KMJ
KUWAIT MEDICAL JOURNAL
The Official Journal of The Kuwait Medical Association

EDITORIAL

REVIEW ARTICLE

ORIGINAL ARTICLES

CASE REPORTS

KU ISSN 0023-5776

Continued inside

Vol. 47 No. 2 JUNE 2015

KUWAIT MEDICAL JOURNAL

CONTENTS

Continued from cover

❊ ❊ ❊

Open access for articles at: ***www.kma.org.kw/KMJ***

Indexed and abstracted in:

EMBASE (*The Excerpta Medica Database*)

Science Citation Index Expanded (also known as **SciSearch®)**

Journal Citation Reports/Science Edition

IMEMR Current Contents (*Index Medicus* for the Eastern Mediterranean Region; available online at: www.emro.who.int/EMRJorList/online.aspx

PUBLISHER: The Kuwait Medical Journal (KU ISSN-0023-5776) is a quarterly publication of THE KUWAIT MEDICAL ASSOCIATION. Address: P.O. Box 1202, 13013 Safat, State of Kuwait; Telephone: 1881181 Fax: 25317972, 25333276. E-mail : kmj@kma.org.kw

INSTRUCTIONS FOR AUTHORS: Authors may submit manuscripts prepared in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals. These Requirements are published in each issue of the Kuwait Medical Journal.

CHANGE OF ADDRESS: Notice should be sent to the Publisher six weeks in advance of the effective date. Include old and new addresses with mail codes.

KUWAIT MEDICAL JOURNAL (previously The Journal of the Kuwait Medical Association) is added to the list of journals adhering to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals", American College of Physicians, Independence Mall West, Sixth Street at Race, Philadelphia, PA 19106-1572, USA, and can be located at http://www.icmje.org/jrnlist.html

Kuwait Medical Journal (KMJ)

Published by the Kuwait Medical Association
Previously known as The Journal of the Kuwait Medical Association (Est. 1967)

EDITORIAL OFFICE

Editorial Manager : Babichan K Chandy
Language Editor : Abhay U Patwari

EDITORIAL ADDRESS

P.O. Box: 1202, 13013-Safat, Kuwait
Telephone: (00-965) 1881181(Ext. 201) - Fax: (00-965) 25317972, 25333276
E-mail: kmj@kma.org.kw
Website: www.kma.org.kw/KMJ

KUWAIT MEDICAL JOURNAL (KMJ)
Instructions for Authors

INTRODUCTION

Formerly known as 'The Journal of the Kuwait Medical Association', the Kuwait Medical Journal (KMJ) was established in the year 1967. It is the official publication of the Kuwait Medical Association and published quarterly and regularly in March, June, September and December.

AIMS AND SCOPE

KMJ aims to publish peer-reviewed manuscripts of international interest. Submissions on clinical, scientific or laboratory investigations of relevance to medicine and health science come within the scope of its publication. **Original articles, case reports, brief communications, book reviews, insights and letters to the editor are all considered. Review articles are solicited.** Basic medical science articles are published under the section 'Experimental Medicine'.

GENERAL

The Kuwait Medical Journal is a signatory journal to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, the fifth (1997) revision of a document by the international Committee of Medical Journal Editors. A description of important features of this document is available on the Lancet website at http://www.thelancet.com. Alternatively, you may consult the following: N Engl J Med 1997; 336:307-315 or order the leaflet "Uniform Requirements for Manuscripts Submitted to Biomdical Journals" by writing to the Editor of the British Medical Journal (BMJ), BMA House, Tavistock Square, London WC1H 9JR, UK.

To present your original work for consideration, one complete set of the manuscript, written in English (only) accompanied by tables and one set of figures (if applicable) should be submitted to the Editor through e-mail to: kmj@kma.org.kw, as attachment files. Authors could also submit the manuscript (in MS word format) on an IBM compatible medium such as a CD or USB flash/pen-drive, if not submitted through e-mail. The KMJ editorial office uses Microsoft 'Office 2007' word processing and 'Excel' programs. Details of the type of computer used, the software employed and the disk system, if known, would be appreciated.

ELECTRONIC SUBMISSIONS

The manuscript submitted **through e-mail should be in word-document (.doc) format, together with a scanned copy or .pdf version of the signed consent letter of the author(s).** The consent letter could otherwise be faxed to the journal office to (+965) 25317972 or 25333276. **Figures/photographs photomicrographs etc, if any, need to be presented in .jpg/jpeg or .bmp format with 300 dpi resolution and illustrations such as graphs, charts etc., as Excel format files.** They should submitted as separate attachments along with the manuscript. Incomplete/ improper submissions will not be processed, and will be returned. Author/s will receive a formal acknowledgment letter with a permanent reference number towards each submission.

Following a peer review process, **the corresponding author will be advised of the status; acceptance/recommendation for revision or rejection of the paper, in a formal letter sent through e-mail.** A galley proof will be forwarded to the corresponding author through e-mail before publication of the accepted paper which must be returned to the journal office within 48 hours with specific comments or corrections, if any. Such corrections in the galley proof, must be limited to typographical errors, or missing contents from the original submission, if any.

ETHICAL CONSIDERATIONS

Where human investigations or animal experiments are part of the study, the design of the work has to be approved by a local ethics committee. A relevant statement of approval should be added in the 'Subjects and Methods' section of the manuscript.

PREPARATION OF THE MANUSCRIPT

The manuscript should be typed as 'normal text' with **no hyphenation** and no hard-returns within paragraphs (use automatic wordwrap) **on A4 size (29.7 x 21 cm) paper in single column format, preferably in font size 12. Cell format** for paragraphs, artwork and/or **special effects** for the text and/or table(s) **are not acceptable.** *Italics* shall be used **only for foreign/Latin expressions and/ or special terminologies such as names of micro organisms.** Maintain a minimum of 2 cm margin on both sides of the text and a 3 cm margin at the top and bottom of each page. No part of the manuscript other than abbreviations and/or subtitles shall be written in **upper case (ALL capital).** Header/foot notes, end notes, lines drawn to separate the paragraphs or pages etc. are not acceptable. **Do not submit articles written/saved in 'Track-change' mode.**

THE ORDER OF THE TEXT

Original Articles: Should contain separate sections such as, Title page, Abstract (structured format for original articles) of no more than 250 words, Key Words (no more than five), Introduction, Subjects (or Materials) and methods, Results, Discussion, Conclusion, Acknowledgment/s (if any)

and References followed by (if relevant), Legends to figures, Tables, and Figures. Details of the section contents are explained below for further adherence.

Review Articles (solicited only): Should contain separate sections such as, Title Page, Abstract of no more than 250 words, Key Words (no more than five), Introduction, Methods/History (if applicable), Literature Review, Conclusion, Acknowledgment/s (if any) and References followed by (if relevant), Legends to figures, Tables, and Figures.

Case Studies: Should contain separate sections such as, Title page, Abstract (a short summary of **not more than 200 words),** Key Words (no more than five), Introduction, Case history/report, Discussion, Conclusion, Acknowledgment/s (if any) and References followed by (if relevant), Legends to figures, Tables, and Figures.

Do NOT paginate the manuscript manually, instead use '**insert page number**' to the document **commencing the title page.** Main headings, introduction, subjects and methods, etc., should be placed on separate lines.

More than six authors are not appreciated for a research article and if listed, the authors may be asked to justify the contribution of each individual author. **For case reports, NOT more than three authors are acceptable.** Regarding contributions of authors over the limit mentioned above, please read the 'Acknowledgment' section.

THE TITLE PAGE

Title page of the submitted manuscript should provide a clear title of the study followed by **full names of all authors, the highest academic degree** and affiliations if any, the name and address of the institution/s where the work was done including **the department, the name and complete address of the corresponding author** to whom proofs and correspondences shall be sent, duly supported with contacts such as telephone, mobile/cell, fax numbers and the e-mail address/es.

STRUCTURED ABSTRACT

A structured abstract (no more than 250 words) is required for studies under the section "Original Articles". It must provide an overview of the entire paper, and should contain succinct statements on the following, where appropriate: Objective(s), Design, Setting, Subjects, Intervention(s), Main Outcome Measure(s), Result(s), and Conclusion(s). (See: Haynes RB, Mulrow CD, Huth AJ, Altman DG, Gardner MJ. More informative abstracts revisited. Annals of Internal Medicine 1990; 113:69-76). Abstract for all other category of submissions shall be a short summary followed by Key words and the report or review.

KEY WORDS

Key Words (maximum five) should be preferably MeSH terms, and shall not duplicate words already in the manuscript title; MeSH terms can be checked at: <http://www.nlm.nih.gov/mesh/>.

TABLES

Tables typed on separate pages using table format should follow the list of references. Tables must be numbered consecutively and provided with appropriate titles. Contents of the table should be simple, and information therein not duplicated, but duly referred to, in the main text. Tables recording only a few values are not appreciated, since such information can be more accurately, usefully and concisely presented in a sentence or two in the manuscript.

DESIGN OF THE WORK

This should be stated clearly. The rationale behind the choice of sample size should be given. Those about to begin randomized controlled studies may wish to study the CONSORT statement (JAMA 1996; 276:637-639).

ILLUSTRATIONS

All illustrations including figures should be saved/numbered as Fig. 1, Fig. 2 *etc.,* in running sequence and submitted as separate attachments along with the manuscript as detailed under the section 'Electronic Submissions'. Photographs should fit within a print area of 164 x 235 mm. Figures where patient's identity is not concealed, authors need to submit a written consent of the patient or of the patient's guardian, in case of minors. **Figure legends should be listed separately after the 'References' section.** If any of the tables, illustrations or photomicrographs have been published elsewhere previously, a written consent for re-production is required from the copyright holder along with the manuscript. When charts are submitted, the numerical data on which they were based should be supplied. Illustrations and figures are printed in black & white colour only.

ABBREVIATIONS

Except for units of measurement, **abbreviations should be defined on their first use** and then applied consistently throughout the article. Non-standard abbreviations or those appearing fewer than three times are not accepted. Use abbreviated units of measure, only when used with numbers. Abbreviations used as legends in Tables and/or figures should be duly defined below the respective item.

NUMBERS AND UNITS

Measurements of length, height, weight and volume must be reported in metric units (meter, kilogram, liter *etc.*) or their decimal multiples. Temperature should be given in degrees Celsius. Blood pressure in mmHg, and hematological and biochemical measurements in Système International (SI) units. For decimal values, use a point, and not a comma, *e.g.*, 5.7. Use a comma for numbers > 10,000

(*i.e.*, 10^3) and do not use a comma for numbers < 9999, (*e.g.*, 6542).

DRUG NAMES

Non-proprietary (generic) names of product should be employed. If a brand name for a drug is used, the British or international non-proprietary (approved) name should be given in parentheses. The source of any new or experimental preparation should also be given.

REFERENCES

Indicate references in the text **in sequence using Arabic numerals within square brackets and as superscripts (*e.g.*,[1, 3-5] *etc*).** Do not quote additional data (like part of the title, year of publication *etc*.) from the references, with citations in the text, unless very important. In the References section, list them in the same sequence as they appeared in the text. **Include the names and initials of all authors if not more than six (< 6), where authorship exceeds six, use *et al* after three author names. Do not use automatic numbering, end notes or footnotes for references.** References to manuscripts either in preparation or submitted for publication, personal communications, unpublished data, *etc*. are not acceptable.

The author's name should be followed by the title of the article, the title of the journal abbreviated in the style of the *Index Medicus*, the year of publication, the volume number and the first and last page numbers. References to books should give the title of the book, followed by the place of publication, the publisher, the year and the relevant pages. References should be limited to those relating directly to the contents of the paper and should be set out in Vancouver style, as shown in the examples below.

