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Sr. No	Faculty	Book	Year	Page No.
1	Dr. Vaishali Korde	Prevention examination and treatment of domestic violence and sexual assault cases	2021	2-24
2	Dr. Virendra Ghaisas	Indian Ethnic Rhinoplasty: A Surgical Guide	2021	25
3	Dr. Vaishali Korde	Obstetrics Algorithms in Clinical Practice - Alok Sharma – 1st Edition 2020 Chapter 40: Transverse Lie	2021	26
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7	Dr. Yash Shah	Hardikar's Orthopedic Operations Text & Atlas	2019	48-58
8	Shashwat S. Banerjee	Cancer Nanotechnology pp 271-281	2017	59-70
9	Dr. Vaishali Korde	Book Chapter- Section -6, 47. Websites on Contraception	2016	71-80

Name of the Faculty	Paper details/Book details Publisher etc.
Dr. Vaishali Korde	SECTION I: Background and Epidemiology Current Scenario of Sexual Violence. CBS publishers and distributors Pvt Ltd ISBN: 978-93-90709-37-3

PET

Prevention, Examination and Treatment of Domestic Violence and Sexual Assault Cases

The training workshop on PET (prevention examination and treatment) of domestic violence and sexual assault by ICOG-FOGSI was conceptualized by us to attempt to plug the gaps in knowledge and improve practices in dealing with violence against women and girls. The book was born out of the effort to collate all the information in concise form which can be referred to by each of you in times of doubt. With the help of this, health professionals can use the guidelines as a day-to-day service document and/or as a tool to guide the development of health services for victims of sexual violence. This can also be used to prepare in-service training courses on sexual violence for health care practitioners and other members of multidisciplinary teams. Our team has made best efforts to collect up-to-date information from global and local experts and prepared a document you must keep as a ready reckoner in your clinics and libraries.

Chief Editor

Mandakini Megh MD, DGO, FICMCH, FICMU, FICOG is Director and Consulting Obstetrician and Gynaecologist, Dr Megh's Gynaeco Care Clinics, Mumbai. Presently she is holding the post of International Vice President, Medical Women's International Association, Central Asia. She is the Chairperson of Indian College of Obstetricians and Gynaecologists: ICOG-FOGSI. She is the Chairperson of AMOGS. She is member of National Monitoring Committee of PCPNDT of Govt. of India. She was National Vice-President, FOGSI in 2012 and National Co-ordinator for 'Advanced Post-Partum Care, Workshop/CME, being held at all India level. She was holding the post of Deputy Director of Health Services, Government of Maharashtra, in charge of Family Welfare RCH, PCPNDT, MTP. She was also advisor to officer on special duty, Family Welfare, Government of Maharashtra, Health Department Mantralaya, Mumbai. She was holding the prestigious post of Superintendent, Cama and Alibless Hospital for Women and Children in Mumbai. She has been maternal health consultant for Government of Maharashtra-UNICEF. She was holding the charge of Imaging Science Committee of FOGSI, Jan-2006 to Jan-2009. She was Dean of Indian College of Medical Ultrasound, past President of Indian Federation of Ultrasound in Medicine and Biology (IFUMB) and also past President of Association of Medical Women in India. She was Hon Visiting Professor of Sawangi Meghe Medical College, Wardha and Hon Associate Professor of PDMC Medical College, Amravati. She has significant contribution in formulation of policies for Government of Maharashtra, specially Maternal Health RCH and Family Welfare.



She was in-charge of World Bank—Clinical Trainings in Maharashtra Health System Development Project (MHSDP). She is the Hon. Professor and fellow guide of Indian College of Maternal and Child Health, Calcutta and also national fellow of Indian College of Maternal Health. She is an executive member of Mumbai OBGY Society.

She is the Chief Editor of *Playing by the Rules—an Update on Government Policies, Regulations and Acts for Practicing Obstetricians and Gynecologists* which was awarded DK Datta best publication award of FOGSI.

Recent Advances in Postpartum Care book edited by her has received best textbook award in "A" category FOGSI publication. She is the Chief Editor of *FOGSI Focus Comprehensive Abortion Care and Post Abortion Contraception* in January 2012. She has published many chapters in various textbooks for PG/UG and many articles in various national and international journals. She is the recipient of Best Fellow Award by Indian College of Maternal and Child Health (Dr C S Dawn), Dr Ganatra Award of MOGS for community services in OBGY. She is recipient of Mahila Gaurav Puraskar of Government of Maharashtra, 1996. She has delivered many prestigious orations in national conference. She is the invited faculty for national and international seminars, workshops, world conferences and FIGO. She is recipient of MOGS "Shailaja Pandit" award for working on woman empowerment, domestic violence, contraception and family welfare.



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PET Prevention, Examination and Treatment of Domestic Violence and Sexual Assault Cases

Megh Wani



PET Prevention, Examination and Treatment of Domestic Violence and Sexual Assault Cases



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Alpesh Gandhi
President, FOGSI



Federation of
Obstetric and Gynaecological
Societies of India

Indian College of
Obstetricians &
Gynaecologists

ICOG 2020

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Indian College of Obstetricians
and Gynaecologists



ICOG 2020

PET

Prevention, Examination and Treatment of Domestic Violence and Sexual Assault Cases



PET

Prevention, Examination and Treatment of Domestic Violence and Sexual Assault Cases



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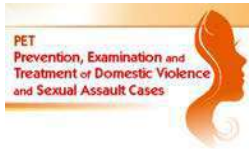


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to

the nameless survivors who have suffered domestic violence or sexual assault, and still have surfaced like the Phoenix, rising from the ashes!



Dr Teertha Shetty, Dr Roshni Khade



Foreword



FOGSI invites you to make a difference!

Violence against girls and women has been prevalent since ages and across all civilizations. In India, the struggle for survival for a girl child begins right from the day she is conceived. We are a country of high maternal mortality and poor gender ratio. The skewed statistics reflect the underlying gender bias, the obsession for a male child, the patriarchy, and the social bigotry prevalent in our society.



A woman in India can barely walk through a street without a nervous glance over a shoulder. Our dreams to call ourselves a progressive nation lies shattered by the NCRB (national crime records bureau) data. It states that the rape vulnerability of women has doubled over the last two decades. This is despite the statutory reforms that came into place post Nirbhaya. Every year thousands of women are victimised. The survivors suffer numerous indignities in their often futile attempts to get justice.

Violence against women and girls (VAWG) is not necessarily sexual, but the consequences are equally damaging. It has a profound impact on physical and mental health, with both immediate and long-term consequences. It also affects the social well-being of the women.

At FOGSI, we are a group of more than 38,000 gynecologists in the unique position to reach out to more than half of the 1.3 billion population of our country ... in fact to ALL since sensitization should be for men and women too! To raise awareness of violence against women; the United Nations General Assembly has designated November 25 as the International Day for the Elimination of Violence Against Women. The UN System's 16 Days of Activism against Gender-based Violence activities, from 25th Nov to 10th Dec took place under the 2020 global theme: ***"Orange the World: Fund, Respond, Prevent, Collect!"*** In a unique initiative, this community connect E-conclave was conducted on 22/11/2020 from 9.30 am–8.30 pm and had about 21,000 viewers across different media including Facebook and Youtube. There were talks from experts in the field and interactive discussions to seek remedy to this grave problem. Results of essay and slogan competitions on the theme, to sensitise the community were released on that day. Our team has also released a booklet "KAVACH" on that day, for which Dr Reena Wani was instrumental in coordination and preparation.

"With Great Power comes Great Responsibility"—dear FOGSIANS, we have to rise to the occasion and translate good intentions into action, even if it may seem to be

a small step, to actually help someone at grassroots level, i.e. when our vision of safety for Indian women will become a reality!! I am very glad that Dr Mandakini Megh, Chairperson, ICOG-FOGSI and her team led by Dr Reena Wani have not only done a certified training workshop on this topic, but have also come out with this book which will be a ready-reckoner for all our members on all aspects of this subject.

Alpesh Gandhi
President, FOGSI

✍ Editorial Note

The various graphics in this section are the E-posters and physical posters prepared by junior doctors and nurses for 25th Nov 2020 competition arranged by the editorial team in HBTMC Cooper Hospital, Juhu, with prizes given by Mumbai Obstetric and Gynecological Society (MOGS) to increase awareness of this critical social issue.

2019 STATS:
4.05 lakhs crimes against women.
1.26 lakhs is highest and it is domestic violence
62.4 crime rate registered per 1 lakh women population

Last night I heard the screaming
Loud voices behind the wall
Another sleepless night for me
It won't do no good to call
The police always come late
If they come at all

And when they arrive
They say, "they can't interfere
With domestic affairs,
Between a man and his wife."
And as they walk out the door
The tears well up in her eyes

Last night I heard the screaming
Then a silence that chilled my soul
I prayed that I was dreaming
When I saw the ambulance in the road
And the policeman said,
"I'm here to keep the peace."
Will the crowd disperse
I think we all could use some
sleep??

- Behind the wall (Tracy Chapman)

NO TO VAWG
PREVENTION AND ELIMINATION OF VIOLENCE AGAINST WOMEN AND GIRLS

STOP VIOLENCE AGAINST WOMEN

Dr Nivedita Pawar (1st Prize)



Foreword

It is with great pleasure that I write the Foreword for an important publication that promises to make a difference to the practice of gynecology.

The title *PET: Prevention, Examination and Treatment of Domestic Violence and Sexual Assault Cases* is itself quite inclusive. It covers all aspects of the problem. It comprises approximately 27 chapters and has included practical as well as theoretical aspects.



For health care system to cater to this problem it is required to have a team of well trained professional with experience. Their approach has to be sensitive, perceptive and compassionate. One has to work with the long-term objective of giving solace and rehabilitation and maintain the dignity of the person.

We have worked on this issue for many years. Violence against women has many forms but has one thing in common that is, it is universal and is seen in all strata of society.

We have also interacted with United Nations which has taken a serious note of the rising incidence. Two of the experts from UN have contributed chapters to this book. Dr Padmini Murthy—MWIA's Representative to UN and Dr Bettina Pfeleiderer—MWIA Past President have both contributed significantly to the alleviation of this problem. The annual UN Conference on "Status of Women" has discussions on this subject and brings out suitable pamphlets and scientific papers. In most countries as also in India the problem is under played. The reported cases are much less than the actual numbers as most women shy away from reporting. Even in the reported cases, the conviction rate is abysmally low.

In India, the law, however has been proactive. Since the famous 'Nirbhaya' case many facilities have been provided to the women in distress and given them shelter and comfort.

I would like to appreciate the tremendous contribution of the Editors Dr Mandakini Megh, Chairperson, FOGSI-ICOG and Dr Reena Wani and their team for their efforts in conducting a certificate course on this subject. This book is a compilation from lectures given by experts, which will help practitioners to give comprehensive care.

If the knowledge gained from this book translate into good clinical practice and helps some women who are in distress the authors will feel that they have not toiled in vain.

We live in interesting times. The future holds many opportunities to strengthen health systems for women and children. New partnerships will develop between Gynecologists, Social Workers, Members of Legal Profession, Public Health and Research Workers. Global players will come in with financing and administrative strategies and policies. In a sense, the world will come together and learn to live with "Gender Harmony".

Congratulations and best wishes to the authors, publishers and all those who have contributed to this magnificent production.

Happy reading and best wishes

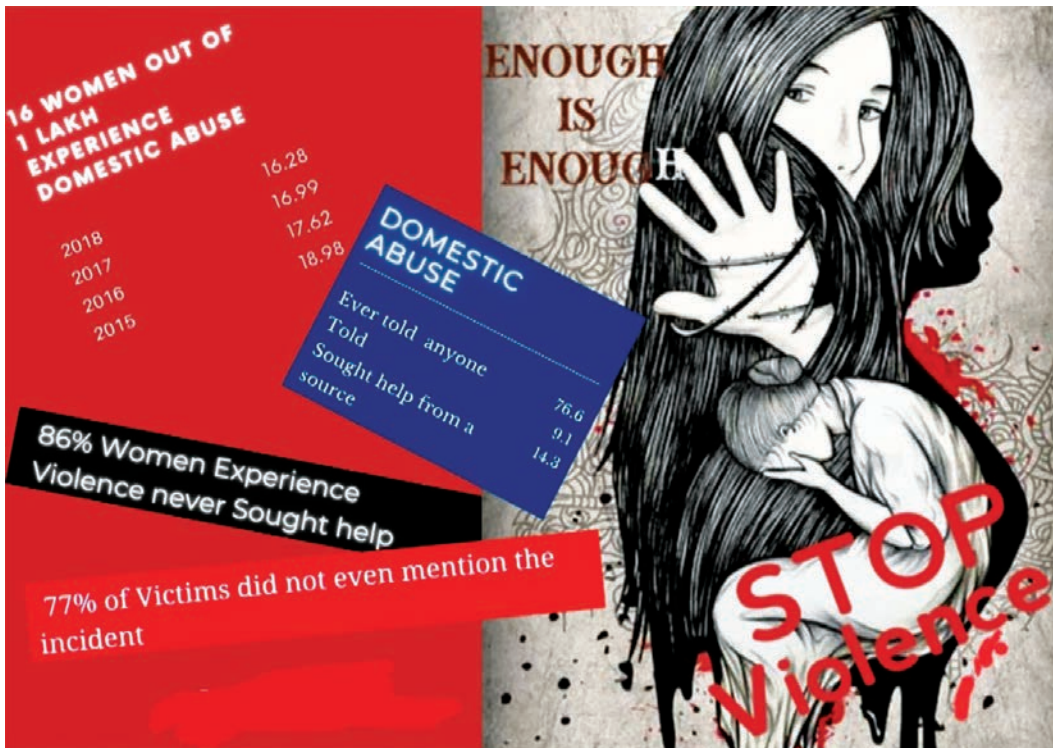
Usha Saraiya

Usha B Saraiya

MD DGO FIAC FICOG FRCOG (UK)

Past President of FOGSI and ICOG (2002)

Past Chairperson of ICOG (2006-2009)



Dr Prashant Telharkar (2nd Prize)



Foreword

Dear FOGSIANS,

I am very happy to present to you the important work that Dr Mandakini Megh, Chairperson, FOGSI-ICOG and her team has created for you.

Sexual violence is a serious public health and human rights problem with both short- and long-term consequences on women's physical, mental, sexual, and reproductive health. According to NRCB data 2019, 88 cases of rape were reported every day in India; which is likely to be just the tip of the iceberg as a fraction of women who are raped file a complaint fearing social stigma.

The physical, financial and social vulnerabilities of women are fundamentally harmful to the future of any society. Violence against women is a crime. Not redressing these vulnerabilities fails to prevent harm to subsequent generations, and contributes to continuing the cycle of violence.

There is a need for wider awareness of the magnitude of the problem of violence against women. Only if this problem is recognized can it be addressed. Physicians, as advocates for women, are uniquely placed to assist in this. There is therefore a duty for professional societies and physicians to publicize information about the frequency of types of violence against women, and the implications for the wider society of allowing this to continue.

Physicians and the health care system can contribute to a great deal in all the three levels of prevention of VAW; which includes creating awareness about violence, identification of violence, providing acute care and long-term rehabilitation of survivors. This requires a team of trained and experienced, tender, sensitive, perceptive, and receptive health care providers who could deliver long-term mental health support and rehabilitation.

