneously enhancing lymph nodes were observed in the left hilar, subcarinal, left retrocrural, and upper paratracheal regions, with the largest measuring 20×20 mm, and a few enlarged lymph nodes in the celiac region.

The positron emission tomography (PET) scan for staging revealed the presence of an irregularly shaped, metabolically active soft tissue mass in the lower left lung lobe. This finding suggests the possibility of primary neoplastic disease (►**Fig. 2**). The mediastinal and left retrocrural regions showed small, inactive lymph nodes, indicating the presence of nearby metastatic lymph nodes. No other abnormal metabolically active lesions were found in the body. A biopsy of the lung mass found a malignant small round cell tumor, suggesting a possible diagnosis of neuroendocrine small-cell carcinoma. Microscopically, the tumor cells were arranged in sheets and occasionally displayed rosette formation. These small, round-to-oval cells exhibited a blue appearance, minimal cytoplasm, inconspicuous nucleoli, and slight overlap with nuclear molding. The stroma was thin and delicate, with occasional mitosis and patchy calcification. Immunohistochemistry (IHC) of the biopsy revealed thyroid transcription factor-1 (TTF-1) and synaptophysin positivity, while CK7 and chromogranin showed focal positivity. The biomarkers CD56, desmin, CD99, vimentin, and NKX 2.2 were found to be negative (►**Fig. 3**). Fluorescence in situ hybridization (FISH) analysis revealed no evidence of EWSR1 gene rearrangement in 100% of the cells studied. Next-generation sequencing did not reveal any clinically relevant pathogenic mutations or fusions.

Based on histomorphology, immunohistochemical features, and FISH reports, a diagnosis of extensive-stage small-cell carcinoma (pure cell type) of the lung was made.

The nature of the disease and the patient's prognosis were explained in detail to the patient's parents, who provided their consent to start chemotherapy. The patient began a chemotherapy regimen consisting of etoposide (100 mg/m^2/dose) and carboplatin (350 mg/m^2/dose) from days 1 to 3, following the latest CHEST recommendations for initial treatment.[9] Chemotherapy led to significant side effects, including intense nausea, vomiting, and neutropenia. He was given oral ondansetron and injectable filgrastim to manage the side effects. During his last follow-up on August 1, 2024, the high-resolution computed tomography of the chest revealed a small atelectatic area adjacent to the pericardium, likely reflecting posttreatment changes (►**Fig. 4**). The patient is currently in remission and continues to be monitored closely.

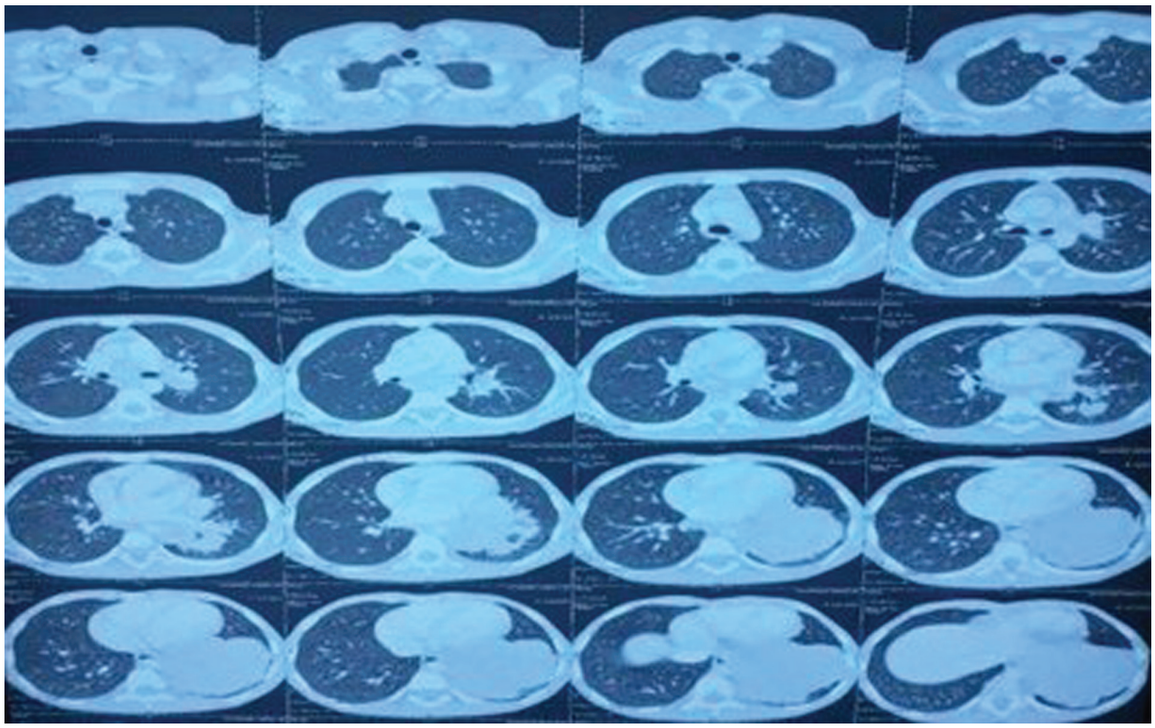

Fig. 1 Contrast-enhanced computed tomography of the chest showing an enlarged mass in the lower left lung.