EXAMPLES

Article

Burrows B, Lebowitz MD. The β agonists dilemma (editorial). N Engl J Med 1992; 326:560-561.

Book

Roberts NK. The cardiac conducting system and His bundle electrogram. New York, Appleton-Century-Crofts, 1981; 49-56.

Book chapter

Philips SJ, Whisnam JP. Hypertension and stroke, In: Laragh JH, Bremner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd Ed. New York: Raven Press; 1995. p 465-478.

Weblinks

U.S. positions on selected issues at the third negotiating session of the Framework Convention on Tobacco Control. Washington, D.C.: Committee on Government Reform, 2002. (Accessed June 4, 2003, at http://www.house.gov/reform/min/inves. tobacco/ index_accord.htm.)

AUTHORSHIP AND CONSENT FORM

All authors must give their signed consent for publication in a letter of submission, which should accompany the manuscript. This letter should contain the following statement *"This manuscript (write the title) is an unpublished work which is not under consideration elsewhere and the results contained in this paper have not been published previously in whole or part, except in abstract form. In consideration of the KMJ accepting my/ our submission for publication, the author(s) undersigned hereby assign all copyrights ownership to the KMJ and shall have no right to withdraw its publication. It is expressly certified that I/we, have done/actively participated in this study and agree to the accuracy of contents of this manuscript. It was conducted in accordance with current ethical considerations and meets with the committee's approval. I/all of us agree to its publication in KMJ and to the authorship as expressed in this declaration and in the title page of our manuscript".* The participation of the authors must include: conception, design, analysis, interpretation, or drafting the article for critically important intellectual content. A change in authorship after initial submission of a manuscript should be duly supported with a documented request from the main author, duly endorsed by the author removed/withdrawn and/or added, in agreement. **A change in authorship is NOT permitted after final acceptance for publication.**

ACKNOWLEDGMENT

The objective of this section is to disclose affiliations with or association of any organization with a direct financial interest in the study. Otherwise, it will be considered as having no such interests. Contributions of others who have involved in the study, such as statisticians, radiologists etc. and/or those who have assisted in the preparation of the manuscript being submitted could also be included in this section.

COPY RIGHT

SUBMISSION OF A MANUSCRIPT

Manuscripts should be submitted to:
The Editor,
Kuwait Medical Journal
P.O. Box: 1202
Code-13013-Safat
Kuwait.
Telephone (965) 1881181, 25333920 extn. 201
Fax (965) 25317972; 25333902
E-mail: kmj@kma.org.kw
Website: www.kma.org.kw/KMJ

OUR GRATITUDE

The Editorial Board of the Kuwait Medical Journal

gladly expresses its gratitude to

The Kuwait Foundation for the Advancement of Sciences

(KFAS)

for the financial support accorded to this journal

during the year 2012

Editorial

Cancer Research and Its Drug Costs

Belle M Hegde
[1]The Journal of the Science of Healing Outcomes, State College, Pennsylvania, USA and Mangalore, India*
[2]Manipal University, Manipal India**
[3]The Middlesex Medical School, University of London, UK#
[4]Northern Colorado University, USA##

Kuwait Medical Journal 2015; 47 (2): 95 - 96

"Everyone should know that most cancer research is largely a fraud, and that the major cancer research organizations are derelict in their duties to the people who support them."

Linus Pauling (Two Nobel Prizes)

"For the most part fraud in the end secures for its companions repentance and shame."

Charles Simmons

Cancer research these days does not seem to set the *Wadi al Batin* on fire. Most of it is being funded by the industry and has thus got to be, per force, positive and productive! Most of the predictive advantages did not stand up to scrutiny or follow-up either. To get positive results researchers even manipulate their basic research statistical analysis methods. The research output does not seem to run parallel with the money spent for cancer research what with cancer research having lots of funds available. Cancer charity is another shady saga; most of the cancer charities spend nearly 90% of their money for administration.

The story elsewhere might not be different. The cost of chemotherapeutic drugs has escalated one hundred times in the last one decade. This made a couple of US researchers to go deep into it. Their paper in the Mayo Clinic Proceedings April 16, 2015 says: "Increasingly high prices for cancer drugs are affecting patient care in the U.S. and the American health care system overall, say researchers. Americans with cancer pay 50 per cent to 100 per cent more for the same patented drug than patients in other countries, says one of the authors. As oncologists we have a moral obligation to advocate for affordable cancer drugs for our patients[1].

What used to be $1000 per year in the year 2000 has become today $100,000! That is not all. Some drugs, especially the recent new drug for CML, a kind of leukaemia costs a vulgarly high price tab. The pharma giant from Switzerland that produces this new tyrosine-kinase inhibitor has been caught with their pants down while bribing the doctors to prescribe their drug only although the same costs almost 10% their price in South Korea where another company makes it locally[2].

US prosecutors have brought civil-fraud charges against one Swiss company for paying kickbacks to doctors to prescribe their drugs. That company has been very generous with Indian doctors too. The company claims that the money was paid for educational activities. "The charges against one company allege speaking fees, lavish dinners, and vacations illegally provided to doctors totalling nearly $65 million. This is shown as drug development cost. Recently, a prominent US drug lord bid for another cancer drug producing company for an outlandish price. The CEO of the buyer company said that their new cancer drug will be the biggest market earner in the next decade. What used to be about $1000 per extra year of life extension in the year 1999 with cancer drugs today has hit the $100,000 per year mark. The drug company bosses feel it will still go up as the rich do not want to die of cancer. Cancer drugs, they feel, is to keep the patient alive from year to year and the patients are prepared to pay any amount[2].

How much of this drug price is ethical is the big debate. There is a debate as to how much it really "costs" a pharmaceutical company to bring a new cancer drug to market. The sum of $1.3 - 1.7 billion

Address correspondence to:
Prof. B M Hegde, MD, FRCP, FRCPE, FRCPG, FRCPI, FACC, FAMS, "Manjunath", Pais Hills, Bejai, Mangalore 575004, India; Tel: +91 824 245 0450, E-mail: hegdebm@gmail.com, website: www.bmhegde.com
**Editor in Chief; **Cardiologist & Former Vice Chancellor (Retd), #Former Visiting Professor of Cardiology, ##Affiliate Professor of Human Health*

is often cited by the companies but independent assessment puts it at $ 60-90 million. This cost includes FDA approval and ancillary expenses such as the cost of conducting the clinical trials, bonuses, salaries, infrastructures, royalties, advertising, and all kinds of perks to the doctors who prescribe the drugs. Once the drug sells for about one billion US dollars, all the initial costs are paid for. For the rest of the drug's life, every penny earned is pure profit. India, and many other countries of the world, will never be able to afford this kind of costs for the next hundred years to come for public hospital distribution. Of course, our corporate giants will be happy that their business will thrive.

The other risk of newer drugs is the unknown side effects of the drug in the long run. The study of statins, the commonest drug used by doctors these days, (an estimated ten million Indians are on it today), has been shown to produce 10% new generation of diabetes per year, adding roughly ten million diabetics annually to the Indian pool for diabetes. A new report warns that the number could go up to 47%, which means annually 47 million diabetics could be added to Indian pool. Now we have a new disease syndrome to contend with, due to drug to drug interaction. That is the ADR syndrome – the adverse drug reaction syndrome. This will tax even the best medical brain, as these are not described in the medical textbooks so far. I teach my students that if a patient presents with any bizarre disease picture that does not fit into any straightjacket, the first differential diagnosis should be ADR and treatment started forthwith by "step-down" regime described by me in the 1990s of cutting down drug by drug gradually to get the patient better[3].

It should be a great worry for the governments all over, the thinking humane doctors of India and the well-meaning citizens to have an effective yet affordable drug regime for the hapless poor patients. One example is very timely here. The latest cancer drug that costs hundreds of dollars per dose is ***basically a tyrosine-kinase inhibitor***. In fact, almost all chemical reductionist drugs are receptor blockers. That is why there is a craze for receptor research all over to hit the jackpot. Going after receptor is not any earth shaking discovery. Many have done it in the past. What we are losing sight of is that our humble house hold TURMERIC (curcumin) is full of ***tyrosine-kinase inhibitors*** and many more anti-cancer chemicals in combination!

While curcumin has not yet been proven to be as specific as Gleevec, imatinib, Tasigna, or nilotinib, (latest expensive anti-cancer chemicals) I have had a few patients rejected by even Christy's in Manchester with leukemia recover completely with turmeric. A PUBMED search of "turmeric and leukemia" reveals multiple mechanisms by which low-cost curcumin may prevent and treat a wide range of cancers. I know a few personally. May I make a fervent appeal to our well-meaning drug giants all over to get into this field of unravelling the hidden potent treasures of nature to the market for the benefit of the common man? God will bless them.

The Government of India has created a new department, AYUSH, precisely for this purpose but even they seem to follow their western methods applied to these holistic chemical sources. The proactive Indian beloved Prime Minister must be informed about this. If he gets to know the truth about these areas, he will take up this indigenous research effort with full force to wake up the sleeping giant, AYUSH. They should not go on the western highway by inventing the reverse Golden triangle to extract the reductionist molecules from our drugs. They do not work. Our drugs are holistic and they work as a whole. I commend some young researchers at the TIFR who did rat studies on Brahmi (Bacopa monniera) leaves as a whole and got wonderful results in that, the rats had significant growth of the Hippocampus major, the memory kit in the brain, with whole Brahmi which one will not get with the extract. Same works with garlic, another one of those wonderful herbal medicines. I can go on and on, but that is for another occasion. "Little do we see in nature that is ours" [4].

Up until the Second World War, when there were small family holding mama and papa drug companies, things were all right. Now that the large corporates have taken over, things have changed. They can dictate drug prices to keep their stakeholders happy. We, the common people, are caught in that cogwheel. So there is no hope of any drug prices coming down in the foreseeable future. May I please appeal to all my colleagues to help us rediscover our own powerful medicines in their holistic form, which are safe for human use at an affordable price range?

"Fraud is the homage that force pays to reason."
Charles Curtis

REFERENCES

1. Hagop Kantarjian, and Vincent Rajkumar. Why are cancer drugs so expensive in the United States and what are the solutions? Mayo Clinic Proceedings 2015; 90:000-504.
2. Editorial: As we see it: Unsustainable drug prices. http://www.lifeextension.com/Magazine/2014/4?checked=1
3. Hegde BM. Hypertension - Assorted Topics. Book 1993, 1997. Published Bharatiya Vidya Bhavan, Mumbai, India.
4. William Wordsworth; The world is too much with us. 1802.

Review Article

Cancer Stem Cells, Therapeutic Implications

Ali Ahmad, Zahraa Habeeb, Mohammed Al-Mousa, Ghyath Al-Shawaf, Mariam Al-Awadi, Ardeshir Algooneh
Department of Internal Medicine, Al-Amiri Hospital, Kuwait

Kuwait Medical Journal 2015; 47 (2): 97 - 114

ABSTRACT

It has been shown throughout the years in numerous studies in cell biology that cancer stem cells have therapeutic implications that can be potentially used to revolutionize treatment of cancer. Using the information available to us from cancer stem cell research, a number of new therapies can be developed to provide a longer and better quality of life for patients suffering from cancer. This review provides an introduction to the biology of stem cells in general, explains the cancer stem cell concept and how stem cells behave in tumors, how this contributes to the thriving of tumor cells, and their heterogeneity. The review also provides examples of the mechanisms of resistance to conventional therapies widely used today, which are present in cells of various cancers such as breast cancer. It also goes through how some tumors acquire different subtypes that enable them to become resistant to therapy and how this contributes to tumor relapse. The various breakthroughs in potential targeted therapies are also explained, such as signaling pathways and tumor markers. Here we address the current issues involving selectivity of therapy and lack of availability of universal tumor markers, based on the pre-established understanding of cancer cell behavior when targeted for therapy.

