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Editorial

Ethics in Publication

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The growth of Medical Sciences is very fast. The core of enrichment of Medical Sciences is dependent on the evidence gathered and published. Performing a well-organized experimental research and final submission of the same as an article is a tedious and painstaking process. Published research article is only the tip of the iceberg. It involves long planning, execution, analysis, and document preparation.[1]

Ethical principles apply at every stage of research starting from planning till the publication of the document. Ethics is derived from the Greek word "*ethikos*," which is derived from the Greek word *ethos*, meaning custom or character. Ethical issues and principles are important for all the pillars of publication, that is, authors (during execution and reporting of research), reviewer (at the time of reviewing the article), and the editor of the journal.

Research misconduct is defined by the Royal College of Physicians of Edinburgh as "any behavior by a researcher, whether intentional or not, that fails to scrupulously respect high scientific and ethical standards."[2] Various types of research misconduct include fabrication or falsification of data, plagiarism, problematic data presentation or analysis, failure to obtain ethical approval by the Research Ethics Committee or to obtain the subject's informed consent, inappropriate claims of authorship, duplicate publication, and undisclosed conflict of interest.

Recently there has been a decline in the ethical principles guiding scientific research. Serious thought has to be given on commercialization of scientific research, which has its effects on the ethical principles and advancement of scientific knowledge. Ethical misconduct done out of ignorance or intentionally has the same consequences, and seriousness of the event remains the same.

Types of Research Misconduct

Research misconducts should be taken on priority to respect the intellectual property rights of others and uphold the standards for academic publishing. Research misconducts can be broadly classified into the following:

- **Plagiarism:** Plagiarism is presenting another person's thoughts, ideas, figures, mythology, words, etc., as if they were author's own work, without giving due credit or acknowledgment.
- **Fabrication:** Fabrication is generation of data without the research being conducted.
- **Falsification:** Falsification is manipulation of data end results intentionally to make them clinically relevant. Selective reporting of data also comes under this heading. Selective reporting is primarily done in pharma industry where the main effects of drugs are highlighted and the other effects are either concealed or given less weightage.
- **Copyright infringement:** It is presenting another person's work of authorship or their ideas as his or her without giving proper acknowledgment.
- **Duplicate (or redundant) publication:** This is the practice of submitting the same article to multiple journals or republishing the same manuscript without reference to a previous publication. When the article republished adds a part of a previously published article, it is known as ***redundant publication***. Publication of single dataset into multiple articles is known as ***salami slicing***.
- **Overlapping publication:** This is the practice of publishing an article that overlaps with the previously published article.
- **Inappropriate authorship:** According to the International Committee of Medical Journal Editors (ICMJE) guide-

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lines, anyone who has made substantial contribution to the conception or design of the work, or the acquisition, analysis, or interpretation of data; drafting or revising the article for intellectual content; or participated in the final approval of the version to be published; or agreement to be accountable for all aspects of the work is entitled to be an author.[3] Authorship dispute arises when an author who has contributed in the manuscript is not included in the author list or a person who had not contributed is given an authorship (guest, ghost, and gifted authorship).

- **Misconduct within the publication process** includes authors submitting manuscripts under fraudulent names or with fraudulent affiliations or reviewer misconduct during the peer review process.

To assess the data regarding falsification and fabrication, Daniele Fanelli conducted 21 surveys that were included in the systematic review and 18 in the meta-analysis. A pooled weighted average of 1.97% of scientists admitted having fabricated, falsified, or modified data or results at least once and up to 33.7% admitted other questionable research practices. In surveys asking about the behavior of colleagues, admission rates were 14.12% for falsification and up to 72% for other questionable research practices.[4]

Fang et al, in a review article, showed that 67.4% of retractions of publications were attributable to misconduct, including fraud or suspected fraud (43.4%), duplicate publication (14.2%), and plagiarism (9.8%). He also stated that the percentage of scientific articles retracted because of fraud has increased approximately 10-fold since 1975.[5] A Chinese journal also finds that 31% of the articles are plagiarized.[6]

Falsification and fabrication were considered as most common and frequent violation (44.9%) in a recent review by Armond et al. Other violations in decreasing order of frequency are non-adherence to laws and regulations (15.7%) such as research and ethics committee approval and lack of informed consent, patient safety issues (11.1%), and plagiarism (6.9%). Most of the cases reported were from Medical and Health Sciences (80.8%), other cases were from Natural Sciences (11.5%), Social Sciences (4.3%), Engineering and Technology (2.1%) and Humanities (1.3%). Most prevalent sanction was paper retraction (45.4%) followed by exclusion from funding applications (35.5%).[7]

An article was published to find out the number of retracted articles from Indian authors, and it showed that there are 508 retracted articles (as on November 2, 2020) authored by Indian authors and account for nearly 6.2% of retracted publications indexed in the PubMed database. However, the number of retracted articles is very low compared with the number of publications contributed by Indian scientists in the database (~0.1%). Twenty-five percent of retracted articles were published in the top 15 journals and 33% were published in the nonimpact factor journals.[8]

Research misconduct leads to many long-lasting consequences. The product developed, based on false and fabricated data, can be unsafe for humanity. This can also mislead the fellow researchers as well as medical practitioners and students. Also, it may destroy public trust on science and misguide the government to implement erroneous health policies.

Fabrication of data for research is a criminal act. Fabrication/falsification leads to wrong conclusions and use of such information may harm the patient and endanger life. The future research may be intended on articles with falsified/fabricated data such as misconduct including redundant publication/salami slicing, and overlapping publication, which can lead to flawed conclusions in a meta-analysis.

Avoiding Accidental Plagiarism

Several steps can be taken to avoid accidental plagiarism. They can be summarized as the following:

- Scrupulously acknowledge prior relevant work.
- Use quotation marks for direct quotes.
- Clearly indicate direct quotation while making notes.
- Use your own words while paraphrasing someone's ideas.
- Provide citations for commonly known facts.

Ethical Issues Arising due to Increasing Use of Artificial Intelligence

It has been advocated that decisions made by artificial intelligence (AI) are based on informed decisions and are devoid of any bias and subjectivity. However, this is not always true, and there are many ethical issues related to it.

- There is lack of transparency of AI tools. Thus, decisions taken are not always comparable to humans.
- AI is not neutral and is susceptible to inaccuracies, discriminatory outcomes, and bias.
- Surveillance practices for data gathering and privacy.
- One must be careful while using AI, and human supervision has to be individualized, or else false information will be disseminated.

Recommendations

World Association of Medical Editors (WAME), ICMJE, and Committee on Publication Ethics (COPE) are the guiding forces to interpret ethical publication appropriately. In any kind of misconduct, COPE guidelines are to be followed. They address various issues of study design, ethical approval, authorship, conflict of interest, data analysis, plagiarism, duplicity, salami publication, and also duties of the editors and reviewers. The following are a few recommendations for ethics in publication:

- Assessing the quality of research by the number of publications and other such metrics, which happens too frequently, should be stopped as this leads to people participating in various forms of misconduct, including augmenting publication numbers most commonly by salami slicing of manuscripts.
- Public universities and public-funded science should not be neglected; researchers in these places are accountable to the public.
- Ethical standards and conduct have to start from school.

- It is essential to have investigative committees with external people who are unbiased.
- Citation should be read critically and selection bias should be avoided.
- The best way to avoid plagiarism is to cite other's work in quotation marks and ask permission from the copyright holder.
- Authorship criteria should be followed as per the ICJME guidelines, and contribution of the authors should be stated.
- Any conflict of interest should be disclosed.
- Research integrity should be encouraged among medical researchers.
- Journal editors must provide WAME, ICMJE, and COPE guidelines to authors as well as reviewers.
- Latest technological support and strong peer review system should be used.

It can be concluded that research is conducted to alleviate human sufferings, so the authors must plan, execute, analyze, and publish their research in an honest way. An ethical environment in the institution will always promote good and ethical publication.

Conflict of Interest
None declared.

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Options of Funding and Ethical Clearance for Medical Researchers in India

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Abstract

Research forms an integral part of present world development and interest. It is the primary source of speculation and outcome-based decision making. Medical research work proves to be a big challenge in low- and middle-income countries due to the constraint of resources and capacity building. The disparities in the distribution of resources, inadequate policy implementation, and lack of prioritization of research make the research challenging. There has been an increase in medical research in India but it is not adequate when compared to other countries or areas. Medical researchers face multiple issues, mainly funding and ethical approval and are stunted by the unacceptance in high-indexed journals. In this paper, we have compiled the options for funding and ethical options and ways available for researchers in India. This will help and encourage researchers pro-actively by providing some guidance on the issues related to finance and ethics required for conducting scientific research.

Keywords
- Medical Research
- challenges
- ethical clearance
- funding

Introduction

Research is the creation of new knowledge and/or the use of existing knowledge to develop new concepts, methodologies and to broaden our understandings.[1] It is a stepwise process which uses collected information to increase our understanding of a topic or issue.[2] Medical research (research related to health) is the stepping stone towards making people healthier and providing quality care. It can be related to the disease trends and risk factors, outcomes of treatment or public health interventions, functional abilities, patterns of care, health care costs and use, and drug or vaccine efficacy and adverse effects.[3] It helps the decision-makers in keeping up with the struggle of rapidly evolving scientific knowledge and applying it to healthcare practice, organisation, and policy. It involves the uptake of clinical procedures, technologies, and organisational models to achieve the best possible healthcare outcomes and to stop practices that are no longer supported by evidence to be beneficial.[4]

The research output from low- and middle-income countries (LMICs) such as India compares poorly with that of high-income countries.[5] There has been an increase in the medical research in India but it is not adequate when compared to other countries or areas.[6,7] This phenomenon, dubbed the '10/90 gap' by the Global Forum for Health Research, refers to the fact that of the over $ 70 billion spent worldwide on health research each year, only about 10% is invested in research into 90% of the Global Burden of Disease (GBD).[8] The inequality rises from macroeconomic, power imbalances and resource drainage to perceived better prospects.[9] Majority of research work in India takes place in the major teaching hospitals wherein the caseload is too high to dedicate a respectable amount of time to genuine research or for discussions. In such a scenario, there is a drill culture, where

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instead of coming up with better or more efficient ideas, healthcare workers are forced to stick to the norms. Risk-taking is not a viable option for the majority of healthcare professionals in India as such initiatives are often seen with suspicion and mistrust, and often lead to communication breakdown. The output of good-quality medical research from India is grossly inadequate, and there is a need for strategic planning. There is a gross low relative research output in several health conditions in India that have a high burden.[10]

A researcher can be affiliated with an academic (or other) institute/organisation or unaffiliated (independent). Affiliated researchers conduct research by using the facilities of their university, libraries, or by collaboration. Unaffiliated researcher (Independent researcher) is not affiliated with any institute/organisation.[11,12] He/she is motivated toward the research by personal interest or passion over the topic. An affiliated researcher can be limited by time constraints, lack of motivation and most importantly independence or freedom. It becomes important in many scenarios wherein an institution or its policies may keep many ideas in check, for the fear of disturbing the homeostasis of the societal fabric. In such a situation, independent research can be the ideal combination for an effective change agent. The independent researcher has the education, expertise, and motivation. He/she can be a change agent, or effective advocate, for an idea or cause.[13] Both types of researchers can face several hindrances in conducting research. One of the major issues is the availability of funds and ethical clearance.[10] There is also a lack of willingness for strategic collaboration, undermining the value of both research and its projected outcome.[14] In such a situation it becomes difficult for the researcher to carry out research effectively and this, in turn, leads to a vicious cycle of losing out in the race for good quality research and study. In this article, we discuss the opportunities and options available for medical researchers in India in terms of funding and ethical clearance. This will ease the process of conducting research and will encourage researchers pro-actively by focusing on the issues related to finance and ethics faced by them.

Funding Options

Not all the studies require funding. Many studies have been done in the past without any financial support. It can be done by utilising the available infrastructure and resources or with the help of volunteers. However, most of the studies require financial support. The majority of the research, if undertaken under the purview of an institution, can be funded by the institute itself (intramural) or from outside the institute (extramural). Researchers who are not affiliated with any institute may face several challenges. The common funding options for an unaffiliated researcher can be through fellowships, grants or awards.

The ways to avail of funding for medical research are enumerated below:

1. Fellowships – Fellowships usually pertain to aids for research in a particular area. Fellowship may refer to just a position and may not include a financial component.
2. Grants – They usually cover expenses related to specific research. There is a funding opportunity followed by applications and decisions regarding the award of the grant. Details are provided in ►**Table 1**.
3. Awards – An acknowledgement or appreciation of the achievements in the respective field. It can be monetary and it can be used for the research.
4. Funding from the Ministries /organisation in India can be obtained under the projects/schemes. Most of this funding is availed through affiliating with the NGOs/associations/societies/bodies/institutes. A list of funding organisations is provided in ►**Table 2**.
5. Availing through registered organisations/societies/trust– Under this, organisations working on the related topic can be approached and research can be initiated in collaboration. Most of the registered organisations get funding for the research or they can apply for grants at various portals. For an independent researcher, working with an organisation makes it easier to get funds for research. From the funder's perspective, the accountability and transparency of the fund are relatively higher in the organisation. Various private companies in India try to invest more in Non-governmental organisations (NGOs) under the Corporate Social Responsibility (CSR).
6. Partnership with the other group of researchers who are working on the same area of interest.
7. Professional societies/associations/trust of medical professions can also be approached; e.g. Indian Medical Association (IMA), Indian Association of Dermatologists, Venereologists and Leprologists (IADVL), Indian Academy of Pediatrics (IAP), Indian Radiology and Imaging Association (IRIA), Indian Association of Preventive and Social Medicine (IAPSM), Indian Public Health Association (IPHA), Association of Physicians of India.

Ethical Clearance

Details about when to approach an Ethics committee have been shown in ►**Figure 1**. For a researcher affiliated with an institution, any research taken up is addressed by the Institutional Ethics Committee (IEC) of the particular institution. In case of collaboration with any other institute, the Ethics Committee of the collaborating institute can be approached for a get-go from their side. For an independent researcher, one of the major challenges faced is getting ethical clearance for their research. Due to the lack of affiliation to an institute /organisation or lack of ethics committee, it becomes difficult to go start any research work. Even during the publication, ethical approval is one of the mandatory requirement.

Thus, the following are the ways for an ethical approval:

1. Approaching the IEC of the affiliated institute
2. An unaffiliated researcher may approach the ethics board of any institution/organisation with the request of overseeing the ethical aspect of their research process. However, in most institutional ethical committees, an independent researcher may not be entertained.

Table 1 List of grants/fellowships for funding related to research

S.No.	Funder	Fellowship/ award/ grants	Eligibility	Duration	Website
Based in India					
1	Lady Tata Memorial Trust	Young Researcher Award	Indian Nationals < 40 years with a PhD with 10 years' experience	3 + 2 years based on the review of progress	https://ladytatatrust.tatatrusts.org/StaticPage/Home/0
2	DBT-Wellcome Trust India Alliance	Margdarshi Fellowship	Strong scientific leadership with more than 10 years' experience running an independent lab	5 years	https://indiaalliance.org/margdarshi-fellowships
3	DBT-Wellcome Trust India Alliance	Team Science Grants	A minimum of three investigators (investigators should have PhD/MD/MBBS-MS/MPH or equivalent, with at least 5 years of experience in running an independent research group or lab).	5 years	https://www.indiaalliance.org/team-science-grants
4	DBT-Wellcome Trust India Alliance	Clinical and Public Health (CPH) Fellowships	Under three categories of early, intermediate and senior category	5 years	https://www.indiaalliance.org/fellowshiptype/clinical-and-public-health-research-fellowships
5	Science and Engineering Research Board	Start-up Research Grant	Indian citizens less than 40 years with a PhD degree	2 years	http://serb.gov.in/srgg.php
6	Human Frontier in Science Program (HFSP)	Young Investigator's grants	Junior Independent PI, within 5 years of obtaining an independent position	3 years	https://www.hfsp.org/funding/hfsp-funding/research-grants
7	Human Frontier in Science Program (HFSP)	Programme grants	Independent PI	3 years	https://www.hfsp.org/funding/hfsp-funding/research-grants
8	Department of Science & Technology (DST)	INSPIRE Faculty Fellow	Indian citizens less than 32 years with PhD degree with more than 60%in all exams since class 12.	5 years	https://online-inspire.gov.in/
9	Science and Engineering Research Board (SERB)	National Postdoctoral Fellowship	Indian citizen less than 35 years with PhD /MD degree	2 years	https://serbonline.in/SERB/npdf
10	Science and Engineering Research Board (SERB)	Ramanujan Fellowship	Indian scientists and engineers working abroad and below the age of 40 years.	5 years	http://serb.gov.in/rnf.php
11	Council Of Scientific And Industrial Research (CSIR)	Fellowship / Associate-ship	Indians less than 35 years with PhD degree/M.D. with one publication in a recognized journal.	1 year	https://csirhrdg.res.in/Home/Index/1/Default/2186/56
12	Department of Biotechnology (DBT)	Ramalingaswami Re-entry Fellowship	Indians working overseas with less than 45 y of PhD or MD and 3 years of PG experience.	5 years	https://dbtindia.gov.in/schemes-programmes/building-capacities/building-critical-mass-science-leaders/ramalingaswami-re
13	Department of Biotechnology (DBT)	Innovative Young Biotechnologist Award	Scientist with PhD less than 35 years of excellent academic career with a PhD and First Class in all academic degrees, along with a track record of high impact peer-reviewed publications	3 years	https://www.indiascienceandtechnology.gov.in/nurturing--minds/s-and-t-awards/national/innovative-young-biotechnologist-award-iyba
14	Sir Ratan Tata Trusts, Bill & Melinda Gates Foundation, and Access Health International	India health policy & systems research fellowships (HPSR)	At least 3 years of research experience in any public health or health systems domain.	18 months	https://indiahpsrfellowships.org//
15	ICMR-DHR	ICMR-DHR International Fellowship Programme for Indian Biomedical Scientists	Applicant should possess an M.D/M.S/Ph.D degree with 3 to 15years of experience	Short term (2wks – 3months) or long term (6 to 12 months)	https://main.icmr.nic.in/content/icmr-dhr-international-fellowship-programme-indian-biomedical-scientists
16	Research Grant in the area of Biological Sciences	Sree Padmavathi Venkateswara Foundation (SreePVF)	Universities, R&D institutions, medical centres and recognized non-profit research organizations in India with PhD/MS/MD	3 years	https://www.lvpei.org/events/2020/sree-ramakrishna-paramahamsa-research-grant/index.html
17	Research Associate Fellowship programme	ICMR	PhD/MD/MDS	3 years	https://main.icmr.nic.in/content/srfra
18	Fellowship Programme for Young Scientists	Department of Health Research (DHR)	MD/ MS/MDS/ or PhD in biomedical sciences	3 years	https://dhr.gov.in/schemes/human-resource-development-health-research-hrd

Table 1 (*Continued*)

S.No.	Funder	Fellowship/ award/ grants	Eligibility	Duration	Website
19	Fellowship Programme for Women Scientists	Department of Health Research (DHR)	M.D./ M.S./ M.D.S. or MBBS/ BDS/ MVSc./ M.Sc./ M.Pharma/ M.Tech with Ph.D.	3 years	https://dhr.gov.in/sites/default/files/Fellowship%20Program%20for%20Women%20Scientist%20.pdf
Based Outside India					
1	Alexander Humboldt Foundation	Fellowship for experienced researchers	Independent PI (Assistant Professor/ Junior Research GL) with < 12 year of experience	6-18 months, can be divided into 3 blocks of a minimum of 3 months each in 3 years.	https://www.humboldt-foundation.de/en/
2	HHMI/ Gates/ Wellcome Trust/ Calouste Gulbenkian Foundation)	International Research Scholars Program	Applicants have outstanding scientific training records and exceptional potential for significant in their independent careers and are trained in the United States or the United Kingdom at the doctoral, medical, or postdoctoral level for at least 1 year.	5 years	https://www.hhmi.org/programs/biomedical-research/international-programs
3	Royal Society, UK	International Exchange Scheme	Postdoc or holding an independent position in a research institute/university	Variable from 3 months, 1 year or 2 years	https://royalsociety.org/grants-schemes-awards/grants/international-exchanges/
4	Wellcome Trust (UK)	Biomedical resource and technology development grants	Researchers in LMIC are eligible to apply if they have a track record of Trust funding or can demonstrate a strong track record of independent research accomplishment.	Upto 5 years	https://wellcome.org/grant-funding/biomedical-resource-and-technology-development-grants-update
5	National Institute of Mental Health (NIMH), USA	**E**nabling translation of **S**cience to **S**ervice to **EN**hance Depression Car**E** (ESSENCE)		2 years	https://sangath.in/essence-3/
6	International Labour Organisation (ILO)	Fellowships and Seed Grants for Junior Researchers	PhD degree holders, PhD students, or Master's degree holders with 5 years of relevant research and teaching experience.	9 to 12 months	https://www.ilo.org/ipec/news/WCMS_774554/lang–en/index.htm
7	An International Association for medical education (AMEE)	Provides awards, grants and fellowships	To be a member of AMEE, hold at least a bachelor's degree.	–	https://amee.org/
8	Trialect	Provides awards, grants and fellowships	Have citizenship in a country other than the U.S, and Hold an academic degree (earned in the U.S. or abroad) equivalent to a U.S. bachelor's degree.	–	https://trialect.com/international-fellowships/grant-details-brief
9	Thakur Foundation	Grants	All applicants must have a college degree or its equivalent and publications	3 months – 2 years	https://www.thakur-foundation.org/grant-announcement.php
10	Yamagiwa-Yoshida Memorial international study grants (any country overseas)	Grants	PhD/MD with min 2 years postdoc experience	3 months	https://www.uicc.org/what-we-do/capacity-building/grants/fellowships/yamagiwa-yoshida-memorial-international-study-grants
11	Primary Health Care Performance Initiative (PHCPI)	Grants	Affiliation with an organisation	–	https://improvingphc.org/

Note: The list is representative, and not comprehensive.