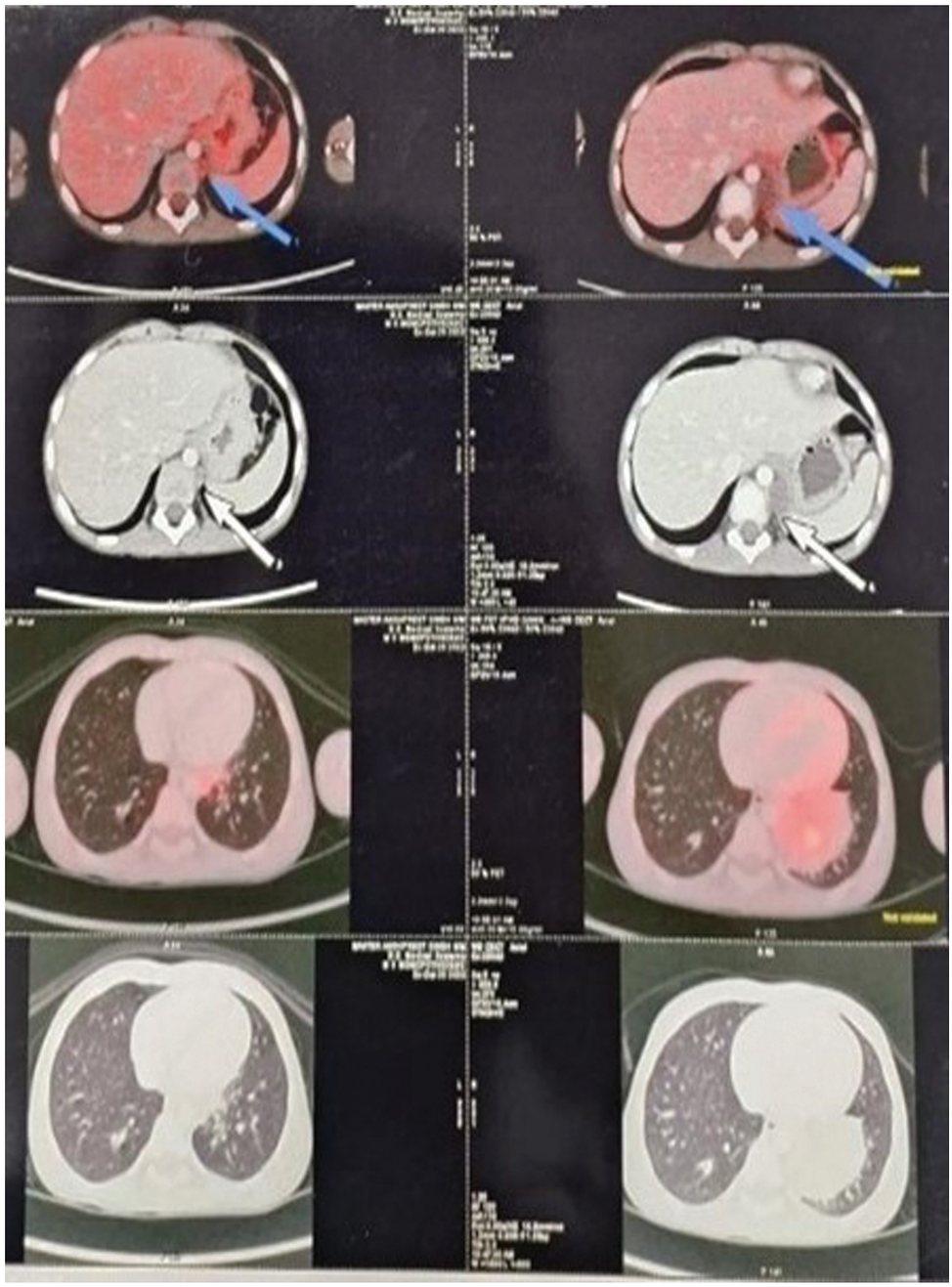

Fig. 2 Positron emission tomography computed tomography scan showing soft tissue mass in the lower left lung lobe.

Discussion

SCLC is predominantly a malignancy seen in middle-aged and older adults, often strongly associated with smoking as a key risk factor. In children, the pathological spectrum of lung cancers is diverse, and SCLC is one of the rarest forms, accounting for only a small fraction of cases.[10] Several reports in the literature highlight that primary malignant pulmonary tumors in children tend to be more aggressive than in adults, often presenting at an advanced stage and with a poor prognosis.[11,12] Delays in diagnosis can occur due to the lack of severe symptoms and low awareness of the disease in pediatric patients. The presenting symptoms are typically nonspecific and may mimic other infectious diseases or congenital lesions.