KEYWORDS: cancer, cancer stem cells, therapeutic implication of cancer stem cells

INTRODUCTION

Stem cells are undifferentiated cells seen in the human body, which can potentially be stimulated to differentiate into more specific cells, with specific functions[1].

Primarily, there are two types of stem cells that reside naturally in the human body, pluripotent and multi-potent stem cells. In 2006 researchers have identified conditions that would allow some specialized adult cells to be genetically manipulated to act like stem cells. These types of stem cells are known as induced pluripotent stem cells[2].

Pluripotent or embryonic stem cells can produce, more or less, all human body cells, like those present in tissues such as the brain, bone, heart, and skin. Multi-potent stem cells reside in adult tissue, as well as fetal umbilical cord. Limitations to capacity of differentiation is explained by the fact that they are specific to the tissue of their origin[1]. For example, it is generally accepted that a hematopoietic stem cell (HSC) found in the bone marrow cannot give rise to cells of different tissue origin such as the brain.

Regardless of their primary source, stem cells share three unique characteristics. First, stem cells are capable of self-renewal for prolonged periods of time. Second, stem cells remain undifferentiated, and the last, their unique ability to generate diverse number of specialized cells.

Tumorogenesis and the cancer stem cell concept

The new approach to the explanation of tumorogenesis differs from the conventional model. The conventional model of tumorogenesis stems from the idea that tumor generation and growth are maintained by the growing number of dividing cells that make up tumors compared to normal tissues. The accumulation of mutations in the cells causes over expression of oncogenes and inhibition of tumor suppressor genes, and their ability to evade the natural process of apoptosis[3]. Bearing in mind that the traditional model of tumorogenesis has been for a very long time, the most widely accepted explanation for how tumors proliferate and thrive in different tissues, it fails to demonstrate how treatments designed to eliminate dividing cells, often fail to cure patients suffering from cancer. Therefore, a newer theory had to be proposed to cover the still unexplained features of tumorogenesis. The "cancer

Address correspondence to:
Dr Ali Ahmad, MB, BCh, BAO (NUI), LRCP and SI, Msc Mol Med. Department of Internal Medicine, Mubarak Al-Kabir Hospital, Jabriya, Kuwait. Tel: 00965-97492104 (Mobile), E-mail: aahmad@tcd.ie/aliahmad@rcsi.ie, ali_ashkanani5@hotmail.com

stem cell (CSC) concept, the most recent theory for tumorogenesis, suggests that a small population of malignant cells is responsible for maintaining tumor growth. These cells exist as part of a heterogeneous tumor and are known as cancer stem cells (CSCs)[4]. The CSC concept proposes that the tumor population is hierarchically arranged with CSCs lying at the apex of the hierarchy[5]. CSCs have been discovered in many hematopoietic malignancies as well as solid tumors, which reside within genetically heterogeneous tumors, along with distinct tumor cells known as sub-clones. Both cell types mentioned compete with the tumor mass. This means that on top of each sub clone resides a genetically distinct population of CSCs, which act as the building blocks of many complex tumors. The CSC concept is currently used to provide a model for the complex process of tumorogenesis and relapse of tumors, by demonstrating the universal traits of tumor cells. The universal traits of CSCs are: arrangement of tumor cells in a hierarchy, potential for unlimited renewal of cells, quiescent or slow-cycling states, and increased resistance to conventional antitumor therapies[3] (Fig.1).

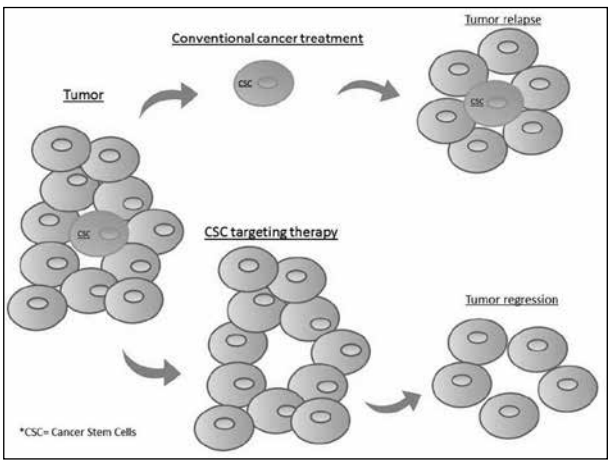

Fig 1: Conventional treatment versus CSC targeting therapy in the treatment of cancer. CSC targeted therapy prevents tumor relapse and promotes its regression

There are a number of CSCs from common tumors such as breast carcinoma that have been reported to show mechanisms of resistance against conventional antitumor therapies. For example, CSCs from breast cancer in locally advanced disease were found after elimination of most of the tumor using cytotoxic agents[6]. Another example is the BCR-ABL-driven leukemic stem cells (LSCs) that are resistant to tyrosine kinase inhibitors. CSCs sustain their resistance to a number of cytotoxic chemotherapies through diverse mechanisms, some of which have been demonstrated in different tumors. An example of one of these mechanisms is increased exportation of the drug from the cells, which is made possible by multidrug resistance (MDR) transporters[7].

The origin of cancer stem cells

When embryonic stem cells were transplanted into recipient mice, they were able to form teratomas (Sell *et al*, 1994)[8]. This suggests that CSCs share some of the properties seen in normal cells and might indeed originate from normal stem cells. It is also possible that CSCs originate from a more differentiated cell progeny, which have accumulated enough genetic abnormalities to allow them to develop stem cell like properties. This implies that each cell, of which the mutation originates, is stem cell in itself. However, different CSCs, which are responsible for the tumorogenic properties seen in the clone, is a more differentiated version[4]. This is the case in acute myeloid leukemia (AML), as it has been demonstrated that the AML-ETO fusion protein was located in normal HSCs.

When the functional LSCs of AML were studied using an *in vitro* colony-forming assay, they were discovered to be in a progenitor cell state with the expression of the Thy1 surface protein[9], suggesting that the disease originated from a normal HSC. However, a contrast to the above case was found when studying CSCs in promyelocytic leukemia (APL), because the MLL-AF9 fusion protein responsible for the disease was not detected in the HSCs[10]. This fusion protein was able to induce leukemia when it was introduced into mice. The occurrence of leukemia in those mice has demonstrated that the cell with the original mutation and the LSCs were more likely to be present in progenitor cells than HSCs[11].

Homing and T-Chimeric antigen receptors

Homing is the transfer of CSCs into new healthy tissue with implantation and possibility of growing new malignant foci. Homing is dependent on the ability of the CSC to implant and burrow in the new tissue as well as the natural features of the grounding tissue such as vascularization and chemotaxis[12,13]. A modality of potential treatment of malignancies is targeting of stem cell homing with the use of T-cell Chimeric Antigen Receptor (CAR). CARs are synthetic, engineered receptors which target surface molecules in their natural conformation. CARs were most heavily studied in hematological malignancies, due to the T-cell innate homing ability with corresponding tissues and ease of sampling of tumor. Research into solid tumors has started and has good reported response in preliminary phases[14,15]. Since the discovery of CARs, there have been three generations of CARs. First-generation CARs comprise of an extracellular portion that is a single-chain F-variable antibody fragment with tailored specificity to targeted population of cells as well as a transmembrane portion. The tests resulted in poor proliferation post-binding to the receptor. Second and third generation CARs were constructed to overcome this obstacle, by

adding intracellular portions of CD3z chain linked to one or two co-stimulatory receptors, for second and third generation CAR respectively[16,17].

The efficacy of CARs lies in its capacity to employ molecular structures independent of antigen processing by the target cell and of MHC. Despite the highly promising results, no comparative studies were conducted to compare different CARs and treatment is still only in phase 1 of testing. There are many reported side effects to the T-cell CARs infusion, such as cytokine release syndrome, tumor lysis syndrome and anaphylaxis. Autologous T-cells can be used to decrease rejection by recipient. Years of research are needed to start considering CARs as first line anticancer therapy[14,16,18].

Methods of evaluation of treatment efficacy

Clinical trials involving antitumor therapies have typically relied on gross tumor regression as an evaluation of efficacy of antitumor therapy[19]. However, it is now accepted that the use of specific CSC tumor markers that can be reliably assigned to each tumor type, and the quantification of remnant CSCs within minimal residual disease, are more reliable in the evaluation of antitumor therapy[19]. The identification of CSC biomarkers has played an important role in showing that a significant inter-patient heterogeneity exists in tumor samples. Various studies involving the profiling of gene expressions along with the use of biomarkers and clinical approaches have deduced that solid tumors can be classified into subtypes of different molecular makeups[20]. This suggests that different CSC phenotypes produced from molecularly diverse tumor types, such as those seen in breast carcinoma, might be linked to different cancer subtypes of the same tumor. With regards to breast cancer a few hundred CSCs from mammary and pleural effusion samples, in the ESA+/CD44+/CD24 population were able to produce the original tumor in non-obese diabetic / severe combined immunodeficient mice, whereas cells from different phenotypes could not[21].

After identifying the CSC biomarkers, specific therapies can be used to target specific surface proteins on CSCs, and restrict tumor proliferation. The problem with this approach of targeted therapy is the severe side effects produced due to the expression of the same surface proteins seen on some normal tissue cells. One such surface protein is CD123, which is produced on LSCs in AML and also on normal HSCs[22].

Molecular pathways and therapy resistance in CSCs

CSC molecular pathways, such as the Wnt signaling pathway, function at different levels in a hierarchy to allow for the differentiation of specific tissues. This implies that it is possible that the self-renewal process in cells controlled by the Wnt signaling pathway in stem and progenitor cells is impaired in cancer cells, enabling malignant growth of cells[23]. This is why the pharmaceutical industry has put the impaired signaling pathways, in tumor cells, as their next target for the development of new treatments. A different pathway, known as the NFkB pathway, has been successfully targeted with a direct IkB kinase inhibitor (parthenolide), and as a result it can be used to selectively target AML and CML LSCs *in vitro* treated leukemic cells[24].

CSC resistance is a major factor in the initiation of several studies that have aimed to make CSCs more sensitive to chemotherapy. Recent reports have provided a useful strategy for targeting dormant CSCs, by demonstrating that quiescent as well as normal stems cells that are resistant to chemotherapy can be induced to become sensitive to such treatments[25]. Colon cancers CSCs are an example of CSCs that were successfully primed and sensitized to chemotherapy, by inhibition of the actions of IL-4[26].

OBSERVED CANCER STEM CELL ACTIVITY IN SOME COMMONLY ENCOUNTERED TUMORS

Cancer stem cells: brain tumors

Gliomas are aggressive incurable carcinomas of glial cells in the spinal cord, or more commonly the brain. They represent around 80% of the total incidence of primary tumors in the central nervous system[27]. In 2007, the World Health Organization (WHO) classified gliomas according to their histological similarities to normal glial cells such as astrocytes, oligodendrocytes, and oligoastrocytomas[28]. Glioblastoma multiforme (GBM, grade III or IV astrocytoma) represent more than half of the overall incidence of gliomas. GBM is a very aggressive cancer, and is known to have an estimated 5% five-year survival rate with multiple therapies of radio, chemo and adjuvant chemotherapy.