3. An unaffiliated researcher may collaborate with another researcher who is affiliated with an institute (having an ethics committee) to get the ethical clearance.
4. Central Ethics Committee on Human Research (CECHR) of Indian Council of Medical Research (ICMR)
5. Independent ethics committees – There are several independent ethics committees in India that a researcher can approach for ethical clearance. Details in ►**Table 3**.
6. Non-governmental Organisations (NGOs) – An NGO/society/trust can have its ethics committee which can be

Table 2 List and website details for funding ministries and organisations in India

Organisation	Name	Website
Ministries in India	Ministry of Ayush	http://ayush.gov.in/#
	Ministry of Health and Family Welfare (MoHFW)	https://www.mohfw.gov.in/
	Ministry of Environment, Forest and Climate change	www.envfor.nic.in
	Department of Scientific and Industrial Research	www.dsir.gov.in
	Ministry of Food Processing	www.mofpi.nic.in
	Ministry of Non-Conventional Energy Sources	www.mnes.nic.in
	Ministry of Power, Central Power Research Institute	http://powerresearch.cpri.res.in
	Ministry of Water Resources, ICID	www.wrmin.nic.in
	Ministry of Earth Sciences	https://www.moes.gov.in/
	Department of Education	www.edudel.nic.in
	Science and Technology: Application for Rural Development	www.scienceandtechnology.dst.org
	Science and Technology: For Weaker Sections.	www.scienceandsociety.dst.org
National Organisations	University Grant Commission (UGC)	www.ugc.ac.in
	Centre for Scientific and Industrial Research (CSIR)	www.csirhrdg.res.in
	Indian Statistical Institute (ISI)	www.isid.ac.in
	Indian Council of Social Science Research (ICSSR)	www.icssr.org.in
	Reserve Bank of India (RBI)	www.rbi.org.in
	National Council of Applied Economic Research (NCAER)	www.ncaer.org
	National Institute of Public Finance and Policy (NIPFP)	www.nipfp.org.in
	Institute of Economic Growth (IEG)	www.iegindia.org
	Indian Council of Social Science Research, New Delhi	https://icssr.org
	Department of Science and Technology (DST)	http://www.dst.gov.in/
	Department of Biotechnology (DBT)	https://dbtindia.gov.in/
	Indian Council of Medical Research (ICMR)	https://main.icmr.nic.in/
	National Health System Resource Centre (NHSRC)	https://nhsrcindia.org/
	Central Pollution Control Board (CPCB)	https://cpcb.nic.in/
	Department of Health Research (DHR)	https://dhr.gov.in/
International organisations	World Bank (WB)	www.worldbank.org
	International Monetary Fund (IMF)	www.imf.org
	Asian Development Bank (ADB)	www.adb.org
	United Nations Conference on Trade and Development (UNCTAD)	www.unctad.org
	United Nations Development Programme (UNDP)	www.undp.org
	World Trade Organization (WTO)	www.wto.org
	Ford Foundation	www.fordfoundation.org
	United Nations Educational, Scientific and Cultural Organization (UNESCO)	www.unseco.org
	International Food Policy Research Institute (IFPRI)	www.ifpri.org
	Bill and Melinda Gates Foundation	www.gatesfoundation.org
	National Institute of Health (NIH)	https://grants.nih.gov/grants/oer.htm
	International Labour Organisation (ILO)	https://www.ilo.org/global/lang–en/index.htm
	United States Agency for International Development (USAID)	https://www.usaid.gov/
	National Institute for Health and Care Research (NIHR)	https://www.nihr.ac.uk/researchers/funding-opportunities/

Table 2 (*Continued*)

Organisation	Name	Website
	Global Alliance for Chronic Diseases (GACD)	https://www.gacd.org/funding
	The Swedish International Development Cooperation Agency (SIDA)	https://www.sida.se/en/for-partners/research-partners/research-calls-and-grants
	Global Challenges Research Fund (GCRF)	https://www.ukri.org/what-we-offer/collaborating-internationally/global-challenges-research-fund/
	Centre for disease Control (CDC)	https://www.cdc.gov/grants/applying/index.html

Note: The list is representative, and not comprehensive.

registered with National Ethics Committee Registry for Biomedical and Health Research (NECRBHR).

The Way Forward

Research today is one of the forerunners of health and development around the world. Research in India has a long and arduous way ahead in terms of galvanizing an entire cohort of Indian minds to the unexplored aspects of healthcare. The most efficient and easy option for an independent researcher is to get funding for research through collaboration with other researchers or organisations, and then apply for funding. To a certain extent, a collaboration provides certain advantages like the pooling of resources and a higher number of inputs to improve the quality of work. However, there are various challenges related to collaboration during sharing the responsibilities, credit, resolving disagreements and decision making.[15] Funding for researchers needs to be made more accessible and flexible. There are very limited organisations that fund unaffiliated researchers. Promoting unaffiliated research should be a part of the policymaking directive. For ethics, we suggest a Central Independent Ethics Review Committee which would guide the nearest ethics committee to approach for approval. Fast-tracking of the research proposal can be done by segregating the research based on types and through undertakings. Promoting free and online training programmes for researchers can be also

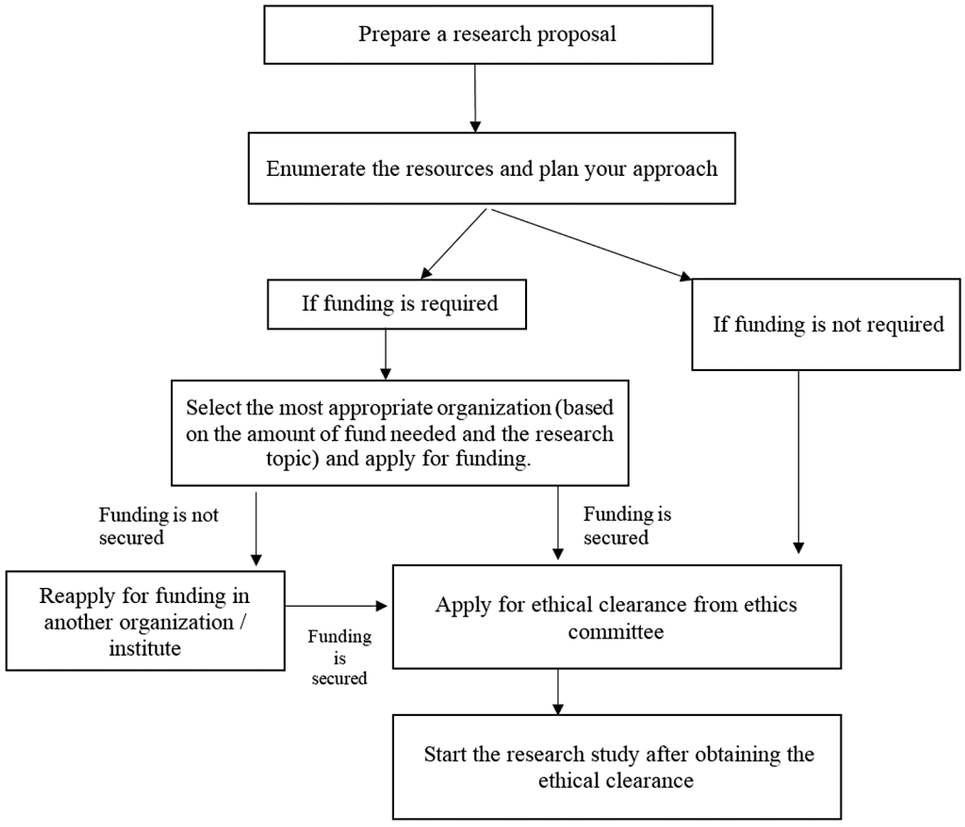

Fig. 1 Process for funding and ethical clearance in conducting a research study.

Table 3 List of Independent Ethics Committees in India

S.No.	Name of Committees	Address
1	Adoni Independent Ethics Committee	4th Floor, Sri Balaji Nursing Home, Opp State Bank of Hyderabad, Adoni-518301, Kurnool District, Andhra Pradesh
2	Deccan Independent Ethics Committee	Quinary Clinical Research Pvt. Ltd.) H.No. 7-1-619/A/3/2, Plot No. 50, Gayatri Nagar Co-Op Housing Society, Ameerpet Hyderabad Hyderabad Telangana
3	Global Independent Ethics Committee	Global Independent Ethics Committee 15/A,16/A,17/A Nandini Enclave Addagutta Society, HMT Hills Road, Serilingampalli Hyderabad Ranga Reddy Telangana
4	Ikon Independent Ethics Committee	Flat No. 104 D, Block Kanchanjunga Apartment, Ameerpet, Hyderabad-500038 Andhra Pradesh India
5	Independent Ethics Committee,	Visakhapatnam, R.R. Towers 2nd Floor, D. No. 17-1-15/24, Flat No.-109, Opp. KGH Maharanipet, Visakhapatnam-530002
6	Maarg Independent Ethics Committee	Sree Nilayam Plot no 38 P&T Colony, Near RTA Office, Trimulgherry, Secunderabad -500015 Andhra Pradesh India
7	Naithika Independent Ethics Committee	305, Sumedha Apartments, Street no.2, Kakathiya Nagar, Habsiguda, Hyderabad-500007
8	S2J Independent Ethics Committee at Holistic Health Care and Research Centre	3 1-230 Nimboliadda Kachiguda Hyderabad-500027 Andhra Pradesh India
9	Independent Ethics Committee	Diabetes Care and Research Centre, G.G-1B, Near Overbridge, Kankarbagh, Patna-800020, Bihar
10	Ethics Committee, entitled as "Independent Ethics Committee"	Academic and Research Department, room no23A, IInd floor, fortis escorts heart institute, Okhla New Delhi.
11	Independent Ethics Committee Society for Research Welfare, New Delhi	New Delhi
12	Independent Ethics Committee, Indian Fertility Society	Department of Obstetrics and Gynaecology, MAMC and Lok Nayak Hospital, Jawahar Lal Nehru Marg, New Delhi-110002
13	Rational Independent Ethics Committee	Registered office: J-36, Saket, New Delhi 110017
14	Aadhya Independent Ethics Committee	1st Floor, 470/41, Ram Vatika, Opp Bank of Baroda, Girdharnagar, Shahibaug-380004, Gujarat
15	Aceas Independent Ethics Committee	Aradhya Ambawadi Ahmedabad
16	Anubhav Independent Ethics Committee	B- Ground Floor, Tirupati Apartment, Behind Old High Court, Navrangapura, Ahmedabad 380009, Gujarat, India
17	Anveshhan Independent Ethics Committee	B- 8, Simandhar Residency, near Gulab Tower, Behind Utopia School, Thaltej, Ahmedabad 380054, Gujarat
18	Bio Smart Independent Ethics Committee	F/12 Avni Complex Naranpura Bus Stop Naranpura Ahmedabad-380013 Gujarat India
19	Ethics Committee, Astha Independent Ethics Committee	502 Shashvat Tower, Near Shyamal Row House-3/B Behind Dhananjay Tower Satellite Ahmedabad 380015 Gujarat India
20	I Biome Independent Ethics Committee	B-501, Krishna Complex, Near Rajpath Club, S.G. Highway, Ahmedabad-380054, Gujarat
21	Independent Ethics Committee	57 Brahmin Mitra Mandal Society B/W Paldi Bus Stop and Jalaram Mandir Paldi Ahmedabad-38006
22	Independent Ethics Committee	Maanav Health Foundation, A-1, Anupam Nagar, B/h, Rajeev Tower, Near Tube Company, O. P. Road, Vadodara 390020, Gujarat
23	Independent Ethics Committee	Aditya, 001, Aradhya, Under Shreyas Flyover, Ambawadi, Ahmadabad-380015, Gujarat
24	Sanjeevani Independent Ethics Committee	GF-28,29 & 44, Avishkar Complex, Nr. G.E.B. Colony, Old Padra Road, Vadodara-390015
25	Siddhant Independent Ethics Committee	210, Umia Vijay Shopping Centre, Satellite Road, Ahmedabad 15, Gujarat
26	South Gujarat Independent Ethics Committee	101, DivyaDarshan Apartment, GhodDod Road, Surat 395007

Table 3 (*Continued*)

S.No.	Name of Committees	Address
27	Spandan-Independent Ethics Committee	B-5, Siddhi Sarjan Apartment, Near Seema Hall, Anandnagar, Satellite, Ahmadabad-380015, Gujarat
28	Imperial life Sciences Independent Ethics Committee ILSEC	463 Pace City II Sector-37 Gurgaon-122001 Haryana India
29	Independent Ethics Committee	Norwich ClinicalServices 147/I First Floor 10th Main 3rd Block Koramangala Bangalore Bengaluru
30	Karesa Independent Ethics Committee	Biosite Research Private Limited 740, Second Floor, 14th Main Kumarswamy Layout, Stage 1 Bengaluru Bengaluru (Bangalore) Urban Karnataka
31	Marthi Independent Ethics Committee	23 12th Cross Palace Guttahalli Main Road Near muthoot Finance Vinayak Nagar Mahheshwaram West Bangalore-560003 India
32	Sri Durgamba Independent Ethics Committee	5th cross 5th stage kamakya layout banashankari same as address line 1 Bengaluru (Bangalore) Rural Karnataka
33	Karkinos Healthcare Independent Ethics Committee	Karkinos Healthcare Technologies Kerala Private Ltd Karkinos Healthcare Technologies Kerala Pvt LTD Kattikaran House 7/3, SRA 30, CRASH Road Ernakulam Ernakulam Kerala
34	Aadhar Independent Ethics Committee	Kitchen Shop, North Shivaji Nagar, Opp Sadanand Hotel, Sangli Miraj Road Sangli
35	Independent Ethics Committee	Prashanti Cancer Care Mission 2, Kapilvastu, Senapati Bapat Road 1, Kapilvastu, Senapati Bapat Road Pune
36	Skinovate Independent Ethics Committee	Skinovate Laser And Cosmetic Surgery Center Office No.303, Royal Avenue, S.No 18, Hissa No.11/6 Rahatani, Pimple Saudagar Pune Pune Maharashtra
37	V Care Independent Ethics Committee	Can Cure Day Care Centre Fr. Agnel College, Sector 9A, Vashi-Navi Mumbai Next To Noor Masjid, Vashi, Navi Mumbai 400703 THANE Thane Maharashtra
38	Veracity Independent Ethics Committee	Veracity Independent Ethics Committee Rock Arcad, Shop No. 55, Camp Road (Block) Tapowan Road Amravati Amravati Maharashtra
39	Vision Independent Ethics Committee	Parth Solitaire Office No. Kalamboli Navi Mumbai Maharashtra India
40	CUTM-Independent Ethics Committee	Centurion University Of Technology And Management - Ramachandrapur, Po- Jatni Khordha Bhubaneswar Khordha Orissa
41	Balaji Independent Ethics Committee	Chettichavaddi Near Zoological Park Salem Tamil Nadu
42	Ripon Independent Ethics Committee	16/34 Veda Nagar 2nd Stage 4th Street Chinmaya Nagar Chennai
43	Sri Rajarajeswari Independent Ethics Pvt. Ltd	Building No. 25, Plot no -2, Door no 3, Ground floor, Vembuli Amman Koil StreetExtn - Palavanthangal Chennai
44	Independent Ethics Committee Narayana Diagnostics	Narayana Diagnostics Plot No.4, H, No- 546/768N, LGF-1, Hardoi Road, Near Era Medical CollegeSarfarazganj, Lucknow Lucknow Uttar Pradesh
45	Independent Ethics Committee CR	India Independent Research Ethics Society 119 Rajdanga Gold Park Tribarna Sangha Kasba Kolkata Kolkata West Bengal

Note: The list is representative, and not comprehensive.

done to enhance the quality of their research. Leveraging the technology for the digitalisation of the process will ease and motivate the researchers. A central online portal with various funding available for the research will increase the opportunities for researchers. It would provide a boost to the researchers who struggle with their ideas in the absence of monetary inputs or a chance for their original work to be recognised.

Author Contributions

S.D.: Methodology, resources, data curation, and writing – original draft, and reviewing; A.C.: Conceptualization, methodology, drafting, writing – original draft, and reviewing; B.N.: Conceptualization, Writing – review and editing, supervision, and visualization

Conflict of Interest

None declared.

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Essential Service Products as Channels for COVID-19 Awareness and Behavior Change: A Narrative Review

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Abstract

Coronavirus disease 2019 (COVID-19) is here to stay. Nonpharmacological interventions such as hand hygiene, social distancing, and respiratory hygiene have remained the mainstay to curb the spread of the virus, and these are sustainable. To understand the information, education, and communication (IEC) efforts undertaken during previous pandemics and successful behavior change strategies that may be applicable to ongoing COVID-19 pandemic, we conducted a narrative review of articles using PubMed search on September 11, 2022. The results highlighted that reinforced IEC is the need of the hour in efforts against COVID-19 pandemic. The communication strategy during a pandemic should be of three stages aligned to the objectives of building a basic understanding and knowledge in the target population: building an awareness of the threat; personal actions to minimize the impact; and reinforcing the need for appropriate actions to minimize disease transmission, in that order. An innovative strategy of displaying IEC on the daily essential products can result in a sustainable solution that might result in a "felt need" in the community to follow COVID-19 appropriate behavior. A broad base of stakeholders' engagement with civil bodies, nongovernmental organizations, private sectors with well-defined responsibilities and accountability would offer an enabling environment for these efforts in ultimately curbing the COVID-19 pandemic.

Keywords
- COVID-19
- essential service products
- behavior change
- narrative review

Introduction

The global pandemic of severe acute respiratory syndrome (SARS) coronavirus 2 (CoV-2) causing novel coronavirus disease 2019 (COVID-19) began in late 2019 affecting more than 214 countries around the world. There were 486 million cases reported globally with more than 6 million deaths reported as of April 1, 2022.[1] India had its first case on January 30, 2020, in Kerala[2] following which the governments initiated information, education, and communication (IEC) activities as a part of the broader infection prevention and control strategy. This included thermal screening at points of entry, isolation at airports, and bans on international flights.[3,4] Nonpharmacological interventions

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endorsed in IEC activities were social distancing (minimum 1 m distance between two persons), staying indoors by avoiding unnecessary travel, frequent handwashing with soap and water or use of alcohol-based hand rubs, and cough etiquettes. There was a nationwide lockdown starting March 24, 2020, which was eventually followed by many countries across the globe.[5]

As appropriate treatment modalities are yet to be discovered and vaccination is providing varied levels of protection because of continuous mutation in the virus (delta, delta plus, omicron),[6] nonpharmacological interventions have remained the mainstay to curb the spread of the virus. In a study reported from China, it was predicted that without nonpharmacological interventions, the number of cases would have been 67-fold higher (interquartile range 44- to 94-fold).[7] In a case study reported from New York City, universal masking was associated with an approximately 7% transmission reduction overall and up to 20% reduction for 65+ years old.[8] Similar measures undertaken by national governments during the complete lockdown period were the suspension of all modes of transport, strict closure of all types of shops, malls, restaurants, saloons, gymnasium and swimming pools, schools, colleges, religious places, ban on religious gatherings, postponement of examinations, interviews, and other meetings involving public gatherings; and work from home policy was implemented by many national and international companies. Educational, official classes/meetings, respectively, were conducted online to support the government guidelines.[9] The use of homemade masks had been mandated for the general public coming outdoors along with hand and respiratory hygiene. The government of India has been engaged in IEC activities through print media, television, caller tunes, banners, posters, and public interviews by national leaders/health professionals with an emphasis on simple interventions such as hand hygiene, social distancing, and respiratory hygiene. Recently, these include efforts to overcome vaccine hesitancy. According to the World Health Organization, communication expertise is as critical as epidemiological and laboratory expertise for the control of an outbreak.[10]

COVID-19 is expected to be with us for a long time and there is no time for complacency.[11] To recover from economic loss in lockdown, India had started reopening the country in a phased manner from June 1, 2020, and is now with zero COVID-19 restrictions. The country has also started international travel effective from March 27, 2022. It is essential to remember that India is still reporting more than 1,500 cases per day amid increasing number of countries reporting fourth spike in COVID-19 cases, reportedly fourth wave.[12,13] It is the need of the hour to develop sustainable interventions that are preferably nonpharmacological to curb to the emerge and spread of COVID-19. The aim of this review was to describe the IEC efforts undertaken during previous pandemics, and successful behavior change strategies that may be applicable to ongoing COVID-19 pandemic.

Methods

A narrative review of articles elaborating on IEC efforts, behavior change strategies in pandemic situations was conducted without restrictions on the type of study. A search was done on PubMed using the keywords that include but not limited to "Information Education and Communication," "behaviour change," pandemic on September 11, 2022. There were no language restrictions. This resulted in 770 articles. After reviewing the titles and abstracts, 285 articles were identified. Finally, 15 studies that were relevant to the objective of this study were identified through full-text screening. The search was not exhaustive, limited to PubMed and the results were narratively summarized. Two review authors (M.K. and K.P.P.) independently screened the titles, abstracts, and full texts; and extracted data. Any disagreements were resolved by discussion or referring to other review authors (V.K.P., S.K.P., and U.R.).

Review

Learnings from Our History

During the great epidemic of the plague in India in 1994, fax service, voice information service, telephone hotlines, and print media were used to disseminate educative materials. During the SARS pandemic of 2003, the travel advisories and other guidelines were primarily circulated through electronic media.[14] During influenza (H1N1) pandemic of 2009, government websites, television, radio, and internet were the major modes of communication.[15] During avian influenza (H5N1) in Thailand, it was an effective communication strategy that brought about behavior change and not just the knowledge about the disease. As behavior change is a complex dynamic process involving both motivators and barriers,[16] it was learnt that increased awareness of the treat of avian influenza was possible due to recent (timing) and regular (frequent) information.[17] This highlights the need to reinforce the general public over and over again to perceive the threat and ultimately change their behavior to prevent the acquisition and spread of COVID-19. South Korea was able to flatten the curve through effective communication, sending infection prevention and control messages to their citizens every day as a text message alert. This was a standout effort by the government as compared with other European countries.[18]

Behavior Change

The purpose of any communication will be to affect behavior change in the target population which in turn depends on the content delivered, channels of communication, factors related to the target population, and existence of feedback mechanisms.[19] Based on the conceptual framework using motivation, opportunity, and ability of the population for management of public health and social behavior, times like COVID-19 provide motivation but lack opportunity, provided ability is considered present. The target population is unable to behave in such situations and requires marketing

strategies to address the same.[20,21] Social marketing is an innovative platform to bring about desired behavior change. As one has to manage his/her life with SARS-CoV-2, the expected behavior change has to be long term at individual, group, or organizational and societal levels. This involves lifestyle changes, organizational changes, and sociocultural changes, respectively.[22,23] The bottom line of any social marketing activity is desired behavior change and in this pandemic situation, it will be the practice of social distancing, frequent hand washing, and cough etiquettes. These are simple acts but should be practiced continuously or frequently.[24]

Need for Reinforcement

Skinner (1938) defined a reinforcer as an experience that raises the frequency of responses associated with it. Reinforcement has its maximum effect when it occurs at the same time as, or just after, the response. Reinforcement not only changes the frequency of the response but also strengthens the association between stimulus and response. Such reinforcements also help in shaping the process (faster when it is continuous) whereby behavior is gradually shifted from one form to another.[25]

Evidence for Reinforcing IEC

In a study by Brewer et al, it was found that smokers whose packs had pictorial warnings attempted to quit more as compared with those whose packs had text-only warnings during the 4-week trial.[26] Having quit smoking for at least 7 days prior to the end of the trial was more common among smokers who received pictorial than those who received text-only warnings. Pictorial warnings also increased forgoing a cigarette, intentions to quit smoking, negative emotional reactions, thinking about the harms of smoking, and conversations about quitting.[26,27] In a meta-analysis conducted by Noar et al,[28] pictorial warnings were more persuading than text-only warnings because pictures were attractive and held attention better; garnered stronger cognitive and emotional reactions; elicited more negative pack attitudes and negative smoking attitudes; therefore, more effectively increased intentions to not start smoking and to quit smoking.