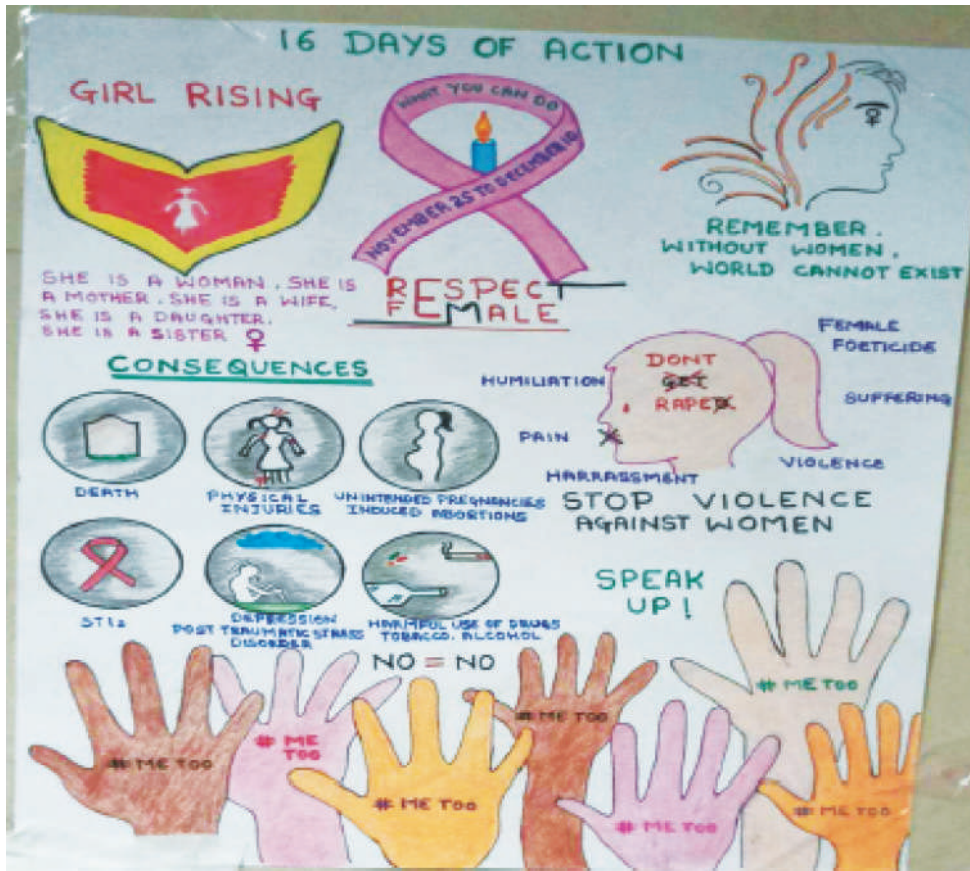
As a medicolegal and ethics person, I would like to appreciate Dr Mandakini Megh, Chairperson, FOGSI-ICOG and Dr Reena Wani and their team for their efforts in conducting a workshop on PET-VAW for practitioners. This book which is a compilation from multidisciplinary experts of OBGYN, psychiatrist law enforcement and NGOs; which will help practitioners to handle medico-legal cases.



Dr Virendra Sutar

Sanjay Gupte

Past President, FOGSI
Chairman, FIGO Committee for
Ethical and Professional Aspects of
Human Reproduction and Women's Health



Dr Poonam Gowda (1st Prize TIE)



Dr Sumaya, Dr Yashshree, Dr Sukeshini (1st Prize TIE)



Preface



Theme of the Chairperson, ICOG 2020-2021
EQUIP_EDUCATE_EMPOWER



MD, DGO, FICMCH, FICMU, FICOG
Chairperson, ICOG

Greetings and Regards!

It is a privilege for me to write the Foreword for *PET: Prevention, Examination and Treatment of Domestic Violence and Sexual Assault Cases*. We are addressing the problems and challenges of very critical social issue of grave importance of today dealing with domestic violence and sexual offences. It is a proud feeling that this publication will help in every step to us, the medical professionals.

“If we are to fight discrimination and injustice against women, we must start from the home for if a woman cannot be safe in her own house then she cannot be expected to feel safe anywhere.”

As we are aware domestic violence, sexual assault affects all parts of society, the responses that arise to combat it are comprehensive, taking place on the individual, administrative, legal, and social levels.

Keeping in mind, how to the prevent, doing examination and treatment of domestic violence and sexual assault cases, the articles have been compiled in this book. With the help of this book health professionals can use the guidelines as a day-to-day service document and/or as a tool to guide the development of health services for victims of sexual violence. The guidelines can also be used to prepare in-service training courses on sexual violence for health care practitioners and other members of multidisciplinary teams.

The guidelines will be useful for a range of professionals who provide care for victims of sexual violence: Health service facility managers, medico-legal specialists, doctors and nurses with forensic training, district medical officers, police surgeons, gynaecologists, emergency room physicians and nurses, general practitioners, and mental health professionals. These guidelines are meant to be adapted to specific local and national circumstances, taking into account the availability of resources and national policies and protocols. This book would be of much help to update knowledge and guide to deal with the situation in time.

In conclusion, I would like to quote the Israeli historian and scholar of the Holocaust; **“Thou shalt not be a victim, thou shalt not be a perpetrator, but above all thou shalt not be a bystander.”**

As a chief editor of book along with co-editor Dr Reena Wani bring this very important and useful publication, *PET: Prevention, Examination and Treatment of Domestic Violence and Sexual Assault Cases*. We are sure this book will help you all in your day-to-day practice.

Wishing you a very happy and healthy coming year.

Dr Mandakini Megh
Chairperson, ICOG

Message from Co-Editor



"We must be the change we want to see in the world" said Mahatma Gandhi.

We are the torch-bearers for women's health and are responsible for them from "Womb to Tomb" being often the primary health providers especially for women who never reach out to the health care system until they get have period problems or get pregnant. Hence, we are often in the privileged position of being first responders in situations of violence against women and children ... but the tragedy is that often the person hesitates to open up, or we fail to do what we should.



Violence against women and girls is a gross violation of human rights and remains largely unreported dues to impunity enjoyed by the accused, and a culture of shaming and blaming the victim. Despite Nirbhaya, Kathua and Hathras incidents, every day there are news reports of domestic and sexual violence. Intensive awareness drives and concerted efforts from every quarter is the need of the hour to quash this evil. It has been a neglected area of research, the available data are scanty and fragmented but after the #MeToo movement, more focus has been put on this area.

Often the health care system is blamed for not responding in a timely or appropriate manner when a girl or woman approaches for help or reporting. Often there is lack of clarity about procedure and protocols to be followed, or hesitancy on behalf of private sector to be involved.

The training workshop by ICOG-FOGSI was conceptualized by us to attempt to plug the gaps in knowledge and improve practices in dealing with violence against women and girls. The book was born out of the effort to collate information in concise form which can be referred to by each of you in times of doubt.

Our pledge and Hippocratic Oath requires us to rise to the challenge of "Saying no to Violence Against Women and Girls" ! If each one of us is able to help even one person, by reporting/facilitating/rehabilitating in a small way, we would have made a difference.

This is just the beginning, but we hope that it will show light at the end of the tunnel.
In the words of Sir Robert Frost

*"The woods are lovely, dark and deep
But I have promises to keep
And miles to go before I sleep,
miles to go before I sleep ..."*

Reena J Wani

Co-Editor and Convenor, FOGSI-ICOG, PET Workshop
Core Committee Member, FOGSI, VAW Cell 2015-2021



Dr Sayesha Patel (1st Prize TIE)



Message

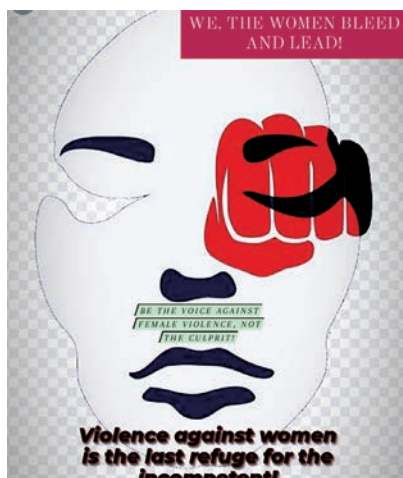
According to National Crime Records Bureau 2019 report, over 32000 women were raped in India, a number which has been steadily rising by 31% in the last decade. The first point of contact for victims of sexual assault are health care providers and the law enforcement. Health care professionals have a dual role to provide the survivor with the required medical and psychological treatment as well as to assist in their medico-legal proceedings.

Doctors in both public and private sectors are hesitant to take up cases of violence against women due to lack of clarity on examination and reporting. Hence, FOGSI-ICOG conducted an online 3-day certificate course and lecture series on prevention, examination and treatment (PET) of domestic violence and sexual assault cases.

The course was the brainchild of ICOG Chairperson Dr Mandakini Megh and Dr Reena Wani. The course coordinators were Dr Hema Relwani, Dr Kruti Doshi and Dr Preeti Deshpande.

With a multidisciplinary panel comprising of 15+ speakers from OBGYN, psychiatry, law enforcement, medico-legal experts and NGOs, the course was an immense success. It was attended by 574 delegates from public and private sector and received highly positive feedback for the practical approach discussed for dealing with domestic violence and sexual assault cases. At the end of the 3 days, 142 attempted and passed the online examination and got certification from FOGSI-ICOG.

In order to summarize the learnings from the course, FOGSI-ICOG is publishing this book on violence against women—prevention, examination and treatment (PET) which is aimed to be a handy manual for practitioners. We are glad to be part of this team preparing this important compilation of up-to-date information shared by our experts.



Dr Priyadarshini Dutta (3rd Prize TIE)



Dr Priyanka Surodwar



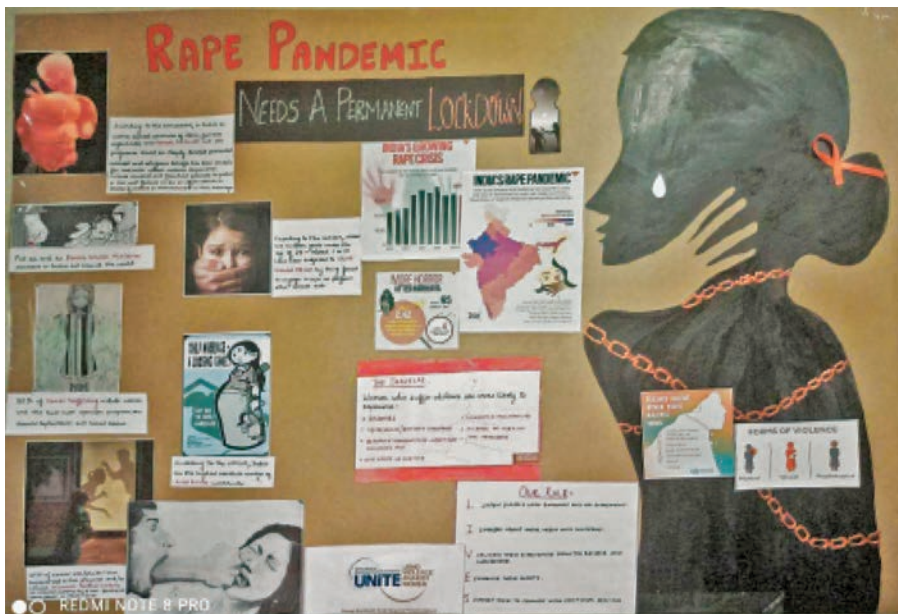
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Preeti Deshpande
Assistant Editor



Student Nurse Divya Save (2nd Prize)



Dr Neha Mathews (3rd Prize)

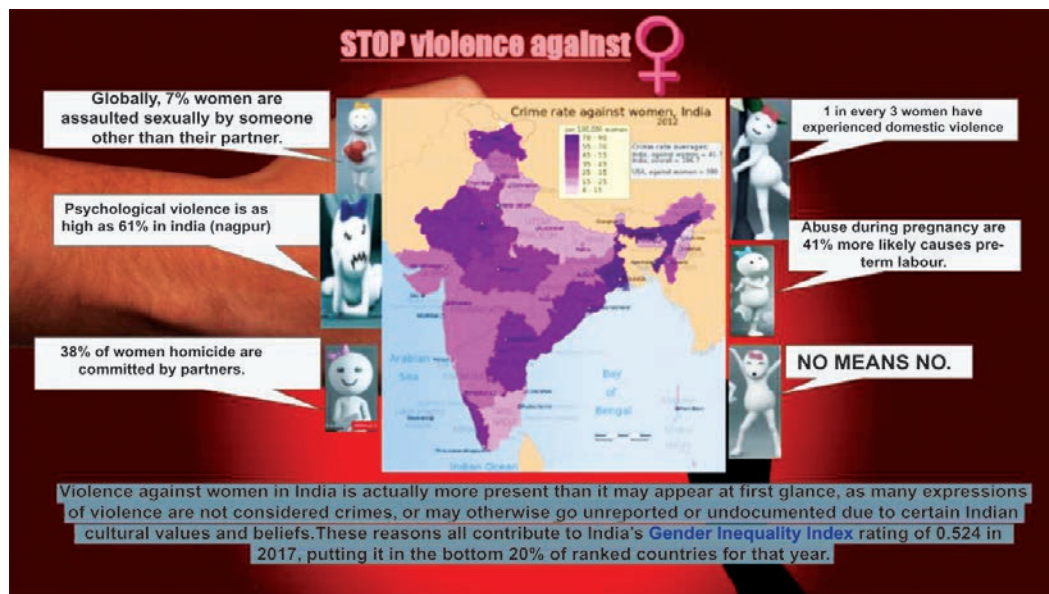


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- FOGSI President Dr Alpesh Gandhi for his vision and mission “Say no to Violence against Women and Girls”.
- Our PET workshop coordinators and faculty who made it happen, despite the Corona Pandemic.
- ICOG office staff, especially Mrs Neelima More, and team of CBS Publications for their timely cooperation and efforts.
- Our spouses, without whose cooperation and adjustment of family time, we (ladies) could not have finished this project in time.
- Our family members ... Special mention of Mr Gorakh Megh, Dr Jatin Wani, Mr Anukool Deshpande and Mr Vinit Shah for being the men behind the strong women.
- A heartfelt thanks to each one of you who made it possible to publish this very important and timely release of the book *PET: Prevention Examination, Treatment of Domestic Violence and Sexual Assault Cases*.

Mandakini Megh
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Preeti Deshpande



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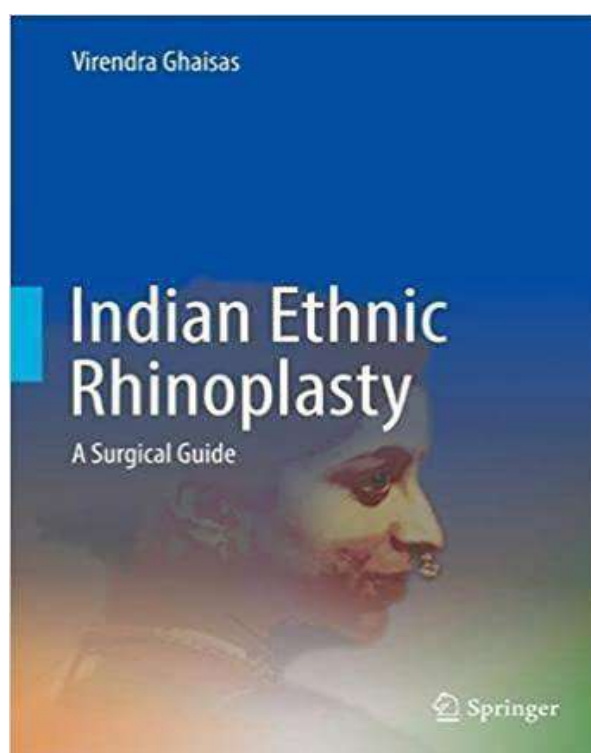
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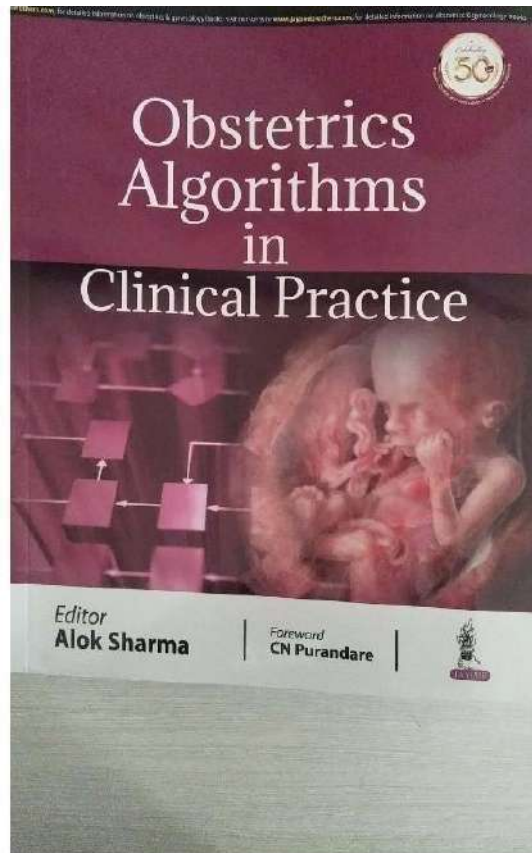
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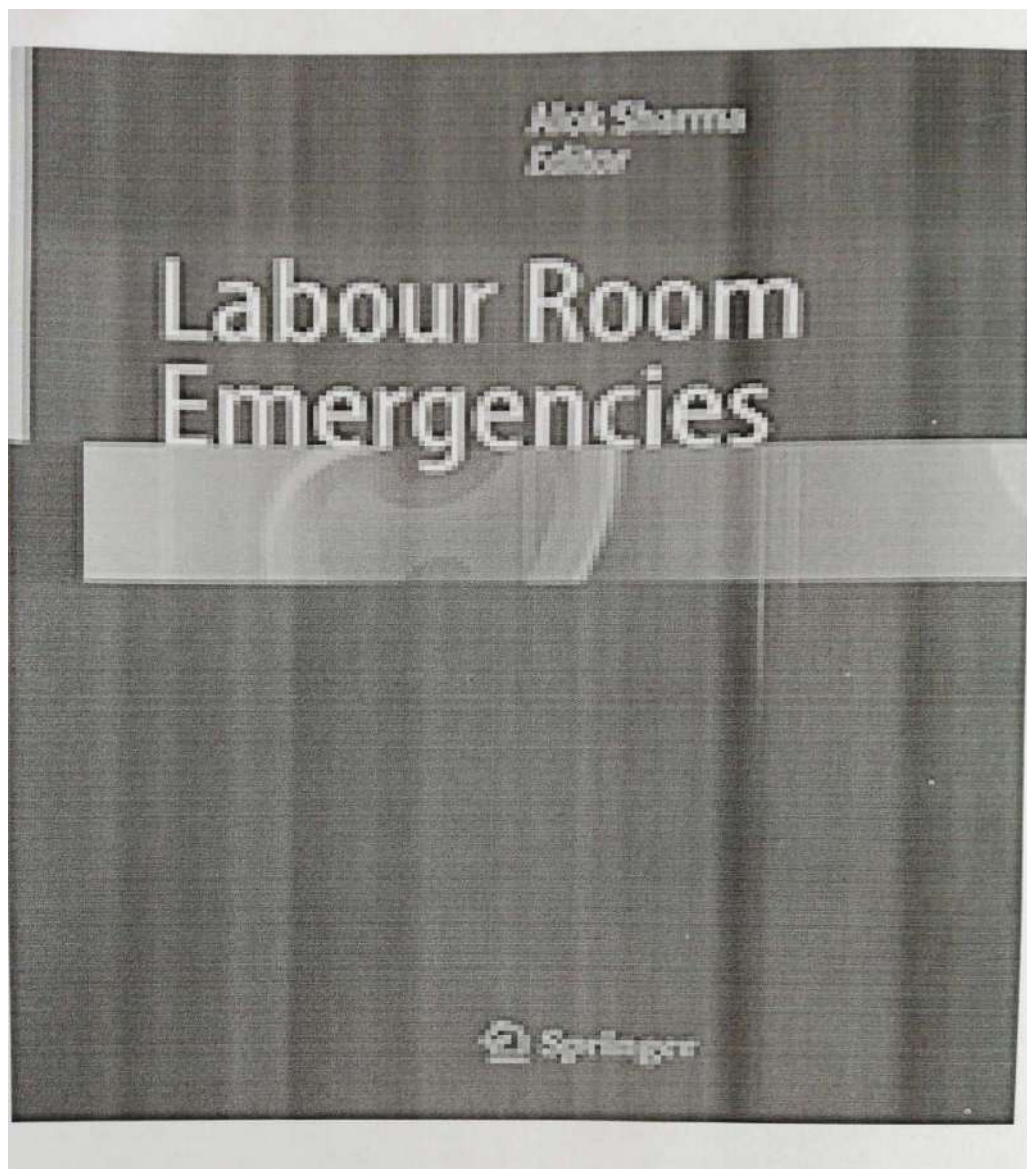