Glioma core signaling pathways and glioma stem cells mechanism of resistance

Three main pathways control the oncogenesis process in gliomas. They are receptor tyrosine kinase (RTK), p53, and retinoblastoma protein (Rb) pathways[29]. Through genetic alteration in the pathways mentioned above, cell proliferation and cell survival enhancement allow the GBM to escape checkpoints, senescence, and apoptosis. RTK pathway activation results in both EGFR and PDGFRA receptor dimerization and cross-phosphorylation, which activates PI3K and Ras, which in turn act on *AKT*, which controls proliferation, migration and survival. Rb binds transcription factors and seizes the trans-activation of primary genes in cell cycle, thus preventing proliferation[30]. The last major pathway is p53. It suppresses the propagation of the cell cycle by

slowing the G1 phase, or the initiation of the apoptosis process[31]. These signaling pathways act on major parts not only seen in the natural evolution of the central nervous system, but also in glioma genesis, making them potential targets for therapy.

GSCs are one of the first kinds of CSCs to be isolated from solid tumors using the tumor marker CD13. When implanting 100 GSCs in immunodeficient mice, the full original tumor can be reproduced, while implantation of 1,000,000 non-GSCs could not achieve the same[32]. This observation could explain the recurrence or relapses of treated tumors. In addition, it was found that GSCs with CD133 positive marker can activate DNA damage checkpoints and enhance the repair pathway through CHK1 / CHK2, therefore, making them more resistant to radiation compared to the CD133 negative cells. In fact, the fraction of CD133 positive cells was increased after radiation. It is suggested that targeting DNA damage checkpoints may be the therapy that overcomes the radio resistance of CD133 positive cells[33]. Moreover, GSCs expressed chemoresistance by exporting chemotherapy agents from the cell such as temozolomide, by overexpression of ATP-binding cassette transporters (ABCG2) through the PTEN / PI3K/AKT pathway[34]. GSCs can be targeted by compounds that affect differentiation promoters such as bone morphogenetic proteins, specifically (BMP4), which expresses the strongest effect on GSCs by inducing astrocytic differentiation of specific cell markers (CD133)[35]. It was pointed out by multiple studies that the essential pathways of GSC maintenance are the Sonic hedgehog, Notch, and Wnt pathways[36-38]. Furthermore, the key characteristic feature of stem cells in GSCs, the stemness, is controlled by the following factors: Olig2, Bmi1 and Nanog[39-41]. When examining 305 GBM samples, the expression of BMI1 was detected in 99% of cases[42]. BMI1 provides GSCs with the features of survival and self-renewal through transcript repression of $p21^{Cip1}$, $p19^{Arf}$ and $p16^{Ink4a}$. A new target for therapy is the JNK pathway, which acts on the processes of self-renewal, stem-like features and GSC differentiation. JNK-1 and JNK-2 were targeted with micro-molecules and JNK inhibitors (SP600125), which depleted the self-renewal feature and inhibited tumor formation, therefore possibly resulting in better prognosis and survival. Experiments involving different doses of SP600125 (such as 40 mg/kg/day) were conducted on nude mice affected with the tumor compared to the use of temozolomide at maximal dose, which displayed no inhibitory effect[43].

Controversies about glioma stem cells

Although many studies identified and isolated GSCs using the transmembrane glycoprotein CD133, the use of CD133 as a universal marker is still controversial. In two different studies the value of CD133 as a marker for GSCs was doubted. Moreover, more than 40% of freshly isolated cells from tumors did not contain CD133 markers; this suggested that CD133 could not be the enhancement marker for GSCs. It was suggested by one study that CD133 positive cell in isolation is insignificant for the progression of tumors. It is the general condition surrounding these cell that are the important determining factors of the tumorgenesis process. It was found that CD133-negative glioma cells subcultured under similar GSCs conditions could also have the characteristics of self-renewal, differentiation and formation of tumors if implanted in a xenograft. All these are features of CSCs in non-CD133 cells[44]. Another observation was the formation of floating spheroids in cultures positive for CD133, whereas negative cultures grew as adhesive spheres with some even giving rise to CD133-positive cells[45]. Other stem cell markers were suggested but not validated such as CD44, CD15 and integrin a 6[46-48]. The niche of GSC consists of endothelial and ependymal cells located in subventricular zone and subgranular layer[49]. The proliferation of endothelial cells increases in cultures with CD133 positive cells in relation to the angiogenesis process and it is slowed when GSC are eradicated[50]. However, GSC themselves can express angiogenic effects by producing SDF-1 and VEGFA factors. This leads to the controversy of what the cause is, and what the effects of it might be. In relation to new research into GBM, CARs are being explored as therapeutic options. This however, is proving difficult as the tumor in its natural habitat is immune-privileged and xenografted human brain tumors are not as invasive and usually would grow in a well-circumscribed manner. In an article published in 2013, two cell lines were yielded and implanted in mice with monitoring of tumor growth. Mice were given T-Cell CARs targeting the specific EGFRvIII+. The results were supportive that there might be a role of adoptive immunotherapy in the future in the case of GBM[14].

Cancer stem cells: breast tumors

Transplant experiments involving CSCs in the 1950s led to the development of two theoretical models that may explain which group of cells within a certain tumor is responsible for tumorigenesis. The hierarchy model predicts that there is a unique group of cells within a tumor that are capable of regenerating the tumor. On the other hand, the *stochastic* model proposed that every cell within a certain tumor has the potential to initiate and sustain a tumor; however, this property is regulated by a number of variables or "stochastic events" that make the cell enter the cell-cycle[51,52]. Research has supported the hierarchy theory which entails that a tumor is composed of heterogeneous groups of cells and only a limited number of cells have the tumor-initiating property.

Pioneering work in the area of CSCs involved studies of hematopoietic malignancies and was later extended to solid tumors such as brain and breast cancer. The study that was carried out in 2003 by Al-Hajj *et al* represents an important landmark in breast CSC research[21]. A summary of the study is mentioned below.

Al-Hajj *et al* used a model of immune-deficient mice called "non-obese diabetic / severe combined immunodeficient (NOD / SCID) mice. Special xenograft assays were prepared in such a way that enabled the implantation of single cell suspensions of human breast cancer tissue into the mammary fat pads of these mice. Samples from both primary and metastatic breast cancers (from pleural effusions) were efficiently implanted and grown in the mammary fat pads of the NOD / SCID mouse model[21].

Breast cancer cells express a variety of cell surface markers. Among these markers are two adhesion molecules called CD24 and CD44. Flow cytometry was used to separate cells that were positive or negative for these markers. It was found that injections of cells which carried the phenotypes $CD44^{+}$ or $CD24^{-/low}$ into the breasts of the NOD / SCID mice led to the formation of visible tumors within two weeks of injection; whereas none of the $CD44^{-}$ cell injections gave rise to tumors and only two out of 12 mice that were injected with $CD24^{+}$ developed palpable growths at injection sites (the authors have provided explanations for the latter)[21].

The human cancer specimens that were used invariably contained some normal cell types like leukocytes and fibroblasts. Several antigens called "lineage markers" were found to be associated with those normal cells, like CD2, CD3, CD18 and others. Cancer cells did not express these antigens. Therefore, one of the main final results of the study by Al-Hajj *et al* was the identification and isolation of the tumorigenic cells which carry the phenotype CD44+ $CD24^{-/low}$ lineage. This was found in eight out of nine patients. Another important observation was that a small number of cells with this phenotype, not even exceeding a hundred cells, were able to initiate and sustain tumors, and the formed tumors were phenotypically diverse and complex. On the other hand, thousands of cells which carried different phenotypes were not capable of forming neoplasms[21]. Pece *et al* suggested that the heterogeneity of breast cancer might be attributed to CSCs. Furthermore, their studies showed that poorly differentiated breast cancers (grade 3) are enriched with CSCs compared to more differentiated tumors[53].

Tumorigenic breast cancer cells referred to as "breast cancer stem cells" (Br CSCs) overexpress a detoxifying enzyme called aldehyde dehydrogenase (ALDH 1). This enzyme is found in both normal stem / progenitor cells of normal human breast as well as the breast CSCs. The function of this enzyme is to regulate the oxidation of intracellular aldehydes and it may be involved in the early differentiation of stem cells[54,55].

Epithelial mesenchymal transition (EMT) is an essential part of normal development through which epithelial cells transform into cells that possess properties that resemble mesenchymal cells[54,56]. Recent studies show that EMT plays a significant role in the pathogenesis of some cancers, including breast cancer, and contributes to the acquisition of malignant and stem cell properties. The acquired mesenchymal features include motility, invasiveness and increased resistance to apoptosis. These features are consistent with high grade malignancy. EMT may also contribute to metastatic dissemination[57].

The "Claudin-low'' is one of the molecular subtypes of breast cancer that have been identified using gene expression analyses. Most of the Claudin-low tumors are triple negative breast cancers (estrogen receptor (ER)-negative, progesterone receptor (PR)-negative and human epidermal growth factor receptor 2(HER2)-negative) and most of them have poor prognosis. A study by Prat *et al* has shown that the Claudin-low breast cancer subtype has characteristic stem cell-like features. These include the CD 44+ / CD24-/ low phenotype which has been found to be enriched in breast CSCs[21]. Also, ALDH-1 enzyme has been noted to be highly expressed in these Claudin-low tumors. This enzyme, as mentioned previously, is found in both normal cells and CSCs. In addition, it was discovered that the *Claudin-low* subtype was enriched with epithelial-mesenchymal transition features[58].

Studies by Korkaya *et al* suggested that the HER2 pathway plays a role in carcinogenesis and tumor growth through its effect on mammary stem / progenitor cells. Their studies showed that HER2 receptor overexpression may contribute to the amplification of the mammary stem cell population. Furthermore, their studies provided evidence that the HER2 pathway may be involved in increasing the population of ALDH1-expressing CSCs. These ALDH1-expressing cells demonstrated increased invasion *in vitro* as well as increased tumorgenesis in immunocompromised mice. In addition, the studies by Korkaya *et al* showed that the effect of transtuzumab (Herceptin), the humanized anti-HER2 antibody, might be mediated through its ability to reduce the number of CSCs in HER2-amplified tumors[59]

The CSC model has important therapeutic implications. Designing therapies that specifically target tumorigenic cells may lead to better treatment results and prevent relapse. Conventional cancer therapies merely lead to tumor regression. According to the stem cell model, these therapies mainly target non-tumorigenic cells which represent the bulk of any tumor, and fail to affect CSCs. This results in tumor

regression; however, in this case there is a strong chance for tumor recurrence due to the ability of the surviving CSCs to create new tumors. Furthermore, recent preclinical studies showed that current breast cancer treatment modalities may actually lead to CSC enrichment and contribute to chemotherapy and radiotherapy resistance[21,54].

A study by Li *et al* used suspension cultures combined with chemotherapeutic agents (paclitaxel and epirubicin) in order to isolate and identify breast CSCs. Their findings showed that after one week of administering this chemotherapeutic combination to a group of breast cancer cells, the majority of the remaining cells which survived had the phenotype CD44+ $CD24^-$, which has been repeatedly shown to be associated with CSCs as mentioned previously. This finding supports the view that current chemotherapeutic agents merely target the rapidly dividing "differentiated" cells and miss the relatively dormant stem cells, therefore contributing to future relapse of cancer due to the ability of CSCs to generate new tumors[60].

Studies carried out by Cicalese *et al* provided evidence that the tumor suppressor factor P53 plays an essential role in regulating self-renewal divisions in mammary stem cells. Their results demonstrated that P53-null SCs (with targeted mutation in P53) are almost immortal in culture, and undergo an unlimited number of symmetric self-renewing divisions thus expanding the pool of stem cells. The role of P53 mutation / loss in different types of cancer has been suggested by many studies over the past decades, and it has been found that a significant proportion of breast cancers involve mutations in P53. Theoretically, restoration of the tumor suppressor P53 function in CSCs is an appealing approach in cancer therapy; however, research is still lacking in this area[61].