Discussion

The review of literature highlighted that reinforced IEC is the need of the hour in efforts against COVID-19 pandemic as:

1. The COVID-19 pandemic necessitates not "only fighting against the epidemic but also the simultaneous infodemic."[29] The spread of misinformation and myths about a disease little known before, in this era of social media, has been unprecedented. To counter this, United Nations organization is already working with search and media companies (Facebook, Google, Pinterest, Twitter, TikTok, YouTube, etc.). The counteraction has mostly been limited to social media, television media, and print media by governments across the world. But these social media along with the television media are also the source of misinformation. The effect of awareness generation in these media is essentially decided by the competitive interest generation and reach by individual IEC material (video/message/pictorial presentation, etc.) as compared with misinformation material available on the same platform.
2. The reinforced IEC through the packaging of essential products can serve as a vital uncompetitive media for delivering awareness messages and can effectively supplement the awareness generation and behavior maintenance of the population during pandemic.
3. The reinforced IEC through the packaging of essential products will broaden the reach of awareness to a wider population because of its doorstep delivery in households. This is important in view of the greater tricking down effect of misinformation and myths among nonsocial media users because of the element of interest and conspiracy attached to it.
4. The reinforced IEC will also help in mitigating the interruption of culture-sensitive communication strategies, for example, community-specific street plays, folk songs, etc. which can be represented pictorially or as poems over the packaging of essential products.
5. These kinds of IEC strategies can help in improving the mental health of the population by dissociating the source of awareness from the source of stressors (e.g., dreadful news of increasing corona deaths on TV, conspiracy regarding virus spread on social media).
6. Awareness generation during the pandemic cannot rely upon the community group awareness strategies because of the social isolation and physical distancing measures advised for curbing the spread of the disease. In these circumstances, individual awareness can reach each household along with the essential products.

A Novel Strategy to Reinforce the Message

The communication strategy during a pandemic should be of three stages aligned to the objectives of building a basic understanding and knowledge in the target population: building an awareness of the threat; personal actions to minimize the impact; and reinforcing the need for appropriate actions to minimize disease transmission, in that order.[15] Accordingly, knowledge about the disease, its symptoms, and preventive measures have to be reiterated to the people time and again, in order to make sure that the interventions, brings about the desired behavior change in them. The information either texts or pictorial representations will be the augmented product, the intended behavior change will be the actual product and protecting themselves from disease acquisition and disease transmission will be the core product. These messages can be delivered with no special costs, making use of regular day-to-day products people purchase or consume. This also saves costs associated with promotional activities. This may necessitate appropriate environmental modifications and policies in addition to delivering health information.

Small messages can be printed out on the daily products which reach the population, underpinning the message newspapers have already started issuing educative materials related to COVID-19 every day. This can further be extended to other printable product covers such as milk packet covers, groceries cover, on cardboard boxes sent out for delivery of products by online delivering companies (►**Figs 1**–**5**). Even the food delivery boxes and covers can contain such informative messages. Printing the essential actions to be followed while boarding public transport, the tickets and boarding passes should contain a reminder message. Private companies can invest in this printing material as a part of corporate social responsibility (CSR) during crisis times.

Fig. 1 COVID-19-related health messages on a water bottle.

Fig. 2 COVID-19-related health messages on train tickets.

Fig. 3 COVID-19-related health messages on a cloth bag.

Fig. 4 COVID-19-related health messages on a milk packet.

Fig. 5 COVID-19-related health messages on cardboard boxes.

Conclusion

The general public by now has a basic level of understanding and knowledge about the threats and risks of SARS CoV-2. It is time to reinforce the need for appropriate actions to be followed continuously to prevent the spread of infection. Government should bring a policy on the grounds of CSR that all the manufacturers will incorporate IEC short messages on the covers of their products for at least the next few months. All forms of media should be actively involved in the desiccation of informative up-to-date guidelines and regulations on COVID-19. It is high time that the desired behavior change brought about in the community would be the most affordable measure to combat the spread of infection and prevent loss of human, material, economic resources, and lives. A broad base of stakeholders' engagement with civil bodies, nongovernmental organizations, private sector with well-defined responsibilities, and accountability would offer an enabling environment and make the governments' proactive efforts meet the desired end.

Ethics Approval
Not applicable.

Authors' Contribution
All the authors were involved in manuscript preparation.

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Conflict of Interest
None declared.

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A Case for Horizontal Distribution of Activities between General Surgery and Surgical Super Specialties

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Abstract

Under the current practice in organizing surgical services, proportionate representation of disciplines is provided in the curricular planning and in corresponding clinical functions. This is based on the level of competence expected by the end of training period. The disciplines as a "whole" are placed in general surgery or super specialty. The system of vertical arrangement has some serious concerns. Paradoxically, patients with diseases of simple and routine nature of discipline categorized as super specialty are neglected. Super specialist is unable to attend on account of preoccupation with serious challenging problems. The general surgeon hesitates because of privileging issues, fear of allegations of negligence and litigation. The system of vertical division is based on premise that some disciplines deal with complex procedures and others with only simple and routine nature. This premise is incorrect. Each discipline is a mix of simple and complex cases requiring specialized treatment. Alternate modified organization of surgical service is proposed. Activities of all disciplines are scrutinized according to the level of expected competence by the end of training. Categorization is shifted from the "discipline' to "activities." Criteria applied for classification of activities are as follows: on completion, the trainee is capable to assume full responsibility-category 1; has gained sufficient experience-category 2; and is conversant with broad understanding of management-category 3. Activities of category 1 from all disciplines are assigned to general surgery and those of category 3 from all disciplines are assigned to respective super specialty. Those in the middle, comprising difficult cases but not requiring specialized training or heavy inputs in equipment, are in category 2. They are assigned to general surgery as additional/optional items, or super specialty, guided by local factors. The scope and practice of general surgery are

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- comprehensive surgery
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- rational organization
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broadened with a shift from "residual" to "comprehensive" discipline. Advantages, concerns, collateral issues of horizontal distribution of activities, its positive impact on research and education are discussed. It is concluded that the proposed organization of surgical services is a rational, logical, and practical strategy for good-quality surgical care in the society. The super specialists need to be convinced that "taking load off" is good for the specialty.

Introduction

Over the past few decades, we have witnessed vast expansion of knowledge and emergence of technologies in medical science. It has resulted in widening the scope of surgical management of complicated cases and undertaking complex procedures. Dedicated departments are established to provide services and for training purposes. The super specialty services achieve better outcomes in complex cases and are here to stay. A number of disciplines have emerged and recognized as surgical super specialty for some disciplines. There is justifiable increasing demand for similar status for more disciplines.

In the back drop of glamour of super specialties, status accorded to the discipline of general surgery does not commensurate with the responsibility bestowed on it. Quite often, opinions are articulated that there is no place for general surgery in the era of specialization and the discipline is dead.[1] On the contrary, it has been opined that the general surgeons have to play much more important and complex role.[2] Occasionally, these disciplines are referred to as subspecialties. The nomenclature whether super or sub is not relevant. Important issue is relative positioning of different disciplines in the overall organization of surgical services with optimal utilization of trained manpower.

The aim of organizing care services for surgical diseases is a challenging task and is dependent on utilization pattern of trained surgical work force. It is contended that assignment of activities/responsibilities between different disciplines is the major issue.

Current Scenario

The department of general surgery is the parent department for conducting training programs for basic certification in surgery for both undergraduate and post graduate studies. The clinical functions are organized to meet the teaching requirements. Teaching/learning activities are carried out as part of patient care, thus ensuring adequate exposure and hands-on-experience. Separate staff for teaching only is not provided. With emergence of multiple surgical super specialties, though not clearly defined, surgical disciplines are assigned to general surgery or superspecialties based on the level of competence expected of the trainees at the end of training. Influenced by this vertical arrangement, there is a trend of reflex referral of patients to superspecialty services without any attempt at initial evaluation or basic investigations. By and large, superspecialty departments do not restrict intake to only referred patients. Usually, there is no facility for prehospital screening. Also, there is no effective linkage between primary care providers and hospitals.

General surgery as a discipline includes knowledge of and management of diseases of organs of wide spectrum of systems. The universities/teaching institutions and the examining bodies follow proportionate representation of different disciplines in their curricular planning. The aim of general surgery training is to address the issue of surgical manpower to best serve the public good.[3] Breadth of training in general surgery is essential for all branches of surgical practice.[4] System of rotation to other disciplines is practiced wherever needed for comprehensive coverage of learning objectives. There are variations in disciplines for and duration of rotation across institutions. Appropriate educational technology is utilized in all disciplines.

There are diverse motivating factors for opting for superspecialties. The prime mover is desire of individuals in accepting challenges of undertaking difficult and complex procedures. Aspirants are willing to devote extra time and finances for further higher training. However, apart from the legitimate reason, there are other important considerations for opting for super-specialties arising from lifestyle and financial issues, and the number of residents opting for careers in general surgery are getting reduced.[5,6]

Shortcomings

The widely practiced current system classifies surgical disciplines as a "whole" comprising care of both complex procedures and those of routine nature. Such a scheme of vertical arrangement of surgical disciplines has certain limitations. A surgeon who has undertaken special training to acquire essential advanced skills in superspecialty discipline is likely to be involved, rightly so, in the care of patients with complicated diseases and complex procedures to the point of exclusion of patients requiring procedures of routine nature, for which no additional training is required but which is involving an organ of superspecialty discipline. In contrast, involvement with routine and simple cases may overwhelm the superspecialist, which may not permit adequate time and attention to complicated cases and challenging problems, for which the superspecialty department is established in the first place. General surgeons may be handicapped in care of such patients with diseases of routine and simple nature belonging to the superspecialty discipline, even though they have the requisite

competence and confidence for the management on account of privileging, professional liability concerns, fear of allegations of negligence and litigation.

Thus, paradoxically, patients with simple diseases of routine nature are likely to be neglected. Overwhelming trend for superspecialization resulting in the shortage of manpower in general surgery has serious implications for the health care delivery.[7–9]

Is there an alternative? Yes. There is.

Alternative

The system of vertical arrangement of disciplines is based on the premise that there are some disciplines dealing with complex procedures and others with procedures of only a simple and routine nature. However, this premise is not correct. Each discipline is a mix of simple routine as well as of complex nature cases requiring specialized treatment.

It is therefore incumbent to consider modification of organization of services to achieve the learning objectives for the purpose providing trained surgical manpower. Activities/procedures within each discipline may be scrutinized to classify according to the level of competence expected from trainees. Criteria applied for the classification of activities are as follows: on completion, the trainee is capable to assume full responsibility-category 1; has gained sufficient experience-category 2; and is conversant with broad understanding-category 3. It may be emphasized that there is a shifting of categorization from " discipline" to "nature/character of the activities of each branch as per defined criteria." Every discipline will have components of category 1, 2, or, 3 in variable proportion.

Activities of simple and routine nature requiring no more than usual training and equipment of all discipline are put in category 1, those requiring additional advanced dedicated training, sophisticated equipment, and infrastructure are categorized as category 3. Those in the middle comprising some difficult cases, which may require some additional orientation but not requiring heavy inputs in equipment or specialized training, are categorized as category 2.

Activities of category 1 of all disciplines are part of general surgery and those of category 3 are in the respective superspecialty. Category 2 activities are assigned to either general surgery as additional/optional activities or superspecialty depending on local factors comprising case load, infrastructure, interest of staff, etc.

The clinical services are organized accordingly aimed at meeting educational requirements. The proposed rational organization is schematically represented in ►**Fig. 1**.

Due attention is merited for authorization for clinical functions including operative work to the staff as per the proposed organization. Privileging is a managerial tool granting permission to staff of the department basically aimed at quality assurance. The process of privileging is not a part of certification or examining bodies but is the responsibility of institutions.[10] A list of privileged specific procedures needs to be reviewed regularly. Addition of new procedures in privileges list is based on careful objective evaluation of competence. Maintaining acceptable level of quality of care is a difficult and challenging task requiring continuing education and organizing workshops, etc. This is true for all branches of surgery. It is to be appreciated that quality and safety issues addressed voluntarily are more effective and reduce chances of intrusive actions.[11]

Changing Scenario: *Shift from* " *general*" *to* " *comprehensive*" *discipline*

The activities in category 1 across all disciplines are included in general surgery as primary constituents of general surgery forming " essential core service," which is indispensable for basic certification. The scope of general surgery is thus broadened. The practice and concept of general surgery are shifted from "residual" to what could be termed "comprehensive" discipline.

Primary responsibilities of parts from each surgical discipline help achieve harmonious synthesis of constituents, leading to integrated comprehensive teaching/learning and training instead of collage of fragments. There may not be necessity for rotation to superspecialty for orientation to basic aspects of the concerned specialty. This arrangement will have desirable impact on undergraduate medical and post graduate studies in surgery, for training of "family physician" and "surgical specialist." It may also provide opportunity for making informed decision in choosing superspecialty training, having been exposed to some aspects of all the disciplines, albeit of simple nature. It is a common observation that candidates apply for more than one superspecialty course.

In addition to discharging responsibility to "core essential," general surgeons may take interest and participate in some of activities in category 2 included as additional/optional activities in general surgery. This will be intellectually and professionally satisfying experience and should be encouraged. However, due caution is needed to ensure that responsibility of essential core primary constituents is not diluted in any way. It is possible that same activity may be opted by more than one staff as per individual's interest. This merits to be accommodated. All staff members may be persuaded to participate in one or more activities of category 2 so as to have maximal coverage of optional items. Desirably, the aim should be to attempt to include all category 2 activities in general surgery in the true spirit of broad-spectrum service.

Planning of infrastructure requirement comprising hospital beds, operation theatres, OPD facilities, office space, staff, ancillary services, etc. should take into account additional commitment.

Progressive specialization of optional category 2 activities within the constituents of general surgery is feasible and will provide breadth of services expected of general surgery.[12,13] Shortened integrated training strategy has been recommended, which may require logistic and financial considerations.[14]

Distribution of activities among disciplines is dependent on many factors such as case load, infrastructure, and training opportunities. Each institution is expected to formulate the list of "core essential" and "advanced/additional" topics. Professional associations/regulatory bodies may provide guidelines. A list of suggestion of some activities/topics is included as example in the proposed curriculum for postgraduate training in surgery submitted to the Medical Council of India.[15]

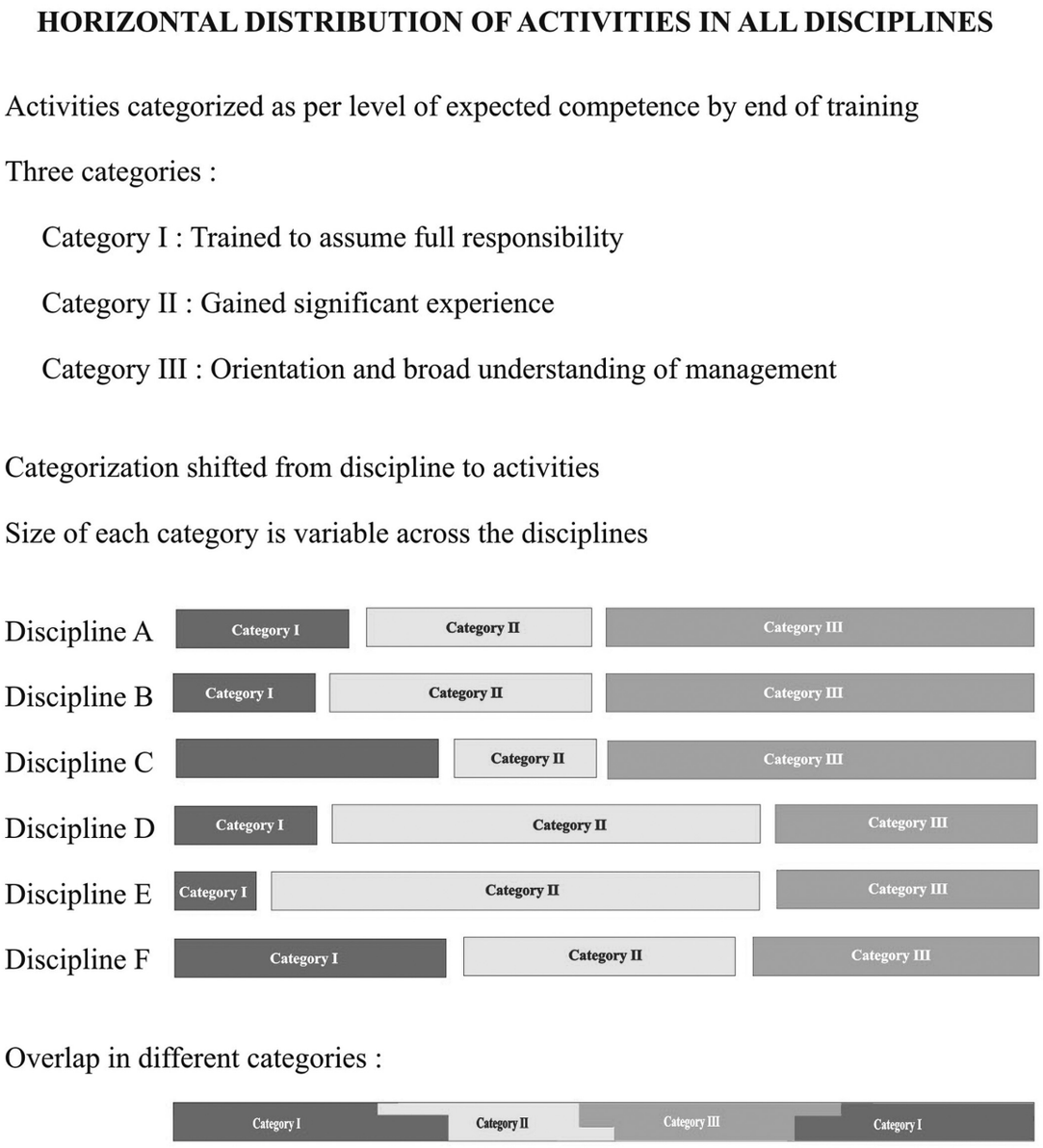

Fig. 1 Horizontal distribution of activities in all disciplines.

Advantages

There are distinct advantages of such horizontally placed activities. The superspecialty departments may be able to devote more attention and time to complex procedures, for which the superspecialty was established in the first place. Patients needing attention of routine nature are taken care of by trained surgeons, and unnecessary referrals are avoided.

The suggested system is useful for providing satisfactory service as well as training commitments. The discipline of general surgery is expected to play an important role in the health care delivery system,[16] and proposed organization of surgical service will facilitate this role.

Collateral Issues

Under the proposed organization, the general surgical practitioners in whatever setting, teaching, community, charity, corporate hospital, or private clinics, are likely to deal with more variety of cases. One direct result is the number of patients in any one condition is small and consequently of reduced experience in every item. This limited experience in a particular procedure may have some apprehension about the competence of the surgeon. This apprehension, however, is unfounded as basic surgical skills are same across all disciplines.

Also, the possibility of large personal/institutional series is reduced, which may be perceived as a negative feature. It actually is a positive feature for satisfactory comprehensive patient care and clinical research. Under the proposed scheme, the research is undertaken by multi-institutional collaborative data collection, and the patient care follows practice guidelines/protocols developed for decision making in the management instead of personal anecdotal observations. It is increasingly being recognized to develop and follow clinical pathways and practice guidelines for efficient and cost-effective patient care.[17]

Advancement within the primary constituents of general surgery will provide breadth of services expected of general surgery requiring complex manpower planning.[12] Shortened integrated training may need logistic and financial support. It must be ensured that "essential core" topics are not compromised while paying attention to acquiring additional skills for optional items.

The aim of surgical research is to encourage curiosity and critical appraisal and helps achieving open and interdisciplinary problem solving approach in improving patient care.[18,19] Research activities by trainees enhance chances for career progression. Properly supervised research reinforces education of future surgeons.[20] Modified organization of surgical services as "comprehensive surgery" departments will have opportunities to engage in relevant research activities to address a wider range of surgical issues prevalent in the society.

Advanced Trauma Life Support (ATLS) course initially designed for rural practitioners is recommended to be included as part of training objectives of general surgery.[21] It is desirable to extend the application of ATLS protocols to all emergency surgical cases or may even be to all patients attending casualty/emergency department pertaining to all departments. It may be worthwhile to modify the term "ATLS'-"Advanced Trauma Life Support" to "ALS"–"Advanced Life Support."

Though trauma is not on focus in this presentation, it may briefly be mentioned that establishing separate "standalone" trauma centers, which are being advocated widely, are of doubtful value in the context of serving all trauma victims in a given community, notwithstanding improved outcomes in the institutional statistics of "Trauma Centre," which may as well be due to better infrastructure and availability of equipment. It may briefly be mentioned that care of trauma victims in the community is likely to be improved more by establishing multi-specialty hospitals not trauma centers, network of peripheral centers for stabilizing, and well-equipped ambulance service. Trauma victims need to be treated as any other emergency patients. Surgeons with broad-spectrum training are better placed in dealing with such situations.