When considering the differential diagnosis for mediastinal masses, it is important to categorize them based on their location. In the anterior mediastinum, the most common masses include lymphoma, thymoma, teratoma, angioma, lipoma, and thyroid tumors. In the middle mediastinum, vascular anomalies such as double aortic arch, right aortic arch, left aortic arch with aberrant right subclavian artery,

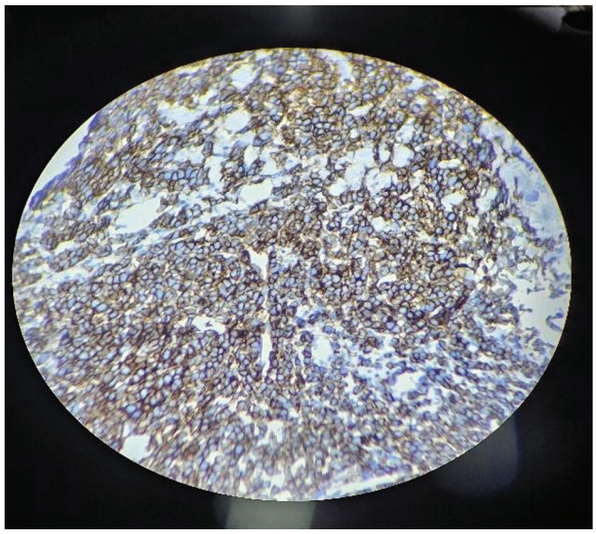

Fig. 3 Immunohistochemistry showing synaptophysin marker positivity.

pulmonary artery sling, and duplicated superior vena cava are often found. Nonvascular middle mediastinal masses can consist of congenital foregut duplication cysts, esophageal duplication cysts, and neuroenteric cysts. Lymphadenopathy can also occur, which may be infectious—typically caused by tuberculosis or histoplasmosis—or neoplastic, as seen in lymphoma, Wilms' tumor, germ cell tumors, and osteosarcoma. In the posterior mediastinum, the most common masses are sympathetic ganglion tumors, such as ganglioneuroblastomas and ganglioneuromas, particularly in older children.[13]

In this case, an 8-year-old boy presented with left-sided chest pain, without typical lung cancer symptoms like cough, shortness of breath, fever, or weight loss complicating the diagnosis given the lack of smoking history. Radiological investigations revealed a large mass in the left lower lung alongside metastatic lymph nodes, prompting further histopathological examination. A biopsy confirmed a malignant small round cell tumor, and FISH analysis ruled out other malignancies, such as Ewing's sarcoma, due to the absence of EWSR1 gene rearrangement.[14]

The treatment of SCLC poses considerable challenges, as only minimal improvements in anticipation have been noted over the past three decades. For adults with early-stage SCLC, surgery may be a viable option aimed at curing the disease. Radiotherapy has diverse effects on both limited-stage and extensive-stage SCLC. The standard initial treatment for SCLC involves antineoplastic therapy, typically combining a platinum-based drug with etoposide or irinotecan.[9]

Given the extensive nature of the disease and the poor prognosis typically associated with SCLC in children, an aggressive treatment approach was adopted. This patient was treated according to the CHEST guidelines with a chemotherapy regimen of etoposide and carboplatin. This treatment plan was selected based on its proven efficacy in adult patients with SCLC and its adaptability to the pediatric population despite the scarcity of specific pediatric treatment protocols for SCLC.

According to existing literature, the youngest reported case of SCLC is in a 14-year-old boy.[15] However, based on our knowledge, the patient in this case report represents the youngest documented case of SCLC to date. The patient's response to chemotherapy was promising, showing considerable improvement, and the patient currently remains asymptomatic. This outcome highlights the potential for effective treatment responses in pediatric SCLC patients, even though these patients are rare and typically have a poor prognosis.