As noted previously, CSCs may play a role in cancer relapse after treatment. There is emerging evidence that CSCs are involved in chemoresistance as well as resistance to radiotherapy. Furthermore, they may contribute to resistance against endocrine therapy.

RESISTANCE TO CHEMOTHERAY

CSCs are not actively dividing cells; this contributes to their resistance against chemotherapeutic agents which mainly target rapidly proliferating cells. Another factor which might explain CSCs chemoresistance is that they are rich in anti-apoptotic proteins which resist cell apoptosis[54]. Recent data suggest that primary breast cancers that contain a higher proportion of Br CSCs are associated with poor clinical response to neo-adjuvant chemotherapy. Furthermore, a high expression of ALDH-1 may be associated with chemoresistance and a poor clinical outcome[62].

Experiments by Shafee *et al* which were focused on Brca-1/$P53^-$-mutated mouse mammary tumors showed that CSCs contribute to resistance against platinum compounds, namely Cisplatin. Their findings showed that cells carrying phenotypes, supposedly expressed by mammary CSCs were enriched in BRCA-1-positive tumors that were refractory to platinum treatment[63].

RESISTANCE TO RADIOTHERAPY

Breast CSCs that express CD44+ and $CD24^{\circledcirc/low}$ were discovered to be resistant to radiotherapy and it has been proposed that this may be due to enhanced activation of the ataxia-telangiectasia mutated kinase (ATMK) signalling pathway which is involved in DNA damage repair. A recent study demonstrated that targeting the ATM kinase with a specific inhibitor called KU55933 has led to increased sensitivity to radiotherapy and decreased survival of CD44+ $CD24^{\circledcirc/low}$ cells after being exposed to radiation[64].

RESISTANCE TO ENDOCRINE THERAPY

A group of steroid receptor-negative cells (namely ER^- PR^- $CK5^+$) that carry the phenotype $CD44^+$ which have the characteristics of CSCs have been identified in ER+ / PR+ breast cancer xenografts. Endocrine therapy in breast cancer targets ER+ / PR+ cells; therefore, this rare population of steroid receptor-negative cells would survive endocrine therapies and lead to future relapse due to their CSC-like property[65].

NOTCH-1 and NOTCH-4 are breast oncogenes which are involved in stem cell self-renewal; hence they play an important role in breast cancer tumorigenesis. NOTCH-1 and NOTCH-4 were found to be over-expressed in triple-negative breast cancers which are considered to have the worst prognosis among the breast cancer subtypes. It has been shown that NOTCH-4 is an essential factor that is involved in breast CSCs survival[66,67].

A recent study demonstrated that PEA3 (polyomavirus enhancer activator 3), an ETS (E-Twenty Six) transcription factor, plays an important role in the transcription and regulation of NOTCH-1 and NOTCH-4 in certain subtypes of breast cancer; therefore, PEA3 represents an important target for future breast cancer therapeutics[66].

NOTCH-1 and NOTCH-4 are activated through several ligand-binding steps which involve enzymatic cleavage. Gamma-secretase is considered to be an essential enzyme that is involved in the activation of NOTCH signalling. The inhibition of gamma-secretase is an appealing approach for targeted cancer therapy. Gamma-secretase inhibitors (GSIs) are currently being investigated in several trials as a novel therapy for certain breast cancer subtypes[66]. A study by Grudzen *et al* supported this novel approach by showing that MRK003, a GSI induced apoptosis is effective in Br CSCs[67].

Interesting recent data provided evidence that metformin, the first line drug used for type 2 diabetes mellitus, may have a role in breast cancer therapy. Studies by Iliopoulos *et al* showed that metformin selectively targets and kills Br CSCs in mammary xenografts[68]. Furthermore, combination therapies which include metformin with different chemotherapeutic agents like doxorubicin, paclitaxel or carboplatin is more effective in suppressing tumor growth and preventing relapse than each of these drugs when used as monotherapy[62]. Another interesting observation is that metformin reduced the dose of the chemotherapeutic agent doxorubicin that is required to delay cancer relapse and prolong survival[62].

Cancer stem cells: Haematological malignancies

CSCs resemble normal HSCs in their cell-surface markers, multipotency of progenitor cells and hierarchical self-renewal properties. This led researchers to hypothesize that leukemic stem cells are derived from normal HSCs, or from more differentiated cells that acquired the normal HSC properties. The point of mutation that confer carcinogenic properties to stem cells, is believed to be a slight alteration in normal signalling pathways acting on specific stem cell niche, resulting in cancerous stem cell phenotype. Understanding the basis of the stem cell carcinogenesis renewal provided two targets for therapeutic agents. The first is the identification of specific leukemic cell surface markers, and the use of anti-monoclonal antibodies acting on surface antigens such as Anti-CD44 and TIM-3, as seen in the treatment for AML[69]. The second is blockade and manipulation of signalling pathways identified in CSC renewal including BMi-1, Wnt, Notch and Hegehog pathways[70].

Retrospective analysis of collective data, concerning surface markers and pathways of stem cells, specifically in myeloid malignancies reveal an organizational hierarchy similar to that of normal myelopoietic cells in terms of progenitor cell formation, self-renewal and maturation. This is mainly due to the shared expression of CD34 and CD45 and the lack of CD38 surface markers, in both normal and malignant myeloid cell phenotypes alike. The discriminative distinction between normal and neoplastic myeloid stem cell lines was made by the discovery of additional surface markers, *i.e.*, CD123 (alpha chain of IL-3 receptor). The year 2006 marked the emergence of many clinical articles confirming that neoplastic stem cells of the CD34+ / CD38- progeny found in AML and MDS patients all express CD123. Furthermore, chronic myeloid leukemia (CML) patients and systemic myelocytosis (SM) patients co-expressed CD123 markers on multi-colour flow cytometry of bone marrow cells, making CD123 a reliable marker for neoplastic progenitor stem cells[71]. Other targeted antigens discovered in the same paper include CD13, CD33 and CD44.

This literature review will examine the molecular targets (CD44, CD33, and CD123 "alpha chain of IL-3 receptor") expressed as surface antigens of CD34+ / CD38-stem cells. The findings of this literature review were refined to highlight the application of these surface markers as targeted pathway therapies for hematological malignancies in general and myeloid neoplasms in particular. Quoted clinical trials are limited to AML patients. Meta analysis of laboratory based articles was restricted to HSCs of the CD34+ / CD38- progeny.

Molecular targets on CD34+/CD38- stem cell surface markers

Minimal residual disease (MRD) cells reside in the bone marrow, and are believed to be responsible for the recurrence of AML relapses after chemotherapy. In fact, MRD parameters are proven to be highly reliable in predicting survival rates in AML. Immunophenotypic detection of MRD frequencies through flow cytometry, helped tailor chemotherapeutic requirements of AML patients, as well as predict morbidity and mortality[72]. According to MRD parameters only 30 - 40% of patients with AML survive despite high dose chemotherapy. Besides, the prognostic value of MRD cells, detection of relapse and survival prediction is also useful in the identification of stem cell-targeted therapy. Some of the stem cell therapeutic agents identified so far include Anti-CD123, Anti-CD44, and Anti-CD33.

CD44+ Leukemic stem cells in AML patients

Studies in 2004 explored the effects of Anti-CD44 monoclonal antibodies (mAbs) on normal myelopoiesis by and large and defective myelopoiesis seen in AML blasts[73]. They illustrated the hierarchical organization of the leukemic clones, through antigenic and cytological features defining the well known AML subtypes (AML 1/2, AML3, AML4, AML5, AML6, and AML7). Like normal HSCs, AML stem cells of all subtypes are found in the primitive CD34+ / CD38-fractions. Thus, they allow Anti-CD44 mAbs to induce terminal differentiation of primary blasts, especially in AML1 to AML5 subtypes. Another mechanisms of action employed by Anti-CD44 mAbs includes inhibition of AML cell proliferation by stabilizing p27kip-1 (a specific cyclin dependent kinase inhibitors, CKI), and finally inducing apoptosis in NB4 cell line and AML3 subtype. Anti-CD44 mAbs have successfully utilized the molecular basis of dysregulated self-renewal features of AML stem cells as a therapeutic target.

Further studies in 2006 questioned the niche dependency aspect of leukemic stem cells (LSC), and utilized the unique requirements of AML LSCs for

interaction with a niche. This resulted in the successful eradication of AML LSCs by solely targeting CD44 receptors[74]. Here, the activation of CD44 by H90 treatment resulted in effective block of the homing of leukemic cells, including primitive CD34+ / CD38-SL-ICs in both bone marrow and spleen. H90 treatment was found to work in two different modes of action; first is alteration of AML LSCs through the manipulation of CD44 function, as the main regulator of leukemic cell proliferation and second, is the abrogation of AML LSCs homing, which resulted in collective eradication.

Receptor CD33 (Siglec-3) expressed in AML LSC

The functional criterion of AML LSCs is their ability to repopulate NOD / SCID irradiated mice with leukemic cells. European Journal of Clinical Investigation experimented with the capabilities of CD33+ progenitor cells to repopulate NOD / SCID mice with leukemic stem cells, and was also successful in presenting evidence demonstrating the co-expression of CD33 cell surface markers in CD34+ / CD38-LSCs in AML patients[75]. However, the study failed to specify the AML-repopulating abilities of all CD34+ / CD38- LSCs that expressed CD33. The importance of this discovery stems from targeting CD33 cell surface receptors for treatment of AML.

Since then, collective meta-analysis of cell surface markers in AML patients confirmed that the expression of CD33 as a myeloid marker is apparent in 75% of AML patients Thus, making CD33 antibodies a frequently used agent in bone marrow (BM) purging strategies and in Ab-targeted therapies. Reports also depict a discrepancy in AML patients response to CD33 Abs based treatments, reflected in variable rates of relapse, and modifiable rates of AML morbidities and mortalities. This was later explained by the CD33+ under expression in CD34- AML primitive leukemic progenitors, when compared to most CD34+ leukemic progenitors[76].

CD123-targeting mAb

The discovery of CD123 cell surface markers explained the extensive rate of proliferation of leukemic progenitors, when compared to normal hematopoietic cells. The significance of CD123 expression comes from its correlation with IL-3 stimulated and spontaneous signal transducer and activator of transcription 5 (STAT5) levels, cycling cell proportions, primitive cell-surface phenotypes and resistance to apoptosis. The over-expression of CD123 in AML LSCs advocates for the efficacy of CD123-targeted treatments in the form of specific monoclonal antibody (7G3)[77]. An extensive body of evidence supports the clinical potential for *in vivo* 7G3 treatment for CD34+ / CD38- leukemic cells. The therapeutic benefit of 7G3 treatments is substantiated by three main lines of evidence.The first, 7G3 specifically targets IL-3 receptor (consisting of CD123- CD131) cell surface markers of LSCs seen in AML. The second line of evidence examines levels of toxicity of the chimeric IgG1 variant of 7G3, and proceeds to emphasize the lack of interference with normal hematopoiesis (confirmed by measured parameters over 70 days). The third and last line of evidence is the application of 7G3 application in phase 1 clinical trials[78], where incidence of adverse events did not increase with escalating doses and reports of adverse events did not correlate with complications and risks of biochemical profile of the infused material[79].

T-Cell Immunoglobulin mucin-3 (TIM-3): Novel therapeutic target for AML

TIM-3 as a cell surface marker uniquely expressed in AML LSCs, is reported to be absent in normal bone marrow HSC. TIM-3 is a negative regulator Th1-T-cell immunity that was recently discovered to synergize with Toll-like receptor signalling *via* cross linking with Galectin-9, when expressed on innate immune cells. It has also been reported to mediate phagocytosis of apoptotic cells by binding phosphatidylserine[80].