It may briefly be mentioned that establishing specialty of "Traumatology," "Emergency Medicine/Surgery," etc., independent of main discipline does not appear logical. Trauma victims are likely to have involvement of multiple organs and are best attended to by a broadly trained surgical team. Experience in elective cold routine cases is useful in emergency situations. Referral to other departments are made selectively for specific problems and advanced interventions to the respective superspecialty service.

Departments of surgery in teaching institutions are mandated to train suitable manpower for future general surgeons, surgical superspecialists, and family physicians. Teaching program of the department is directed to undergraduate medical students, interns, postgraduate students and senior residents in surgery, continuing education, incorporating new procedures, and maintaining learnt skills. Curriculum planning and strategy to address the wide spectrum of teaching role needs to be planned holistically. The challenging task for the academic departments is to train manpower in general surgery for mentoring medical students and residents may require opportunities for acquiring teaching skills and technologies.[22]

Separate staff for teaching exclusively is not required, and the teaching is carried out as part of regular clinical responsibility. Proper infrastructure is required to ensure that both functions are carried out without disturbing each other.[23] Unfortunately, teaching is accorded low priority and the faculty is not rewarded adequately for the time and effort in teaching. It needs to be emphasized that patient care is not an addendum to but essential constituent of medical teaching. It has rightly been pointed out that teaching activities contribute to patient care.[24]

Practice currently in vogue for holding an all India examination aimed at reducing stress of multiple examinations, with objective-type question system, for uniformity for admission to MS/MD during internship and to M.Ch/DM during senior residency has serious implications on training opportunities.[25,26] Admission process to these courses for candidates with wide variations in the qualifying examinations merits to be revisited.

The system of rotation to superspecialties departments currently in vogue for exposure to diseases in category 3 activities with expected competence of "only broad understanding of management" is dispensed with. Cognitive aspects of specialized disciplines can easily be covered by lectures/discussions and other educational tools. The learner

gets more time in the main department and has larger opportunity for observation, hands-on experience, and participation in the management of wide range of surgical diseases including from the disciplines of subspecialty sections. Innovations and application of modern educational technology contribute to improving educational outputs. There are programs for providing adequate exposure and competence in medical educational technology in distance learning and institutional formats.[27]

Simulation as a teaching and assessment tool for imparting skill training is being recognized.[28,29] The aim is that the learner acquires minimal level of competence on simulators before actually performing on human patients. Wide range of skills can be practiced including basic clinical skills of injections, blood sampling; dressing and splinting, etc.; essential surgical skills of knotting, suturing, anastomoses etc.; advanced surgical procedures such as endoscopy and laparoscopy etc. Simulators consisting of models and mannequins, synthetic material mimicking tissues, animal tissues, training boxes for laparoscopy, virtual reality, etc. are being increasingly used for skill learning and assessment for core competencies.[30] Surgical skill laboratory, which may be considered an essential component of teaching department, is still underutilized.[31]

Training in general surgery is a pre-requisite for recruitment to superspecialty courses. Approximately less than half of the trainees are estimated to continue in the discipline of general surgery.[32] Organization of services with inclusion of parts of all disciplines provides opportunity to prospective candidates for informed decision in identifying area of interest and choosing courses for further training. It is a common observation that most candidates apply for more than one course.

Apprehensions and Critiques

The scheme of broadening the scope of general surgery by including some activities from disciplines recognized as super/subspecialization is feared with apprehensions. It may be perceived as a retrograde step and is not considered a viable proposition. It may be alleged that surgical care is being provided by semi-/untrained surgeons, which may have serious professional liability concerns. Nevertheless, it is emphasized the scheme is a step in right direction. Broad-based surgical training is effective in achieving high-quality surgical services without compromising on the overall quality and containing costs. It is good not only for developing countries such as India but also has a global appeal.

It is perceived by some that surgical care in the hands of general surgery is not cost-effective or efficient in quality.[33] These criticisms are unfounded and are based on the consideration of territorial interests of the "super specialty" departments instead of adopting an appropriate problem-solving approach; utilizing the talent is in the best interest of care. Perception that general surgeon is a multiple superspecialist rolled into one is erroneous. As explained earlier, only some selected items are included in the general surgery, which do not require special equipment or detailed further training, and the trained general surgeon is competent to discharge the responsibility. Superspecialists need to be convinced that "taking off load" is good for the specialty.

Objective of better outcomes and reducing defective service is a reasonable goal; however, the concern is how to achieve this. Super specialization may appear to be an obvious simple solution intuitively. However, fragmentation and lack of continuity are not in the best interest of patient care.[34]

A part of resistance in accepting the concept arises from the use of value-loaded term "super," indicating higher status to some disciplines as compared to others in terms of social hierarchy and financial compensation in both organized service and self-employed. This distortion needs to be corrected.

Conclusion

Horizontal distribution of activities between general surgery and surgical super specialties is a rational, logical, and practical strategy for organizing good-quality surgical services covering full-spectrum of surgical diseases with wide reach out in the community.

Authors' Contributions

V.R.M.: Contributed to initiating the concept, developing it further, drafting manuscript, revising to include comments, finalizing, and submission. S.G.: Contributed to concept, comments on the draft and finalizing manuscript. A.G.: Contributed to concept, comments on the draft and finalizing manuscript.

Conflict of Interest

None declared.

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Evaluation of Clinical, Demographic, and Biochemical Profiles of Trinidadian Patients Undergoing Coronary Angiography

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Abstract

Background Trinidad and Tobago ranks number 45 in the world for total deaths due to coronary heart disease. Predictive tests for coronary angiographic results set the basis for earlier monitoring of the disease before additional complications become obvious.

Aims and Methods This study aimed to evaluate the anthropometric and biochemical parameters of 124 patients with suspected coronary artery disease (CAD) in Trinidad and how these parameters correlate to the findings at angiography.

Results The biochemical parameters showed statistically significant correlations with CAD severity by Spearman's rank-order correlation. Two clinical parameters showed significant associations with CAD severity—ethnicity (χ^2 (4) = 12.925, $p = 0.012$) and presence of type 2 diabetes at baseline (χ^2 (4) = 21.483, $p < 0.001$).

Conclusion Biochemical parameters such as fasting blood sugar, N-terminal pro B-type natriuretic peptide, creatinine, and hemoglobin A1c were well correlated and well associated with the severity of CAD after diagnosis by the process of coronary angiography. Hence, these factors can be taken into consideration to predict the severity of CAD.

Keywords
- coronary angiography
- coronary artery disease
- glycated hemoglobin
- diabetes mellitus
- NT-proBNP

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Introduction

Coronary artery disease (CAD) has become the most common type of heart disease worldwide. It is the major cause of morbidity and mortality globally and has therefore become a major public health burden.[1] CAD has caused substantial economic costs on the governments of various countries for monitoring and treatment.[2] In Trinidad specifically, 21% of the population dies of cardiovascular causes annually, with heart disease being the number 1 cause of death. Trinidad and Tobago also ranks number 45 in the world for total deaths due to coronary heart disease.[3] Despite the efforts of medical research and governments in reducing this health burden, recent trends have shown that the disease incidence has escalated and has started affecting a younger age group.[4] A variety of tests can assist in the diagnosis of CAD electrocardiography, stress testing, echocardiography, chest X-rays, and laboratory blood tests. Coronary angiography is considered to be the "gold standard" for the diagnosis of CAD as it is used to identify the precise location and severity of the disease.[5] It has also been acknowledged that in spite of successful treatment achievements in recent decades, the mortality of patients with heart disease continues to be elevated.[6] Early detection and control of known and emerging risk factors of CAD will enable better monitoring of the disease at an earlier stage. Hence, further study of specific cardiac factors may be able to generate predictive tests for coronary angiographic results and set the basis for earlier monitoring of the disease before additional complications arise. This study aimed to examine various clinical, demographic, and biochemical profiles of Trinidadian patients undergoing coronary angiography and determine how they correlate to findings at angiography. The objective is to better target parameters related to CAD and its severity. Correlating such parameters with patients' angiographic results has not been conducted in Trinidad or the Caribbean region thus far. This study is therefore of much significance and is a vital step in assessing ways to diagnose and control CAD at an earlier stage.

Materials and Methods

Study Design and Setting

The research method was of a quantitative orientation, with the research design as experimental with patient questionnaires. As such, this research method emphasized measurements and the statistical analysis of the data collected through questionnaires or by manipulating preexisting numerical data to explain the phenomenon.

Study Participants and Sample Size

Since this was a quantitative research methodology, a sample size calculation was not followed. Sample size above the minimum requirement for statistical analysis was provided. One hundred and twenty-four ($n = 124$) patients age range 17 to 70 years were included in this study. All patients were required to fast for 10 to 12 hours overnight. The included patients were referred for angiography at the catheterization laboratory of the Eric Williams Medical Sciences Complex hospital. This hospital was selected on the basis that it is centrally located in Trinidad and is the only public (government funded) cardiac catheterization laboratory in the island. Its referral base for cardiac catheterization is unselected and comes from the entire country's public health network. The patient pool therefore is representative of most of the country's population and was not possible to be controlled by the authors. The protocol for the study was approved by the Ethics Committee of the Faculty of Medical Sciences, The University of the West Indies, Ref: CEC107/12/15.

Collection of Clinical and Biochemical Data

After getting informed consent, patient age, self-reported ethnicity, smoking status, diet, medical history, and family history were collected by administering the questionnaire to the study participants. Blood pressure, waist and hip circumferences, and body weight were measured using standard procedures. Measurement of the waist and hip circumferences was obtained using a tape measure: the patient was first asked to stand upright with arms relaxed at their sides, feet evenly spread apart at approximately shoulder width, and body weight evenly distributed. The waist measurement was made at the midpoint between the top of the upper edge of the pelvic bone and the lowest point of the ribcage that can be palpated. Once this location was obtained, the tape measure was wrapped snugly around the body ensuring that the abdominal muscles were relaxed. For hip circumference, the tape measure was wrapped around the hips at the widest circumference of the buttocks. The patients' weights in kilograms were measured using a medical scale and heights were obtained in meters using a stadiometer.

The 10 mL of fasting blood sample was collected from each patient and further used to analyze fasting blood sugar (FBS) (sodium fluoride/potassium oxalate tube), lipid profile, creatinine, uric acid, interleukin-6, insulin, creatine kinase, creatine kinase MB izoenzyne, N-terminal pro B-type natriuretic peptide (NT-proBNP), and soluble ST2. All these parameters were analyzed on an automated dry multichannel analyzer, except for soluble ST2, which was analyzed using Presage ST2 Assay Elisa Kit due to equipment and resource availability. Interleukin-6, insulin, and NT-proBNP were analyzed using the Cobas e601 machine and the other parameters were measured using the Vitros 4600 analyzer. Whole blood obtained from the K_2EDTA tube was used to evaluate glycated hemoglobin using the Arkray A1c analyzer.

Coronary Angiographic Assessment

Patients were grouped according to the severity of CAD after undergoing coronary angiography. These patients were grouped as having no CAD, mild or nonobstructive CAD, single-vessel disease (SVD), double-vessel disease (DVD), and triple-vessel disease (TVD), according to the number of vessels involved and the degree of stenosis. Stenosis of a vessel was characterized as significant once the vessel was more than or equal to 50% narrowed. CAD was therefore defined as 50% or more reduction in the luminal diameter by obstruction in one or more coronary arteries as assessed by

using angiography by a cardiologist. The cardiologist then suggested the appropriate revascularization strategy (percutaneous coronary intervention or coronary artery bypass grafting) to be performed or optimal medical therapy to treat the patient.

Statistical Analysis

Statistical analyses were performed using SigmaPlot 11 and IBM SPSS Statistics 20. Normally distributed data were presented as mean ± standard deviation, whereas data with nonnormal distributions were expressed as median (interquartile range). Categorical data were presented as percentages and coded before statistical analysis. The presence of CAD was coded as 1 and absence as 0. Additionally, severity of CAD was coded as follows: no disease—0, mild CAD—1, SVD—2, DVD—3, and TVD—4. Correlations between nonnormal parameters and CAD presence and severity were assessed using a nonparametric test: Spearman's rank-order correlation coefficient. Chi-square test was also used to compare the associations between categorical variables. Statistical significance was accepted when the p-value was less than 0.05. In addition to these tests, the eta coefficient of nonlinear association was evaluated between some of the intervals and categorical variables, that is, type 2 diabetes (T2D) at baseline and coronary angiography severity, and between alcohol status and CAD severity, to further support the previous results obtained. Finally, multinomial logistic regression was conducted to show the significant predictors of CAD presence and severity after adjustment for possible confounding factors.

Results

Baseline Characteristics

►**Table 1** depicts the baseline descriptive characteristics of the patients analyzed in this study.

The number of patients falling into each category of CAD normal (9.7%), mild CAD (18.5%), SVD (26.6%), DVD (15.3%), and TVD (29.9%) were categorized. It was observed that out of 124 patients, most were males. Majority of these patients were of East Indian descent (Indo-Trinidadians) with an omnivorous diet and hypertensive at baseline.

Correlations between the clinical and biochemical parameters with the presence and severity of CAD were analyzed. Among the parameters analyzed, age, FBS, NT-proBNP, and hemoglobin A1c (HbA1c) showed strongly positive statistically significant relationships with CAD ($p < 0.001$). There was a moderately positive correlation with creatinine ($p = 0.006$) as shown in ►**Table 2**. These positive correlations suggest that as both of these parameters, NT-proBNP and HbA1c, increased in the blood, it was likely that the severity of CAD would increase, from no disease to TVD. There was no statistically significant relationship between soluble ST2 and CAD severity ($r = 0.029$, $p = 0.745$).

►**Table 3** reveals the results of the chi-square test association between categorical clinical variables and CAD presence and severity. A statistically significant relationship between gender and presence of CAD (χ^2 (1) = 4.978, $p = 0.026$) with an odds ratio of 3.895 at 95% confidence interval (1.102, 13.763) was observed suggesting that CAD is approximately four times more likely to be present in males than females. A statistically significant relationship was also found between male gender and CAD severity (χ^2 (4) = 11.894, $p = 0.018$).

An association was also observed between the presence of T2D before angiography and the presence of CAD with statistical significance (χ^2 (1) = 5.091, $p = 0.024$) and an odds ratio of 5.182 at 95% confidence interval (1.086, 9.548). This indicated that the odds of a patient with T2D presenting with CAD is 5.182 times more likely than individuals without T2D. A statistically significant relationship was also observed between presence of T2D at baseline and the severity of CAD (χ^2 (4) = 21.483, $p < 0.001$).

No statistically significant associations were established between smoking and alcohol consumption with the presence

Table 1 Baseline descriptive characteristics of patients referred for coronary angiography

Characteristic	Cohort ($n = 124$)
Age (y)	56 ± 11
Male	78 (62.9%)
Female	46 (37.1%)
Systolic blood pressure (mm Hg), median	135
Diastolic blood pressure (mm Hg), median	80
Body mass index (kg/m^2), median	27
Waist:hip, median	0.94
Smokers	27 (21.8%)
Alcoholics	12 (9.7%)
Diet	
Omnivorous	111 (89.5%)
Vegetarian	13 (10.5%)
Family history	
Heart disease	80 (64.5%)
Diabetes mellitus	73 (58.9%)
Stroke	24 (19.4%)
Self-reported ethnicity	
Indo-Trinidadians	93 (75%)
Afro-Trinidadians	19 (15.3%)
Others (mixed)	12 (9.7%)
Chronic conditions before angiography	
STEMI	18 (14.5%)
NSTEMI	23 (18.5%)
Hypertension	80 (64.5%)
Hyperlipidemia	65 (52.4%)
Type 2 diabetes	59 (47.6%)

Abbreviations: NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.

Table 2 Results of Spearman's rank-order correlation between clinical and biochemical parameters with severity of CAD

Parameter	Cohort ($n = 124$)	Correlation coefficient	*p*-Value
Age (y)	56 ± 11	0.387	<0.001
Systolic blood pressure (mm Hg)	135 (30.8)	0.141	0.119
Diastolic blood pressure (mm Hg)	80 (17)	−0.069	0.446
Waist:hip	0.94 (0.1)	0.126	0.165
Body mass index (kg/m^2)	26.7 (7.4)	−0.063	0.490
FBS (mg/dL)	97 (37.5)	0.418	<0.001
Trigs (mg/dL)	2.1 ± 0.19	0.150	0.097
HDL (mg/dL)	1.6 ± 0.13	−0.157	0.082
LDL (mg/dL)	81 (70.5)	0.158	0.080
TC (mg/dL)	2.2 ± 0.15	0.133	0.142
CK (mg/dL)	1.9 ± 0.31	−0.136	0.133
CKMB (mg/dL)	12 (8)	0.077	0.393
Creatinine (mg/dL)	1 (0.36)	0.244	0.006
Uric acid (mg/dL)	5.7 (2.3)	0.099	0.274
IL-6 (pg/mL)	4.5 (19.2)	0.095	0.295
NT-proBNP (pg/mL)	2.4 ± 0.8	0.307	0.001
Soluble ST2 (ng/mL)	30.3 (28)	0.029	0.745
IR (HOMA)	4.2 (3.6)	0.078	0.388
HbA1c (%)	6.1 (1.4)	0.488	<0.001

Abbreviations: CAD, coronary artery disease; CK, creatine kinase; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; IL, interleukin; IR, insulin resistance; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro B-type natriuretic peptide; TC, total cholesterol; Trigs, triglycerides.

and severity of CAD. None was also observed between the presence of hypertension and hyperlipidemia at baseline with the presence or severity of CAD.

Indo-Trinidadians formed 75% of this cohort of patients. Ethnicity, represented as Indo-Trinidadians or not (mixed and Afro-Trinidadians), was found to have a statistically significant correlation with CAD severity (χ^2 (4) = 12.925, $p = 0.012$). The relationship between CAD severity and number of patients of the various ethnic groups is shown in ►**Fig. 1**.

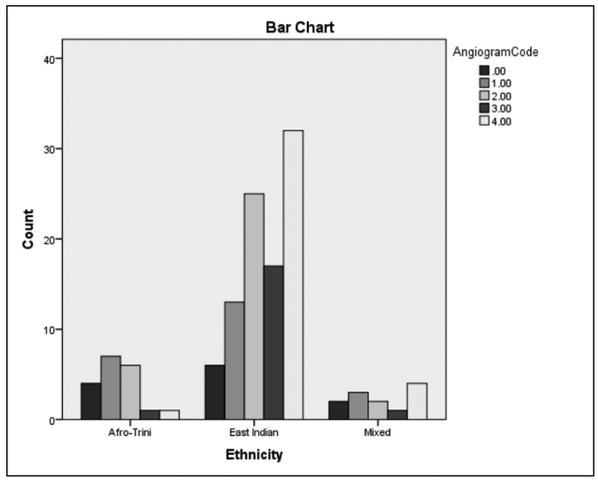

Fig. 1 Relationship between coronary artery disease severity and number of patients of the various ethnic groups.

Another test was performed to confirm these results, called the eta measure which is part of an analysis of variance test. This found moderate associations with these same categorical variables—T2D presence at baseline and ethnicity with CAD severity. The presence of T2D before coronary angiography resulted in an eta value of 0.413 (eta$^2 = 0.171$) and ethnicity (Indo-Trinidadian/not Indo-Trinidadian) presented an eta value of 0.300 (eta$^2 = 0.09$). When ethnicity was categorized as Indo-Trinidadian, Afro-Trinidadian, and other (mixed), a moderately strong association was also found, with an eta value of 0.333 (eta$^2 = 0.111$) (not shown in the table).

Another regression was performed using gender, smoking status, alcohol consumption, and ethnicity as possible confounders with CAD severity and the biochemical parameters. The results of this regression showed that age, FBS, interleukin-6, and NT-proBNP were all significant predictors of the severity of CAD (►**Table 4**).

Discussion

The focus of this study was to evaluate the anthropometric and biochemical parameters and to determine how these parameters correlated to the findings of coronary angiography. Angiographic findings were grouped by the number of diseased coronary vessels and the percentage of coronary stenosis. The results observed were statistically significant

Table 3 Chi-square test associations between categorical clinical variables and CAD presence and severity

Parameter	Cohort ($n = 124$)	With CAD presence			With CAD severity	
		Chi-square	*p*-Value	OR (95% CI)	Chi-square	*p*-Value
Gender—male	78 (62.9%)	(1) = 4.978	0.026	3.895 (1.102, 13.763)	(4) = 11.894	0.018
T2DM presence (at baseline)	59 (47.6%)	(1) = 5.091	0.024	5.182 (1.086, 9.548)	(4) = 21.483	<0.001
Ethnicity (Indo-Trinidadians)	93 (75%)	(1) = 4.429	0.072	3.480 (1.032, 11.739)	(4) = 12.925	0.012

Abbreviations: CAD, coronary artery disease; CI, confidence interval; OR, odds ratio; T2DM, type 2 diabetes mellitus.

Table 4 Multinomial logistic regression results after adjustment for gender, smoking status, alcohol status, and ethnicity

Parameter	*p*-Value		Exp (B) (95% CI)
	Before	After	
Age	0.037	0.009	0.851 (0.753, 0.961)
FBS	0.004	0.042	0.910 (0.832, 0.996)
IL-6	0.009	0.005	1.126 (1.037, 1.224)
NT-proBNP	0.068	0.038	0.998 (0.997, 1.000)

Abbreviations: CI, confidence interval; FBS, fasting blood sugar; IL, interleukin; NT-proBNP, N-terminal pro B-type natriuretic peptide.

correlations between the severity of CAD and age, FBS, glycated hemoglobin, creatinine, and NT-proBNP levels.

A more refined approach to risk assessment may include the use of additional biological markers of pathophysiological processes, for example, those of myocardial fibrosis and stretch. An enhanced risk assessment would therefore be of significant clinical value to precisely identify heart failure patients who are at risk of death. These patients could therefore be targeted for more intensive treatment and monitoring at earlier stages. In addition to the already-established risk factors of CAD, a few emerging parameters were incorporated into the study to enhance the risk assessment of the patient. The early identification and treatment of these risk factors will therefore aid in accelerating disease monitoring and prevention and in turn improve the morbidity rate.