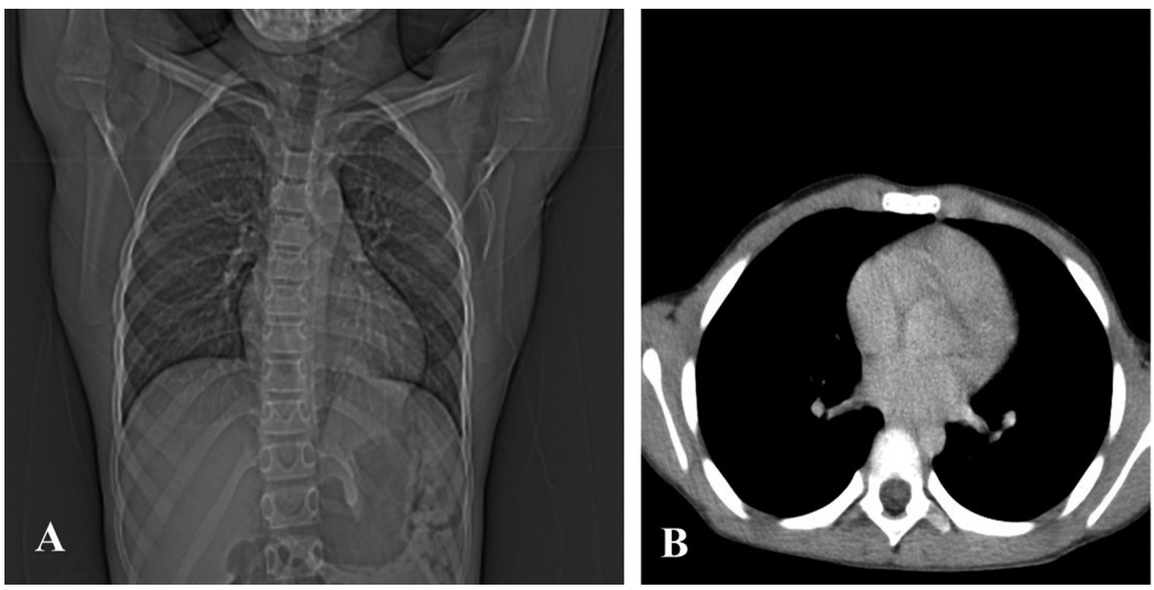

Fig. 4 (**A**, **B**) Posttreatment follow-up chest high-resolution computed tomography images demonstrating a small area of atelectasis in the left lower lobe.

Conclusion

This case emphasizes the importance of early and accurate diagnosis, aggressive treatment, and comprehensive follow-up in managing pediatric SCLC. It also highlights the necessity for further research and development of pediatric-specific treatment protocols to improve outcomes for young patients with this rare and aggressive cancer. The successful management of this case provides hope and valuable insights into the potential for treating similar cases in the future.

Authors' Contributions

M.G., S.B., and G.M.B. worked toward managing and following up with the patient. M.G. and R.R. had the inception of the idea of writing the case. S.D. and M.G. wrote the manuscript. G.M.B. edited and fine-tuned the manuscript. S.B. helped in the diagnosis of the disease.

Ethical Approval

The current study was approved by the institutional review board (IEC/P-835/2024) at the NIMS University, Rajasthan, Jaipur. Written informed consent for publication of this case report and any accompanying images was obtained from the patient's legal guardians.

Patient Consent

Informed consent was provided by the guardian's of the patient.

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Conflict of Interest

None declared.

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An Uncommon Case Report of Malignant Epithelial Ovarian Neoplasm in the Pediatric Age Group with a Brief Literature Review

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Abstract

Epithelial ovarian neoplasm is the most common gynecological malignancy causing mortality in adults, but is a rare diagnosis in children and adolescents. The majority of the malignancies found in adolescents are of germ cell origin. Low-grade serous ovarian malignancies may arise de novo or may follow already diagnosed case of serous borderline tumor. Clinically, patients present with nonspecific symptoms like lower abdominal pain. Apart from radiology, it is equally important to carry out histopathology, which shows numerous micropapillary structures having scant fibrovascular cores. We report a rare case of low-grade papillary serous ovarian neoplasm in a 5-year-old girl, wherein we discuss the need to do thorough clinical, radiological, pathological, and biochemical investigations for all women despite age category so as to provide adequate management on time.

Keywords

- case report
- epithelial ovarian neoplasm
- low grade
- pediatric
- serous cystadenocarcinoma

Introduction

Ovarian neoplasms are a significant cause of mortality among malignancies in developed countries, ranking just after breast cancers in females.[1,2] While epithelial ovarian neoplasms predominantly affect adults, germ cell tumors are more frequently observed in children and adolescents. Epithelial ovarian neoplasms are exceedingly rare in females below the age of 17 years.[3] Among these, low-grade serous carcinomas (LGSCs) are seen in only 2 to 5% of all ovarian tumors and 5 to 10% of all ovarian cancers.[4] In this report, we present an exceptionally rare case of low-grade papillary serous cystadenocarcinoma of the ovary in a 5-year-old girl.

Case Report

A 5-year-old girl presented to the pediatric outpatient department (OPD) with periumbilical abdominal pain for 9 months. Recently, she also experienced loss of appetite and few episodes of nonprojectile vomiting; however, she denied history of fever. Her bowel and urine habits were normal. Family history was unremarkable. General examination revealed no abnormality. She showed normal growth and development according to her age and sex. On per abdominal examination, there was mild abdominal distension with tenderness in the periumbilical region. The rest of the systemic examinations were within normal limits. With the clinical differential of persistent chronic infection, she was referred for ultrasonography (USG) of the abdomen and pelvis, which showed the presence of a 15×10 cm complex abdominal mass suspicious of ovarian neoplasm. The rest of her basic biochemical parameters were within normal parameters. All the serological markers performed were within normal limits, including Alpha fetoprotein (AFP), Lactate dehydrogenase (LDH), Human chorionic gonadotrophin (β-HCG), Cancer antigen 125 (CA125) preoperatively. Computed