The successful reconstitution of Anti-TIM-3 monoclonal antibody, as the established clone (ATIK2a) was effective in eliminating TIM-3 expressing cell lines *via* both antibody dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). The therapeutic effect of ATIK2a on normal and leukemic AML hematopoiesis was found to be substantial in leukemic development in NOD / SCID mice, with near complete sparing of normal hematopoietic monocytes. Collective data indicates the combination of novel Anti-TIM-3 and known Anti-CD44 or Anti-CD33 as a successful practical approach to eradicate AML LSCs, with minimal incidence of relapse. This is currently an active area for clinical trial design, and patient recruiting is currently ongoing[81].

Manipulation of signalling molecules in AML pathways

Another strategy to induce longer remissions, in AML patients with higher rates of relapses, is leukemic hematopoiesis pathway blockage and manipulation. A prominent example of application in this field is Notch pathway manipulation, a pathway strictly involved in CSC renewal. Recent interest in Hes1 induction as a critical stem / progenitor cell signalling molecule potentiated by hypoxia inducible factor (Hif1-alpha), lead to the discovery of Hif1-alpha as a therapeutic target in hematological malignancies[82]. This is achieved by the Hif1-alpha dose dependent autoregulation of Hes1 *via* blockade of its expression by blocking a negative-feedback autoregulation loop. Researchers continued identifying pharmacological

agents for Hif1-alpha knockdown such as echinomycin. However, the limitations of these studies were delineated by utilizing an exogenic model of human AML and from the short-term nature of echinomycin therapeutic effects reported.

Other hematological malignancies and possible therapeutic pathways

CARs have been greatly explored in the region of hematological malignancies. However, study designs in all available data are variable in many aspects including CAR design, *in vivo* preparation and pre-administration of lymph depletion mechanisms used[83].

Both chronic and acute lymphocytic luekemias (CLL and ALL) have been looked at as models to use T-cell CARs The most used is anti-CD19 to bind receptor CD19 which is expressed on almost all B-cells, whether they were normal or B cells ALL[83-85].

With all data available, we conclude that the higher the CAR activity preinfusion and the longer its residence *in vivo*, the more effective is the treatment. However, with the higher titers in the body, there is a greater chance of exhibiting toxicity. Results were also more promising in ALL than in CLL[83-85].

A small study has shown a good response in 30 patients with different types of B-cell malignancies who received second generation CARs anti-CD19 with two subjects achieving complete remission[15].

A case report published in NEJM, reported a patient with refractory CLL who exhibited positive results with low dose T-cell with anti-CD19 combined with CD137 antigen infusion[83].

A brief report was published by Grupp and colleagues in 2013, in which they studied CD19 in a patient populace of B-cell malignancies reaped good results in CLL with no need of concomitant chemotherapy to achieve remission. A subsection of relapsed ALL patients developed CD19 blast cells and speculations were that this is specific to a subset of ALL patients[84].

However, due to the nature of CD19 antigen being present on all B cells, B cell deficiency is expected. It cannot be speculated, if normalization of cell population would occur or remain deficient. Further studies are required to assess both therapeutic and complication profile of CARs in hematological malignancies[14,83,84].

Cancer stem cells: Colorectal tumors

Colorectal cancer (CRC) is one of the most well understood and researched cancers. Yet, it still remains as one of the leading causes of deaths worldwide. It was the third highest estimated incidence of cancer in the United States in 2012. Over 50,000 estimated deaths makes colorectal cancer, the 2nd highest of cancer related deaths[86]. Methods of treatment and therapy such as chemotherapy, radiotherapy and surgery do not cover the metastatic nature of the tumor in the later stages of cancer growth.

The mechanism of tumor development still remains one of the most elusive. With better understanding, we may one day be able to create a targeting therapy to disrupt cancer growth and metastasis. The CSC theory provides a new base of research in understanding colorectal cancer mutagenesis. The CSC theory attempts to open new insight regarding mutagenesis, discovery and the treatment of colon cancer. This review will show the developments and setbacks of recent research in colorectal CSC targeting.

The CSC model states that malignancies are derived from cancer cells with stem cell characteristics[3]. This model puts into perspective that existing stem cells within the human body are mutated and in those stem cells, there is a mechanism in which cancer cells may proliferate. Colonic stem cells are located at the bottom of the colon crypts. These cells normally replicate and produce progenitor cells for goblet cell, enterocyte, and endocrine cell formation. There is also a belief that these cells exist around the base sparsely placed throughout paneth cells, possibly implicating the surrounding tissues.

Stem cells have the ability to regenerate themselves as well as the ability to form differentiated specialized cells. This pluripotency may enable colorectal stem cells to form the malignant nature of tumor development. These mutated stem cells would then be capable of mass proliferation and creation of other progenitor cells. The colorectal stem cells are normally capable of asymmetric proliferation. This asymmetric division allows for a division in which a stem cell regenerates as a new cell, while the other cell develops as a progenitor cell. Due to this, the stem cell is more constant through the cell cycle. This provides a location in which targeted therapy may benefit as now it is understood that CSCs are implicated in the development, metastasis and resistance of cancer[87]. It was previously thought that six mutations were needed to cause a cell to become cancerous. Now it might possible that only one mutation is needed[88]. Due to the long lasting and proliferative nature of the stem cells, a mutation in those cells provides a strong foothold for the tumor to develop.

Chemoradiotherapy (CRT) tends to target rapidly growing mutated cells. It may be plausible that the therapies don't target the CSC. This could be the reason behind recurrence of tumor growth or resistance to treatment post CRT[89]. Many studies have investigated different ways to target tumorgenesis. One focus of recent research has been usage of markers. Markers are molecules on the cell surfaces of these mutated cells. These markers are used in attempts to identify CRC and potentially target therapy.

Implications of tumor markers in colorectal tumors

CD133, one of the more commonly known markers has been used to note multiple different types of cancer growths. It is a 5- transaminase glycoprotein found on cell surface on many cells, but more prominently and in greater numbers on CSCs. The function of CD133 is still unknown. It came with promise for radio or cytotoxic targeting. However, targeting cells with CRT using CD133+ would not be applicable as the CD133 expression is found within normal tissue as well[90]. It has also been shown to exist in normally functioning cells such as acinar and islet cells[91]. Although it has been shown not to be a sensitive marker for targeting therapy, CD133+ cells have been able to produce and maintain tumor growth on xenografts, while CD133- were not capable of initiating tumor growth[92]. CD133 serves as a prognostic factor for colorectal cancers as it becomes over expressed in metastasis. Although the majority is present in mature cancer cells, identifying CD133+ in patients with colorectal cancer served to show a grim prognosis as opposed to CD133-[93]. The CD133+ has also been shown to be of poor prognostic outcome post-CRT for colorectal cancer[92]. This may be due to resistance of the cells caused by cancer proliferation. CD133+ cells have also been shown to have significant correlation with liver metastasis from colorectal carcinomas[94,95]. Another issue with usage of the CD133 as a marker is the inability to mark undifferentiated cells.

CD44 is another one of the renowned markers in targeting CSCs. It is a cell surface hyaluronic acid receptor. CD44 is usually expressed as a surface marker in CSCs, but may also extend to luminal areas in CRC. It may be used on its own or in conjunction with other markers like CD24, LGR5 and ALDH-1. This works synergistically with the other markers to provide a higher specificity towards targeting CSC, much like CD133[83,96]. Another important point about this marker is CD44 surface marker targeting has been shown to be successful in treatment of AML, one of the first cancers that kick-started the CSC theory[5]. It has also been shown that CD44+ cells have been able to replicate the heterogenic nature of the CSC hierarchy. The CD44+ cells when isolated have been seen to produce tumorgenisis[97]. New studies have isolated variants of the CD44 that contribute to the tumorgenesis and metastatic nature of CRC. These cellular prion proteins have been used to locate CD44+ colorectal CSCs[97]. As CRC becomes resistant, it becomes increasingly hard to treat. New studies on OCT4 show it's prominence in primary colon cancer. OCT4 has shown that it is capable of inhibiting cell differentiation[98]. It has been thought that the OCT4-AKT-ABCG2 is what enables OCT4 to have a role in chemotherapy resistance[99]. The anti-apoptotic effects of OCT4 are believed to be due to the STAT3 / surviving pathway. These effects may give rise to resistance to chemotherapy[100]. In a study by Kunming Wen, two chemo-resistant cell lines were found. Both of these lines had an over expression of OCT4. These lines were characteristically similar to CSCs. The study also showed that when the OCT4 gene was not expressed, there was an increase of apoptotic rate within cells. It was also discovered that higher levels of STAT3 and surviving proteins were also found in these cell lines[100]. This provides new evidence to prove that OCT4 has a hand in anti-apoptotis in CSCs.

Targeting colorectal cancer stem cells signaling pathways

Another form of targeting cancer growth is *via* pathways. The regulation of the stem cells survival is through the Wnt pathway. Markers for the mentioned pathways may enable us to locate stem cells and monitor progression. To understand tumor development we must understand the signaling pathway. Disturbing this pathway has shown to affect the stem cells' ability to replicate[101]. The Wnt pathway is the pathway in which stem cells are capable of regenerating, or differentiating. Destabilizing this pathway, along with TGF pathway is what is believed to be the CSC's ability to proliferate and cause tumor growth[102]. ALDH is seen to be more specific towards CSCs than CD44 and CD133 towards cancers, although all are raised in malignant cells.

Some are even thought to be used in combination to increase monitoring ability and to determine more specific targeting. These have only offered a minute effect in understanding tumor initiation[96]. ALDH is proving to be beneficial for marking metastasis progression[96]. Another marker with potential promise is BCL-2. BCL-2 acts as an anti-apoptotic gene. When tested on mice lacking the gene, rates of apoptosis were significantly higher. This gives rise to the idea that the gene is resistant to apoptosis[103,104].

Cancer stem cells: Lung tumors

Lung cancer is the second most commonly encountered cancer in the United States as well as the major cause of cancer-related deaths. Lung cancer makes up the world's highest incidence of malignant tumors, and has become a burden because of the increase in mortality rate[105]. In men, the age-adjusted incidence of lung cancer is 84.9 in every 100,000 men. Regarding cancer deaths, the age-adjusted incidence is 68.8 in every 100,000 men. In women, the age-adjusted incidence is 55.6 in every 100,000 women, while the age-adjusted lung cancer deaths incidence is 40.6 in every 100,000 women[106]. About 95% of all lung cancer cases have been reported in men that are above 40 years old, especially those who are between the ages 55 and 65 years. In non-smokers, the male and female incidence

is similar[107]. Treatment failures occur whenever the cancerous cells in the affected tissues develop resistance to chemotherapy. Ideally, the function of the chemotherapy interventions is to induce apoptosis in the affected area. Whenever this is unachievable at clinically relevant drug concentrations, resistance can be said to have set in[108]. Apoptosis fails to happen when there is increased DNA repair in resistant cells, increased cytoplasmic detoxification, and reduced cellular drug accumulation[108]. Specific cancer cell populations that are resistant to chemotherapy are able to produce progenitor cells, metastasize and repopulate. These cells are known as CSCs. In recent years, when the concept of stem cells was introduced in oncology research, in addition to a variety of tumor tissues, and cancer cell lines, tumor stem cells have been successfully isolated and CSCs identified[109].

CSCs are characterized by self-renewal and proliferation similar to normal somatic stem cells, which makes them resistant to chemotherapy and radiotherapy. Therefore, they prevent chemo treatment from inducing apoptosis. This leads to the hypothesis that chemotherapy is not as effective in killing CSCs as compared to a treatment that specifically targets CSCs. Therefore more studies are needed to find out the origin of lung CSCs as well as new markers, to lead us to a new target therapy.