Glycemic control is a known risk factor associated with both the presence and severity of CAD as supported by the results of this study. It was observed that there was a general increase in fasting blood glucose levels over the spectrum of CAD severity from normal patients to those diagnosed with the TVD. In a study conducted by Park et al in which the objective was to characterize the dose–response relationship between fasting glucose levels below those diagnostic of diabetes with cardiovascular events and when the levels rose above the normal value, the risk also gradually increased.[7] The presence of T2D further augments these factors and can therefore induce adverse functional and structural changes to the vessel wall. It has been suggested that increased concentrations of free fatty acids may lead to the induction of inflammatory responses, worsen insulin resistance, and impair endothelium-dependent vasodilation.[8]

HbA1c levels have been able to identify individuals with prediabetes as having a worse cardiometabolic risk profile and more severe CAD as compared with those patients with normal glycemic levels.[9] HbA1c levels have been known to be associated with an increased risk for vascular complications and have also been observed to be strongly correlated with CAD severity.[10] The present study of this cohort of Trinidadian patients produced results that followed the same patterns as reported in the previous studies. High fasting blood glucose and increased HbA1c levels have been observed to be potential risk factors for cardiovascular events.[11] Elevated glucose levels in the blood tend to change the structure and viscosity of the blood, causing it to potentially adhere to the sides of veins and arteries. This, therefore, causes blood vessels to become thicker and blood flow is then impaired. This process can then lead to atherosclerosis and other heart issues.[12] These data were additionally supported by the strong positive correlation produced by HbA1c and CAD severity, suggesting that as these levels increase in the blood, so does the severity of CAD from normal to TVD. As stated by Dutta et al, with increasing levels of glycated hemoglobin, a significant increase was observed in the mean number of diseased vessels involved in CAD. They also reported a linear correlation between HbA1c and the number of vessels involved.[10] The current study in the cohort of Trinidadian patients, therefore, added support to the results of the studies in the past.

Creatinine is a chemical waste product that is generated from muscle metabolism. Mild forms of creatinine elevation are also associated with poor cardiovascular outcomes. Korkmaz et al stated that patients in the highest creatinine group also had the highest total stenosis irrespective of their age and gender.[13] Their study aimed to show whether creatinine has any influence on angiographically shown stenosis and extension of coronary atherosclerosis in patients with stable CAD.[13] Another study found that small increases in the creatinine levels of patients with systolic dysfunction after myocardial infarction, over a specified period, were defined as worsening renal function. Creatinine was then characterized as a measure of renal function.[14]

Another group recently demonstrated that NT-proBNP is independently predictive of the severity of coronary disease diagnosed by coronary angiography. It was observed that the diagnostic value of NT-proBNP seemed to have increased with the severity of atherosclerotic lesions, especially those of TVD and left main artery disease. Levels of natriuretic peptides in the blood were detected to be increased across the CAD severity spectrum.[15] The NT-proBNP patterns in our study patient population were found to be consistent with the above-mentioned literature. NT-proBNP levels were also seen to be correlated with the number of vessels involved in CAD, from no disease to TVD. NT-proBNP is the inactive fragment of its prohormone and is released by the cardiac atria and ventricles in response to elevated volume and filling pressures.[16] This marker is released into the circulation in response to ventricular stretch and cardiac ischemia and has therefore emerged as a biochemical measurement of cardiac performance.[15] Since this is a physiological hormone and is actively secreted under ischemic conditions, it serves as a marker for myocardial ischemia. Previous studies have shown that expression of the BNP gene is upregulated by myocardial hypoxia and this may be the probable mechanism.[17] Thus, NT-proBNP has been characterized as a relevant emerging biomarker of CAD, and heart disease in general. It is predictive of CAD presence and severity, and this Trinidadian population of CAD patients seemed to follow a very similar pattern.

Blood concentrations of soluble ST2 are elevated in inflammatory and heart diseases, and this marker can be considered as a valuable prognostic variable in these

conditions. Increases in soluble ST2 at baseline have been found to be associated with long-term all-cause and cardiovascular mortality in subjects having stable CAD.[18] Soluble ST2 has also been observed to be a marker of myocardial fibrosis and remodeling.[6] In addition to this, soluble ST2 has been explored to be able to predict mortality in heart failure patients and may also be able to identify heart failure patients at a higher risk of sudden cardiac death.[6] This may be a possible explanation of why this parameter produced no significant pattern or correlation with the spectrum of CAD severity in our study. Our patients were not categorized based on the presence or absence of heart failure, stable or unstable CAD. Further study is therefore required in this area of these Trinidadian cardiac patients.

It has been suggested that natives of South Asians tend to have increased mortality rates with clinical appearance of CAD at early life due to their lifestyle and genetic susceptibility. Due to individuals of Indian descent having more traditional risk factors at earlier ages, this can explain the prevalence of premature CAD in this group. In addition, it has been found that the presence of abnormal lipid profile has a higher characteristic of CAD risk in South Asians when matched to other ethnic groups.[19] Moreover, the association between alcohol consumption and cardiovascular mortality and morbidity has been debated for a long time. Several international comparisons show a negative association between alcohol usage and mortality due to coronary heart disease. Nevertheless, in the Regional Heart Study of 22 towns in Great Britain, a positive correlation was observed between the population of heavy drinkers in a town and mortality from coronary heart disease.[20] Our observation of CAD status with respect to alcohol consumption in this study was in line with the previous reports that found no significant association between the two. Changes in various ethnic groups may be due to their risk factors and intensity of CAD. Various ethnic groups are subjected to develop CAD to an extent, causing a raise in frequency or intensity of disease in those populations. This trend has been shown in both developed and developing countries.[21]

For several decades, it has been believed that coronary heart disease is distinctly more common in men than in women. In both sexes, however, coronary heart disease increases with age.[22] According to a study conducted by Hertzer et al, the overall occurrence of severe CAD is significant in all age groups; however, a higher incidence of severe CAD determined by coronary angiography was observed with each decade of advancing age and is also supported by the findings in this study.[23] Another study has reinforced that women had a tendency to develop cardiovascular diseases 7 to 10 years later than men.[24] Studies have explored the possible reasons why heart disease was more frequent in men. Gender differences were discovered in psychosocial and behavioral coronary risk factors in men, including excessive alcohol consumption and smoking. The dealing ability of men with stressful situations seems to be less adaptive physiologically. This possibly increased their risk for coronary heart disease.[25]

Given the unselected nature of the patient selection, the authors could not control for the possibility of referral bias inflating the rates of males versus females in our patient population. The hospital at which the study was conducted performs cardiac catheterization procedures for the entire island's public health care system, and it is therefore reasonable to assume that the patient population is representative of the island's CAD population.

Conclusion

This study investigates whether the anthropometric, biochemical, and metabolic profiles of patients undergoing coronary angiography predict the presence and severity of CAD in a Trinidadian cohort. There is a paucity of data for the Caribbean as it pertains to noncommunicable diseases in general and cardiovascular disease specifically. The economic challenges of the region as a whole mean that earlier risk stratification of patients potentially at risk of severe CAD can mean more targeted utilization of costly resources with a potentially greater return on investment for developing countries.

A few parameters were well correlated and well associated with the severity of CAD after diagnosis by the process of coronary angiography. These biochemical parameters included FBS, NT-proBNP, creatinine, and particularly, glycated hemoglobin. The clinical parameters observed to be well associated with CAD severity in this population were presence of T2D, ethnicity (Indo-Trinidadian), and age. One variable which was expected to produce similar results to that of NT-proBNP and HbA1c was soluble ST2. However, this marker showed no statistically significant correlations with CAD severity. Further study with this population will therefore need to be conducted. Overall, FBS, glycated hemoglobin, NT-proBNP, and creatinine could be used as a panel of potential prognostic biomarkers of CAD severity. Our study will provide the basis for longer term studies on larger Caribbean patient populations with perhaps more diverse ethnic groups. This will provide more insights on these key biomarkers that predispose to CAD.

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Conflict of Interest
None declared.

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Identification of Bacterial Isolates and Their Antimicrobial Susceptibility Pattern from Wound/Pus Sample in a Tertiary Care Hospital, Gwalior, India

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Abstract

Objective The goal of this investigation was to look at the frequency and dispersal of bacteria isolated from pus/wound, as well as their susceptibility patterns.

Materials and Methods A study was conducted on 175 patients who provided pus and/or wound discharge samples in different wards (outpatient department or inpatient department). MacConkey agar and blood agar plates were immediately inoculated with samples and incubated at 37°C for 24 hours. The Gram stain and biochemical tests were used to identify all isolates after incubation. Kirby–Bauer's disc diffusion method was used to perform sensitivity tests on Mueller–Hinton agar plates.

Results This study covered 175 patients, with a bacterial isolation rate of 102 (58.28%). Males outnumbered females in the samples (M:F = 1.8:1), with a median age of 45 years as majority were in the age group of 40 to 60 years which was 41 (40.20%). Total 90.1% samples showed monomicrobial infection, whereas 9.8% showed polymicrobial infection, and total 112 bacterial strains were isolated.

Conclusion *Escherichia coli* was the most prevalent isolate in present investigation, followed by *Pseudomonas aeruginosa*. Chloramphenicol is the only antibiotic which is effective for both gram-negative bacilli and gram-positive cocci. This report's susceptibility statistic may be worth considering for developing empiric treatment regimens for pyogenic infections.

Keywords
- pus sample
- wound infection
- antimicrobial susceptibility
- pyogenic
- multidrug resistant
- GNB
- GPC

Introduction

A wound is a break in the skin or tissues integrity, which can result in structural and functional disturbances.[1] Infection of the wound can be pyogenic (pus forming) or nonpyogenic, depending on the causative organism. The majority of the organisms in wounds are aerobes, includes gram-positive cocci (GPC) such as *Enterococci*, *Staphylococcus epidermis*, *S. aureus*, *Streptococcus pyogenes*, and gram-negative bacilli (GNB) such as *Pseudomonas aeruginosa*, *Klebsiella*

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pneumoniae, *Escherichia coli*, *Proteus*.[2] A wound infection is more likely to occur in situations with higher wound class (dirty-infected wound) and higher bacterial load.[3] Microbes are the unseen adversaries of humans, wreaking havoc on the human body as well as other living organisms.[4,5] Bacterial illnesses continue to be the predominant factor in morbidity and mortality.[6] Various bacterial species reside on human skin, in the nasopharynx, in the gastrointestinal system, and other areas of the body, but they have a lower risk of causing disease due to the body's first line of defense.[7] Microbial pathogens cause human skin and soft tissue infections (SSTIs) during or after trauma, burns, bites, abrasions, minor cuts, lacerations, crush injuries, gunshot injury, and surgical procedures. Compromising in front line of defense leads to bacterial contamination, resulting in the generation of pus, a white or yellow fluid containing dead leukocytes, cellular detritus, and devitalized tissue.[8,9] Infection can be either endogenous or exogenous.[10] The loss of skin integrity due to a variety of reasons creates an environment conducive to the colonization and proliferation of microorganisms.[11] Humidity, heat, and nutrition in the wound attract pathogen from the cutaneous surface, environment, or the patient's own flora, which grow and release various virulence factors, resulting in wound infection.[12] Immune cells are recruited to the infection site by the body's defense mechanism to fight pathogens.[13] Pyogenic infection results from the build-up of these cells inhibits wound healing and can lead to complications such as wound dehiscence or wound disintegration.[14] Fungus, in addition to bacteria, can induce wound infection, and they might coincide with more than one bacteria in a single lesion.[15] Infectious diseases are a major threat to human health and life.[16] Antimicrobial agents or medications are substances that have the ability to kill bacteria or stop them from multiplying.[17] Knowing the susceptibility of a certain bacteria to an antibiotic helps you to treat the patient empirically until the culture report is generated. The choice of antibiotics is then determined by the results of the culture.[13] Inadvertent and inappropriate antibiotics use results in the establishment of a drug-resistant bacteria, which leads to a lengthy hospital stay, a significant financial loss, and serious medical complications.[18] During a prolonged hospital stay, a patient may spread drug-resistant microorganisms to other patients, family, or even health care workers.[19] The antibiotics susceptibility of these organisms in a given environment change over time as bacteria evolve and as antibiotic use or misuse patterns change.[20] The rise of antibiotic-resistant pathogenic microorganism is regarded as a severe hazard to global public health.[21]

Materials and Methods

Study Design and Sampling Process

The study was conducted in the microbiology department at Birla Institute of Medical Research (BIMR) Hospital, Gwalior from September 2021 to April 2022 for a period of 8 months. The pus samples were taken from individuals who were examined in the outpatient department and were admitted to the hospital's inpatient department, using sterile cotton swabs, a syringe, or a sealed capillary tube. It was labeled and immediately sent to microbiology laboratory. The study population consisted of all individuals who had SSTIs.

Inclusion and Exclusion Criteria

As part of standard patient treatment, nonduplicated specimen was taken and cultured. The pus sample from one location is included, unless it was taken from the other location. One patient underwent susceptibility testing only once.

The study excluded patients with missing antibiotic sensitivity results, inadequate data, prior exposure to antibiotics, or repeated culture results during the last 6 months.

Isolation and Identification

The isolation and identification of microorganisms from the sample of pus were performed by streaking sample on MacConkey agar and blood agar plates, and incubating them at 37°C for 24 to 48 hours. Following incubation, bacterial colonies showing different characteristics were chosen for further investigation. The colonies grown were identified with the help of Gram staining which differentiate gram-positive and -negative bacteria followed by biochemical test such as coagulase, catalase, indole, Voges–Proskauer, methyl red, oxidase test, urease, and citrate which were performed as per standard protocol.

Catalase enzyme estimation aids to distinguish *Streptococci* from *Staphylococci* colonies. The coagulase test distinguishes *S. aureus* (which is coagulase positive) from *S. epidermis* and *S. saprophyticus* (which is coagulase negative). Oxidase test were used to distinguish Enterobacteriaceae from other GNB.

Samples considered to be negative when no growth was observed on blood agar and MacConkey agar media only after 48 hours of incubation.

Antimicrobial Agents

GNB were tested with antibiotic discs such as amikacin (30 µg), gentamycin (10 µg), ertapenem (10 µg), meropenem (10 µg), imipenem (10 µg), ceftazidime (30 µg), cefazolin (30 µg), cefepime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), cefoxitin (30 µg), ampicillin (10 µg), piperacillin–tazobactam (10 µg), ampicillin–sulbactam (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), trimethoprim–sulfamethoxazole (25 µg), ceftazidime–avibactam (30 µg), chloramphenicol (30 µg).

GPC were tested with antibiotic discs such as cefoxitin (30 µg), cefazolin (30 µg), ampicillin (25 µg), penicillin-G (2 units), erythromycin (15 µg), fusidic acid (30 µg), vancomycin (30 µg), clindamycin (2 µg), ciprofloxacin (5 µg), moxifloxacin (5 µg), mupirocin (5 µg), doxycycline (30 µg), daptomycin, quinupristin–dalfopristin (15 µg), rifampin (5 µg), chloramphenicol (30 µg), linezolid (30 µg), trimethoprim–sulfamethoxazole (25 µg).

Antimicrobial Susceptibility Testing

The antibiotic susceptibility testing was done as per Clinical and Laboratory Standards institute (CLSI)

guidelines using Kirby–Bauer's method.[22] Inoculum was prepared for each bacterial isolate by matching the turbidity to 0.5 McFarland standard and spreading on Mueller-Hinton agar (MHA) plate. Paper disc which contains antibiotics were kept on the top of the MHA plate and incubate at 37°C for 24 hours. According to CLSI M100 Guideline 2022, the size of the zones of inhibition was classified as sensitive, moderate, or resistant to the antibiotics tested.[22]

For accurate identification of pathogen and their susceptibility pattern, automated BD Phoenix M50 machine were used as per manufacturer's instruction.

Quality Control

Pseudomonas aeruginosa American Type Culture Collection (ATCC) 27853, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922 strains are used as quality control for the identification and susceptibility test (►**Table 1**).

Results

A total of 175 pus samples were received in the department of microbiology from September 2021 to April 2022. Out of total 175 pus/wound swab samples processed, 102 (58.28%) samples were culture positive, whereas 73 (41.71%) samples

Table 1 Quality control data for antibiotics

Antimicrobial agent	Diameter of zone of inhibition in mm		
	Escherichia coli ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923
Amikacin	19–26	18–26	–
Gentamicin	19–26	17–23	–
Ertapenem	29–36	–	–
Imipenem	26–32	20–28	–
Meropenem	28–35	27–33	–
Cefazolin	21–27	–	29–35
Cefuroxime	20–26	–	–
Cefoxitin	23–29	–	23–29
Ceftazidime	25–32	22–29	–
Ceftriaxone	29–35	–	–
Cefepime	31–37	25–31	–
Ampicillin	15–22	–	27–35
Ampicillin–sulbactam	15–22	–	–
Piperacillin–tazobactam	21–25	21–25	–
Ciprofloxacin	29–38	25–33	22–30
Levofloxacin	29–37	19–26	–
Trimethoprim–sulfamethoxazole	23–29	–	24–32
Ceftazidime-avibactam	21–25	21–25	–
Chloramphenicol	21–27	–	19–26
Penicillin-G	–	–	26–37
Vancomycin	–	–	17–21
Clindamycin	–	–	24–30
Erythromycin	–	–	22–30
Moxifloxacin	–	–	28–35
Doxycycline	–	–	23–29
Quinupristin–dalfopristin	–	–	21–28
Fusidic acid	–	–	24–32
Linezolid	–	–	25–32
Mupirocin	–	–	18–24
Rifampin	–	–	26–34

Abbreviation: ATCC, American Type Culture Collection.

Table 2 Age- and gender-wise distribution of bacterial growth from pus/wound sample

Age group	No. of male (%)	No. of female (%)	Frequency (%) ($n = 102$)
< 20 y	7 (6.86)	3 (2.94)	10 (9.80)
20–40 y	22 (21.56)	8 (7.84)	30 (29.41)
40–60 y	27 (26.47)	14 (13.72)	41 (40.20)
> 60 y	10 (9.80)	11 (10.78)	21 (20.59)
Total	66 (64.70)	36 (35.29)	102 (100)

were negative for growth. Out of 102 positive samples, monomicrobial infections were seen in 92 (90.19%) samples, whereas polymicrobial infections with growth of two pathogens in 10 (9.80%) samples, and total 112 bacterial strains were isolated. Among 112 isolates, 83 (74.10%) were GNB, 23 (20.53%) were GPC, and 6 (5.35%) were *Candida*. Among 102 (58.28%) culture positive, mostly in the age of 40 to 60 years, it was 41 (40.20%) cases, subsequently 20 to 40 years, >60 years and then <20 years which was 30 (29.41%), 21 (20.59%) and 10 (9.80%) instances, respectively (►**Table 2**).

Discussion

Infection of the wound is the common cause of patient's impairment and if it is not cured in early stage, then it increases the hospital stays. Severe wound infections can lead to sepsis, which can be fatal, especially if the bacteria are multidrug resistant. Any wound has the potential to get infected as infection of the wound becomes commonest hospital-acquired infection. In the present study, pus samples from a tertiary care hospital were analyzed to determine the etiological agents and their pattern of antibiotic susceptibility.

The majority (58.28%) of the samples in this study revealed positive growth. This is due to the fact that suppurative infections of the eye, ear, and skin are frequently seen in both inpatient and outpatient departments. Furthermore, among surgical patients, wound infection is the most prevalent hospital-acquired infection. It has been linked to more trauma care, longer hospitals stay, and treatment. The results revealed 58.28% positivity rate of total sample that correlate with the studies of Rai et al[23] (59%), Trojan et al[8] (60.1%), and Khanam et al[21] (61.8%); however, it exceeded a study conducted by Singh et al[13] (52.73%) and less than a research conducted by Muluye et al[7] (70.2%) and Batra et al[24] (85.02%).

According to sex, the predominance of males (64.70%) is higher than females (35.29%) in the present study (►**Table 2**). It is most likely related to increased exposure to the environment and the increased risk of accidents when earning a living, as well as social behavior in which males are treated as superior to female and are given preferential biased treatment when compared with females.

Table 3 Frequency/percentage of the isolates (monomicrobial) after aerobic culture from pus/wound sample

Isolated organisms	Frequency ($n = 86$)	Percentage
Escherichia coli	20	23.25
Pseudomonas aeruginosa	18	20.93
Staphylococcus aureus	15	17.44
Klebsiella spp.	12	13.95
Acinetobacter spp.	10	11.62
Enterobacter cloacae	2	2.32
Morganella morganii	2	2.32
CoNS	2	2.32
Micrococcus	2	2.32
Enterococcus spp.	1	1.16
Burkholderia spp.	1	1.16
Stenotrophomonas maltophilia	1	1.16
Total	86	100

Abbreviation: CoNS, coagulase-negative staphylococci.

In the present study, monomicrobial infections predominated (90.19%), while polymicrobial infections were observed (9.80%) (►**Tables 3** and **4**). The study by Sudhaharan et al[11] found that monomicrobial infection was 93.2% and polymicrobial infection was 6.8%; this result is consistent with our findings.

In the present study, GNB were the predominant isolates which was 74.10% compared with GPC which was 20.53% and *Candida* which was 5.35%. A research done by Bankar et al[25] also recorded predominance of GNB which was 51.97%, whereas GPC was 47.36% and *Candida* was 0.65%.