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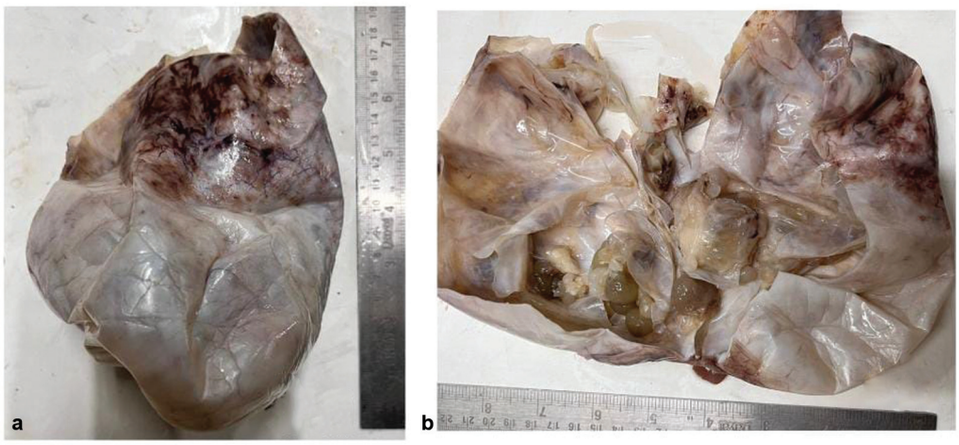

Fig. 1 (a, b) Gross image shows an ovarian mass measuring 18 × 15 × 10 cm, with the cut section showing clear cystic areas and focal solid areas.

tomography (CT) scan of the abdomen and pelvis revealed a hypodense solid-cystic mass coming from the pelvis measuring 15 × 10 cm possibly indicative of malignant ovarian neoplasm. With a radiographic diagnosis of right-sided complex tubo-ovarian mass, exploratory laparotomy was planned. Contralateral left side ovary was normal. Excised tubo-ovarian mass was then sent for histopathological examination. No nodal involvement was seen intraoperatively. Grossly, we received an ovoid mass measuring 18 × 15 × 10 cm. The cut section showed clear fluid with complex cysts and focal warty areas (►**Fig. 1**). Microsection from the solid and cystic areas showed focal papillary projections lined by neoplastic epithelial cells with the surrounding fibrocollagenous stroma (►**Fig. 2a, b**). Solid nodular areas showed hyperchromatic pleomorphic neoplastic epithelial cells, with mild to moderate nuclear pleomorphism having prominent central nucleoli at few places (►**Fig. 2c–f**). The mitotic index was 10/10 HPFs (high power fields). However, there was no evidence of necrosis, or high-grade nuclear features, hence indicative of a low-grade neoplasm. With the above histomorphological features, a diagnosis of low-grade papillary serous cystadenocarcinoma was made. Immunohistochemistry (IHC) was done for confirmation of the same findings. It showed nuclear positivity for Wilms tumor protein (WT1), estrogen receptor (ER) and wild type expression of *P53* (►**Fig. 3a–c**) and negative *P16* expression (►**Fig. 3d**). The patient was referred to a higher oncology center for further treatment; however, she was lost to follow-up.

Discussion

Epithelial ovarian cancers represent 90% of all the ovarian tumors.[3] According to the latest fifth edition of the WHO classification of ovarian malignancies, epithelial ovarian malignancies are broadly categorized into serous tumors, mucinous tumors, endometrioid tumors, clear cell tumors, and seromucinous and Brenner's tumors. Serous tumors are further subcategorized into serous cystadenomas, adenofibromas, serous borderline tumors, LGSCs, and high-grade serous carcinomas (HGSCs). LGSCs have a unique clinical presentation and molecular behavior and distinguishes them from their close counterpart, that is, HGSCs. LGSCs usually present at a young age and may show gradual progression with resistance to chemotherapy. They usually get detected at a later stage. Identification of risk factors apart from genetic mutation is too difficult to assess because of its presentation at the young age.

Shih and Kurman, in 2004, gave a "two-pathway theory" of ovarian neoplasms based on the histopathological, clinical, and genetic analysis.[5] Type 1 ovarian neoplasms associated with *K-RAS* and *BRAF* mutations show the benign nature of progression as seen in our case. In contrast, type 2 ovarian neoplasms associated with *BRCA* 1/2 present aggressively.[3] Malpica et al described a two-tier system that differentiates LGSCs from HGSCs based on categories of nuclear pleomorphism and mitotic activity/HPFs. LGSCs show mild to moderate nuclear pleomorphism and have mitosis up to 12/10 HPFs.[6]

Papillary serous cystadenocarcinoma of the ovary is most commonly seen among all ovarian neoplasms, constituting nearly 50% of all malignant ovarian tumors. They usually show bilateral involvement of the ovaries.[7] Few case reports have been documented in adolescents, and none have been reported in a child as young as 5 years. The most common site of metastases includes the contralateral ovary, peritoneum, and para-aortic and pelvic lymph nodes.[8] Whenever ovarian neoplasm suspected, it is ideal to carry out nonspecific markers like CA-125, which acts as an adjunct to the diagnosis.[4]