The lung CSC research breakthrough occurred in 2007. Ho *et al*[110], found for the first time that Hoechst dye efflux method from a variety of human lung cancer cell lines and human lung cancer clinical samples in the separated side populations cells showed high tumorgenicity *in vitro* rate, fine ABC transporter cell surface protein expression, human telomere terminal transferase, and enzyme expression increased similar to the rate of stem cells. This indicated that this part of the side population had cells with CSC characteristics. The signalling pathways of Hedgehog (Hh), Notch, and Wnt / β-catenin signalling pathways all play an important role in tumorgensis by acquisition of unregulated self-renewal[111]. These unregulated pathways are involved in inappropriate proliferation, tumor occurrence, invasion, and the metastasis process. They can potentially account as targets for lung cancer treatment.

The Hh / Patched pathway is taking part in embryonic development and cell fate determination. Throughout lung growth, Hh signalling is a major factor in lung bud branching morphogenesis. Hh signalling has been identified in regulating self-renewal of cancers cells in a number of solid tumors, like hematopoietic stem cells[112] and myeloid leukemia cells[113]. Hh overexpression may result in out of control proliferation of tissue stem cells, producing a sufficient number of target cells for further oncogenic occurrences, resulting in acquisition of CSCs. Modifications in the Hh pathway have been documented in a number of cancers such as glioma, stomach, colon, breast, prostate, and lung cancer[114].

The Notch family of transmembrane signalling proteins (Notch-1 , -2 , -3 , and-4) determine cell fate and are identified in stem cells[115] . Notch signalling stimulation is involved in the up keep of self-renewal and plasticity *via* Jagged-1, for hematopoietic stem cells[116]. Inappropriate stimulation of Notch signalling induces proliferation, limits differentiation and inhibits apoptosis in cancer cells, and is associated with a variety of human cancers including lung cancer[117]. Furthermore, Notch signalling has been proven to maintain lung somatic stem cells in an undifferentiated form, blocking terminal epithelial differentiation.

The Wnt pathway is responsible for cell fate determination in a number of organs for the period of embryonic growth. The Wnt pathway consists of an enormous range of proteins in a stream that eventually results in regulating the quantity of β-catenin that reaches the nucleus to stimulate gene expression. Wnt signalling has been demonstrated to control self-renewal in stem cells[118]. Data from transgenic mouse models showed Wnt signalling pathway stimulation in stem cells resulting in epithelial cancers[119]. This indicates the contribution of Wnt signalling pathway members in the deregulation of stem cells into CSCs. Wnt signalling might have a part in lung tumorgenesis[120].

Lung cancer stem cell surface markers: CD133 and CD44

CD133 is a five-transmembrane glycoprotein also known as prominin-1. The function of CD133 has not been clearly explained and its ligand is unidentified at this point. At first it was described as a marker for isolating CD34+ human hematopoietic progenitor cells[121]. It has been documented that CD133 might contribute to cell cycle regulation and proliferation of cells, but not always tumor initiation[122]. CD133 has been used as a marker to analyze lung CSCs.

Chen *et al* showed larger expression of Oct-4 in CD133+ cells in comparison to CD133− cells in a research involving the examination of CD133 in lung cancer cells from patients and cell lines[123]. In that research, CD133+ cells were also reported to have enhanced resistance to conventional treatments and enhanced *in vivo* tumor-restoration capability and proliferation compared to CD133− cells. Also Oct-4 expression was shown to be important for keeping stem cell-like characteristics, for instance self-renewal capacity and invasiveness. Eramo *et al* showed that the expression of CD133 ranged between 0 .32% and 22% of tumor cells. In that research, the CD133+ cells isolated

from lung cancer patient samples can mature and have a tumorigenic capability that was not present in CD133− cells[124]. CD133+ cells have been documented to have a high rate in most lung cancers, with a relatively less rate in normal lung cells (< 1%) samples. Cells expressing CD133 have been identified as having a self-renewal capacity, chemotherapy resistance *in vitro* and *in vivo*, and greater tumorgenicity[125]. In spite of this, CD133 is probably not a widely used marker for all lung CSCs. In one of the studies, CD133 was not identified in most of the non-small cell lung cancer cell lines[126]. Likewise, another study showed that both CD133+ and CD133− sub-populations of two human lung cancer cell lines have similar CSCs features[127]. The unique reliance of this marker needs to be further evaluated.

CD44 is a cell-surface glycoprotein receptor that is involved in cell-cell interaction, cell adhesion, migration, and is associated with multi-drug resistance[128]. Cancer associated factors such as chemokines, cell adhesion molecules, genes associated with WnT signalling and TGF-β have been found to be up-regulated in cells expressing CD44[129,130]. CD44, by means of its actions as a co-receptor with EGFR and ErbB family receptor tyrosine kinases, can influence cell proliferation[131]. Furthermore, *via* the PI3K / AKT cascade CD44 has been associated with increasing anti-apoptosis[132]. Leung *et al* showed that CD44 expressing sub-populations of some lung cancer cell lines have stem cell like properties. More studies are needed to justify the function of CD44 in tumor cell renewal and proliferation in the *in vivo*[126].

The regeneration of tumors after initial effective treatment is considered as resistance to chemotherapy and radiotherapy. Resistance to conventional therapies of cancer seen in CSCs compared to non-stem cells is likely because like their slow division, they have decreased apoptosis rate and the enhanced capability for DNA repair[7]. This resulted in the concept that treatment targeting differentiated cells, but missing CSCs, results in tumor relapse, while new therapies focusing on CSCs could leads to tumor elimination. Discovering new therapies is a dedicated concept. The lack of specific and universal markers for lung CSCs makes it difficult to tackle.

Targeting lung cancer stem cells self-renewal pathways

There is rising curiosity to study the self-renewal pathways used by lung CSCs as therapeutic targets. These pathways include Hh, Notch, and Wnt. There is also an increased interest in using a combination of targeted lung CSC therapies with traditional therapies.

Taipale *et al* showed that the use of cyclopamine, a plant veratrum alkaloid, inhibits the stimulation of Hh abnormal cell growth and the response pathway in basal cell carcinoma, rhabdomyosarcoma, and medulloblastoma. Other tumors with abnormal Hh signalling pathways may benefit from the use of Cyclopamine[133]. The primitive features of pulmonary neuroendocrine cells are found in small cell lung cancer (SCLC) and activation of Hh signalling pathway in SCLC stimulates tumor growth. In the growth of SCLC and in the normal differentiation of pulmonary neuroendocrine precursor cells, Hh signalling is a major factor. Cyclopamine has shown to inhibit Hb signalling pathway as result enhanced apoptosis for SCLC[134]. A new novel semi-synthetic cyclopamine analogue, IPI-926, with enhanced pharmaceutical properties and potency has been identified. It is now being subjected to clinical trials for numerous tumors[135].

Inhibition of Notch signalling pathway in lung cancer has demonstrated confirmation of reducing tumor growth and enhancing apoptosis *via* inhibition of the self-renewal effectiveness of CSCs[136,137]. Konishi *et al* showed that a gamma-secretase inhibitor, MRK-003, decreased growth and enhanced apoptosis of human lung cancer cell lines *in vitro* and *in vivo*, from utilizing xenograft models and inhibiting Notch-3 signalling[136]. Haruki *et al* showed that a dominant-negative Notch-3 receptor was useful in decreasing soft agar growth of human lung cancer lines by inhibiting the Notch-3 pathway[137]. Tumor inhibition and inhibition of self-renewal in progenitor cells / adult stem cells by antibodies directed towards Notch signalling pathway is an undergoing investigation[138].

A wide range of mechanisms can be used to inhibit Wnt pathway. Down-regulation of β- catenin signalling has been achieved by using retinoic acid (RA) and tyrosine kinase inhibitors[139,140]. A good result has been achieved against an NSCLC cell line using monoclonal antibodies targeted towards Wnt-1 by preventing the Wnt / β-catenin signalling pathway as well as inducing apoptosis[141]. The monoclonal antibody may have effects on normal cells. For this reason, the benefit has to be weighed against treatment efficacy with CSCs.

The use of monoclonal antibodies (mAbs) targeting CSCs is a fairly new method. Direct mAb therapy particularly aiming towards lung CSCs has not been clearly studied. In spite of this, two of the markers (CD133 and CD44) expressed by lung CSCs, do have the possibility of being treated by mAb therapy. AC133 is a monoclonal antibody that identifies CD133. Display of tumor growth inhibition in hepatocellular cancer cells *in vitro* and *in vivo* has been achieved by the use of AC133 conjugated to a cytotoxic drug in a pre-clinical study[142]. Likewise, H90 is another mAb that identifies CD44. H90 has shown to be effective in an AML model by being particularly aimed towards

CSCs, resulting in inhibition of tumor proliferation and niche localization[74].

CONCLUSION

The identification of CSCs represents a turning point in cancer research. Breast CSCs have been studied widely and recent studies have been successful in isolating Br CSCs that bare distinctive phenotypes like CD44+ and $CD24^{\oplus/low}$. Breast CSCs-targeted therapy represents a new era in breast cancer therapeutics. These targeted therapies have potential advantages in preventing relapse and prolonging survival, compared to conventional therapies.

Our understanding of CSCs has completely revolutionized how new treatments aimed at curing cancer patients should be designed. As is seen with many conventional anti-tumor therapies and as mentioned before, it is a fact that they merely target the proliferating population of cells that make up the tumor. This results in a failure to cure many patients suffering from cancer, and necessitates the use of approaches that not only target the feature of proliferation, but also other unique characteristics seen in cancer cells like resistance and novel survival strategies.

Although research has uncovered multiple new vulnerable features in cancer cells which can be exploited for new treatments, there is still the issue that mainly involves the selective and direct targeting of cells. The CSC concept has even allowed us to understand and model resistance to conventional therapies, which is an important factor that influences treatment outcome. Using the information gathered from analysis of CSCs, the mechanisms of resistance to CRT and endocrine therapy are now better understood.

The mechanism of how cancer cells are generated as progeny from stem cells and the fact that they can be reproduced, when transplanted into other hosts, is further proof that stemness is a property exhibited by cancer cells, and plays a role in survival and tumor relapse. The uncovering of the stem cell signalling pathways is breaking new ground in the designing of anti-tumor therapies by providing us with a better understanding of how tumor cells thrive, and new targets to focus treatment on. By exploiting such signalling pathways, it is also possible to make cancer cells less resistant to conventional treatments, making it possible to combine newer emerging treatments with current cytotoxic therapies. Finally, the identification of tumor markers has provided us with other explanations as to how cancer cells confer resistance, because with such markers we now know that cancers such as breast carcinoma exhibit subtypes which are more resistant to treatments than other subtypes due to differences in their molecular make-up.

As promising as the these markers seem to be towards potential monitoring progression of tumors and targeting cancer cells, the outlook for many offers a grim reality. For example, CD133, although a marker of colonic tumor cells, has shown an increase post-CRT, not only in the crypts but throughout the intestine. This tends to show a dimmer outlook toward effective targeting. CD44, OCT4, ALDH, BCL2 and CD133 have all shown to have potential as CSC markers. They shed light into the novel world of colorectal CSCs.

Colorectal cancer can be treated in the early stages *via* surgery, however if not diagnosed and treated in time, the rate of survival diminishes rapidly. All colorectal CRT treatments target the proliferative nature of the cancer cells in an attempt to minimize the growth. It seems as if the stem cells are not being targeted or perhaps provide resistance to the therapies themselves. The CSC may be capable of regenerating cells resistant to the therapy. The future of treatment of colorectal cancers lies in the new and upcoming research of CSCs. The potential in CSC targeting *via* markers is vast in account of the benefits regarding treatment. If these CSCs can be found and pathways better understood, CRT resistance cancers can be targeted, offering a better prognosis than the current situation.