In the present study, the most common isolates were *E. coli* (GNB) and *S. aureus* (GPC) (►**Table 3**). The present findings correlate with the research done by Trojan et al,[8]

Table 4 Frequency/percentage of mixed isolates (polymicrobial) after aerobic culture from pus/wound sample

Mixed isolated organisms	Frequency	Percentage
Escherichia coli + *Pseudomonas aeruginosa*	3	30
Escherichia coli + *Proteus mirabilis*	2	20
Staphylococcus aureus + *Klebsiella* spp.	1	10
Staphylococcus aureus + *Pseudomonas aeruginosa*	1	10
Enterococcus spp. + *Klebsiella* spp.	1	10
Pseudomonas aeruginosa + Klebsiella spp.	1	10
Pseudomonas aeruginosa + *Morganella morganii*	1	10
Total	10	100

Table 5 Frequency/percentage of antibiotic sensitivity pattern of GNB isolated from pus/wound sample

Antibiotics	*Acinetobacter* spp. ($n=10$)	*Burkholderia* ($n=1$)	*Enterobacter cloacae* ($n=2$)	*Escherichia coli* ($n=25$)	*Klebsiella* spp. ($n=15$)	*Morganella morganii* ($n=3$)	*Proteus mirabilis* ($n=2$)	*Pseudomonas aeruginosa* ($n=24$)	*Stenotrophomonas maltophilia* ($n=1$)
Amikacin	0 (0%)	0 (0%)	2 (100%)	22 (88%	3 (20%)	3 (100%)	2 (100%)	11 (45.83%)	IR
Gentamicin	0 (0%)	0 (0%)	1 (50%)	15 (60%)	2 (13.33%)	2 (66.66%)	2 (100%)	12 (50%)	IR
Ertapenem	IR	IR	0 (0%)	14 (56%)	2 (13.33%)	2 (66.66%)	2 (100%)	IR	IR
Imipenem	0 (0%)	0 (0%)	0 (0%)	16 (64%)	2 (13.33%)	0 (0%)	NA	11 (45.83%)	IR
Meropenem	0 (0%)	0 (0%)	0 (0%)	18 (72%)	2 (13.33%)	2 (66.66%)	2 (100%)	12 (50%)	IR
Cefazolin	IR	IR	0 (0%)	3 (12%)	0 (0%)	0 (0%)	2 (100%)	IR	IR
Cefuroxime	IR	IR	0 (0%)	3 (12%)	0 (0%)	0 (0%)	2 (100%)	IR	IR
Cefoxitin	IR	IR	0 (0%)	10 (40%)	0 (0%)	3 (100%)	2 (100%)	IR	IR
Ceftazidime	0 (0%)	0 (0%)	0 (0%)	4 (16%)	2 (13.33%)	2 (66.66%)	2 (100%)	11 (45.83%)	0 (0%)
Ceftriaxone	0 (0%)	0 (0%)	0 (0%)	4 (16%)	2 (13.33%)	1 (33.33%)	2 (100%)	IR	IR
Cefepime	0 (0%)	0 (0%)	0 (0%)	3 (12%)	2 (13.33%)	1 (33.33%)	2 (100%)	7 (29.16%)	0 (0%)
Ampicillin	IR	IR	0 (0%)	3 (12%)	0 (0%)	0 (0%)	2 (100%)	IR	IR
Ampicillin–sulbactam	0 (0%)	IR	0 (0%)	5 (20%)	0 (0%)	0 (0%)	2 (100%)	IR	IR
Piperacillin–tazobactam	0 (0%)	0 (0%)	0 (0%)	11 (44%)	2 (13.33%)	3 (100%)	2 (100%)	12 (50%)	IR
Ciprofloxacin	0 (0%)	0 (0%)	0 (0%)	3 (12%)	1 (6.66%)	0 (0%)	0 (0%)	7 (29.16%)	0 (0%)
Levofloxacin	0 (0%)	0 (0%)	1 (50%)	5 (20%)	1 (6.66%)	0 (0%)	0 (0%)	8 (33.33%)	1 (100%)
Trimethoprim–sulfamethoxazole	2 (20%)	1 (100%)	0 (0%)	11 (44%)	2 (13.33%)	1 (33.33%)	1 (50%)	IR	1 (100%)
Ceftazidime–avibactam	0 (0%)	0 (0%)	0 (0%)	18 (72%)	5 (33.33%)	3 (100%)	2 (100%)	16 (66.66%)	0 (0%)
Chloramphenicol	0 (0%)	0 (0%)	2 (100%)	25 (100%)	5 (33.33%)	2 (66.66%)	1 (50%)	IR	1 (100%)

Abbreviations: GNB, gram-negative bacilli; IR, intrinsic resistant; NA, not available.

Bankar et al,[25] Sudhaharan et al,[11] and Singh et al,[13] in which *E. coli* (GNB) and *S. aureus* (GPC) were the highly prevalent bacterial isolates in the cases of wound infection.

According to present research, chloramphenicol (100%) was the most effective antibiotic against *E. coli*, followed by amikacin (88%), meropenem and ceftazidime–avibactam (72%) (►**Table 5**). Meropenem sensitivity was comparable to research conducted by Trojan et al[8] (68%); however, the results were not in synchronization with the studies of Khanam et al[21] (50%). *Pseudomonas aeruginosa* showed higher sensitivity to gentamicin, meropenem, and piperacillin–tazobactam (50%), *Klebsiella* showed higher sensitivity to ceftazidime–avibactam and chloramphenicol (33.33%). Amikacin, cefoxitin, and piperacillin–tazobactam (100%) were highly sensitive against *Morganella morganii*. Amikacin and chloramphenicol (100%) were highly sensitive against *Enterobacter cloacae*.

In the present study, *Acinetobacter* spp. and *Burkholderia* spp. are 100% resistant to multiple antibiotic except trimethoprim–sulfamethoxazole which shows 20 and 100% sensitivity, respectively (►**Table 5**). It is due to the evolution of bacteria with the passage of time.

The antibiotic sensitivity pattern of *S. aureus* in the present study shows 100% sensitivity to vancomycin, doxycycline, and linezolid (►**Table 6**). It was equivalent to the study conducted by Batra et al[24] and Trojan et al[8] which shows 100% sensitivity to linezolid and vancomycin, but it was inconsistent with the study done by Khanam et al[21] which shows 31.2 and 18.5% sensitivity to linezolid and vancomycin, respectively.

In the present study, vancomycin, doxycycline, and linezolid were 100% sensitive to *S. aureus*, coagulase-negative staphylococci (CoNS), and *Enterococcus*. Daptomycin are 100% sensitive to CoNS and *Enterococcus* (►**Table 6**). The current results are consistent with Batra et al[24] who recorded linezolid and vancomycin were 100% sensitive for *S. aureus* and CoNS.

The incidence of pyogenic isolates of bacteria and their patterns of antibiotic resistance vary widely depending on geographic location and atmospheric conditions. Due to the rising incidence of isolates that are resistant to multiple drugs-resistant bacteria, it is more prevalent in wound infections. Thus, the present study indicates to patient neglect, inadequate treatment plans, antibiotic usage, self- and mis-prescription, a lack of regional antibiogram data, and clinician's weak understanding of multidrug-resistant isolates and antimicrobial resistance. Controlling antibiotic overuse and implementing infection–prevention measures from primary to tertiary care would aid in the prevention of infections caused by resistant bacteria. Antibiotics should be used rationally, at the appropriate dose and duration.

Table 6 Frequency/percentage of antibiotic sensitivity pattern of GPC isolated from pus/wound sample

Antibiotics	*Staphylococcus aureus* ($n = 17$)	*Enterococcus* ($n = 2$)	CoNS ($n = 2$)
Cefazolin	6 (35.29%)	IR	0 (0%)
Cefoxitin	6 (35.29%)	IR	0 (0%)
Ampicillin	2 (11.76%)	0 (0%)	0 (0%)
Penicillin-g	2 (11.76%)	2 (100%)	0 (0%)
Vancomycin	17 (100%)	2(100%)	2 (100%)
Clindamycin	11 (64.70%)	IR	0 (0%)
Erythromycin	4 (23.52%)	1 (50%)	0 (0%)
Ciprofloxacin	1 (5.88%)	1 (50%)	0 (0%)
Moxifloxacin	14 (82.35%)	0 (0%)	2 (100%)
Doxycycline	17 (100%)	2 (100%)	2 (100%)
Daptomycin	16 (94.11%)	2 (100%)	2 (100%)
Trimethoprim–sulfamethoxazole	6 (35.29%)	IR	0 (0%)
Quinupristin–dalfopristin	15 (88.23%)	1 (50%)	2 (100%)
Chloramphenicol	15 (88.23%)	2 (100%)	2 (100%)
Fusidic acid	16 (94.11%)	IR	2 (100%)
Linezolid	17 (100%)	2 (100%)	2 (100%)
Mupirocin	15 (88.23%)	2 (100%)	1 (50%)
Rifampin	14 (82.35%)	NA	0 (0%)

Abbreviation: CoNS, coagulase-negative staphylococci; GPC, gram-positive cocci; IR, intrinsic resistant; NA, not available.

Conclusion

The current research highlights that GNB are the most frequent microorganisms which cause the infection of wound, and it is due to the fact that the organisms causing wound infections are frequently present in hospital environments. For GNB, amikacin, gentamicin, and chloramphenicol are the most effective antibiotics, whereas for GPC, doxycycline, linezolid, and chloramphenicol are the most effective antibiotics. Thus, the present study exhibited that increase in bacterial resistance as compared with other studies is due to the modification or evolution of bacteria with the time or irrational use of antibiotics.[26]

Ethical Approval

The study was approved by the head of the institute where study was done.

Funding

None.

Conflict of Interest

None declared.

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Detection of Antibiotic Resistance and Biofilm-Producing Ability of *Staphylococcus* Species in Clinical Isolates

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Abstract

Background Staphylococci are responsible for life-threatening infections in hospitals and community. Their ability to produce multiple virulence factors and antibiotic resistance is an important reason of high mortality in staphylococcal infections. Biofilm production by these organisms makes it difficult to treat. Most of the treating antibiotics are failing and making it a matter of concern.

Aims This study aims to detect the increased antibiotic resistance in biofilm-producing *Staphylococcus* and to compare the performance of three potential methods of detection.

Methods A total of 81 isolates of staphylococci including coagulase negative staphylococci (CoNs), methicillin resistant *S. aureus* (MRSA), and methicillin sensitive *S. aureus* (MSSA) are included in this study. After the identification, an antibiotic sensitivity test was performed. Biofilm detection was done by three different methods: Congo red agar method, tube adherence method, and microtiter plate method.

Result Out of the 81 samples, 37 CoNs, 17 MRSA, and 27 MSSA were identified. Out of them we got 43 (53%) biofilm producers by Congo red agar method, 40 (49%) by tube adherence method, and 52 (64%) producers by tissue culture plate/microtiter plate method. Most of the biofilm producers showed multiple drug resistance.

Conclusion We found out that the microtiter plate method is sensitive and reliable as compared with the other two methods. Antibiotic resistance was found to be very common in biofilm producers. This was due to the resistance developed as a result of the matrix that does not let the antibiotic bind with the organisms. This can make the treatment of *Staphylococcus* very difficult in the future as the rate of drug resistance is faster as compared with newly emerging antibiotics.

Keywords
- biofilm
- antibiotic resistance
- MRSA
- MSSA
- CoNs

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Introduction

Staphylococcus aureus is a pathogen that can infect human as well as animals. The infections can range from skin and soft tissue infection to serious life-threatening illnesses like endocarditis, septicemia, and toxin-mediated shock syndrome in human, and skin infections and mastitis caused by *S. aureus* are reported in animals.[1] *Staphylococcus aureus* has many virulence factors that help the organism survive and initiate infection inside the host. These factors act in different ways to colonize, invade, and spread themselves. This is the primary reason for *S. aureus* being able to attack immunologically competent hosts.[2] The ability of the organism to develop biofilm enhances the severity of infections caused by *S. aureus* as well as increases the antibiotic resistance.[3] The steps of biofilm formation involve adhesion of the organism to the surface, which is aided by the expression of various microbial surface components that recognize adhesive matrix molecules. These molecules can bind to various extracellular matrix components, including elastin, fibronectin A and B, laminin, collagen, fibrinogen, and clumping factors. These proteins can connect to the cell wall or other surfaces by using similar signal sequences. They can cover medical equipment and start the creation of proteins like biofilm matrix proteins in a bacterial matrix.[4,5] Several *Staphylococcus* species now exhibit significant virulence traits due to the production of biofilm.[5] The biofilm serves as a protective cover that provides a favorable environment to the bacteria and allows them to survive against adverse conditions.[6] In addition to its ability to form biofilm on medical devices, which leads to the removal of devices, *S. aureus* can form biofilm inside the human body. This leads to infection associated with resistance to antimicrobial agents and failure of the treatment resulting in removal of the infected tissue to control the infection.[7] The resistance caused by biofilm is due to the presence of bacteria inside the biofilm; they remain dormant during the antimicrobial therapy and later emerge as resistant strains. Another mechanism that leads to resistance to antibiotics is the inability of antimicrobials to penetrate through the layers of biofilm.[8] Numerous coagulase negative staphylococci (CoNs) species, including *S. epidermidis*, have emerged as the primary carriers of nosocomial infections, particularly those that affect newborns, people with immunological disorders, and patients receiving care in the intensive care unit who utilize catheters or other prosthetic devices.[9] Catheter-associated infections may be caused by CoNs that produce biofilm that affects immunocompromised patients, including premature infants, neutropenic cancer patients, elderly people with serious underlying diseases, hospitalized patients undergoing invasive procedures, and people with permanent plastic devices.[10] Methicillin resistant *S. aureus* (MRSA) is largely found to be resistant to common available antibiotics. One of the common reasons for high mortality and morbidity in MRSA infections are biofilm producers.[11] In hospitals, the biofilm-producing pathogens are becoming a serious concern, and the existing antimicrobial agents are failing to treat these pathogens. The increase in multidrug-resistant organisms with biofilm is weakening the health care policy.[12] Therefore, the development of clinically applicable biofilm experimental modalities is urgently required since biofilm plays a significant role in the slow healing of wounds.[13]

In our study, 81 isolates belonging to the genus *Staphylococcus* were investigated for the presence of biofilm forming activity by three different methods such as the tube adherence method, Congo red agar method, and tissue culture plate method. Antibiotic resistance was compared between the biofilm producers and nonproducers. We found that the multidrug resistance was common among the biofilm producers and not all the strains showed presence of biofilm by all the methods used in this study.

Materials and Methods

Sample Collection

A total of 81 strains of staphylococci isolated from various clinical samples were collected. We included organisms that were clinically significant pathogens and not contaminants or those with insignificant growth. The samples included were pus, throat swab, urine, nasal swab, tracheal secretion, ear swab, vaginal swab, etc.

Identification of *Staphylococcus* Species

Gram staining and other biochemical tests such as slide and tube coagulase tests, catalase test, and other identification tests were used to distinguish between different species of staphylococci. After an overnight incubation, *S. aureus* is recognized by its golden yellow β-hemolytic colonies on blood agar, catalase production, and coagulase production. Staphylococci that are coagulase negative are CoNs and were further identified up to the species level.[14] All the *S. aureus* strains were further tested for the identification of MRSA using Cefoxitin disc (30 mcg) by the Kirby–Bauer disc diffusion method. The results were given as per the CLSI (Clinical laboratory Standard Institute) guidelines.[15]

Antibiotic Sensitivity Testing

The antibiotic sensitivity test was performed by the Kirby–Bauer disc diffusion method and interpreted based on the CLSI guidelines. The antibiotic discs tested were gentamycin (G), erythromycin (E), tetracycline (TE), ciprofloxacin (CIP), trimethoprim/sulfamethoxazole (TS), linezolid (L), teicoplanin (TE), tetracycline (TE), ampicillin (AM), cefuroxime (CXM), clindamycin (CD), levofloxacin (LV), amoxicillin-clavulanic acid, ceftazidime [CFT], nitrofurantoin [NF], tigecycline [TG] and doxycycline [DX], cefoxitin (Cx). The test was performed on Mueller–Hinton agar (Himedia India).

ATCC 25923 and ATCC 43300 were used as the controls for methicillin sensitive *S. aureus* (MSSA) and MRSA, respectively.[15–17]

Detection of Biofilm

The detection of biofilm production was done by three different methods to detect their efficacy and also to compare the findings along with the antibiotic resistance.

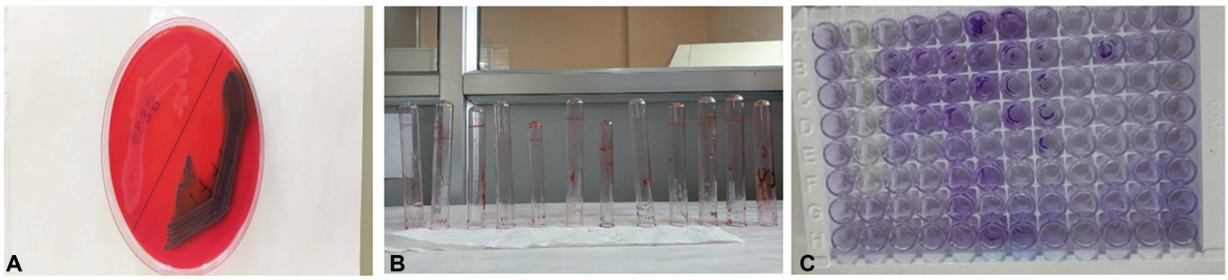

Fig. 1 (**A**) Biofilm producers show black metallic colonies on Congo red agar. (**B**) Biofilm producers show material adhering to the bottom. (**C**) Tissue culture plate method.

Congo Red Agar Method

The detection of biofilm by the Congo red agar method was done by using the method given in a previous study. The test organisms are inoculated into a special media prepared using brain–heart infusion medium supplemented with sucrose (5%) and Congo red dye. After the inoculation, the plates were incubated at 37°C for 24 to 48 hours. The test was interpreted positive for biofilm if the colonies were black in color with dry crystalline consistency. Weakly positive showed pink colonies with occasional black color in the center of the colonies (►**Fig. 1A**).[18]

Tube Adherence Method

The bacterial suspension of the test organisms was incubated at 35°C in glass test tubes containing brain–heart infusion broth for 48 hours. After the incubation was over, the supernatant was discarded and the glass tube was stained with 0.1% of safranine solution. The tube was washed three times with distilled water and then air dried. Any material adhering to the test tube surface was considered as positive and presence of only a ring was considered negative (►**Fig. 1B**).[19]

Microtiter Plate Method/Tissue Culture Plate Method

The detection of biofilm was also done by the tissue culture plate method using the procedure given by Christensen et al in 1995.[20] Fresh growths of the test isolates were inoculated into 5 mL of Trypticase soy broth and then incubated for 24 hours at 37°C. After 24 hours of incubation, the culture was further diluted to 1:100 using fresh Trypticase soy broth. A 96-well polystyrene plate was used; each well was filled with 0.2 mL of the bacterial inoculums and incubated for 24 hours at 37°C. After the incubation the bacterial suspension was removed from all the wells by gently tapping the plate. The wells were washed two times using phosphate-buffered saline (pH 7.2) and then allowed to incubate for 1 hour at 37°C. After that the wells were stained for 10 minutes using 0.2 mL of Crystal violet (0.1%).[21] Any excess amount of stain is removed by washing with distilled water twice. After washing the plates were kept to dry. Then 200 µL of glacial acetic acid was added to each well and then the optical density of the isolate was determined by an ELISA Microtiter plate reader (Alere AM2100) at 570 nm wavelength and the interpretation of the result was done as strong, moderate, and weak or nonbiofilm producers (►**Fig. 1C**). This interpretation was based on a previous study conducted by Stepanović et al (►**Table 1**).[22]

Table 1 Interpretation of the biofilm production by optical density (OD)[17]

OD value (average)	Biofilm production
<0.17	Negative
0.17–0.34	Weak positive
0.35–0.68	Moderate positive
>0.68	Strong positive

Result

In this study we included 81 isolates of *Staphylococcus* isolated from various clinical samples. The distribution of *S. aureus* and CoNs among the different types of samples is shown in ►**Fig. 1**. Of the 81 samples, *S. aureus* was isolated from 44 (54%) samples and CoNs from 37 (45.6%) samples. *Staphylococcus aureus* was mostly isolated from pus ($n = 17$) and CoNs were present mostly in the nasal swabs ($n = 17$).

Out of the 44 *S. aureus* found 27 were MSSA and 17 were MRSA and out of the total 37 CoNs, 18 were identified as *S. epidermidis*, 16 as *S. haemolyticus* and 3 as *S. saprophyticus* (►**Fig. 2**).

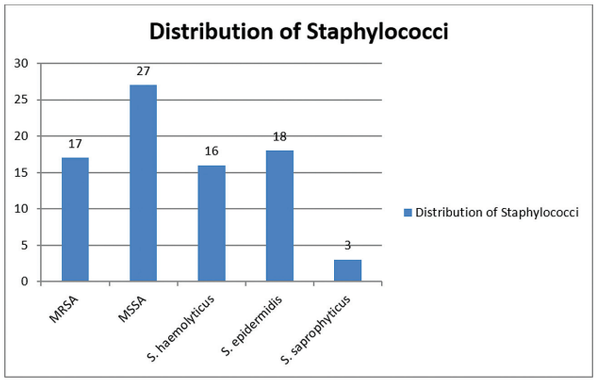

Fig. 2 Distribution of staphylococci isolated from various clinical samples.

Antibiotic Resistance Pattern

The antibiotic resistance pattern of the isolates was determined by Kirby–Bauer disc diffusion method. The resistance pattern of the isolates against antibiotics is given in ►**Table 2**. Many strains of MRSA, MSSA, and CoNs showed multiple drug resistance.

Biofilm Formation

The biofilm detection of all the strains was done by total three methods. The findings of presence of biofilm by different methods are given in ►**Table 3**.

The table above shows the numbers of strains showing biofilm production by different methods used. All the 81 strains of *S. aureus* were subjected to testing for biofilm by three methods. The tissue culture plate method was one of the most promising of the three methods, since it showed a large number of isolates ($n = 52$) that are positive for biofilm followed by Congo red agar ($n = 43$), and tube adherence (40). Many strains showed positive by one or two methods only. Only 13 CoNs, 9 MRSA, and 13 MSSA were positive by all the three methods used in this study.

Discussion

Staphylococcus aureus is present on the skin and mucous membrane of the nasal cavity of human beings. It can cause multiple infections like septicemia, pneumonia, urinary tract infection, and skin infections in human. Production of biofilm by them is an important virulence factor that allows the bacteria to adapt to the adverse conditions and difficult to treat. This is also responsible for antimicrobial resistance. This calls for the need of removal of biofilm-producing organisms from medical devices and for the detection of biofilm-producing ability of the organism.[21]

In our study, we included 81 isolates of *Staphylococcus* species. The isolates were tested for their ability to produce biofilm as a virulence factor by three different methods. With regard to these three techniques, the Congo red agar method detected 43 (53%) biofilm producers among the isolates and 38 nonproducers; the tube adherence method detected 40 (49%) positive biofilm detections and 41 nonproducers, and the tissue culture plate method detected 52 (64%) producers and 29 nonproducers. Looking at the positivity rate given by various methods, the microtiter plate method is highly efficient. In a study conducted by Amita Jain in 2009, the microtiter plate method is used as the gold standard test for biofilm to evaluate the sensitivity and specificity of Congo red agar method.[23] Tube adherence on the other hand has given the least rate of positivity; this could be due to weak biofilm producers which fail to adhere well to the tube. Most of the biofilm positive strains showed resistance to multiple antibiotics. CoNs have shown resistance against many antibiotics like clindamycin, levofloxacin, and trimethoprim-sulfamethoxazole. MSSA and MRSA also showed significant degrees of resistance to antibiotics like ciprofloxacin, erythromycin, and amoxicillin- clavulanic acid.