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Broad Ligament Haematoma

Vaishali Korde-Nayak and Parag Biniwale

41.1 Introduction

During pregnancy the uterus, vagina and vulva have rich vascular supplies. Any significant trauma during birth process may result in formation of a haematoma. Puerperal genital haematomas are relatively uncommon causes of PPH but can lead to serious morbidity and even maternal death [1]. The reported incidence of significant postpartum haematoma is around 1 in 500–700 deliveries [2].

Suprlevator haematomas also known as broad ligament haematomas are rare, with widely varying incidence of between 1:500 and 1:20,000 deliveries [3]. As the symptoms are nonspecific and size dependent and bleeding is often concealed, most of them are difficult to diagnose.

Whitridge Williams was the first person to report broad ligament haematoma in 1904 [4]. He reported a series of cases in a monograph on subperitoneal haematoma. He studied 33 cases of spontaneous broad ligament haematomas and ascribed them to capillary bleeding [4] (Fig. 41.1).

41.2 Types of Puerperal Genital Haematomas

1. **Infralelevator Haematomas**
 - (a) Location—Below the levator ani muscle
 - (b) Include the vulva, perineum and lower vagina, episiotomy site
 - (c) More common than suprlevator variety
 - (d) Occur following vaginal birth/instrumental deliveries/big baby/any other obstetric trauma
2. **Suprlevator Haematomas**
 - (a) Location—Above the levator ani muscle, in the leaves of broad ligament
 - (b) Mostly due to an extension of cervical tear, forniceal tear or uterine incision and uterine rupture
 - (c) Less common than infralelevator haematomas
 - (d) Can be associated with spontaneous vaginal birth, but commonly occur following instrumental vaginal deliveries, difficult caesarean section or vaginal birth after caesarean (VBAC), etc.

Broad ligament haematoma occurs secondary to lacerations/tear in the upper vagina, cervix or uterus that extends into uterine or vaginal vessels or vessels of the broad ligament. The engorged vessels of pregnancy bleed profusely in the space between the leaves of broad ligament accommodating significant blood collection.

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SECTION 7

GENERAL ORTHOPEDICS

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CHAPTER 62

Nonunion of Fracture Clavicle: Plate Fixation and Bone Grafting

Yash Shah

INTRODUCTION

Clavicular fractures are common and nonunion of unoperated mid shaft fractures are rare, with a reported incidence between 1% and 4%. These reports are predominantly in adults, but nonunion has also been described in children. Several factors have been hypothesized to increase the risk of nonunion developing. They include—inadequate immobilization, severity of trauma, re-fracture, location of fracture, degree of displacement, and primary open reduction. The rate of nonunion following primary internal fixation is quoted to range from 0.1% to 4%, but in one group has been quoted as high as 13%. Management of patients who have symptomatic nonunion has involved use of various surgical techniques, the most common being either intramedullary fixation or rigid internal fixation with plates. There has been a report of achieving 100% union at 10 weeks postoperatively using open reduction and internal fixation with bone grafting. However, Jupiter reported on clavicular nonunions treated surgically with a success rate of 90% in achieving union.

RISK FACTORS

The risk factors for nonunion include increasing age, female sex, fracture displacement, and comminution. However, the majority of fractures occur in a younger, predominantly male population, and most nonunions are therefore encountered in this population.

SYMPTOMS

Shaft nonunions in active individuals are usually symptomatic, causing pain, and a clicking sensation on movement. Restriction of shoulder movement, weakness, cosmetic deformity, neurological symptoms, thoracic outlet syndrome, and subclavian vein compression has also been reported. Patients may also report disturbed sleep, an inability to perform manual work, difficulty in driving, enforced absence from normal sporting activities, and a reduction in sexual activities due to pain.

INVESTIGATIONS

- X-ray clavicle anteroposterior (AP) view is advised.
- Computed tomography (CT) scan (in equivocal cases).

MANAGING NONUNION OF FRACTURES

Asymptomatic nonunion of midshaft clavicle fractures can be left alone without treatment, given the fact that the patient is ready to accept a mild cosmetic deformity and functional restriction.

Symptomatic nonunion of midshaft clavicular fractures can be treated surgically with either an intramedullary device or plate fixation, and often combined with iliac crest bone graft. Success of which is around 89–100%. There is little written about the subsequent management of those who do not unite with this surgery.

The treatment options are:

- Plating and autograft
- Plating and allograft
- Plating and vascular fibular graft
- Intramedullary fixation
- External fixator
- Bone morphogenetic proteins
- Electrical stimulation.

Plating and Autograft

Bone defects that do not heal are frequently treated with bone graft harvested from the iliac crest. Majority of the cases have been treated by this method. However, the supply of autograft bone is limited and has a risk of donor site morbidity.

Plating and Allograft

Allograft has several attractions, but has limited osteoinductive capacity and a limited capacity to incorporate.

Plating and Vascular Fibular Graft

Vascularized fibular and medial femoral condyle grafts have also been used successfully, although they are probably indicated only in revision cases in which the initial operative treatment of the nonunion has failed.

Intramedullary Fixation and External Fixation

Intramedullary fixation and external fixation have been used in small series. Although these techniques produce more cosmetically acceptable incisions and disturb the soft-tissue envelope less, they provide less rigid fixation and thus are not commonly used. External fixation with use of Papineau technique has been utilized to treat infected pseudarthroses, but it is not widely used.

Bone Morphogenetic Proteins

More recently, osteoinductive materials such as bone **morphogenetic proteins** have been shown to induce new bone formation, these are part of the transforming growth factor-B superfamily. While recombinant human osteogenic protein-1 (OP-1) combined with type-1 collagen has been shown to promote the healing of segmental bone defects in animals.

Electrical Stimulation

Electrical stimulation has been used with mixed acceptance for a long time, with some reports of success over 150 years ago. However, because of inconsistent results, its use was dwindled. It was repopularized following Fukuda and Yasuda's report of inducing bone growth.

NONUNION OF LATERAL-END CLAVICLE FRACTURES

The rate of nonunion after nonoperative treatment of lateral-end fractures is higher than the rate after nonoperative treatment of shaft fractures, but there is some debate regarding its exact prevalence, with some authors reporting higher rates than others. In some small case series, the rate was reported to be between 18% and 40%, whereas more recently, a larger prospective study of 263 lateral-end fractures demonstrated a nonunion rate of 11.5%.

The treatment options for an established symptomatic nonunion include excision of the lateral end of the clavicle, or fracture fixation with or without bone graft. Excision is usually preferred, if the lateral fragment is small and the coracoclavicular ligaments are intact, whereas fixation is used when there is a larger fragment, with good bone stock, and there is a reasonable chance of the procedure successfully promoting union. The methods of internal fixation that have been used are similar to those described previously for acute fractures. The results of treatment have been assessed only in small numbers of patients, in studies mainly focusing on the treatment of midshaft nonunions.

NONUNION OF LONG BONES CLAVICLE—SURGICAL STEPS



Fig. 1: X-ray showing a nonunion of the shaft of clavicle. Sclerosis can be observed at the ends of the clavicle.



Fig. 2: Position of the patient. Head can be slightly elevated or patient can be fully supine. Make sure that if the patient is intubated the endotracheal tube is on the opposite side of the fracture.



Fig. 3: Instruments required for clavicle plating. In addition, the instruments needed for iliac crest bone graft harvesting must be there. And if the bone graft harvest is planned simultaneously, we must have two sets of mastoid retractors, forceps, osteotomes, etc. (1) General surgical instruments (artery forceps, Allis forceps, Mollison mastoid retractor), (2) Toothed and plain forceps, and No. 15 and No. 23 sterile surgical blades on BP handles, (3) Right angle retractors of various sizes, (4) Bone spikes, (5) Bone nibbler, (6) Plier, (7) Sharp osteotomes, (8) Periosteum elevator, (9) Mallet, (10) Clavicle dynamic compression (DC) plates, (11) Needle holders and dissecting scissors, (12) Cordless power drill.



Fig. 4: After painting and draping, the skin incision is marked with a sterile skin marking pen.



Fig. 5: A skin incision of about 8-10 cm is made.



Fig. 6: Two self-retaining retractors reduce the work of the assistant. Care should be taken to relieve the pressure of the skin in case the surgery is taking too much time.



Fig. 7: One of the fragments may be hooked out with a roller bandage and negotiated, so that it becomes easy to curette and clean the sclerotic ends of the fracture.



Fig. 8: Identify the fractured ends and freshen the edges.



Fig. 9: Use a periosteum elevator, if need be, for freshening the fracture.



Fig. 10: Use a bone nibbler to nibble out the sclerotic bone ends.



Fig. 11: Apply the plate with sleeve on the clavicle.



Fig. 12: One-by-one apply the locking screws.

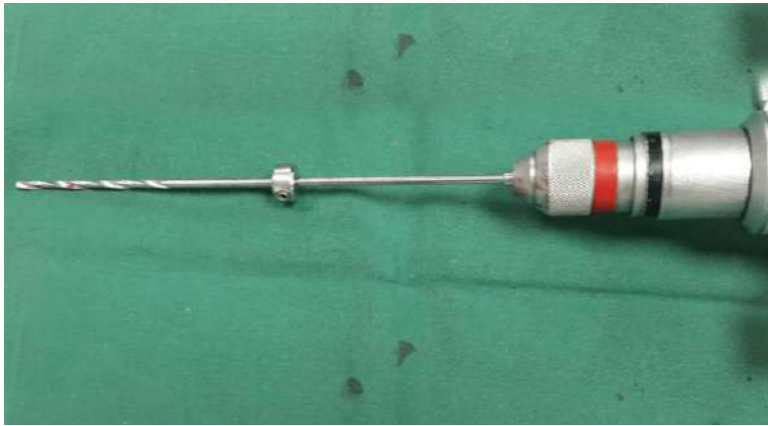


Fig. 13: It is always better to use such a drill bit where you can control the depth, so as to avoid injury to the nerves and vessel inferior to the clavicle. Care should be taken to avoid slippage of drill guard.



Fig. 14: After inserting all the screws, yet a gap can be seen.

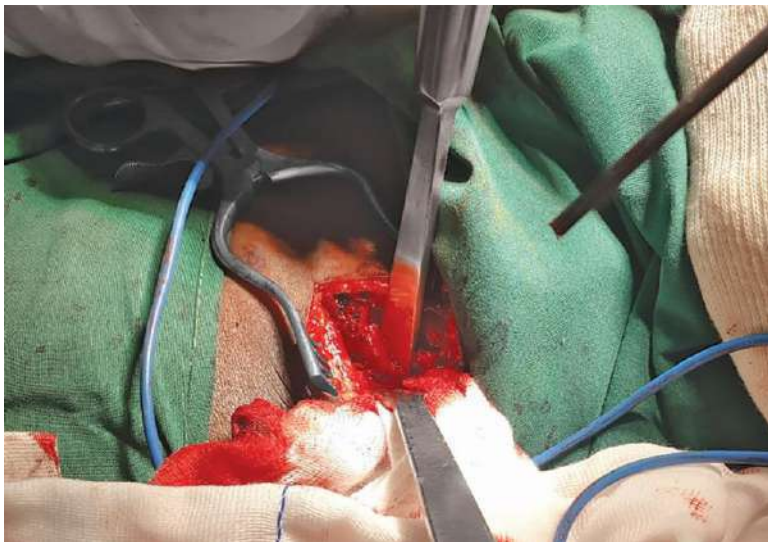


Fig. 15: An Iliac crest bone graft is harvested.



Fig. 16: Bone graft, which is harvested from the Iliac crest. Blood, which oozes should also be inserted at the fracture site as it has a lot of osteoprogenitor cells.



Fig. 17: Intraoperative C-arm picture before inserting the bone graft at the fracture site.

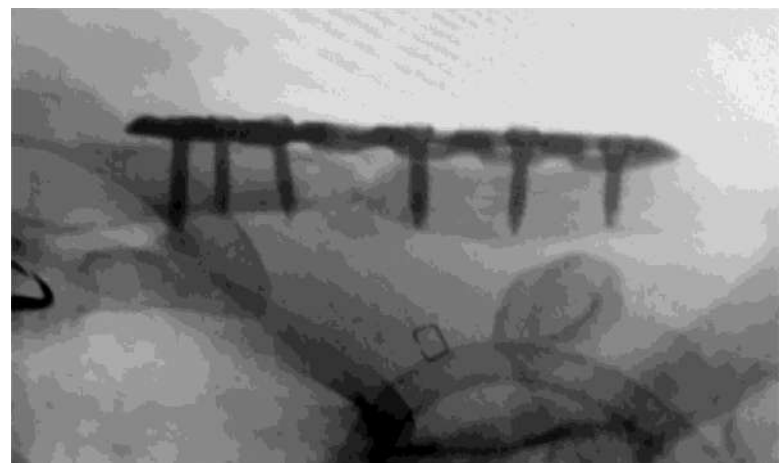


Fig. 18: Intraoperative C-arm picture after inserting the bone graft at the fracture site.



Figs. 19A and B: (A) After suturing the subcutaneous layer with absorbable suture (polyglactin); (B) Skin can be closed with either staples or sutures.



Fig. 20: Final postoperative X-ray showing the bone graft in place.