Macroscopically, LGSCs are bilateral and exhibits a papillary growth. Microscopically, the noninvasive subtypes show a nonhierarchial architecture having micropapillae and a cribriform pattern in an expansile growth. Micropapillary papillary projections have a length at least five times the width with scant fibrovascular cores. Individual tumor cells are cuboidal to polygonal in shape, having scant eosinophilic cytoplasm, high nucleocytoplasmic ratio, with mild to moderate nuclear pleomorphism and small centrally placed atypical nuclei.[9] Invasive subtypes are seen with noninvasive components based on the absence of destructive infiltrative growth like either serous adenofibroma or serous borderline

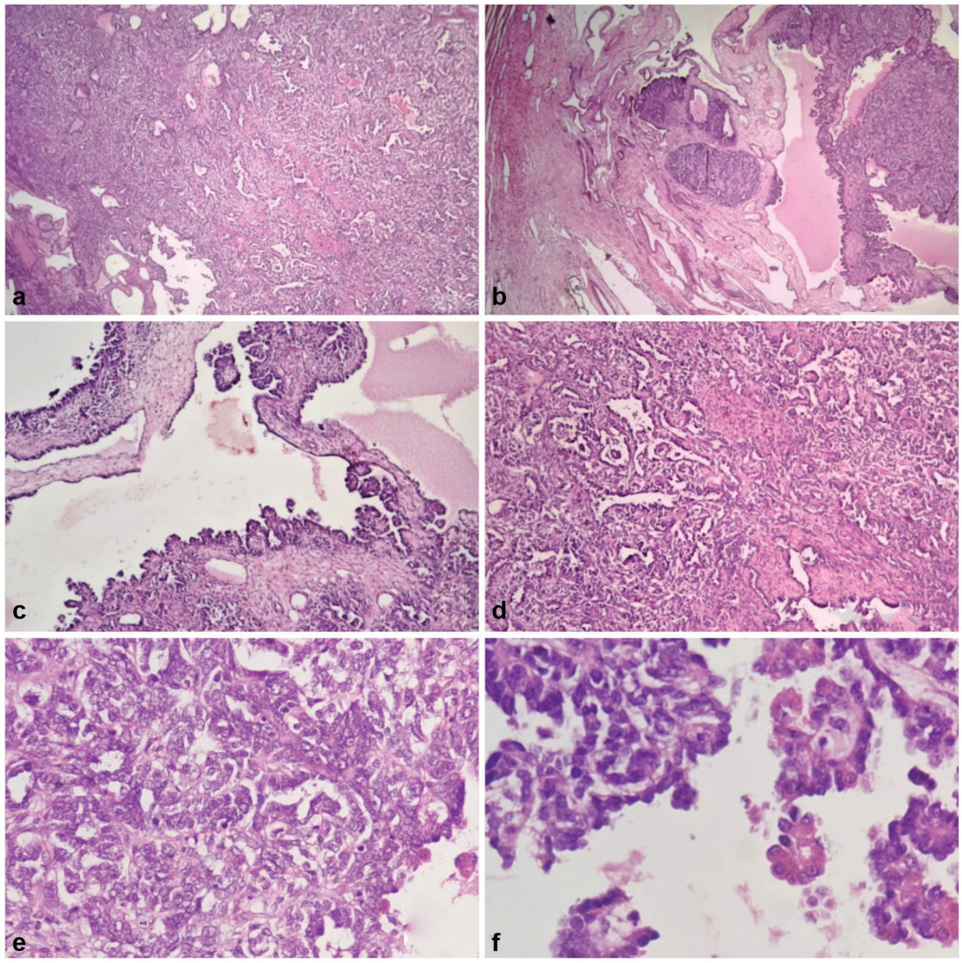

Fig. 2 (**a, b**) Microphotograph shows tumor cells arranged in diffuse sheets surrounded by fibrocollagenous stroma and some areas showing pale eosinophilic cystic areas in between (hematoxylin and eosin [H&E], 40x). (**c, d**) Micropapillary architecture with low mitotic rates less than 12/10 high-power fields (HPFs; H&E, 40x,100x). (**e, f**) Individual tumor cells are cuboidal to polygonal in shape, with scant eosinophilic cytoplasm, centrally placed round nucleus with prominent nucleoli at few places with no features of stromal invasion (H&E, 400x).

tumors.[3] Numerous psammoma bodies are commonly seen in LGSCs. The differentials include serous borderline tumor, which does not show invasion or prominent nucleoli.[10] HGSCs, according to Malpica et al, show increased nuclear pleomorphism with moderate to severe nuclear pleomorphism, and mitosis more than 12/10 HPFs.[6]

Apart from radiological investigations, IHC plays a crucial role in making the final diagnosis and differentiating from other ovarian neoplasms. On IHC, LGSCs show strong nuclear positivity for WT1, a marker of serous differentiation, PAX-8 which ensures mullerian origin, ER, PR, Her2neu, P16 is diffusely positive in high grade serous ovarian cancers, whereas it is negative or patchy positive in low grade serous cancer cases. P53 shows a wild-type pattern in LGSCs in contrast to HGSCs, which show an aberrant heterogenous pattern.[11] So the characteristic feature of this entity is diagnosis at comparatively young age and relative chemoresistance in comparison to high-grade serous ovarian neoplasms.[12]