Table 2 The resistance pattern of *Staphylococcus*

	Cefoxitin	Benzyl-penicillin	Ampi-cillin	Amoxicillin/ clavulanic Acid	Cefuro-xime	Gentamicin	Ciprofloxacin	Levofloxacin	Erythro-mycin	Clindamycin	Linezolid	Dapto-mycin	Teico-planin	Doxy-cycline	Tetra-cycline	Tigecy-cline	Nitro-furantoin	Trimethoprim/ sulfame-thoxazole
MRSA (17)	17	16	17	17	17	6	10	10	12	5	1	0	1	0	2	0	1	7
MSSA (27)	0	23	25	2	3	3	22	23	18	9	0	0	0	0	0	0	0	2
CoNs (37)	19	24	4	3	3	3	17	17	14	23	0	0	0	0	10	0	0	10

Abbreviations: CoNs, coagulase negative staphylococci; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin sensitive *S. aureus*.

Table 3 Positive strains of biofilm by different methods

No. of isolates ($N = 81$)	CoNs (37)	MRSA (17)	MSSA (27)	Total positive	Total negative
Congo red agar	18	9	16	*43*	38
Tube adherence	15	10	15	*40*	41
Tissue culture plate method	22	13	17	*52*	29
Positive by all the methods	*13*	*9*	*13*	*35*	

Abbreviations: CoNs, coagulase negative staphylococci; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin sensitive *S. aureus.*

Conclusion

Clinically isolated strains of *S. aureus* recovered from infection in hospitalized patients show high levels of biofilm formation. Higher rates of antibiotic resistance were demonstrated by biofilm manufacturers compared with nonbiofilm manufacturers. The biofilm formation of *S. aureus*, MRSA, and CoNs can lead to high rates of antibiotic resistance, making treatment difficult. Biofilm screening must be done to prevent it. This study detects the phenotypic ability of *Staphylococcus* species to produce biofilm; a genotypic detection of associated genes can give better results.

Funding
None.

Conflict of Interest
None declared.

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Chondromyxoid Fibroma of Clavicle Presenting as Radiological Disappearance of Bone

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Abstract

Case Presentation Chondromyxoid fibroma (CMF) is a relatively rare bone tumor of cartilaginous origin and it comprises less than 1% of all primary bony tumors. Clavicle is an unusual site of involvement for any bone tumor and may produce diagnostic dilemma. Approximately only 1% of all primary bone tumors may involve the clavicle. The literature on clinical features and outcome of CMF clavicle remains sparse.

Conclusion We present an unusual case of CMF clavicle in which the medial aspect of the clavicle gradually disappeared on radiographs. CMF should be included in the differential diagnoses of disappearing bone disease.

Keywords
- chondromyxoid fibroma
- clavicle
- disappearing bone

Introduction

Chondromyxoid fibroma (CMF) is a relatively rare bone tumor of cartilaginous origin and comprises less than 1% of all primary bony tumors.[1] Clavicle is an unusual site of involvement for any bone tumor and may produce diagnostic dilemma. Approximately 1% of all primary bone tumors may involve the clavicle.[2,3] In a review of 48 cases of clavicular tumors over a 50-year period, Smith et al did not observe even a single case of CMF in the clavicle.[3] Another review of 12 patients with primary tumor or tumorous lesions of the clavicle over 10 years did not feature CMF.[2] The present case was unusual as the lesion initially involved diaphysis of the clavicle and this part of the bone gradually disappeared on radiographs. We suggest that CMF should be included as one of the differentials of disappearing bone disease.

Written, informed consent was obtained from the parents authorizing radiological and photographic documentation and they were also informed that data concerning the case might be published in print and/or electronic form.

Case Report

A 13-year-old Indian girl presented with the complaints of pain and swelling over the left clavicle for past 8 months. Pain was insidious in onset, gradually progressive, non-radiating, and dull aching in nature. No diurnal variation was observed. It used to get relieved on rest and taking oral analgesics, and aggravated with activities of the left arm. The parents also noticed diffuse swelling/fullness in the region that was limited to middle and medial third of the clavicle. These complaints were associated with fever around 2 months before presenting to us. The episode necessitated admission in the medicine ward with the diagnosis of pneumonitis with subcutaneous emphysema. Chest radiographs at that time showed bilateral upper zone

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opacities in the lungs with hilar lymphadenopathy; however, left clavicle appeared normal. High resolution computed tomography revealed bilateral multiple cavitary lesions in lungs. She was given intensive care with intravenous antibiotics through central line and was discharged after 1 month once she improved. One month later, she reported back with rapid aggravation of the pain over the left clavicular region. Fresh radiographs were obtained, but patient refused admission for further management. They came back after 3 weeks for admission. There was no history of weakness in the affected limb, discharge from the involved region, recent weight loss, anorexia, hemoptysis, pruritus, seizures, or similar swellings elsewhere in the body. It was not associated with any preceding history of trauma. Her family history was noncontributory.

On examination, the vitals were stable and there was no evidence of lymphadenopathy. Her physical status was good. Local examination revealed tenderness and diffuse swelling originating from medial two-third of left clavicle. Local temperature was normal. Overlying skin was freely mobile. There were no scars or sinuses. Bony gap with abnormal painful mobility in the middle of clavicle was also appreciable. Rest of the organ systems were normal.

Plain radiograph obtained during her stay in intensive care unit was reported as having no bony abnormality (►**Fig. 1A**). The radiograph (obtained 2 months later) revealed areas of lysis in the middle third of left clavicle (►**Fig. 1B**). The latest radiograph (obtained 2 months and 3 weeks later) revealed a pathological diaphyseal fracture with complete destruction of the middle third of clavicle with surrounding osteopenia especially in the medial aspect (►**Fig. 1C**). Though on retrospective analysis, it was noted that ill-defined lytic lesion was appreciable in the first round of radiograph, which was missed. Computed tomography revealed irregular outline of the medial two-third clavicle and lytic lesion in the mid-shaft along breach in cortical continuity (►**Fig. 2A–C**). There was no evidence of calcification. Surrounding osseous structures were unremarkable. MRI revealed markedly irregular medial two-third clavicle that displayed altered signal intensity appearing hypo- to isointense on T1-weighted imaging (T1WI) and hyperintense on T2WI and short tau inversion recovery. Postgadolinium images revealed heterogenous contrast enhancement along with ill-defined adjacent soft tissue component and surrounding edema (►**Fig. 3A, B**). There was no evidence of hemorrhage or necrosis. Laboratory examination revealed leucocyte count (13,800/cumm), polymorphs 63%, lymphocytes 33%, monocytes 2%, and eosinophils 2%. The erythrocyte sedimentation rate was 39 mm at the end of first hour and C-reactive protein level was 2.3 mg/L. Kidney and parathyroid functions were normal. Sputum examination was negative for acid fast bacilli. Enzyme-linked immunosorbent assay test for human immunodeficiency virus I and II antibody was negative. The patient's immune status was normal with no other focus of infection.

She underwent open biopsy under general anesthesia. Soft tissue component with overlying pseudocapsule was dissected and excised. It was a fibrous structure without areas of hemorrhage or necrosis. Curettage of the bone ends was performed. The entire tissue was submitted for histopathological examination and culture. The histopathological examination revealed predominantly low cellularity (►**Fig. 4A**). Bland tumor cells were observed, at the periphery occupying the intertrabecular spaces, with collagenous tissue (►**Fig. 4A, B**). The tumor was composed of areas of fibrous tissue, chondromyxoid tissue in varying proportions (►**Fig. 4C–D**). The hypocellular areas were chondromyxoid that had multiple stellate shaped cells with minimal pleomorphism (►**Fig. 4D**). However, sclerotic shell at the periphery, haemosiderin laden macrophages, hyaline cartilaginous tissue, and unlined blood filed spaces were not observed. There was no evidence of calcification, necrosis, or hemorrhage. Cultures for pyogenic organisms and microscopic examination for acid fast bacilli were negative. These findings were considered consistent with fibrous predominant CMF.

The patient underwent resection of the medial two-third of the clavicle with preservation of the lateral third clavicle along with acromioclavicular complex. No reconstruction was done. Resected specimen confirmed the biopsy diagnosis. Gradual shoulder mobilization was started at 4 weeks and she was kept under regular follow-up on outpatient basis. She was asymptomatic during latest follow-up at 22 months and had good shoulder function. The repeat radiograph did not reveal any further disappearance of bone (►**Fig. 5**). She had mild discomfort only on carrying heavy weights (like bucket full of water).

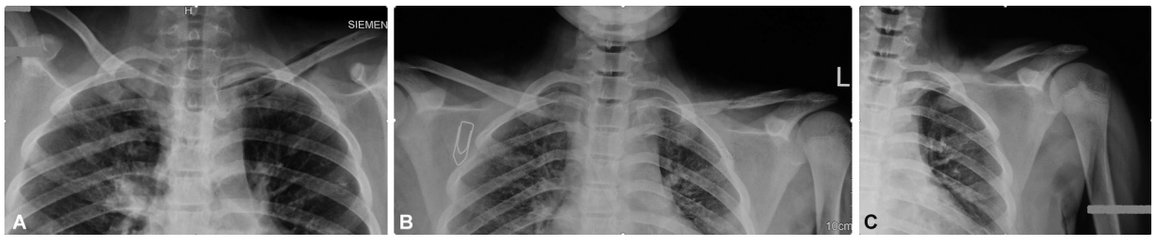

Fig. 1 (**A**) Plain radiograph taken in medical intensive care unit did not reveal obvious bony abnormality. (**B**) Plain radiograph (taken 2 months later) showing areas of lysis in the middle third of left clavicle. (**C**) The radiograph (taken 2 months and 3 weeks later) revealed a pathological diaphyseal fracture with complete destruction of the middle third of left clavicle. There was also presence of surrounding osteopenia especially in the medial third clavicle.

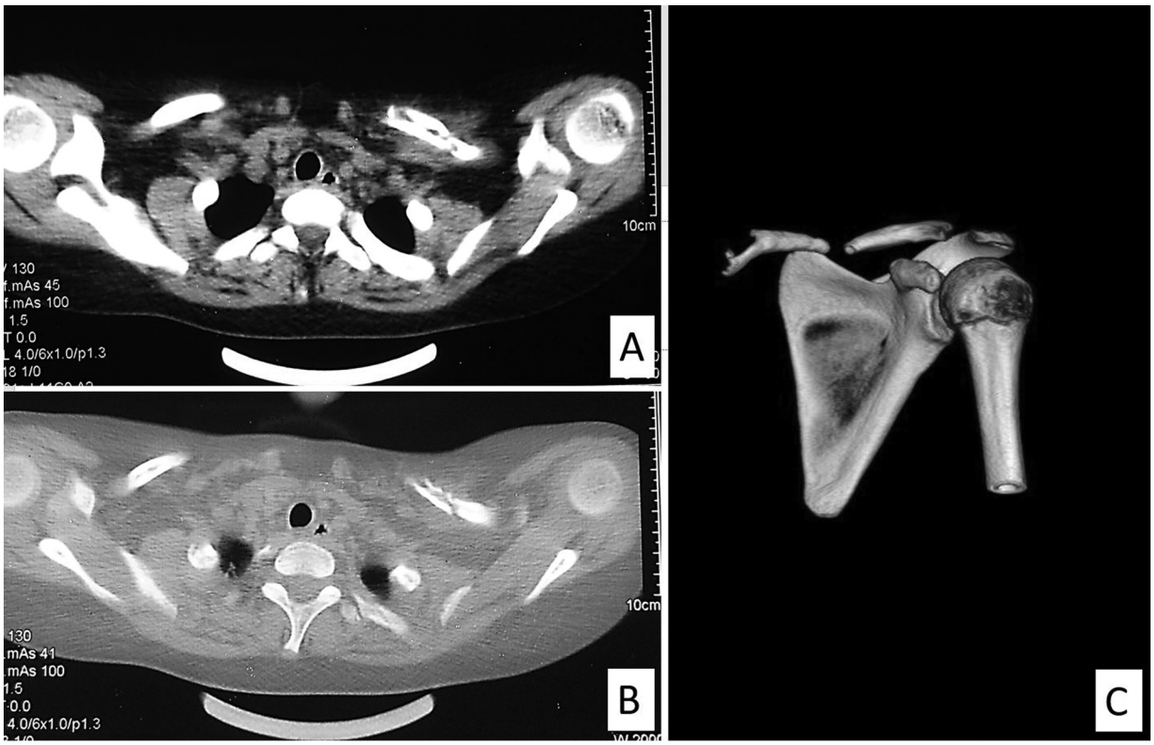

Fig. 2 Computed tomographic image: (**A**) axial section of soft tissue window; (**B**) axial section of bony window; (**C**) three-dimensional reconstruction image showing irregular outline of the clavicular diaphysis, and lytic lesion with pathological fracture and soft tissue swelling.

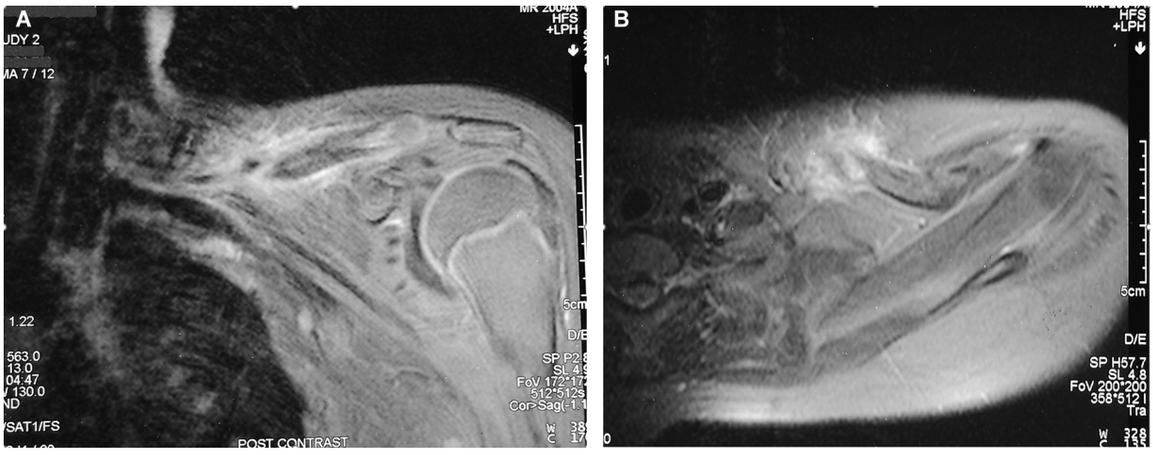

Fig. 3 (**A**) T1-weighted magnetic resonance imaging (MRI) coronal image acquired following administration of intravenous Gadolinium agent showing heterogenous contrast enhancement along with ill-defined adjacent soft tissue component; (**B**) T2-weighted MRI axial image showing hyperintense signal intensity. Soft tissue component was seen abutting the mediastinal fat in the prevascular space.

Discussion

CMF of the clavicle is exceedingly rare and only seven cases bearing this diagnosis have been reported in literature, to the best of our knowledge[4–11] (►**Table 1**). This includes a case out of total 36 cases of CMF reported by Zillmer and Dorfman over 22 years,[9] and another out of 278 cases of CMF reported by Wu et al over the period of 88 years.[8]

A careful clinical, radiological, and pathological correlation is necessary to arrive at the correct diagnosis. CMF often display local aggressiveness radiologically, such as cortical thinning, expansion, erosions, and destruction,[8–10] as was evident in our case.

The differential-diagnosis based on clinical and radiographic data included chronic osteomyelitis, tuberculous osteomyelitis, malignant neoplasm (Ewing's sarcoma), benign neoplasm (CMF/nonossifying fibroma), Synovitis, Acne, Pustulosis, Hyperostosis, Osteitis (SAPHO) syndrome, and chronic recurrent multifocal osteomyelitis. Considering the radiological disappearance of bone, we additionally considered differential diagnosis of Gorham's disease, osteolysis

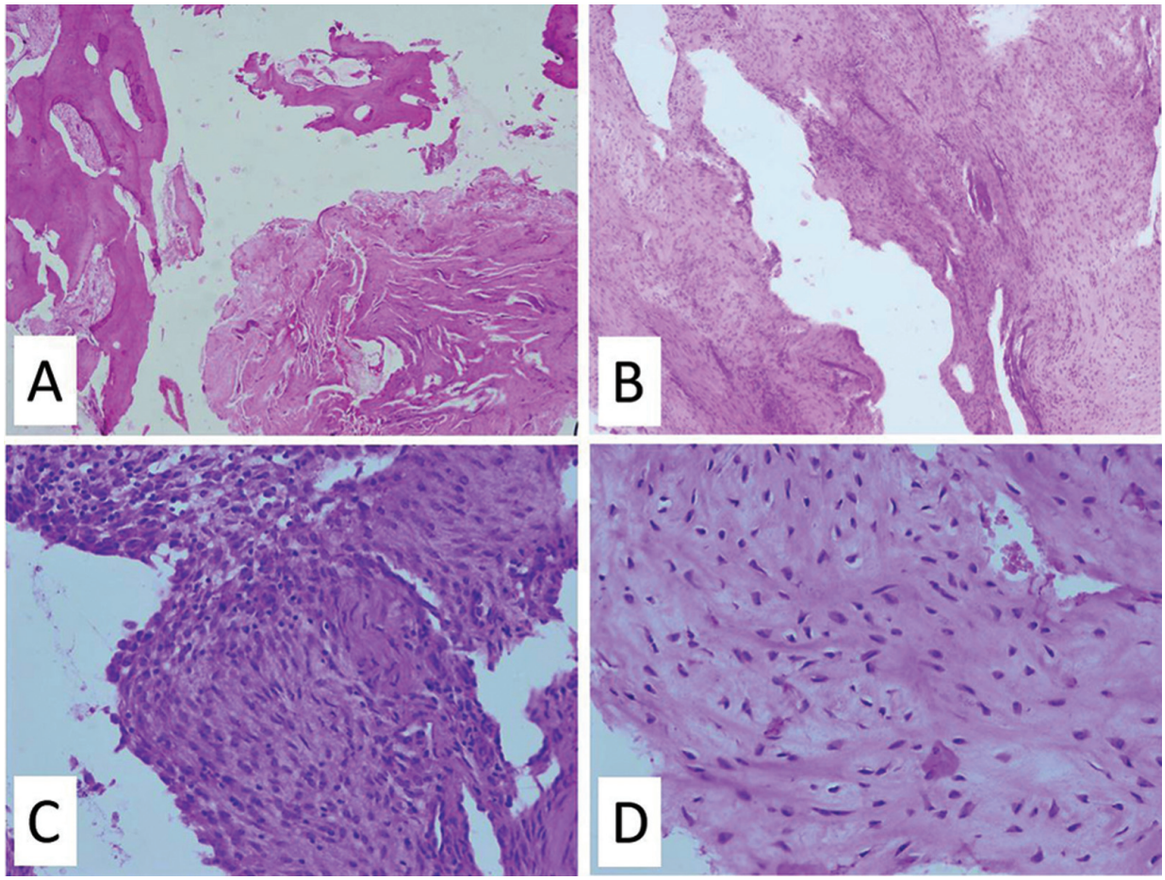

Fig. 4 (**A**) Photomicrograph (stain, hematoxylin and eosin, original magnification, ×100) showing the presence of hypocellular myxoid areas and scattered stellate cells. (**B**) Photomicrograph (stain, hematoxylin and eosin, original magnification, ×200) showing predominantly low cellularity. Bland tumor cells are seen at the periphery occupying the intertrabecular spaces, with collagenous tissue. (**C**) A high-power photomicrograph (stain, hematoxylin and eosin, original magnification, ×400) showing focal ABC like areas with increased cellularity. However, hemosiderin laden macrophages, and unlined blood-filled spaces are not present. (**D**) A high-power photomicrograph (stain, hematoxylin and eosin, original magnification, ×400) showing lobulated chondroid areas with chondroid cells displaying minimal pleomorphism.

with nephropathy, hyperparathyroidism, and eosinophilic granuloma.

Chronic infective etiology displays osteolytic lesions surrounded by sclerotic rim.[12] The absence of sequestrum, negative cultures, typical histopathological features excluded the infective etiology. Other bony neoplasms could be ruled out by the absence of typical histopathological features.[1,13] Other differentials of disappearing bone[14] could be ruled out by laboratory tests and the absence of typical histopathological features.

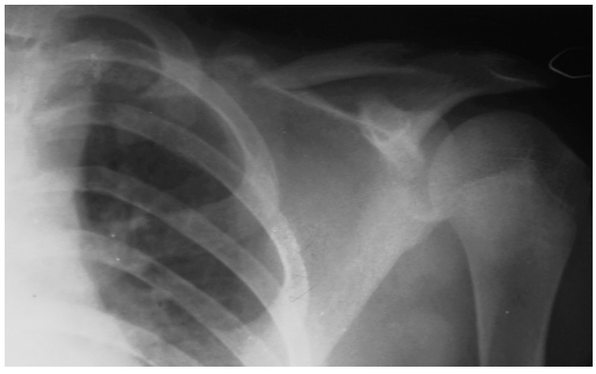

Fig. 5 Follow-up radiograph at 22 months postoperatively showing resected medial aspect of clavicle.