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SECTION 8

TRAUMATOLOGY

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CHAPTER 77

Fracture Clavicle: Plate Fixation Middle Third/Distal Third

Yash Shah

INTRODUCTION

As viewed in the coronal plane, the clavicle is a slender bone, wider medially at its sternal articulation and noticeably thinner at its lateral third. Viewed axially, however, the three-dimensional structure becomes more apparent. The clavicle assumes a gentle S-shape, the medial end convex forward, and the lateral end concave forward. This shape has been likened to the musical symbol the *clavicula*, thus the name. In the axial projection, the clavicle is also noted to have both medial and lateral flat expanses, linked by a thin, tubular middle. This central transitional area represents a weak link in clavicular structure. The mid clavicle, therefore, is the most common site of fracture.

FRACTURE BIOMECHANICS

For Midshaft Clavicle

For a midshaft fracture, the displacing forces are as follows (Fig. 1):

- Stabilizing on the medial segment by the sternoclavicular ligaments.
- Superior on the medial segment through the sternocleidomastoid.
- Inferior and medial on the lateral segment through the pectoralis major.
- Inferior on the lateral segment through the weight of the arm pulling through the coracoclavicular ligaments.

For Lateral End Clavicle

For more lateral fractures, the displacing forces are as follows (Fig. 2):

- Superior on the medial segment through the sternocleidomastoid and trapezius.
- Medial on the distal segment through the pull of the pectoralis major, pectoralis minor, and latissimus dorsi.
- Inferior on the distal segment through the weight of the arm.

CRAIG'S CLASSIFICATION

- *Group I*: Fracture of the middle third.

- *Group II*: Fracture of the distal third. Subclassified according to the location of coracoclavicular ligaments relative to the fracture as follows:
 - *Type I*: Minimal displacement—interligamentous fracture between conoid and trapezoid or between the coracoclavicular and acromioclavicular ligaments.
 - *Type II*: Displaced secondary to a fracture medial to the coracoclavicular ligaments—higher incidence of nonunion.
 - *IIA*: Conoid and trapezoid attached to the distal segment
 - *IIB*: Conoid torn, trapezoid attached to the distal segment.
 - *Type III*: Fracture of the articular surface of the acromioclavicular joint with no ligamentous injury—may be confused with first-degree acromioclavicular joint separation.
- *Group III*: Fracture of the proximal third:
 - *Type I*: Minimal displacement
 - *Type II*: Significant displaced (ligamentous rupture)
 - *Type III*: Intra-articular
 - *Type IV*: Epiphyseal separation
 - *Type V*: Comminuted.

INDICATIONS FOR OPEN REDUCTION AND INTERNAL FIXATION OF DISPLACED MIDSHAFT FRACTURES (TABLE 1)

TABLE 1: Indications for open reduction and internal fixation of displaced midshaft fractures.

Absolute	Relative
<ul style="list-style-type: none"> • Shortening of >20 mm • Open injury • Impending skin disruption and irreducible fracture • Vascular compromise • Progressive neurological loss • Displaced pathologic fracture with associated trapezius paralysis • Scapulothoracic dissociation 	<ul style="list-style-type: none"> • Displacement of >20 mm • Neurological disorder • Parkinson's • Seizures • Head injury • Multitrauma • Expected prolonged recumbency • Floating shoulder • Intolerance to immobilization • Bilateral fractures • Ipsilateral upper extremity fracture • Cosmesis

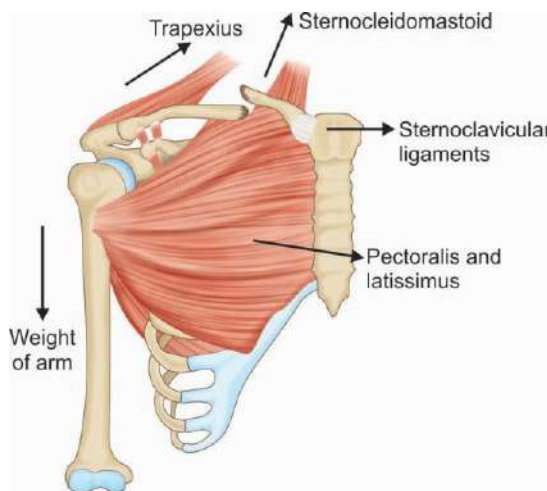


Fig. 1: Displacing forces for a midshaft fracture.

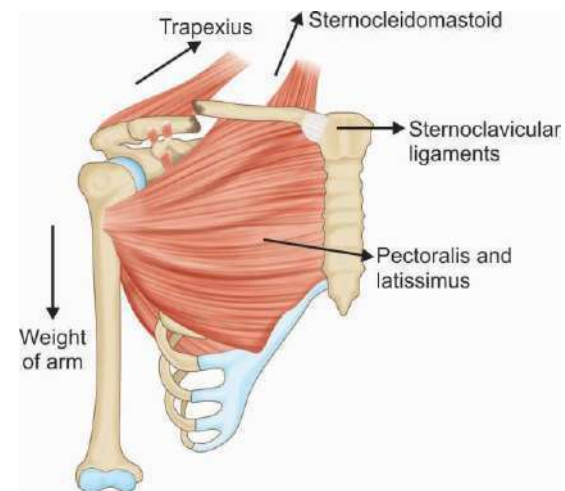


Fig. 2: Displacing forces for lateral fracture.

INDICATIONS FOR DISTAL CLAVICLE FRACTURE

Yet, the literature is more controversial for this fracture type than for the midclavicle. But Neer observed that when the coracoclavicular ligament is also disrupted Type II distal end clavicle, there is more chance of nonunion. Hence, such distal end fractures must be operated.

OPERATIVE OPTIONS: MIDDLE THIRD CLAVICLE

- Open reduction and plate fixation is favored over closed reduction and closed nail fixation.
- Commonly used plates are reconstruction plates and anatomical locked plates.
- Six to eight cortices hold seems sufficient for noncomminuted fractures.

OPERATIVE OPTIONS: DISTAL CLAVICLE

Kirschner Wire Fixation

Migration rates of up to 50% and failure of Kirschner wire fixation have led several authors to recommend that it not be used as a primary fixation technique.

CC Screw Fixation

- Its use is limited by the fracture location and extent of comminution.
- Screws must be routinely removed because they can limit shoulder girdle motion.

Plate or Hook Plate Fixation

Plate fixation can also be used in circumstances where the distal fragment allows sufficient fixation.

Hook Plate Fixation

Similar to plate except that distal fixation is achieved by placing the “hooked” end of the implant under the acromion to maintain a satisfactory reduction.

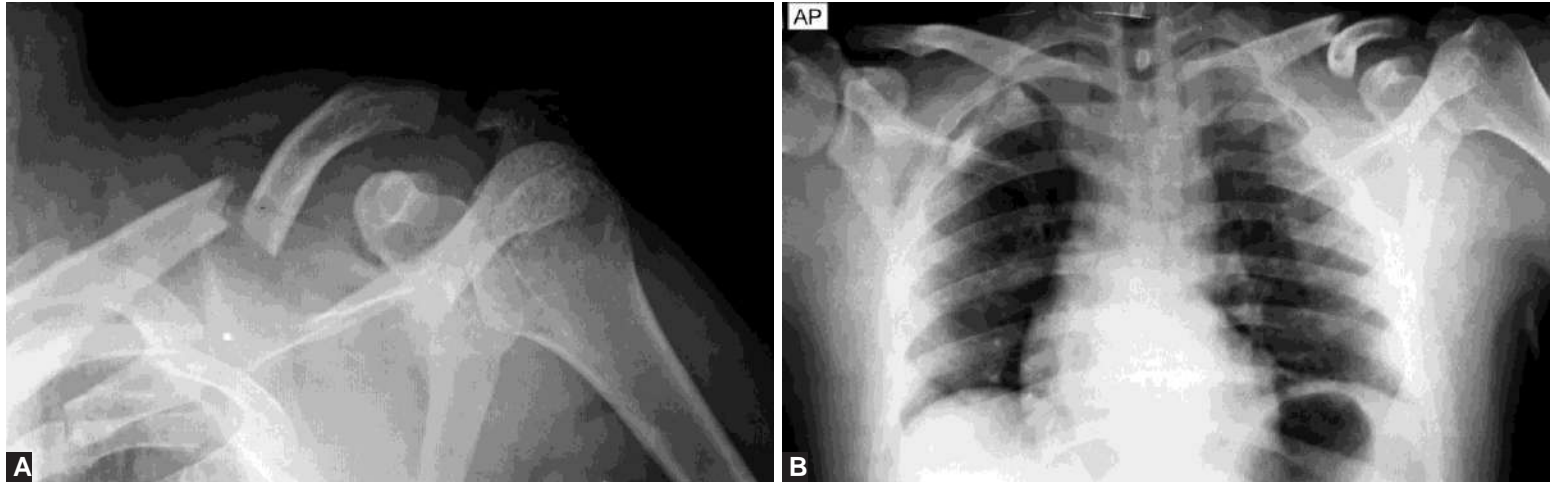
Suture and Sling Technique

Finally, suture and graft sling techniques can be used to reconstruct CC ligaments in a manner similar to anatomic acromioclavicular joint reconstruction. These techniques can be used to reinforce other fixation techniques or as the primary mode of reconstruction.

TIMING OF SURGERY

The timing of surgery for lateral-end fractures seems more important for patient outcome when compared with medial-third fractures. Although the union rate does not seem to be influenced by acute or delayed treatment, the complication rate may be higher when the surgical treatment is delayed (7% vs 36%). Lateral clavicle fractures that exhibit intra-articular extension may result in an increased risk of acromioclavicular joint degeneration. If acromioclavicular arthritis occurs, the patient may require a late distal clavicle excision.

SURGICAL STEPS: FRACTURE MIDSHAFT CLAVICLE



Figs. 3A and B: Preoperative X-ray of a patient with a left clavicle fracture. Always the X-ray of the chest is taken as well to rule out any other injuries.



Fig. 4: Instrument trolley. Always keep various types and sizes of bone holders and spike retractors at disposal. Specially contoured locking plates are to be used.



Figs. 5A and B: Position of the patient. Head end is slightly elevated.



Figs. 6A and B: Draping of the patient should be such that the arm should be able to freely externally and internally rotate.



Fig. 7: Always better to mark your incision as many a times the patient is obese and it is easy to veer off into the wrong plane.



Fig. 8: A skin incision of about 8-10 cm is made.

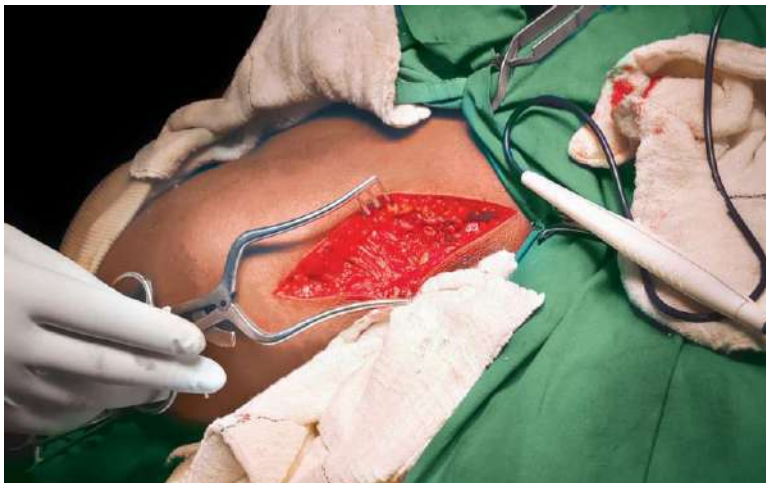


Fig. 9: A self-retaining retractor reduces the work of the assistant. Care should be taken to relieve the pressure off the skin in case the surgery is taking too much time.



Fig. 10: Dissect out the supraclavicular nerves.



Fig. 11: Identify the fractured ends and freshen the edges.



Fig. 12: Position the plate on the medial end of the clavicle using locking sleeve.



Fig. 13: Apply two screws, first being cortical to pull bone to plate on the medial fragment. Application of two screws ensures that all screws placement is in center of shaft. This is important for medial fragment as it is comparatively narrower.



Figs. 14A and B: Patient's head comes in way of drill for more medial screws. The sleeve can be lifted up after application of first screw to create space for the drill. The drill bit length outside sleeve to be only slightly longer than bone width to prevent accidental plunge into adjacent vital structures.

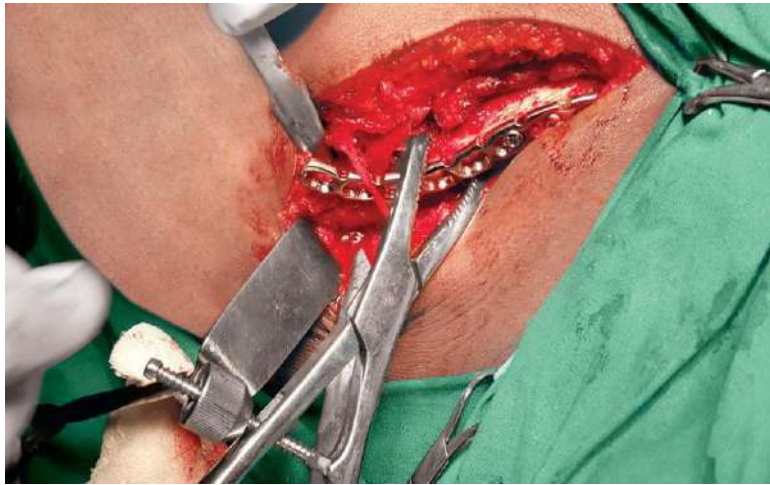


Fig. 15: Reduce the fracture with the help of a bone holding forceps.

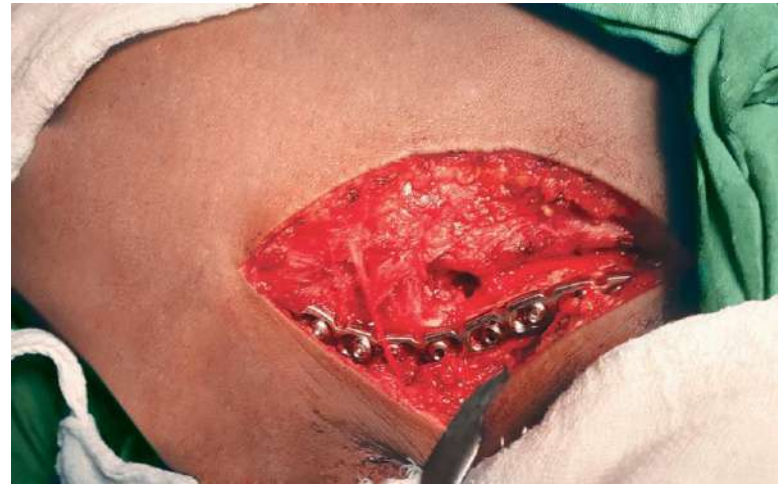
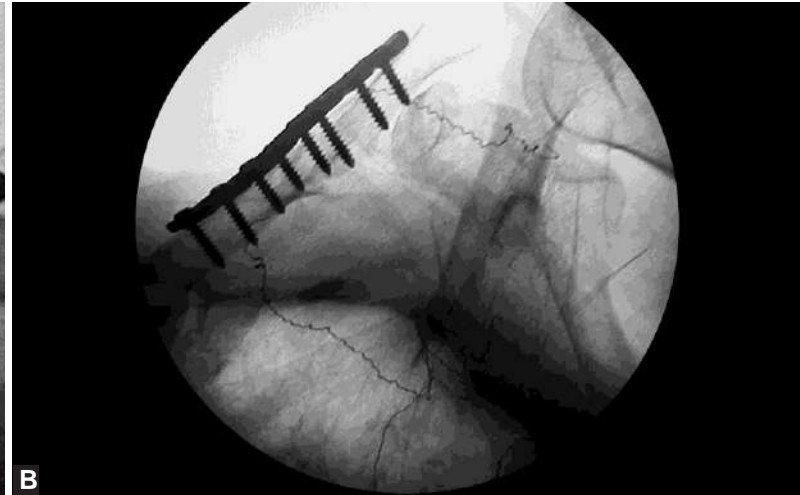
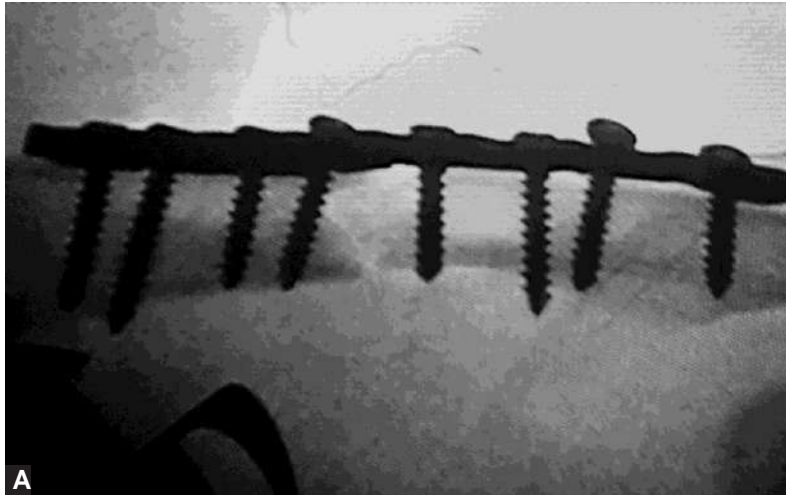


Fig. 16: Apply the remaining screws on the lateral end of the clavicle as well.