Nasioudis et al conducted a study on females below the age of 19 years between 1988 and 2013 to assess the diagnosis of 114 cases of borderline and 140 cases of malignant epithelial ovarian cancer (EOC). Their study showed a median age of 17 years, with the majority diagnosed as mucinous neoplasms followed by serous neoplasms. Most of them had undergone fertility-sparing surgery and had grade I neoplasm.[13]

However, malignant epithelial tumors, especially, serous cystadenocarcinomas, are rarely present in the literature. Gupta et al[14] conducted a systematic search of literature from 1980 onward from the PubMed database including the following words: "ovarian tumor," "epithelial," and "children/pediatric groups." Their search showed the majority of epithelial tumors occurred alone in all age groups, with 0.04% of epithelial neoplasms in children, most of which were cystadenomas followed by malignant adenocarcinomas, small cell carcinomas, and squamous cell carcinoma with two case reports of papillary serous cystadenocarcinomas (one in a15-year-old adolescent girl and another in a 4-year-old girl).[15] In the past 10 years, only one case of serous papillary cystadenocarcinoma in 17-year-old adolescent girl has been reported in the literature.[3]

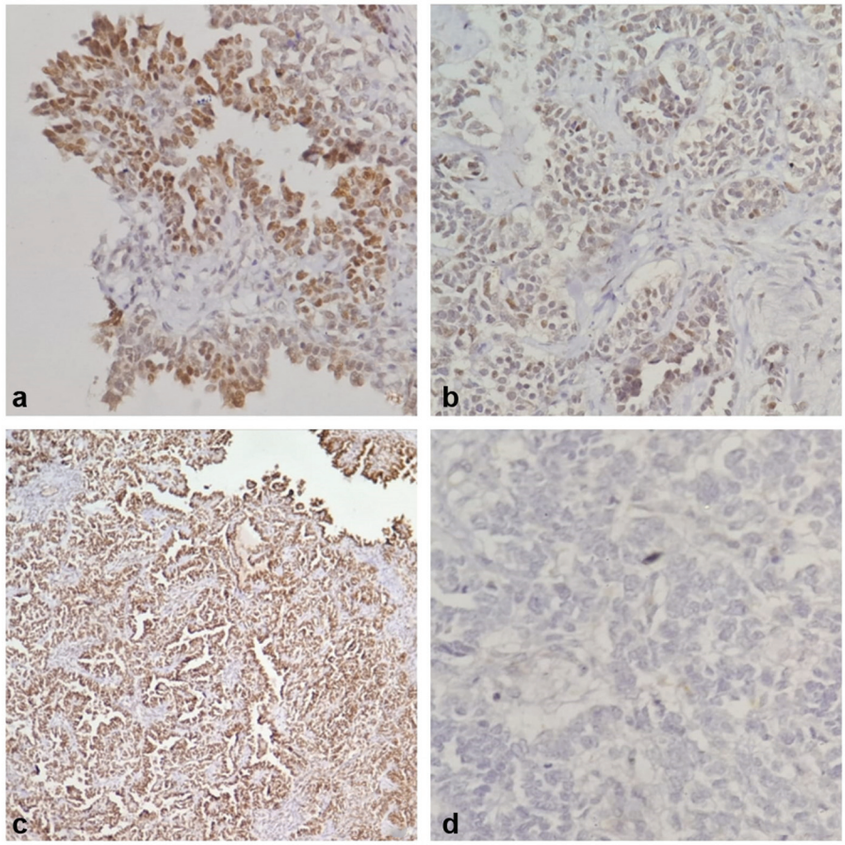

Fig. 3 (**a**) Estrogen receptor (ER) shows strong nuclear positivity in tumor cells (immunohistochemistry [IHC], 400x). (**b**) p53 shows wildtype expression in tumor cells (IHC, 400x). (**c**) WT1 shows strong nuclear positivity in tumor cells (IHC, 100x). (**d**) p16 shows negative in tumor cells (IHC, 400x).

Among adolescents, the prognosis is favorable, so a conservative approach can be followed to preserve the fertility.[4] The role of chemotherapy is limited although it is ideal to be given along with surgical debulking. Singer et al[9] suggests *MEK* enzyme inhibitors as target therapy for the mitogen-activated protein kinase pathway (*MAPK*), which is the known pathway involved in the causation of low-grade serous ovarian neoplasms.[12] However, surgical debulking along with a combination of cisplatin and paclitaxel chemotherapy is an ideal management approach for a case of low-grade serous cystadenocarcinoma of the ovary according to Babaier et al.[16] Schmeler et al tried six cycles of platinum-based neoadjuvant chemotherapy in 25 patients, of which 88% were stable following six cycles.[17]

Conclusion

The rarity of such cases in the literature poses challenges in studying disease frequency and prognosis. Furthermore, to date, no other cases of LGSC have been diagnosed in children aged 2 to 5 years. This case underscores the critical need for comprehensive clinical examination, including proper history taking, thorough physical examination, and meticulous analysis of radiological, biochemical, and histopathological investigations. IHC studies should also be considered essential, regardless of the patient's age. Early diagnosis and proper decision regarding surgery and additional chemotherapy methods provide a good life to the patient.