The prognosis of CMF of clavicle has not been established because of the paucity of literature[4–11] (►**Table 1**). It, however, appears good with early diagnosis and appropriate treatment as presented in various case reports. Conventionally, it has been treated with curettage with or without bone grafting/clavicular reconstruction. Approximately 20 to 25% cases of the CMF of the various sites may recur following curettage and the figure may be slightly higher in pediatric age-group.[1] However, such recurrences have not been observed in any of the reported cases of CMFs of clavicle that may be attributed to the facts that clavicle is a subcutaneous bone; its entire length and reconstruction may not be necessary following bone resection. Phenol has been used as an adjunct to curettage to reduce local recurrence.[7]

Table 1 The details of reported cases of chondromyxoid fibroma of the clavicle

Authors	Age/ sex	Involved region in clavicle	Radiological features	Treatment	Follow-up
Zillmer and Dorfman[9] (1989)	One out of 36 reported cases of chondromyxoid fibroma had involvement of clavicle. However, a detailed workup of the case was not published				
Wu et al[8] (1998)	One out of 278 reported cases of chondromyxoid fibroma had involvement of clavicle. However, a detailed workup of the case was not published				
Nakazora et al[5] (2003)	34/ F	Diaphysis	Radiographs and CT: an osteolytic lesion with cortical thinning and expansion with partial destruction at the diaphysis MRI: a homogeneous iso-signal intensity mass in T1WI and a heterogeneous high-signal intensity in T2WI		
Pattamapaspong et al[6] (2006)	23/M	Distal end clavicle	Radiograph: an expanded osteolytic lesion at distal end of the clavicle with well-defined margins and internal septa NCCT: expanded osteolytic lesion at the distal end of the clavicle with endosteal scalloping, thin sclerotic rim and erosion of the inferior surface MRI: not available Tc-99m MDP bone scan: focal tracer uptake in distal end of the clavicle	Curettage with bone grafting	No recurrence at 37 months follow-up
Sakamoto et al[7] (2006)	17/M	Lateral end of clavicle	Radiograph and NCCT: osteolytic lesion with cortical thinning, expansion and destruction. High density areas of calcification were present MRI: hypointense signal intensity on T1W and hyperintense signal intensity on T2WI in intramedullary space with soft tissue extension	Extended curettage with phenol followed by bone grafting	No recurrence at 12 months follow-up
Khan et al[4] (2008)	6/F	Medial end of clavicle	Radiograph: large osteolytic area involving whole of medial end of clavicle with resorption of superior cortical margins of medial end NCCT: extensive lytic lesion in medial end of clavicle with breach in posterior cortex and a soft tissue shadow MRI: globular soft tissue component with no involvement of the underlying neurovascular bundle	Wide excision	No recurrence at 2 years follow-up
Aggarwal et al[10] (2012)	84/M	Lateral end of clavicle	Radiograph: eccentrically placed osteolytic lesion in the lateral end of clavicle MRI: $4.3 \times 3.7 \times 3$ cm cystic solid lesion involving the lateral end of clavicle and acromion process with cortical erosions and scalloping extending to acromioclavicular joint.	En-bloc excision	No recurrence at 18 months follow-up
Hope et al[11] (2018)	7/F	Lateral end of clavicle	Radiograph: expanded, radiolucent, osteolytic lesion in the lateral end of clavicle CT: thin sclerotic rim with endosteal scalloping measuring $2.9\,\text{cm} \times 1.3\,\text{cm}$. No calcification or soft tissue mass	Curettage with phenol followed by synthetic processed bone grafting	No recurrence at 5 years follow-up
Present case	13/F	Medial two-third	Radiograph: a pathological diaphyseal fracture with complete destruction of the middle third of the clavicle CT: irregular outline of the medial two-third clavicle and lytic lesion in the mid shaft along with breach in the cortical continuity MRI: markedly irregular medial two-third clavicle which displayed altered signal intensity appearing hypo- to isointense on T1WI and hyperintense on T2WI. Heterogenous enhancement was evident on postgadolinium contrast images	Excision	No recurrence at 22 months follow-up

Abbreviations: CT, computed tomography; MDP, methylene diphosphonate; MRI, magnetic resonance imaging; NCCT, noncontrast computed tomography; T1WI, T1-weighted imaging.

High index of suspicion may be required for early diagnosis and treatment in such patients. The diagnosis may be delayed owing to its indolent course, nonspecific symptomatology, and unfamiliarity among orthopaedic surgeons with such atypical presentation. CMF should be considered as a differential diagnosis of disappearing bone disease.

Note

Each author certifies that he has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements) that might pose a conflict of interest in connection with the submitted article.

Each author certifies that his institution approved the reporting of this case report, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.

This work was performed at The Maulana Azad Medical College, New Delhi-110002, India.

Conflict of Interest

None declared.

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Solid Pseudopapillary Epithelial Neoplasm of the Pancreas: A Rare Entity with Diagnostic Dilemma

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Abstract

The solid pseudopapillary epithelial neoplasm (SPEN) of the pancreas is a relatively uncommon entity. The aim of the present study was to summarize our experiences with regard to diagnostic dilemma, surgery, postoperative follow-up, and management. This retrospective data were collected during the period from January 1, 2018 to December 31, 2020. A total of four patients (three females and one male) were identified within an age range of 13 to 25 years. All the patients were presented with nonspecific symptoms such as abdomen lumps, swelling in the abdomen, and abdominal pain. To reach a definite diagnosis, imaging studies were conducted along with endoscopic ultrasound fine-needle aspiration (EUS-FNA) and biopsy. After confirmation of SPEN on biopsy, all the patients underwent surgery without any complications. Patients are on follow-up, and to date, no metastasis has been detected. SPEN is a rare pancreatic tumor with unusual pathological features leading to a diagnostic dilemma. The pathologist should be familiar with SPEN and its salient histological characteristics that differentiate it from other look-alike pancreatic tumors and can help in timely surgery and management.

Keywords

- pancreas
- solid pseudopapillary epithelial neoplasm
- diagnostic dilemma
- immunohistochemistry
- surgery

Introduction

Solid pseudopapillary epithelial neoplasm (SPEN) was first reported in 1959 by Dr. Frantz as a "papillary cystic tumor of the pancreas" and later described by multiple names in the literature reflecting its biology and histogenesis. In 2010, the World Health Organization (WHO) for the first time defined it as SPEN. It is a rare pancreatic tumor accounting for only 2 to 3% of all pancreatic neoplasms and 1 to 3% of exocrine pancreatic neoplasms.[1] SPEN is most commonly observed in young women (~90%) with a median age of ~30 years.[2] Despite unknown etiopathogenesis, its incidence was observed to be increasing rapidly in the past 10 years due to technological advancement. Due to such low incidence, its clinical and pathologic features have not been extensively studied. Even its etiology and differential status remained challenging. In practice, diagnosis of SPEN was also found to

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be very difficult due to its vague and nonspecific abdominal symptoms.[3]

In the present study, we bring our experience with SPEN in Indian patients with its clinical, radiological, histopathological, and surgical findings to provide a reference for the management of this rare disease.

Case History

In this retrospective study, a total of four patients have undergone surgery for SPEN during the period from January 1, 2018 to December 31, 2020. All these patients' demographic, clinical, imaging, surgical, pathological, survival, and follow-up data were extracted from hospital medical records and evaluated. All the necessary ethical approvals were procured from the institutional ethics committee prior to study commencement.

From the records, a total of four patients were identified; three were females (75%) and 1 (25%) was male. The patients' age ranged from 13 to 25 years. Symptoms on presentation were largely varied and nonspecific. However, the most common symptoms were found to be pain and swelling in the abdomen with a palpable abdomen mass (in one patient). In all the patients, serum tumor marker tests like Ca19–9, carcinoembryonic antigen (CEA), and Ca-125 (in female patients) were done and reported as normal. Preoperative radiological examinations such as transabdominal ultrasonography and computed tomography (CT) were also performed. In these imaging studies, large complex solid cystic lesions in various regions of the pancreas were revealed. Axial contrast-enhanced CT images also revealed a large, enhancing solid heterogeneous, well-circumscribed mass originating in the pancreatic body and tail. No perilesional fat stranding and calcification were noted.

Later a positron emission tomography (PET) scan was done, revealing an abnormal and high F-18 fluorodeoxyglucose (FDG) uptake in the solid, enhancing part of the pancreatic lesions (►**Fig. 1**). The tumor location in two patients (50%) was found to be the head of the pancreas, followed by the head and body, and the tail of the pancreas, one in each patient. The mean diameter of the tumor was ~12 cm. Further, to determine the radiological findings in SPEN, all the suspected patients underwent endoscopic ultrasound fine-needle aspiration (EUS-FNA) and biopsy of the pancreas through the transduodenal approach or transgastric route. The cytology was reported as papillary neoplasm of the pancreas, while the biopsies were reported as SPEN. To confirm the diagnosis and to avoid any diagnostic dilemma, immunohistochemical staining (IHC) was performed on all biopsies. After confirmation of SPEN on IHC, three patients (75%) with tumors on the head, neck, and body of the pancreas underwent pancreaticoduodenectomy. The remaining one patient (25%) with a tumor on the tail of the pancreas underwent a distal pancreatectomy. The perioperative and postoperative periods were uneventful

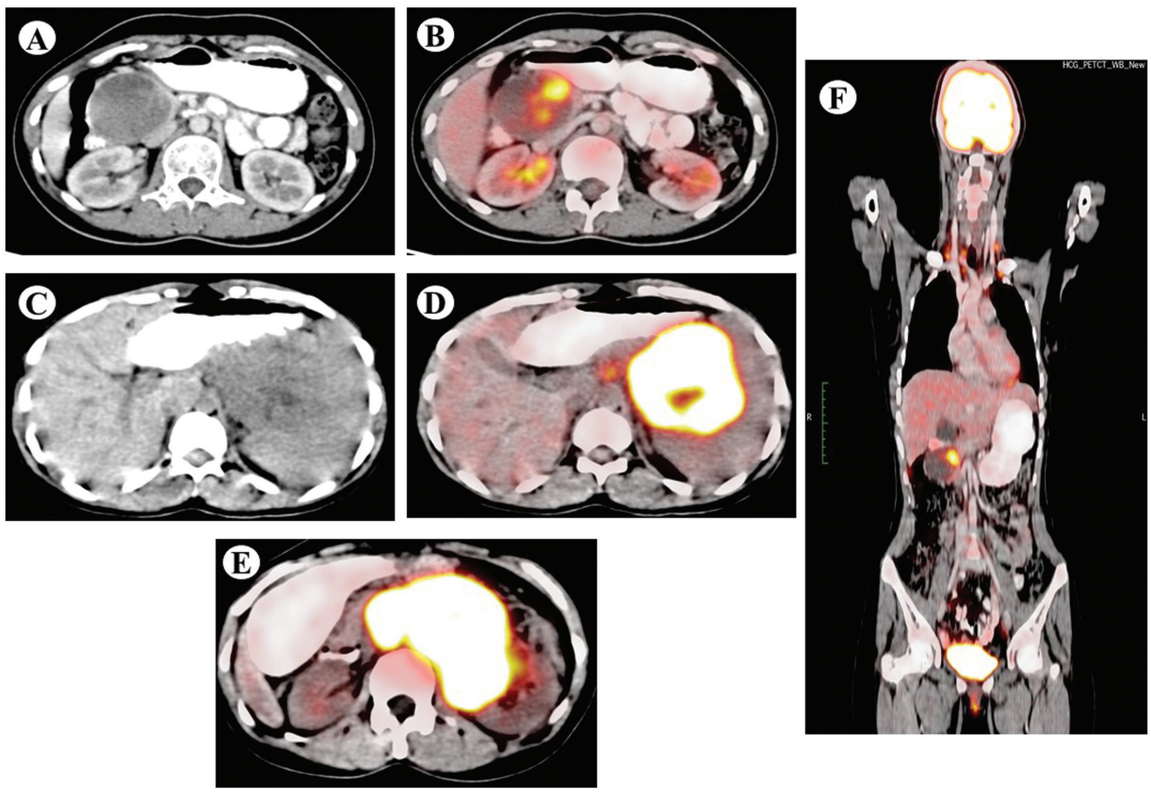

Fig. 1 **A** and **B**: FDG avid mixed solid cystic lesion in head of pancreas. **C** and **D**: FDG avid mass in pancreatic tail infiltrating into splenic hilum. **E**: Large FDG avid mass in body and tail of pancreas. **F**: FDG avid mass in body of pancreas.

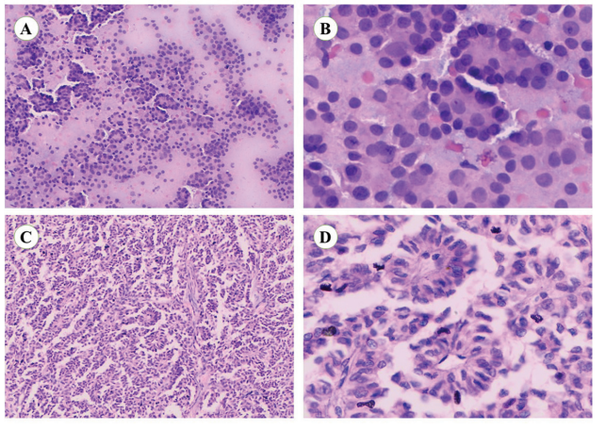

Fig. 2 A and B (PAP Stain): Cellular smear showing tumor cells with minimal cytological atypia forming rosettes. Cells are also showing eccentric nuclei with prominent granular cytoplasm. **C** (10x H and E): Solid nests of poorly cohesive tumor cells forming a cuff surrounding blood vessels, resulting in a pseudopapillary architecture. **D** (40x H and E): Tumor cells arranged around thin fibrovascular cores forming pseudo-rosettes. Tumor cells showing a moderate amount of eosinophilic cytoplasm with large intracytoplasmic hyaline globules and perinuclear vacuoles.

in all the patients. The radical specimens were reported as SPEN on final histopathology report. IHC revealed strong positivity for pancytokeratin (Pan CK), β-catenin, CD56, and synaptophysin. All the data related to clinical and pathological features of the patients with SPEN of the pancreas are presented in ►**Table 1**.

Papanicolaou's staining (Pap stain) of cellular smears showed tumor cells with minimal cytological atypia forming rosettes (►**Fig. 2A** and **2B**). Cells were also showing eccentric nuclei with prominent granular cytoplasm. Histology of the tissues (hematoxylin and eosin [H&E] staining) with 10X magnification revealed pseudopapillary architecture (►**Fig. 2C**). Whereas on 40X magnification, tumor cells were observed to be arranged around thin fibrovascular cores forming pseudorosettes (►**Fig. 2D**). Tumor cells also showed perinuclear vacuoles, large intracytoplasmic hyaline globules, and eosinophilic cytoplasm in moderate amount.

Discussion

With the advancement of technology and imaging modalities, early identification and accurate diagnosis of pancreatic tumors is helping patients to live longer compared with patients from the previous decade. Non-neoplastic and neoplastic pancreatic tumors present as encapsulated masses with a variable amount of hemorrhagic, cystic, and solid components. Pancreatic cystic lesions are further divided into four groups: intraductal pancreatic mucinous lesions, SPENs, mucinous cystic lesions, and serous cystic lesions.[4] Among all the pancreatic cystic lesions, SPEN is extremely rare.

Among these pancreatic tumors, non-neoplastic lesions include the intrapancreatic accessory spleen, congenital anomalies (annular pancreas, heterotopic pancreas, pancreatic lobulation, nesidioblastosis, and rare miscellaneous conditions), cysts, pseudocysts, granulomatous inflammation, and pancreatitis, whereas neoplastic lesions include metastatic tumors, mesenchymal tumors, lymphoid tumors, pancreatoblastoma, solid pseudopapillary tumor, acinar cell tumors, pancreatic intraepithelial neoplasia, intraductal papillary mucinous neoplasms, cystic pancreatic lesions, anaplastic carcinoma, ductal adenocarcinoma, pancreatic neuroendocrine tumor, pancreatic lymphoma, other epithelial exocrine tumors, and rare miscellaneous neoplasms.

Among all those cystic neoplasms, SPEN is an uncommon, indolent, low-grade malignant tumor of unknown etiology. SPEN was frequently reported in young females and the female-to-male ratio was generally observed to be 10:1.[5] In a systematic review conducted by Law et al,[6] the mean age of the patients was found to be 28.5 years (SD ± 13.7 years). Results from our study are in agreement with the previous studies, where 75% of our patient cohort were females. The average age was also observed to be 20.5 years. On clinical presentation, nonspecific symptoms were reported such as early satiety, vomiting, nausea, bloating, weight loss, palpable abdominal mass/discomfort, and abdominal pain. In many patients, SPENs are often identified incidentally. It was also reported that there is no correlation between tumor size and symptoms leading to patients presenting themselves to clinics in later stages of the disease. With respect to tumor localization, they were often found in the tail, followed by the head and body. However, in our patient series, the head followed by the tail and body of the pancreas was reported to be the most common tumor location. In some exceptional cases, multicentric tumors and extrapancreatic sites such as the duodenum, liver, omentum, retroperitoneum, and mesocolon were also found to show these tumors representing synchronous tumor spread. However, such multicentric, extrapancreatic tumors were not reported in our patients. Often, SPEN is misdiagnosed and usually there is no evidence of an endocrine syndrome, elevated pancreatic enzymes, cholestasis, abnormal liver function tests, serum tumor markers, and pancreatic insufficiency to determine it. Therefore, clinicians should always consider a differential diagnosis of SPEN, especially when the patient is young.[3]

Regular laboratory parameters, tumor markers, and clinical and radiological findings are proven to be of no help/unremarkable. In such scenarios, to reach a definite preoperative diagnosis, preoperative percutaneous biopsies, tissue sampling with EUS-FNA, and cytology should always be considered. Except for the tumor cell dissemination, EUS-FNA was proven to be a reliable tool for accurate diagnosis of SPEN by characterizing the cytomorphological features.[7] The characteristic features of SPEN can be diagnosed readily based on characteristic cytological and histological features.[3]

On cytology, cellular smears showed tumor cells with minimal cytological atypia forming rosettes. Cells had eccentric nucleus with prominent granular cytoplasm. On histology, SPENs can demonstrate various microscopic patterns such as solid, cystic, and pseudopapillary arrangements. In many patients, cells demonstrate solid nests of

Table 1 Clinical and pathological characteristics of patients with solid pseudopapillary neoplasms of the pancreas

Gender	Age (y)	Symptoms	Tumor location on pancreas	Tumor size (mm)	Postoperative complication	Therapy/ intervention	IHC findings	Tumor markers	Metastasis
Female	25	Upper abdominal pain radiating to back	Tail	12.2 × 8.5 × 6.0 cm	None	Distal pancreatectomy	Pan CK: positive LCA: negative CD99: negative Beta catenin: positive CD56: positive Chromogranin: negative AR: negative ER: negative TFE 3: negative Synaptophysin: negative	Ca 19–9- 46.19; CEA: 1.1; AFP: 4.16	No
Female	20	Pain in abdomen	Head	5.4 × 5.2 × 8.3 cm	None	Whipple's surgery	AE1/AE3: positive Beta catenin: positive (nuclear), Synaptophysin: negative Chromogranin: negative, CD56: positive	Ca 19.9 <3.0 Ca125 5.31, CEA 1.67	No
Female	13	Pain and lump in abdomen	Head	11.1 × 7.5 cm	None	Whipple's surgery	Pan Ck: positive LCA: negative Synaptophysin: positive Chromogranin: negative CD56: positive Beta catenin: positive.	Ca 19.9 <3.0 CEA 1.67	No
Male	24	Pain and swelling in the abdomen	Head and body	14 × 15 × 17 cm	None	Whipple's surgery	CK7: negative CK20: negative PAX-8: negative Beta-catenin: positive ER: negative PR: negative	Ca 19.9–1.25	No

Abbreviations: AR, androgen receptor; CD, cluster of differentiation; CEA, carcinoembryonic antigen; CK, cytokeratin; ER, estrogen receptor; LCA, leukocyte common antigen; Pan CK, Pancytokeratin; PR, progesterone receptor; TFE3, transcription factor E3.

uniform, polygonal cells with abundant cytoplasm (clear to granular). In our patient cohort, tumor cells have showed perinuclear vacuoles and large intracytoplasmic hyaline globules with moderate amount of eosinophilic cytoplasm. The characteristic pseudopapillary architecture or pseudorosettes were clearly visible in all our patients in accordance with the previous studies.[3,8] Such rosette formations generally contain degenerated cells, tumor cells, and viable cells arranged around the thin fibrovascular cores giving that typical pseudopapillary architecture. To confirm the diagnosis further, IHC tests were performed, where IHC analysis of the specimens was reported to have shown strong positivity for Pan CK, β-catenin, and CD56. Whereas synaptophysin was positive in a single case, progesterone receptor (PR) and estrogen receptor (ER) were negative in all cases. β-catenin localization was also reported to be strongly positive in these patients due to SPEN somatic point mutations in exon 3 of CTNNB1.[9] Runjan and Stefano[10] have also emphasized the importance of the β-catenin pathway to diagnose and differentiate SPEN from look-alike pancreatic endocrine tumors and have also confirmed its presence in 90% of cases.[8,10] On the other hand, with PR, all the patients in our study were reported negative contrary to multiple studies.[1,11,12] Although there is a female preponderance for SPEN, ER positivity is very uncommon and it was negative in all our patients.

After confirmation of SPEN, as a standard of care in the management protocol, all our patients have undergone a complete R0 resection (distal pancreatectomy or pancreaticoduodenectomy: Whipple's surgery). Generally, in unresectable cases, patients were recommended to undergo radiotherapy, chemotherapy, transarterial chemoembolization, alcohol injection, and/or liver transplantation. Postoperative samples have shown no metastasis or invasion to regional lymph nodes and our reports are consistent with previous studies.[13] Multiple studies have reported the prognosis, and the 5-year survival rate is excellent in ~97% of the patients even with metastasis, if treated from time to time. The strong prognostic factors for patients' prolonged survival include the level of surrounding tissue invasion, lymph node involvement, and vascular and perineural invasion, unbalanced translocation between chromosomes 13 and 17, trisomy of chromosome 3, double loss of X chromosomes, DNA aneuploidy, dedifferentiation, nuclear pleomorphism, high mitotic count, significant nuclear atypia, extensive tumor necrosis, and diffuse infiltrative growth pattern.[14] Regular follow-up is also a key for early detection of disease and prolonged survival. All the patients in our study were on regular follow-up and have shown no signs of recurrence or metastasis.

Conclusion

To summarize, SPEN is an uncommon, asymptomatic, low-grade malignant tumor that was typically seen in young women. Due to its diagnostic dilemma, preoperative percutaneous biopsies, tissue sampling EUS-FNA, cytology, and IHC should be strictly considered for a definite diagnosis. A multidisciplinary team approach will always improve treatment accuracy and will help in timely management. Complete R0 resection is the only effective option in all stages of the disease and the prognosis is also proven to be good. All the time, the pathologist should be familiar with the SPEN's salient clinical, microscopic, cytopathological, histopathological, and immunohistochemical features to differentiate them from other circumscribed pancreatic neoplasms such as neuroendocrine lesions. Finally, a minimum of 5-year follow-up after the surgical resection is highly recommended to identify the possible signs of recurrence of the SPEN.

Ethical Approval

The study followed the ethical guidelines of the Declaration of Helsinki. Written informed consent was taken from the patients for publication in the journal.

Funding

None.

Conflict of Interest

None declared.

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