Figs. 17A and B: An intraoperative radiograph image is used to verify the correct length of the screws. Too long screws can damage the neurovascular structures underneath.



Figs. 18A and B: Sometimes, a butterfly fragment is attached to the plate by a sling suture of strong nonabsorbable material.



Fig. 19: Closure is performed in layers in a routine manner.

SURGICAL STEPS: FRACTURE OF DISTAL CLAVICLE



Fig. 20: Preoperative anteroposterior radiograph showing a fracture in the distal end of the clavicle.



Fig. 21: Axillary view of the shoulder of the same patient.



Fig. 22: Position of the patient. Anesthetist is at the foot end of the patient. The head end of the patient is raised by a pillow or the table is put in a beach chair position. The head of the patient is securely strapped to the table. Prior to painting and draping, a test shoot is taken to see if a proper view of the clavicle is obtained.



Fig. 23: Draping of the patient. The arm of the patient is draped in a sterile stockinette and a waterproof seal with a sticky drape is applied.



Fig. 24: An incision approximately 6-7 cm long is taken on the clavicle at the fracture site. A cautery is used to coagulate bleeders if any. Avoid injury to the supraclavicular nerve.



Fig. 25: Retraction is done by a mastoid/self-retaining retractor and the supraclavicular nerve is identified.



Fig. 26: Sometimes, reduction has to be done by using strong nonabsorbable sutures or similar to pull up the distal end of clavicle. This suture application has to be done at exposure prior to plate application as later on it is difficult.



Fig. 27: A specially contoured anatomical locked plate is applied to the clavicle and screws are applied to the medial fragment.



Fig. 28: Plate position is checked on C-arm image.



Fig. 29: After reduction is confirmed, the remaining screws are applied in the similar fashion.

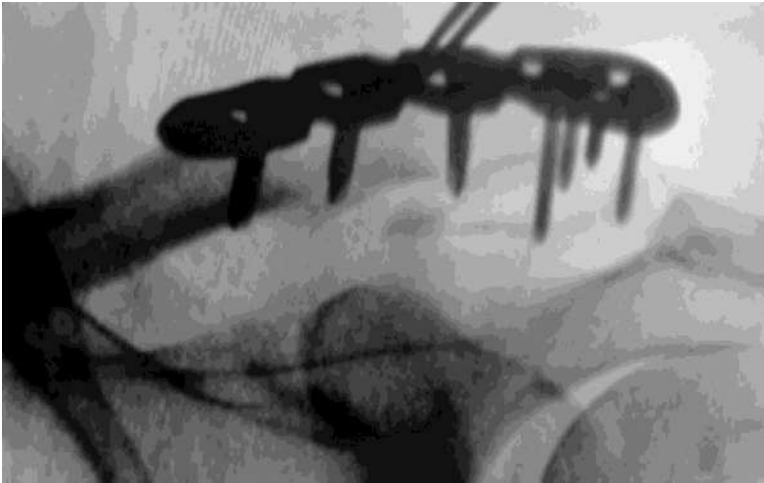


Fig. 30: After all screws are applied a final C-arm image is taken to confirm the placement and reduction. Lateral screws are carefully checked to ensure that they are not encroaching on joint.



Fig. 31: The nonabsorbable sutures, which were passed under the distal fragment, are now tied securely over the plate. Care should be taken to cut them short after the knot else there will be irritation to the overlying skin.



Fig. 32: Suturing is done in layers. Subcutaneous tissue is sutured with either 2-0 or 3-0 absorbable and skin is sutured with either 3-0 nylon or Staples.

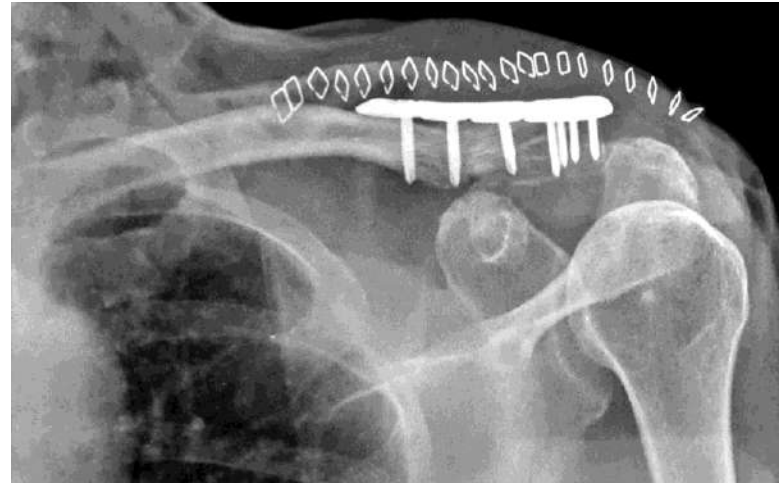


Fig. 33: A postoperative radiograph shows good placement of the plate and reduction.

COMPLICATIONS

Infection

As the clavicle is subcutaneous, the soft tissue envelope available for closure over implanted hardware is relatively thin, likely contributing to rates of wound complications. In a recent randomized trial, there was a wound complication rate of approximately 5%; however, all patients were successfully managed with local wound care, antibiotics, and hardware removal after fracture union.

Malunion

All clavicle fractures treated nonoperatively unite with some amount of angulation. Recent studies show some amount of functional loss as well. Clavicular shortening of more than 15 mm has been associated with shoulder discomfort and dysfunction and can change shoulder dynamics. Malunion may also be symptomatic, producing pain, neurovascular compromise, and upper extremity weakness.

Nonunion

Nonunion rates, however, are much greater for displaced fractures. **The rate of midshaft clavicle fractures** is less than 1–15% for displaced fractures and for distal end clavicle from 11% to 40%, though not all radiographic nonunion are symptomatic. Risk factors for nonunion include female sex, older age, degree of displacement, and comminution. Symptomatically, distal and shaft nonunion are similar and are associated with pain, restriction of shoulder movement, weakness, and neurovascular symptoms, including thoracic outlet syndrome and subclavian vein compression. Plate fixation **vs** bone grafting is the primary treatment for symptomatic nonunion of a clavicle shaft fracture. Treatment options for nonunion of a distal clavicle fracture depend on the size of the distal fragment: if the fragment is small and the CC ligaments are intact, distal fragment excision is recommended; however, if the distal fragment is large enough, internal fixation has been shown to be effective in promoting healing. Methods of internal fixation for nonunion of distal clavicle fractures are similar to primary operative treatment of distal clavicle fractures, as described previously.

Refracture

- It is reported at rate of between 1% and 2%.

Hardware Prominence

Positioning the hardware along the anterior surface of the clavicle, as opposed to the more traditional superior position, may reduce the rates of hardware irritation, which is often caused by backpacks or bra straps. The rates of removal of hardware for prominent hardware are reported to be around 8%.

Pain

- Restriction of shoulder movement
- Weakness and neurovascular symptoms
- Thoracic outlet syndrome
- Subclavian vein compression.

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Name of the Faculty	Paper details/book details publisher etc.
Dr. Yash Shah	Chapter 102; Fracture Neck Femur Bipolar Hip Replacement Hardikar's Orthopedic Operations Text & Atlas ISBN 978-93- 5270-697-6

CHAPTER 98

Fracture Neck of Femur Bipolar Hip Replacement

Dudhani Baldev, Yash Shah

BIPOLAR HIP REPLACEMENT (UNCEMENTED)

Dudhani Baldev

SURGICAL TECHNIQUE FOR MODULAR UNCEMENTED BIPOLAR ARTHROPLASTY



Fig. 1: Radiograph showing intracapsular fracture in elderly. Note gentle anterolateral bowing in this case.



Fig. 2: Instruments on trolley: (1) Mallet, (2) Cork screw, (3) Box cutter, (4) Canal finding reamer, (5 and 6) Rasps of various sizes with handle, (7) Charnley self-retaining retractor, (8) Head sizer, (9) Different sizes of trial heads, (10) Head pusher.



Fig. 3: Primary preparation of the surgical site with povidone-iodine in lateral position under spinal anesthesia.



Fig. 4: Marking of incision centering on tip of greater trochanter, for posterolateral approach.



Fig. 5: Subcutaneous fat and fascia being cut in line with incision to expose the gluteus maximus fibers; after which splitting of gluteus maximus fibers is done.

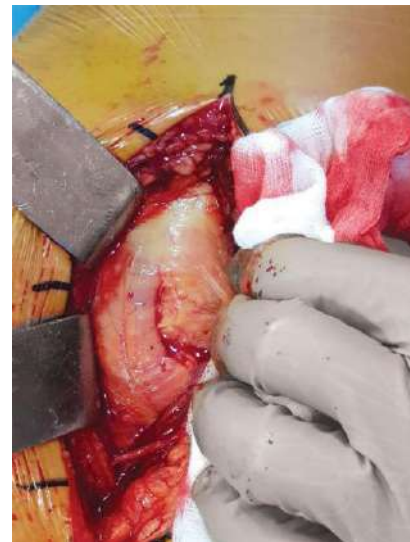


Fig. 6: Trochanteric bursa is being identified and cut.



Fig. 7: Neck femur is being visualized after erasing short external rotators from the trochanteric crest.



Fig. 8: Neck is being cut with the oscillating saw/osteotome, at 45° angle with longitudinal axis of femur, one finger breadth above the lesser trochanter with the leg in internal rotation at hip with knee flexed 90° till sole of foot is facing toward ceiling.



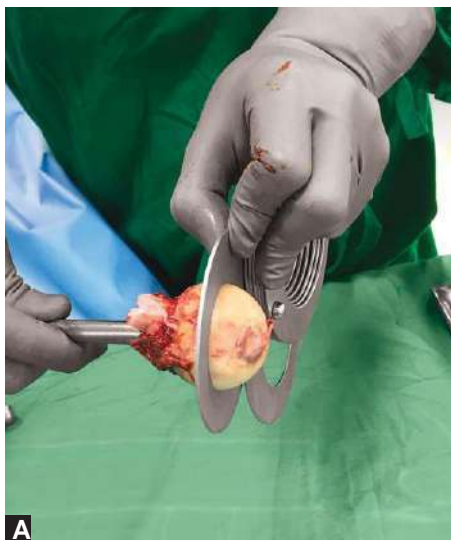
Fig. 9: Head of femur is visualized.



Fig. 10: Head is being extracted from the acetabulum after using cork screw and cutting ligamentum teres from acetabulum fovea to deliver head out.



Fig. 11: Extracted head and loose pieces of bone.



A



B

Figs. 12A and B: (A) Sizing of head is done and same size for final prosthesis is chosen. (B) The cut neck femur is properly exposed.



Fig. 13: Canal finder is used to assess direction of femoral canal.



Fig. 14: Initial proximal rasping of canal is done with small size rasp starting from most lateral side.

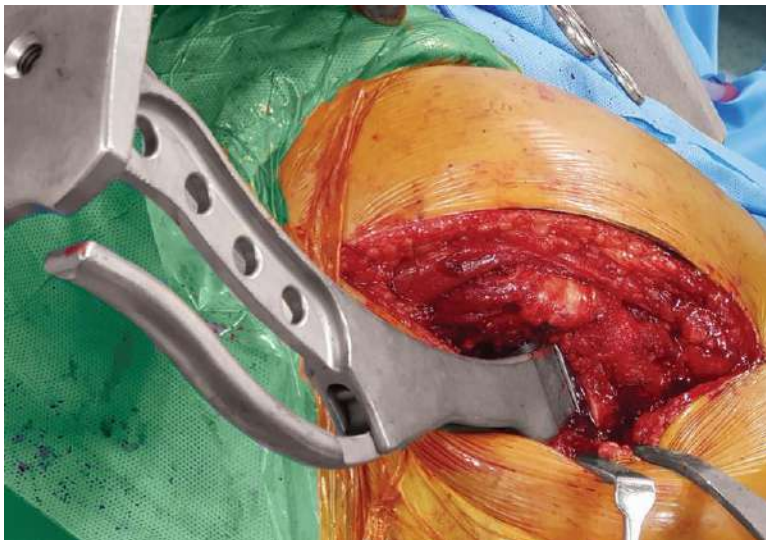


Fig. 15: Different sized rasps are used till one is chosen which fits snugly in canal. While rasping, care is taken to insert rasp in 10-15° of anteversion using either the position of the lesser trochanter as a reference; or sole of foot facing the ceiling as another.

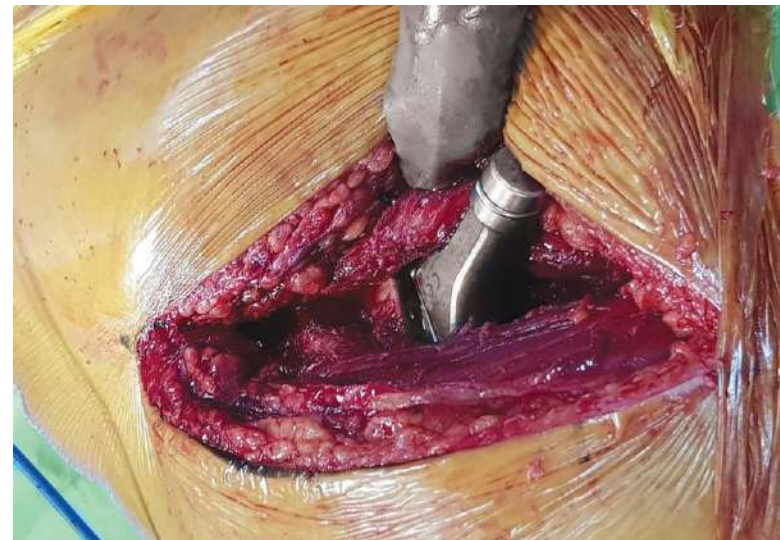


Fig. 16: Chosen trial prosthesis (femoral stem) is inserted.



Fig. 17: Trial is done with chosen trial head and neck components and stability checked by flexing, adducting, and internally rotating hip 30°. Leg length is checked by comparing with knee and malleolar level of contralateral side.



Fig. 18: Final prosthesis is being pushed in the canal.



Fig. 19: Final hammering and seating of prosthesis in 10-15° anteversion.



Fig. 20: Bipolar cup.



Fig. 21: The inner head.

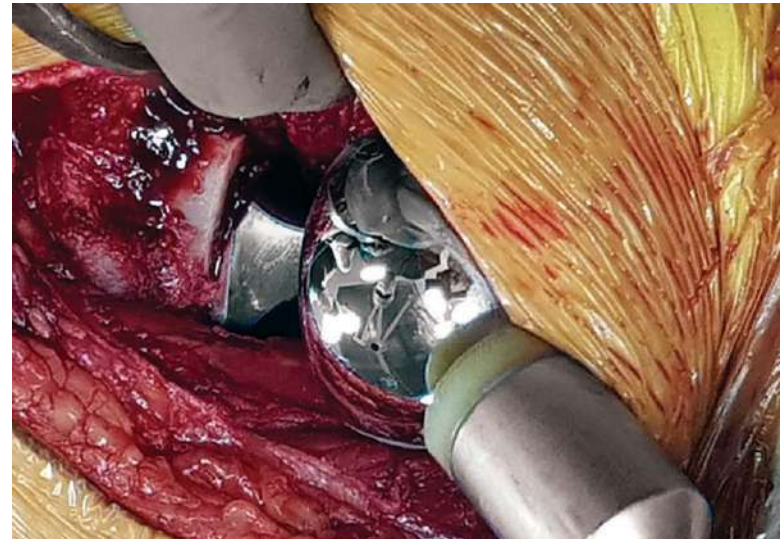


Fig. 22: The cup and the head assembly mounted on the prosthesis with gentle hammering.