Authors' Contributions

P.M. made significant contributions to literature search, data acquisition, and manuscript preparation. S.K.H. made significant contributions to concepts, design, definition of intellectual content, and manuscript review, and is a guarantor. S.S. made significant contributions to concepts, design, definition of intellectual content, and manuscript review. R.N.M. has made contribution to intellectual content and manuscript review. P.N. made significant contributions to manuscript review and concepts.

Patient Consent

Informed consent was taken from the patient party.

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Conflict of Interest

None declared.

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Nirogacestat in Desmoid Tumor Management: Painful to Painless?

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Desmoid tumors are comparatively rare, locally aggressive, and nonmetastatic tumors leading to significant morbidity in the patient but rarely leading to death. Local aggressiveness of the tumor can lead to a variety of symptoms like pain, bowel obstruction, functional impairment, nerve damage, and perforation of the bowel. Management of desmoid tumors is difficult owing to variable presentation and unpredictable course of the disease, with 20 to 30% cases showing spontaneous regression. Hence, the management of desmoid tumors ranges from active surveillance to surgery, tyrosine kinase inhibitors (TKIs), and radiation therapy with no fixed modality of treatment as it varies depending on the location of tumor and the symptoms of the patient.[1]

Nirogacestat, which has been recently approved by the Food and Drug Administration (FDA) on November 27, 2023,[2] is a promising drug that holds immense potential in fighting desmoid tumors. It is the first-ever approved drug for desmoid tumors.[2] Nirogacestat was initially developed for Alzheimer's disease but later on was proved to be effective in desmoid tumors.[3]

Nirogacestat (brand name: OGSIVEO)[4] is an oral, selective, small molecule gamma secretase inhibitor that blocks prophylactic activation of Notch receptor. A Notch receptor, when dysregulated, can activate pathways that contribute to tumor growth.

This approval was based on the DeFi trial.[5] Studies demonstrated that patients with desmoid tumors have genetic mutations in Wnt–adenomatous polyposis coli–β-catenin pathway (*CTNNB1* and *APC*). In addition, desmoid tumors overexpress Notch1, which is responsible for progression of these tumors, which can thus be regulated with the help of gamma secretase inhibitors, through selective inhibition of gamma secretase–mediated cleavage of Notch receptors.

The DeFi trial is a phase 3, international, double-blind, randomized and placebo-controlled trial with an aim to determine the safety as well as efficacy of nirogacestat in adult patients of histologically proven progressive desmoid tumor. A total of 141 patients were randomized in 1:1 ratio to placebo versus nirogacestat (150 mg) twice daily continuously in 28-day cycles. The primary endpoint was progression-free survival. Imaging-based progression was determined by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria 1.1. Secondary endpoints were objective response, quality of life, physical functioning, and role functioning. The median duration of exposure to nirogacestat was 20.6 versus 11.4 months for placebo. Adverse events included diarrhea, nausea, maculopapular rash, fatigue, hypophosphatemia, stomatitis, headache, dermatitis acneiform, and vomiting. Ninety-five percent of the adverse events were either grade 1 or 2. Dose reduction due to adverse events was seen in 42% patients receiving nirogacestat. Ovarian dysfunction was seen in 27 out of 36 women of childbearing age. However, 74% patients had resolution of symptoms.[5] It was observed that at cycle 10, nirogacestat showed significant benefits over placebo in terms of pain, symptom severity related to the disease, and physical functioning. Forty-one percent of patients in the nirogacestat group showed objective response, whereas only 8% in the placebo group showed the same. Complete response was seen in 7% in the nirogacestat group and was not seen in the placebo group.[5]

Gamma secretase cleaves multiple transmembrane proteins, including Notch, that are believed to play a role in activating pathways that contribute to the growth of desmoid and ovarian granulosa cell tumors. Therefore, in another phase 2 trial, the role of nirogacestat in the management of ovarian granulosa tumor is being evaluated at present. In addition, nirogacestat's usefulness in the management of multiple myeloma is being observed in preclinical models.[4]

Currently, nirogacestat is recommended for those patients who are inoperable or have been refractory to at least one line

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of therapy. The recommended dosage is 150 mg twice daily till disease progression or unacceptable toxicity. Based on the findings of animal studies, nirogacestat can cause fetal harm and hence it is not recommended in pregnancy at present. Females and males of reproductive potential are advised to use effective contraception during treatment with nirogacestat. Adverse events including ovarian dysfunction have been observed in females of reproductive age group, which was reversed on discontinuation of therapy.[6]

Another randomized trial with the drug AL102, another gamma secretase inhibitor, is enrolling patients with advanced desmoid tumors.[3]

For dose and prescription information of nirogacestat, see U.S. FDA.[6]

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Conflict of Interest
None declared.

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