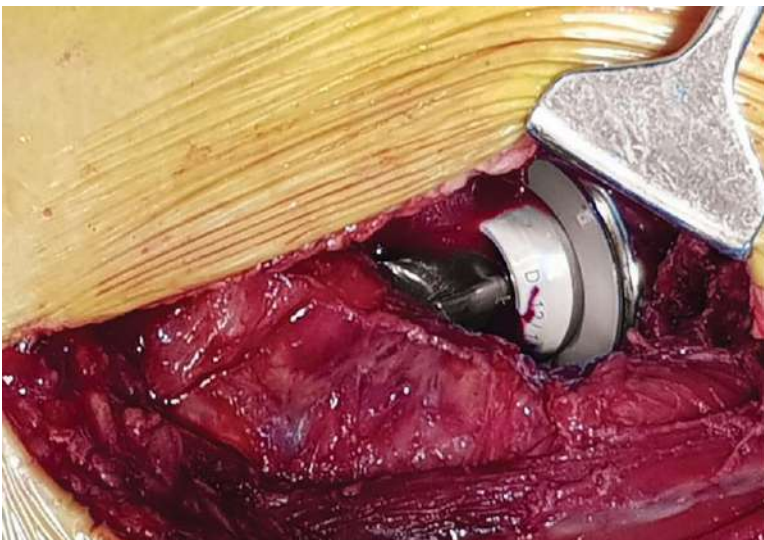


Fig. 23: Prosthesis is seated properly. Stability is again checked and shuck test is performed.

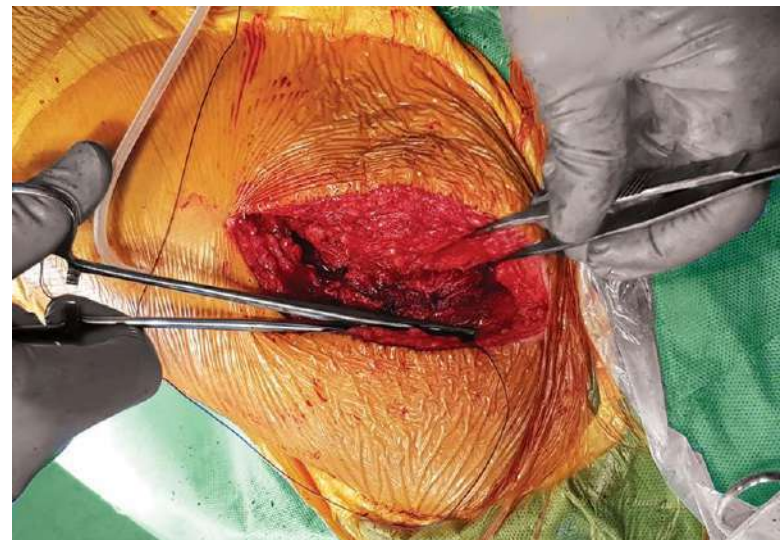


Fig. 24: The wound closure done in layers.



Fig. 25: Skin closure.

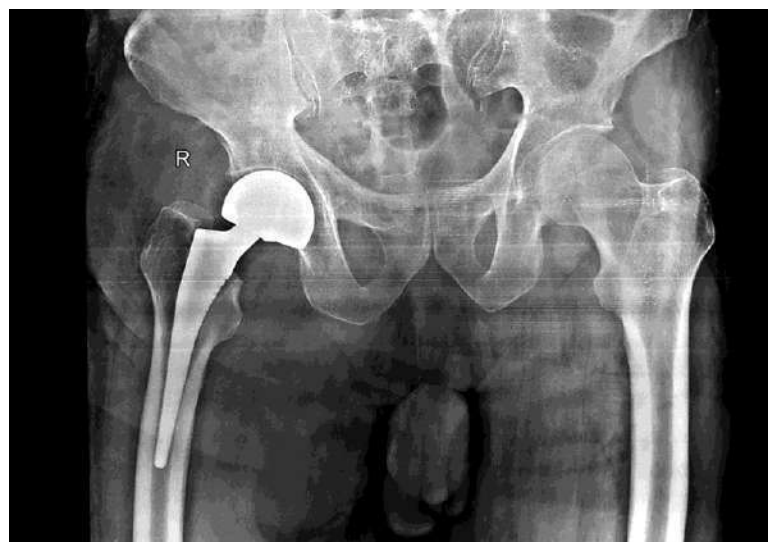


Fig. 26: Postoperative X-ray.



Figs. 27A and B: Bone cement and compatible prosthesis should be kept ready as a backup in case of problems encountered for uncemented prosthesis.

BIPOLAR HIP REPLACEMENT (CEMENTED)

Yash Shah

CEMENTED BIPOLAR: SURGICAL STEPS



Fig. 28: The approach in this case is by the posterior approach. Lateral position is given and painting and draping are done. It is imperative to check the preoperative range of motion. If there is any restriction of abduction, due to adductor contracture, adductor tenotomy may be required at the end of the surgery after checking the postoperative range of motion.



Fig. 29: Sterile stockinette is applied.



Fig. 30: Sterile povidone-iodine plastic sheet is applied and incision is marked.



Fig. 31: Flex the hip at 90° and take straight. Incision started from 6-8 cm above and posterior to greater trochanter (GT), it runs from this point to posterior border of GT continue distally over shaft about 5-7 cm from GT.

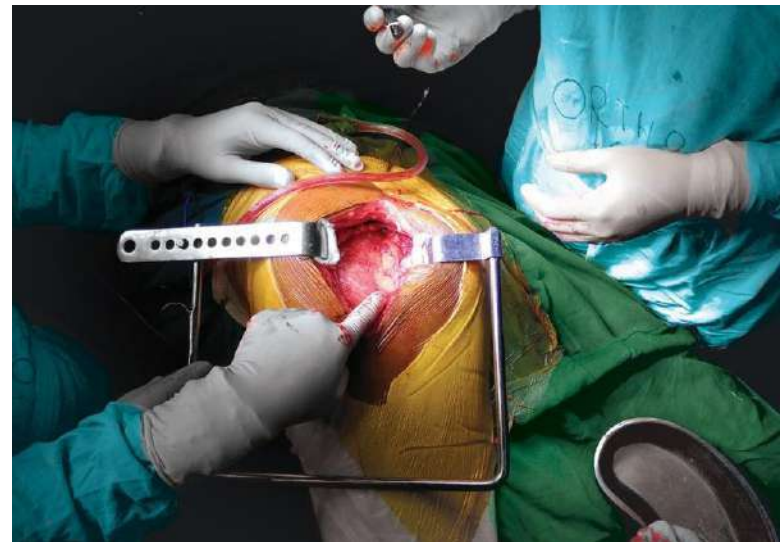


Fig. 32: Retraction is done using a Charnley retractor.



Fig. 33: Soft tissues are cut, viz gluteus medius, trochanteric bursa. It is better to abduct the hip while cutting the fascia so that it gets relaxed and gets cut better.



Fig. 34: Head is extracted using a cork screw extractor. Many a times, there is difficulty in extracting the head if there is an intact ligamentum teres. In those cases, it must be cut either with a cautery or a curved pair of scissors.



Fig. 35: Sizing of the extracted head is done using a readymade template.



Fig. 36: Neck to be cut is marked by a cautery pen. This avoids unnecessary errors while using the saw.



Fig. 37: Femoral neck is cut using a saw. One tip is that the saw machine can be started before the saw touches the bone. This avoids slipping of the blade in the hands of a newcomer.

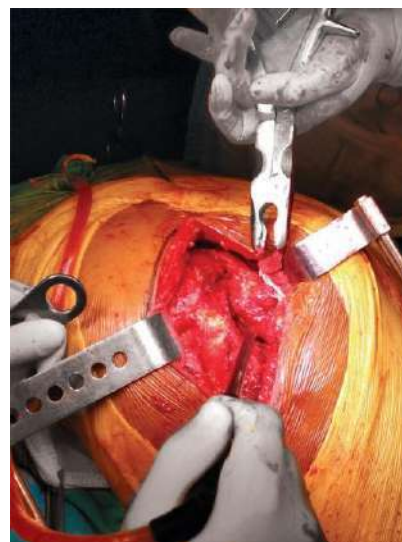


Fig. 38: Some bone near the piriform fossa is nibbled out. This helps in enabling as lateral access as possible in the piriform fossa.



Fig. 39: A box cutting tool is used to make the initial box cut. Care must be taken to go as lateral as possible in the piriform fossa. This avoids a varus positioning of the stem. This is done in 15° anteversion. This can either be achieved by positioning the box cut in 15° or positioning the box cut in horizontal position and the assistant holding in limb in 15° internal rotation.



Fig. 40: A canal finder is used to judge the direction of the canal. This helps in wrong broaching and prevents disastrous penetration of the broach through the shaft.

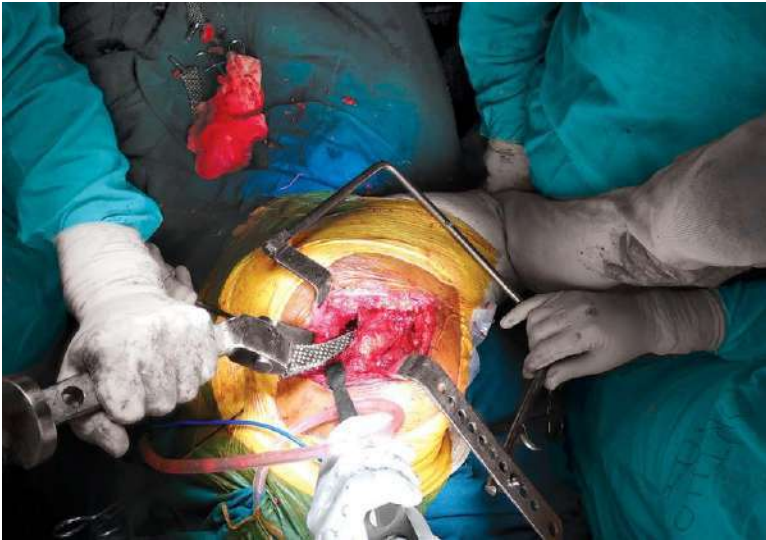


Fig. 41: Broaching of the femoral canal is done. Serial broaching is done. The systems may vary from company to company. Better to stop broaching once a tight feeling is felt and/or the sound changes. This may prevent an inadvertent fracture of the shaft of femur.



Fig. 42: Trial prosthesis is inserted. Length and version are checked.



Fig. 43: Stability is checked. A shuck test is performed.



Fig. 44: An assistant is told to take the hip joint through all the normal ranges of motion to check the fit of the prosthesis.



Fig. 45: The length as how deep to insert the cement restrictor is judged from either the trial stem/broach/actual prosthesis and the cement restrictor is inserted.



Fig. 46: The canal is cleared of all debris and blood by a suction. Any blood in-between the cement and bone is a cause of loosening.



Fig. 47: Roller gauze/absorbent gauze is inserted in the femoral canal. This keeps the canal dry.



Fig. 48: Acetabulum pack is inserted. This prevents cement being impacted in the joint later on.



Fig. 49: Cement is mixed in a bowl. An assistant is told to keep track of the time and he/she is told to remind everyone of the time since mixing the cement till it hardens. Mixing can be done either with a spoon or a curette.



Fig. 50: After the cement turns into a semisolid state, it can be inserted in the cement syringe attached to cement gun.



Fig. 51: The cement is then inserted into the femoral canal after removing the femoral pack while keeping the acetabulum pack. Any excess cement can be removed with an artery forceps after incising the excess with a scalpel/with nibbler/osteotome, if it hardens.

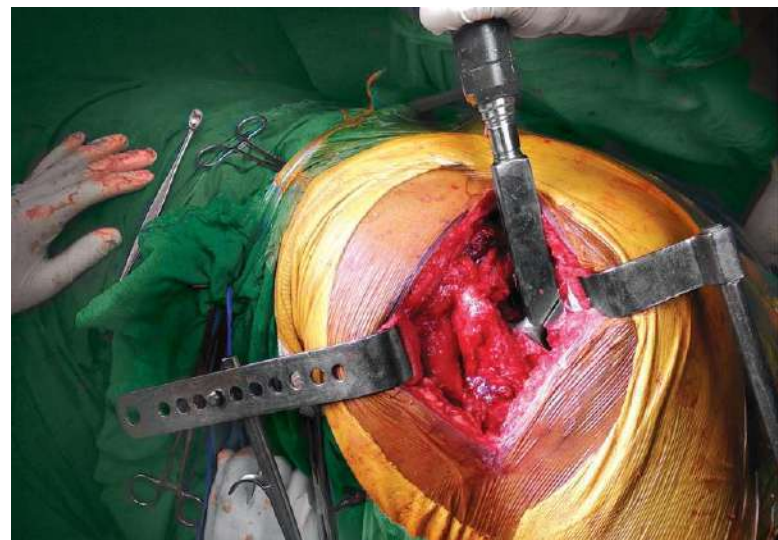


Fig. 52: The final prosthesis is inserted in around 15° of anteversion.

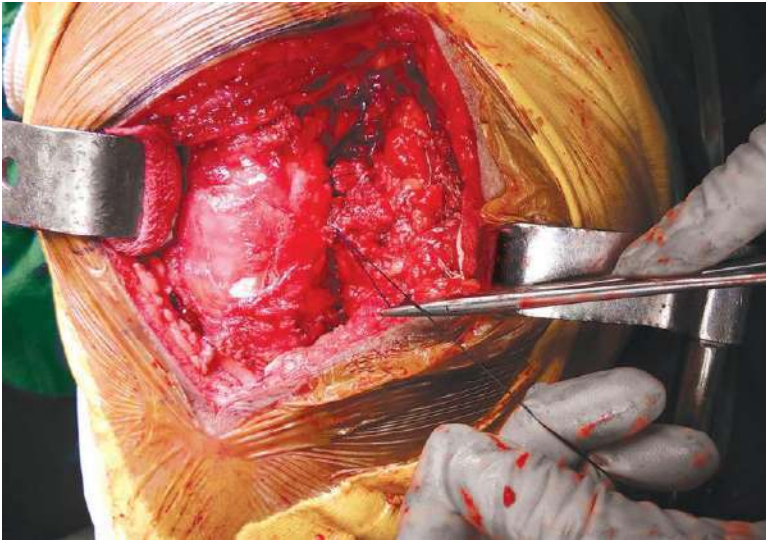


Fig. 53: Generally three sutures are taken on the short external rotator muscles of the hip. In any difficulty in passing the sutures directly through the bone, holes can be made in the bone either by a thick K-wire or drill so as to aid passage of the suture. 0 or 1 No. absorbable suture can be used here.

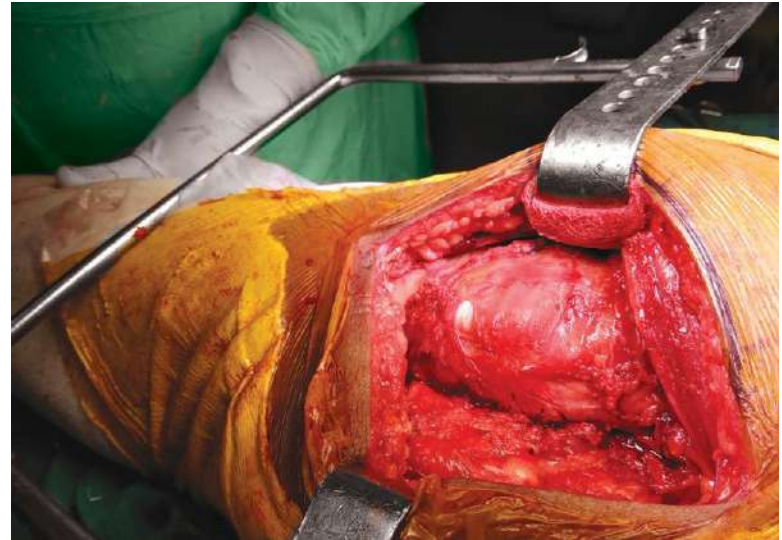


Fig. 54: The final picture after the suturing of these muscles to the trochanter.

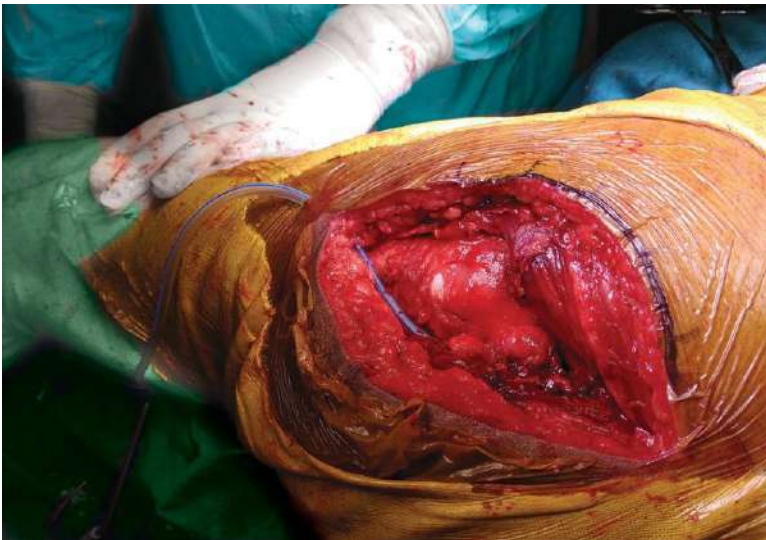


Fig. 55: Drain is inserted. It is clamped for 2 hours and then opened. This causes a tamponade and reduces the postoperative blood loss.



Fig. 56: Suturing is done in layers. 0 or 1 No. absorbable suture can be used for suturing Gluteus maximus and No. 2-0 absorbable suture can be used for suturing fascia, and fat and subcutaneous layers. Skin can be closed with either staples or 2-0 or 3-0 nonabsorbable sutures.



Fig. 57: Final postoperative radiograph. It shows a good cementing and a good placement of the stem.

Name of the Faculty	Paper details/ book details publisher etc.
Shashwat S. Banerjee, Vrushali Khobragade, Jayant J. Kandhare	Designing Multicomponent Nano systems for Rapid Detection of Circulating Tumor Cells Cancer Nanotechnology pp 271-281 Print NO(1868- 6958) Online NO (1868-6966) SPRINGER

Designing Multicomponent Nanosystems for Rapid Detection of Circulating Tumor Cells

Shashwat S. Banerjee, Vrushali Khobragade, and Jayant Khandare

Abstract

Detection of circulating tumor cells (CTCs) in the blood circulation holds immense promise as it predicts the overall probability of patient survival. Therefore, CTC-based technologies are gaining prominence as a “liquid biopsy” for cancer diagnostics and prognostics. Here, we describe the design and synthesis of two distinct multicomponent magnetic nanosystems for rapid capture and detection of CTCs. The multifunctional Magneto-Dendrimeric Nano System (MDNS) composed of an anchoring dendrimer that is conjugated to multiple agents such as near infrared (NIR) fluorescent cyanine 5 NHS (Cy5), glutathione (GSH), transferrin (Tf), and iron oxide (Fe_3O_4) magnetic nanoparticle (MNP) for simultaneous tumor cell-specific affinity, multimodal high resolution confocal imaging, and cell isolation. The second nanosystem is a self-propelled microrocket that is composed of carbon nanotube (CNT), chemically conjugated with targeting ligand such as transferrin on the outer surface and Fe_3O_4 nanoparticles in the inner surface. The multicomponent nanosystems described here are highly efficient in targeting and isolating cancer cells thus benefiting early diagnosis and therapy of cancer.

Key words Cancer diagnosis, Circulating tumor cell, Nanosystem, Multicomponent, Chemical conjugation

1 Introduction

Cancer is the second leading cause of death worldwide and metastasis causes more than 90 % of deaths in cancer patients [1]. Cancer metastasizes due to the migration of tumor cells from the primary site and spread to other parts of the body, via lymph system or bloodstream, where they form a secondary tumor [1]. Circulating tumor cells (CTCs) are imperative biomarkers for examining cancer prognosis as well as a real-time diagnosis and detecting them supports monitoring of drug resistance during the anticancer treatment and in clinical trials [2–4]. However, the detection of CTCs from blood has been challenging because of their extreme low occurrence (10–100 per mL) among a high number (10^9 cells per mL) of hematologic cells in blood. Thus, to efficiently obtain CTCs from

peripheral blood, various platforms have been developed such as immunomagnetic beads, microfluidic-based platforms, and microfilter devices [1, 5, 6–14]. At present, CellSearch[®] technology (Veridex, LLC, Raritan, NJ, USA) is the only FDA approved technology for enumeration of CTCs from cancer patients. This technology relies on the immunomagnetic separation of cells based upon their expression of cell surface antigens, such as epithelial cell adhesion molecule (EpCAM). However, most of these approaches encounter drawbacks of slow capture, prolonged processing and less efficacy [15].

Here, we describe the methods for synthesis of two different immunomagnetic separation-based nanosystem platforms namely MDNS [16] and a CNT based magnetic microrocket system (Tf-CNT-Fe₃O₄) [17] for specific targeting and rapid isolation of CTCs (Fig. 1). The MDNS nanosystem consists of five functional components: (1) transferrin (Tf), to specifically target transferrin receptors (TfRs) overexpressing cancer cells (2) iron oxide (Fe₃O₄) nanoparticles for magnetic isolation, (3) imaging probe cyanine 5 *N*-hydroxysuccinimide (Cy5 NHS) to enable high-resolution imaging of the isolated CTCs, (4) fourth generation (G4) dendrimers to facilitate simultaneous attachment of multiple functional groups (e.g., Tf, Fe₃O₄ nanoparticles and Cy5) and also to increase the dispersibility of the nanosystem, and (5) glutathione (GSH) to act as a multifunctional reactive linker. This versatile MDNS system exhibited: (1) rapid (~5 min) and efficient (~80 %) capture of TfR^p cancer cells from an artificial CTC-like suspension, (2) magnetic

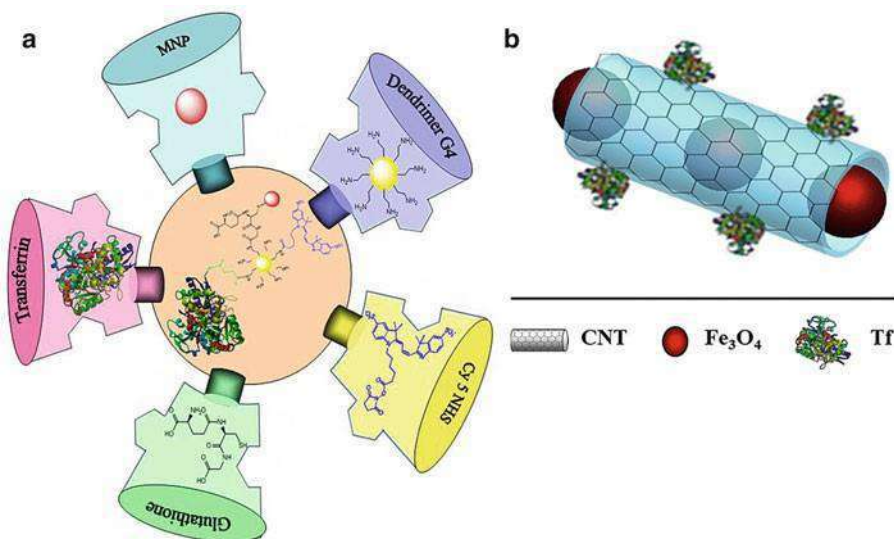


Fig. 1 Schematic illustration of (a) MDNS is designed by using multi-components, such as PAMAM G4 dendrimer, Tf, iron oxide nanoparticles, and imaging probe cyanine 5 (reproduced from [16]) and (b) Tf-CNT-Fe₃O₄ nanosystem is designed by using multi-components, such as CNT, iron oxide nanoparticles, and Tf (reproduced from [17])

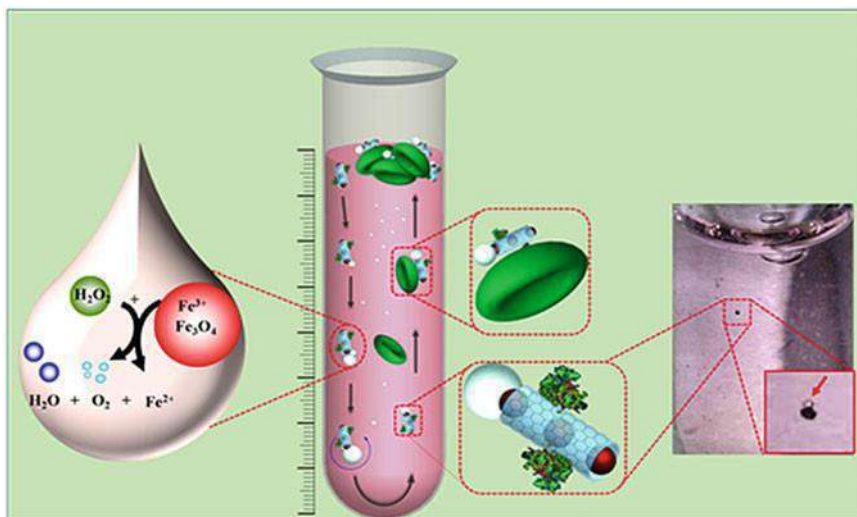


Fig. 2 Self-propulsion and CTC capture mechanism for the Tf-CNT-Fe₃O₄ microrocket. *Left side inset* shows the formation of O₂ bubble and Fe^{2b} due to Fe^{3b} reduction by the surrounding H₂O₂ which propels Tf-CNT-Fe₃O₄ microrocket. *Right side inset* shows Tf-CNT-Fe₃O₄ microrocket adhered with O₂ bubble (indicated by the red arrow) that influences upward motion (reproduced from [17])

isolation of the captured cells from peripheral blood cells and (3) subsequent high-resolution imaging. While the microrocket system is fabricated by conjugating three functional components: (1) CNT, (2) iron oxide (Fe₃O₄), and (3) the Tf ligand (Tf) (Fig. 2). The microrocket platform possesses unique advantages such as: (1) instantaneous propellant motion of microparticles (downward and upward) due to the formation of O₂ bubbles which helps in rapid recognition and capture (~5 min) of TfR⁺ overexpressing (TfR^b) cancer cells, (2) specific targeting ability due to presence of Tf ligand, (3) magnetic isolation of the captured cells owing to the presence of Fe₃O₄ nanoparticles, and (4) large surface area and internal volume allows multiple components to be loaded onto the nanotube.

2 Materials

2.1 Chemicals

1. Poly(amidoamine) (PAMAM)-G4-NH₂ dendrimer (M_w. 14214.7 Da, 64 end groups, 10 wt.% in methanol)
2. *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloric acid (EDC.HCl)
3. Succinic anhydride
4. *N,N*-diisopropyl ethylamine (DIPEA)
5. Transferrin (Tf)
6. 4-(dimethylamino)pyridine (DMAP)

7. Glutathione (GSH)
8. Cyanine 5 *N*-hydroxysuccinimide ester (Cy5) probe
9. Ferric chloride tetrahydrate
10. Ferrous chloride hexahydrate
11. Multiwalled carbon nanotube (CNT; outer diameter of 10–20 nm; length 10–30 μm ; and purity >95 %), Fe_3O_4 nanoparticle (average size: 23.7 nm).

2.2 Biologicals

1. Green Fluorescence Protein (GFP) labeled human colon cancer cells (HCT 116) or any other TfR^p cancer cell line.
2. *Dulbecco's Modified Eagle Medium (DMEM)*: 10 % fetal bovine serum, 100 unit/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin in DMEM medium.
3. *DMEM complete media with H_2O_2* : 10 % fetal bovine serum, 100 unit/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin, 4 % hydrogen peroxide (H_2O_2) in DMEM medium (see Note 1).
4. *Roswell Park Memorial Institute medium (RPMI) media*: 10 % fetal bovine serum, 100 unit/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin in DMEM medium.
5. Whole blood from healthy volunteer, heparinized vacutainer tubes, Ficoll-Hypaque solution, sterile PBS 1 \times , sterile 15 and 50 mL tubes, sterile 25 mL pipettes, sterile pipette, Neubauer chamber or hemocytometer, trypan blue (see Note 2).
6. MDNS, Tf-CNT- Fe_3O_4 , magnet (with a surface magnetization of 6000 G).

3 Methods

3.1 Synthesis of MDNS

3.1.1 Synthesis of PAMAM G4 Dendrimer-Cy5 Conjugate

1. Dry 500 μL of 10 wt. % PAMAM-G4-NH₂ dendrimer solution in methanol to obtain 50 mg of PAMAM G4 NH₂ in a vial.
2. Dissolve 50 mg of PAMAM G4 NH₂ in 2.5 mL of ultrapure water and add 400 μL of 1000 ppm Cy5 NHS in water along with 100 μL of 1000 $\mu\text{g}/\text{mL}$ DIPEA.
3. Adjust the final solution pH to 7.8. Stir the reaction mixture continuously at room temperature for 24 h.
4. Purify the reaction mixture by gel filtration through Sephadex G-10 with >700 molecular weight cut off size (see Note 3) to remove the unreacted Cy5 NHS.
5. Dry the product at room temperature under vacuum.

3.1.2 Synthesis of PAMAM G4 Dendrimer-Cy5-GSH Conjugate

1. Dissolve 5.4 mg GSH in 1.0 mL of ultrapure water and activate the carboxyl group of GSH for 1 h using 3.5 mg of EDC.HCl and DMAP as catalysts (see Note 4).

2. Mix the reaction mixture with the PAMAM G4 dendrimer-Cy5 (35 mg) conjugate previously dissolved in 1.0 mL of ultrapure water.
3. Continue stirring the reaction mixture at room temperature for 24 h.
4. Purify the conjugate by both membrane dialysis followed by Sephadex G-10 column with >700 molecular weight cut off size to remove the unreacted GSH.
5. Dry the reaction mixture at room temperature under vacuum.

3.1.3 *Conjugation of Transferrin Succinate (TfS) with PAMAM G4 Dendrimer-Cy5-GSH*

1. First convert Tf to TfS by reacting with succinic acid using EDC.HCl coupling.
2. Dissolve 10 mg of TfS in 2 mL of ultrapure water and add 30 mg of the PAMAM G4 dendrimer-Cy5-GSH (30 mg) conjugate.
3. Add EDC.HCl to the reaction mixture (adding the same number of moles of EDC.HCl as TfS) and constantly stir at room temperature for 4 h.
4. The reaction time is limited to 4 h to avoid any possible intramolecular cross linking.
5. Purify the reaction mixture by membrane dialysis followed by Sephadex G-10 column with >700 molecular weight cut off size to remove unreacted EDC.HCl and dry at room temperature under vacuum.

3.1.4 *Synthesis of Fe₃O₄ Nanoparticles*

1. To prepare Fe₃O₄ magnetic nanoparticles (MNP), first dissolve ferric and ferrous chlorides (molar ratio 2:1) in water at a concentration of 0.3 M iron ions.
2. Chemically precipitate at 25 °C under vigorous stirring by adding ammonium hydroxide till the pH of the solution attains 10.
3. The precipitate is then washed several times with deionized water and ethanol,
4. Finally dry in a vacuum oven at 70 °C.

3.1.5 *Anchoring of Fe₃O₄ with PAMAM G4 Dendrimer-Cy5-GSH-TfS Conjugate (MDNS)*

1. Heat the precipitates at 80 °C on a water-bath for 30 min, then wash several times with ultrapure water and ethanol, and finally dry in a vacuum oven at 70 °C.
2. Then disperse 5 mg of Fe₃O₄ nanoparticles in 0.15 mL of ultrapure water and 0.05 mL of methanol.
3. Sonicate for 15 min and then mix with 22 mg of G4 dendrimer-Cy5-GSH-TfS conjugate dissolved in 1.8 mL of ultrapure water and again sonicate the suspension for 2 h.

4. Isolate MDNS by magnetic separation to remove unreacted TfS, Cy5, PAMAM G4 dendrimer, and PAMAM G4 dendrimer-Cy5-GSH-TfS conjugate.
5. Wash MDNS with repeated cycles of excess deionized water, and dry under vacuum.

3.2 Synthesis of Tf-CNT-Fe₃O₄

3.2.1 Functionalization of CNT (AO-CNT)

1. Add 5 mg CNTs to concentrated HCl in a screw top vial.
2. Sonicate for 2 h with the vial sealed and ensure that the exterior of the vial does not contain any concentrated HCl. Also ensure that the vial has airspace above the solution to avoid any popping of the vial.
3. Then keep the solution standing for 24 h at room temperature.
4. Dilute the solution with deionized water and filter through Whatman 41 filter paper and collect the treated CNT using a spatula.
5. Sonicate the CNTs collected from filter paper in 2 mL of 98 % H₂SO₄:65 % HNO₃ (v/v 3 : 1) for 1.5 h.
6. Dilute the mixture with 25 mL of distilled water and filter. Collect the CNTs off of the filter paper using a spatula. Dry the treated CNTs at room temperature under vacuum and sonicate in a mixture of 98 % H₂SO₄/H₂O₂ (v/v 4:1) for 10 min and then keep standing for 2 h.
7. Wash the mixture thoroughly with deionized water, filter and dry under vacuum at room temperature.

3.2.2 Synthesis of CNT-Fe₃O₄ Microparticle

1. Mix 2 mg CNTs (AO-CNT) with 0.5 mL ethylene glycol (M. Wt. 62.07) and 1 mL deionized water.
2. Add FeCl₃ · 6H₂O and FeCl₂ 4H₂O (molar ratio 2:1 and concentration of 0.3 M iron ions) to the mixture and sonicate for 2 h, followed by vigorous stirring for 4 h.
3. Afterward, adjust the pH value to ~10 using concentrated NH₃ solution. Isolate the resulting CNT-Fe₃O₄ microparticles by magnetic separation, wash with deionized water and dry overnight under vacuum.

3.2.3 Conjugation of Tf with CNT-Fe₃O₄

1. Dissolve 2 mg of Tf in 2 mL of ultrapure water and add 1 mg of the CNT-Fe₃O₄ to it.
2. Add EDC.HCl to this reaction mixture (adding the same number of moles of EDC.HCl as Tf) and stir constantly at room temperature for 4 h.
3. Limit the reaction time to 4 h to avoid any possible intramolecular cross linking.

4. Isolate the resulting Tf conjugated CNT-Fe₃O₄ microparticles by magnetic separation.
5. Wash with deionized water and dry at room temperature under vacuum.

3.3 Isolation of Human Peripheral Blood Mononuclear Cells (hPBMC)

1. Collect blood from a subject in a sterile heparinized blood-collection tube.
2. Add 15 mL of Ficoll-Hypaque solution in a sterile 50 mL test tube. Mix blood and phosphate buffered saline PBS in 1:1 proportion in a separate sterile 50 mL test tube.
3. Overload this blood-PBS mixture on to 15 mL of Ficoll-Hypaque solution.
4. Centrifuge at 1500 RCF for 40 min at 18 °C.
5. After centrifugation remove the upper layer containing plasma and platelets with a sterile pipette.
6. Transfer the white-colored buffy coat layer containing hPBMCs into a fresh tube using a sterile pipette.
7. Wash this buffy layer with 20 mL PBS twice at the interval of 5 min.
8. Finally resuspend it into complete RPMI 1640 culture media.
9. Count the cells using Neubauer chamber or hemocytometer and trypan blue exclusion test for viability of cells.

3.4 Spiking of hPBMC with Green Fluorescence Protein (GFP) Labeled Human Colon Cancer Cells (HCT 116)

1. Culture GFP labeled HCT116 cells in complete DMEM culture media and incubate at 37 °C.
2. After 24 h of incubation, count numbers of cells per mL of media under the microscope using trypan blue exclusion test for viability of cells.
3. To prepare synthetic CTC samples, mix GFP labeled HCT116 cells and hPBMC in (HCT116:hPBMC 1:50, 1:200, 1:1000, 1:2000, 1:10000, 1:100000), respectively.

3.5 Detection of CTCs Using MDNS and Capture of Cancer Cells from Synthetic CTC Samples

1. Mix and incubate the artificial CTC sample with 100 µg/mL MDNS for 5 min at room temperature.
2. Expose it to a magnetic field for 1 min or till the cell pellet is separated due to strong magnetic field.
3. Separate the cell pellet from rest of the cell suspension containing hPBMCs and uncaptured cancer cells (Fig. 3).
4. Wash the captured cell pellet using DMEM complete media and resuspend it in the media.
5. Visualize both captured and uncaptured cell fraction under the microscope and count the cells using ImageJ cell counter plugin.

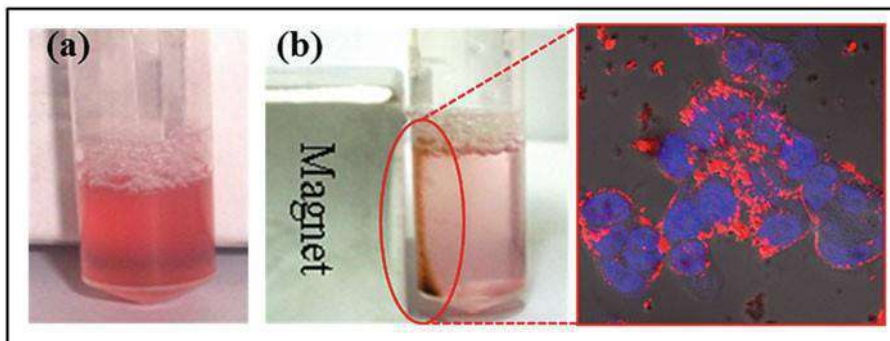


Fig. 3 (a) The synthetic CTC sample having HCT116 (TfR^P) cells and hPBMCs (TfR⁻ blood cells in 1:50 ratio. (b) MDNS captured and magnetically isolated HCT116 cells (*red circle*) from synthetic CTC sample. *Right side figure inset* shows localization of MDNS particles on the cell membrane confirming the rapidity and specificity of the MDNS–cell interaction after just 5 min of exposure

6. Calculate the capture efficiency of MDNS system by the formula as mentioned in note.

3.6 Detection of CTCs Using Tf-CNT-Fe₃O₄ Nanosystem and Capture of CTCs from Synthetic CTC Samples

1. Seed 2×10^5 cells/mL of HCT116-GFP labeled cells on glass coverslips in 35 mm culture dishes.
2. Incubate at 37 °C for 24 h; add 1×10^6 cells per ml in DMEM complete media with 4 % H₂O₂ (see Note 1).
3. Transfer the cells in NMR tube (see Note 5) and treat them with 500 µg/mL of Tf-CNT-Fe₃O₄ for 5 min.
4. Expose the NMR tube containing the cells treated with the material to the strong magnet for isolation of immuno-labeled cells.
5. One minute after exposure to the strong magnetic field, remove the rest of the cell suspension containing uncaptured cancer cells to a separate tube in order to separate the cell pellet formed.
6. Resuspend the captured cell pellet in 500 µL PBS.
7. Visualize the captured and uncaptured fraction of GFP labeled HCT116 cells under the fluorescence microscope and count the number of cells using ImageJ cell counter plugin.
8. Calculate the capture efficiency of MDNS system by the formula mentioned in Note 6.
9. Figure 4 shows the magnetically isolated GFP-labeled HCT116 cells (green) as well as number of cells captured by MDNS and Tf-CNT-Fe₃O₄ nanosystems.

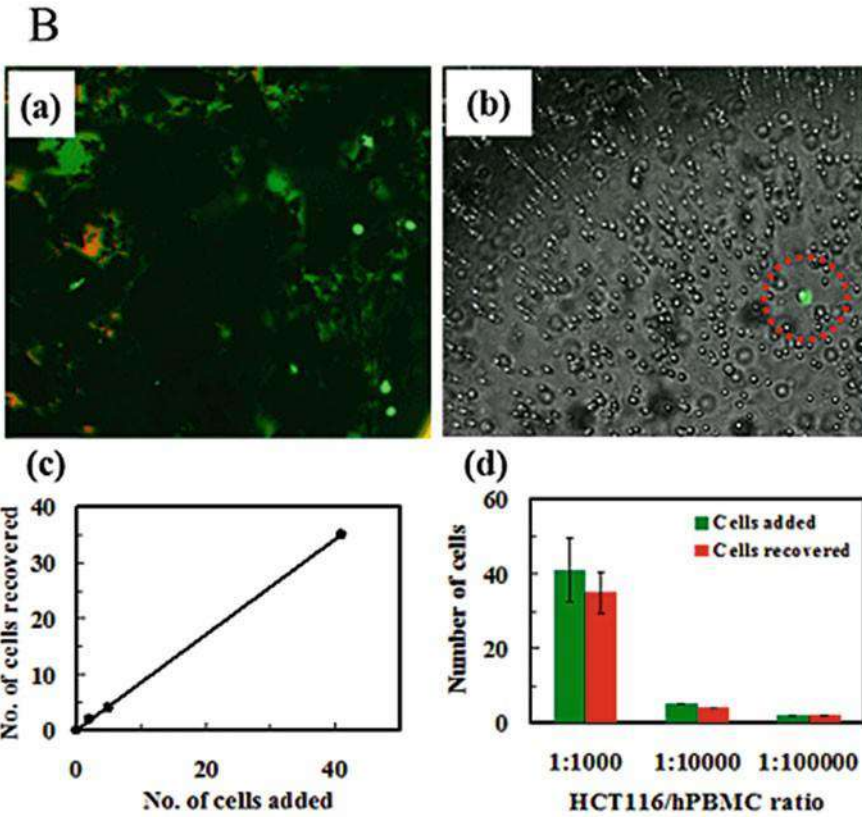
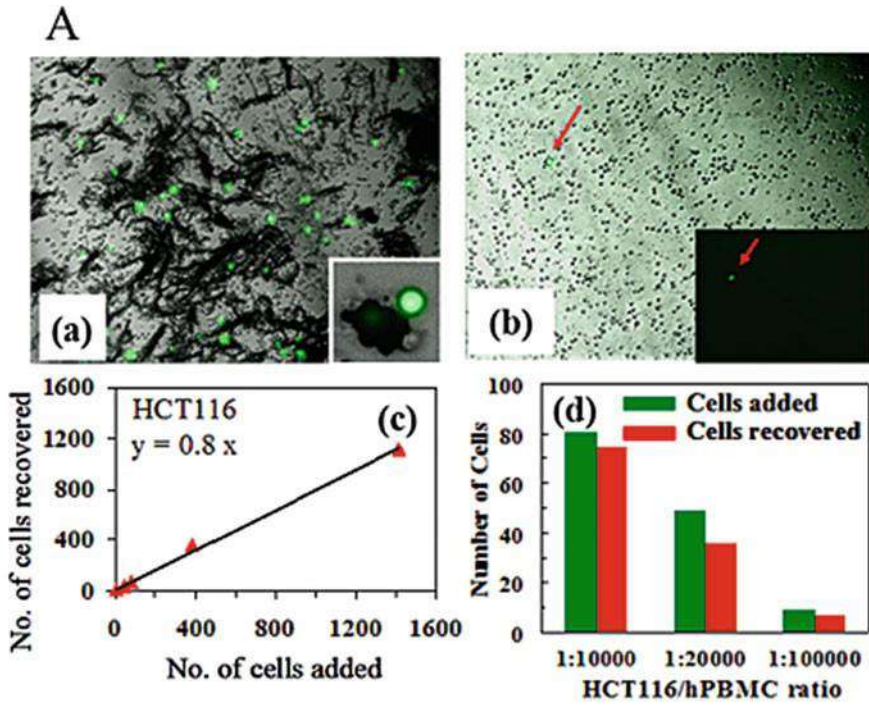


Fig. 4 (A) (a) Image of MDNS captured (black particles) and magnetically isolated GFP-labeled HCT116 cells (green) from an artificial CTC suspension of HCT116 and hPBMNC in 1:10³ ratio. The inset is magnified image of

4 Notes

1. Presence of H_2O_2 helps in propulsion of Fe_3O_4 nanoparticles as it reacts with $\text{Fe}^{3\text{p}}$ in Fe_3O_4 to generate $\text{Fe}^{2\text{p}}$ and O_2 bubbles. The formation of O_2 bubbles propels the nanoparticles resulting in motion. Furthermore, the speed of micro-rocket is dependent on H_2O_2 concentration.
2. During isolation of hPBMC add 15 mL of Ficoll-Hypaque solution slowly in a sterile 50 mL test tube since the solution is very viscous.
3. Sephadex G-10 column helps in removing small molecules > 700 molecular weight from the reaction mixture.
4. EDC.HCl coupling method helps in conjugating PAMAM G4- NH_2 with GSH. EDC.HCl is generally used as a carboxyl activating agent for the coupling of primary amines to yield an amide linkage between the carboxylic-group and the amine group.
5. Performing CTC capture experiments in NMR tube is beneficial as it is easier to capture the motion of the Tf-CNT- Fe_3O_4 nanoparticles using high magnification camera.
6. *Estimation of capture efficiency*
Calculate the capture efficiency (C.E.) by using the equation:

$$\text{C.E.} = \frac{1}{4} \frac{N_C}{(N_C + N_U)}$$

Where, N_C is number of captured cells, N_U is number of uncaptured cells.

Fig. 4 (continued) such single cell isolated using magnet and captured by MDNS. (b) Image of the residual cell fraction after magnetic isolation of the HCT116 cells, which contains a single GFP labeled HCT116 cell (*green*, as indicated by *red arrow* and magnified in the inset). (c) Graph indicates number of HCT116 cells recovered from the artificial CTC suspension vs. the number of spiked HCT116 cells. Capture efficiency determined using slope of the regression line is 80 %. (d) Plot indicating capture efficiency of MDNS for three different synthetic CTC samples having hPBMC and HCT116 in ratios of 10000:1, 20000:1 and 100000:1, respectively. Green indicates initial cell concentration and red denotes the final cell concentration. (B) (a) Image of Tf-CNT- Fe_3O_4 captured (*black particles*) and the magnetically isolated GFP labeled HCT116 cells (*green*) from an artificial CTC suspension of HCT116 and hPBMC in 1:10³ ratio. (b) Image of the residual cell fraction after magnetic isolation of the HCT116 cells, which contains a single GFP labeled HCT116 cell (*green*, as indicated by *red circle* and magnified in the *inset*). (c) Graph indicates number of HCT116 cells recovered from the artificial CTC suspension vs. the number of spiked HCT116 cells. Capture efficiency determined using slope of the regression line is 85 %. (d) Plot indicating capture efficiency of Tf-CNT- Fe_3O_4 microrocket for three different synthetic CTC samples having hPBMC and HCT116 in ratios of 1000:1, 10000:1, and 100000:1, respectively. Green indicates initial cell concentration and red denotes the final cell concentration

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14

Shaping Interprofessional Education – Educators in Action

Payal Bansal, Rashmi Vyas, Pramila Menon, Seema Patrikar and Arun Jamka (India)

Introduction

The World Health Organization long ago recognized the value of inter-professional education (IPE) as a strategy to promote *Health for all by the year 2000* (WHO, 1978). This led to endorsement by other agencies such as the World Federation of Medical Education (WFME, 1988), encouraging countries to adopt it at a national level (Oandasan and Reeves, 2005). Projects and initiatives to tackle the fragmented healthcare practices at the public health delivery level resulted in its expansion. Countries adopted interprofessional education to varying extents, with the best emerging examples from a Canadian government initiative and the United Kingdom (Oandasan and Reeves, 2005). Over the past two decades, what started as a ‘top-down’ initiative from these government agencies has become established as a well conceptualized educational strategy, informed by a rich variety of experiences, models, evaluation evidence and research, towards collaborative care for improved patient and healthcare outcomes.

Today there is an abundance of literature on all aspects of IPE in the form of country initiatives (Cook, 2005; Barr, 2005; Oandasan and Reeves, 2005), projects in community settings, and tertiary care settings, involving clinicians, non-clinicians and all types of health professionals. (Nandan and Scott, 2014; De Los Santos et al., 2014; Menard and Varpio, 2014).

The Indian context

Health Science Universities in India developed in response to a call to reorganize human resources for health in the education and healthcare

delivery sectors in order to overcome manpower deficits and improve processes for better healthcare delivery and outcomes. The recommendations for this reorganization were documented as the Bajaj Committee Report (1987), and were motivated by the WHO imperative to streamline human resources for health, including educational reform. The health university concept was proposed in this report and later implemented.

Preparing the ground for interprofessional education

In the previous edition of this book (Forman et al., 2014), we described the birth and development of the Maharashtra University of Health Sciences (MUHS) (Bansal and Jamkar, 2014) with over 300 affiliated health professions colleges and the department of medical education which is its key arm for educational growth through faculty development. The interprofessional faculty development program in education methods, run by the institute of medical education, has created a faculty which we believe has the requisite skills to launch an IPE initiative.

In this chapter the preparatory phase of this initiative is described and consists of a review of the relevant literature, development of the educator team's perspective, a study of the faculty's readiness for IPE using a modified version of RIPLS (Readiness for Interprofessional Learning Scale) (Parsell and Bligh, 1999), and a case study of an MUHS initiative in public health.

The vision of the leadership, the organizational advantages of a university and a dedicated faculty development program are the key strengths that we believe can lead and guide successful implementation of interprofessional education at the prequalification level.

Theoretical frameworks

A review of relevant literature was carried out with the aim of better understanding the concept of IPE and to determine the feasibility of its implementation. The term interprofessionality was first introduced by D'Amour and Oandasan (2005) who define it as 'an education and practice orientation, an approach to care and education where educators and practitioners collaborate synergistically', with the ultimate goal of improving patient care and healthcare delivery outcomes. They emphasized the link between IPE and Inter Professional Collaborative Practice (IPCP) and the interdependency of the two spheres.

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