This review article is a retrospective review of medical theory and clinical practice style, utilizing all medical publications excluding historical novelty references. The following databases are quoted: BMJ publishing Group Ltd, Cochrane Library, New England Journal of Medicine (NEJM), Medline (EBSCO), and PubMed.

REFERENCES

1. What are stem cells?: Center of bioethics, University of Minnesota. Available from: http://www.ahc.umn.edu/bioethics/prod/groups/ahc/@pub/@ahc/documents/asset/ahc_75703.pdf
2. Stem Cell Basics: Introduction. In Stem Cell Information [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2002 [cited Thursday, August 29, 2013] Available at <http://stemcells.nih.gov/info/basics/pages/basics1.aspx>
3. Boman BM, Wicha MS. Cancer stem cells: a step toward the cure. J Clin Oncol (Official journal of the American Society of Clinical Oncology) 2008; 26:2795-2799.
4. Baccelli I, Trumpp A. The evolving concept of cancer and metastasis stem cells. J Cell Biol 2012; 198:281-293.
5. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997; 3:730-737.
6. Li X, Lewis MT, Huang J, *et al*. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst 2008; 100:672-679.

7. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005; 5:275-284.
8. Sell S, Pierce GB. Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. Laboratory investigation. Journal of Technical Methods and Pathology 1994; 70:6-22.
9. Miyamoto T, Weissman IL, Akashi K. AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. Proc Natl Acad Sci USA 2000; 97:7521-7526.
10. Turhan AG, Lemoine FM, Debert C, *et al*. Highly purified primitive hematopoietic stem cells are PML-RARA negative and generate non-clonal progenitors in acute promyelocytic leukemia. Blood 1995; 85:2154-2161.
11. Krivtsov AV, Twomey D, Feng Z, *et al*. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. Nature 2006; 442:818-822.
12. Patel LR, Camacho DF, Shiozawa Y, Pienta KJ, Taichman RS. Mechanisms of cancer cell metastasis to the bone: A multistep process. Disclosure Future Oncology 2011; 7:1285-1297.
13. Mishra A, Shiozawa Y, Peinta KJ, Taichman RS. Homing of cancer cells to the bone. Cancer Microenviron 2011; 4:221-235.
14. CAR T-Cell Therapy: Engineering patients' immune cells to treat their cancers. National Cancer Institute at the National Institutes of Health. June 12th- 2013 http://www.cancer.gov/cancertopics/research-updates/2013/CAR-T-cells.
15. Miao HD, Choi B, Carter MS, *et al*. EGFRvIII-specific chimeric antigen receptor T cells migrate to and kill tumor deposits infiltrating the brain parenchyma in an invasive xenograft model of glioblastoma. PLoS One 2014; 9: e94281.
16. Panagiotis T, Avichai S, Aron A. The expanding horizon of immunotherapy in the treatment of malignant disorders: Allogeneic hematopoietic stem cell transplantation and beyond. Ann Med 2014; Early Online: 1–13 DOI: 10.3109/07853890.2014.918463.
17. Donald BK, Gianpietro D, Renier B, *et al*. CARs on track in the clinic workshop of the Blood and Marrow Transplant Clinical Trials Network Subcommittee on Cell and Gene Therapy, Washington DC. Molecular Therapy 2011; 19:432-438. doi:10.1038/mt.2011.1.
18. Marcela VM, Andrew RH, Gregory LB, Steven MA, Bruce LL, Xiaojun L. T-cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer Immunol Res 2013; 1:26. doi: 10.1158/2326-6066.CIR-13-0006.
19. Blagosklonny MV. Target for cancer therapy: proliferating cells or stem cells. Leukemia 2006; 20:385-391.
20. Perou CM, Sorlie T, Eisen MB, *et al*. Molecular portraits of human breast tumours. Nature 2000; 406:747-752.
21. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 2003; 100:3983-3988.
22. Taussig DC, Pearce DJ, Simpson C, *et al*. Hematopoietic stem cells express multiple myeloid markers: implications for the origin and targeted therapy of acute myeloid leukemia. Blood 2005; 106:4086-4092.
23. Holland JD, Klaus A, Garratt AN, Birchmeier W. Wnt signaling in stem and cancer stem cells. Curr Opin Cell Biol 2013; 25:254-264.
24. Guzman ML, Rossi RM, Karnischky L, *et al*. The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. Blood 2005; 105:4163-4169.
25. Essers MA, Trumpp A. Targeting leukemic stem cells by breaking their dormancy. Mol Oncol 2010; 4:443-450.
26. Francipane MG, Alea MP, Lombardo Y, Todaro M, Medema JP, Stassi G. Crucial role of interleukin-4 in the survival of colon cancer stem cells. Cancer Res 2008; 68:4022-4025.
27. CBTRUS (Central Brain Tumor Registry of the United States) (2009). CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in Eighteen States in 2002–2006.
28. Fuller GN, Scheithauer BW. The 2007 revised World Health Organisation (WHO) classification of tumours of the central nervous system: newly codified entities. Brain Pathol 17: 304-307.
29. Furnari FB, Fenton T, Bachoo RM, *et al*. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes & Development 2007; 21:2683-2710.
30. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. Cancer Cell 2002; 2:103-112.
31. Vousden KH. Activation of the p53 tumor suppressor protein. Biochimica et Biophysica Acta 2002; 1602:47-59.
32. Singh SK, Hawkins C, Clarke ID, *et al*. Identification of human brain tumour initiating cells. Nature 2004; 432:396-401.
33. Bao S, Wu Q, McLendon RE, *et al*. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006; 444:756-760.
34. Bleau AM, Hambardzumyan D, Ozawa T, *et al*. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. Cell Stem Cell 2009; 4:226-235.
35. Piccirillo SG, Reynolds BA, Zanetti N, *et al*. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 2006; 444:761-765.
36. Po A, Ferretti E, Miele E, *et al*. Hedgehog controls neural stem cells through p53-independent regulation of Nanog. The EMBO Journal. 2010; 29:2646-2658.
37. Stiles CD, Rowitch DH. Glioma stem cells: a midterm exam. Neuron 2008; 58:832-846.
38. Zheng H, Ying H, Wiedemeyer R, *et al*. PLAGL2 regulates Wnt signaling to impede differentiation in neural stem cells and gliomas. Cancer Cell 2010; 17:497-509.
39. Bruggeman SW, Hulsman D, Tanger E, *et al*. Bmi1 controls tumor development in an Ink4a/Arf-independent manner in a mouse model for glioma. Cancer Cell 2007; 12:328-341.
40. Ligon KL, Huillard E, Mehta S, *et al*. Olig2-regulated lineage-restricted pathway controls replication

competence in neural stem cells and malignant glioma. Neuron 2007; 53:503-517.
41. Zbinden M, Duquet A, Lorente-Trigos, *et al*. NANOG regulates glioma stem cells and is essential in vivo acting in a cross-functional network with GLI1 and p53. The EMBO Journal 2010; 29:2659-2674.
42. Hayry V, Tynninen O, Haapasalo HK, *et al*. Stem cell protein BMI-1 is an independent marker for poor prognosis in oligodendroglial tumours. Neuropathol Appl Neurobiol 2008; 34:555-563.
43. Matsuda K, Sato A, Okada M, *et al*. Targeting JNK for therapeutic depletion of stem-like glioblastoma cells. Scientific Reports 2012; 2:516.
44. Beier D, Hau P, Proescholdt M, *et al*. CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Res 2007; 67:4010-4015.
45. Chen R, Nishimura MC, Bumbaca SM, *et al*. A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. Cancer Cell 2010; 17:362-375.
46. Anido J, Saez-Borderias A, Gonzalez-Junca A, *et al*. TGF-beta receptor inhibitors target the CD44(high) / Id1(high) glioma-initiating cell population in human glioblastoma. Cancer Cell 2010; 18:655-668.
47. Lathia JD, Gallagher J, Heddleston JM, *et al*. Integrin alpha 6 regulates glioblastoma stem cells. Cell Stem Cell 2010; 6:421-432.
48. Son MJ, Woolard K, Nam DH, Lee J, Fine HA. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell Stem Cell 2009; 4:440-452.
49. Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. Cell 2008; 132:645-660.
50. Calabrese C, Poppleton H, Kocak M, *et al*. A perivascular niche for brain tumor stem cells. Cancer Cell 2007; 11:69-82.
51. Dick JE. Breast cancer stem cells revealed. Proc Natl Acad Sci USA 2003; 100:3547-3549.
52. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001; 414:105-111.
53. Pece S, Tosoni D, Confalonieri S, *et al*. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell 2010; 140:62-73.
54. Economopoulou P, Kaklamani VG, Siziopikou K. The role of cancer stem cells in breast cancer initiation and progression: potential cancer stem cell-directed therapies. Oncologist 2012; 17:1394-1401.
55. Ginestier C, Hur MH, Charafe-Jauffret E, *et al*. ALDH1 is a marker of normal and malignant human mammary Stem Cells and a predictor of poor clinical outcome. Cell Stem Cell 2007; 1:555-567.
56. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 2009; 9:265-273.
57. Larue L, Bellacosa A. Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3′ kinase/AKT pathways. Oncogene 2005; 24:7443-7454.
58. Prat A, Parker JS, Karginova O, *et al*. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast cancer research: BCR 2010; 12:R68.
59. Korkaya H, Paulson A, Iovino F, Wicha MS. HER2 regulates the mammary stem/progenitor cell population driving tumorigenesis and invasion. Oncogene 2008; 27:6120-6130.
60. Li HZ, Yi TB, Wu ZY. Suspension culture combined with chemotherapeutic agents for sorting of breast cancer stem cells. BMC Cancer 2008; 8:135.
61. Cicalese A, Bonizzi G, Pasi CE, *et al*. The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. Cell 2009; 138:1083-1095.
62. Gong C, Yao H, Liu Q, *et al*. Markers of tumor-initiating cells predict chemoresistance in breast cancer. PloS One 2010; 5:e15630.
63. Shafee N, Smith CR, Wei S, *et al*. Cancer stem cells contribute to cisplatin resistance in Brca1/p53-mediated mouse mammary tumors. Cancer Res 2008; 68:3243-3250.
64. Yin H, Glass J. The phenotypic radiation resistance of CD44+/CD24(-or low) breast cancer cells is mediated through the enhanced activation of ATM signaling. PloS One 2011; 6:e24080.
65. Horwitz KB, Dye WW, Harrell JC, Kabos P, Sartorius CA. Rare steroid receptor-negative basal-like tumorigenic cells in luminal subtype human breast cancer xenografts. Proc Natl Acad Sci USA 2008; 105:5774-5779.
66. Clementz AG, Rogowski A, Pandya K, Miele L, Osipo C. NOTCH-1 and NOTCH-4 are novel gene targets of PEA3 in breast cancer: novel therapeutic implications. Breast Cancer Res 2011; 13:R63.
67. Grudzien P, Lo S, Albain KS, *et al*. Inhibition of Notch signaling reduces the stem-like population of breast cancer cells and prevents mammosphere formation. Anticancer Res 2010; 30:3853-3867.
68. Iliopoulos D, Hirsch HA, Struhl K. Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. Cancer Res 2011; 71:3196-3201. http://dx.doi.org/10.1073/pnas.97.13.7521
69. Kikushige Y, Akashi K. TIM-3 as a therapeutic target for malignant stem cells in acute myelogenous leukemia. Ann New York Acad Sci 2012; 1266:118-123.
70. Lam BS, Adams GB. Blocking HIF1alpha activity eliminates hematological cancer stem cells. Cell Stem Cell 2011; 8:354-356.
71. Florian S, Sonneck K, Hauswirth AW, *et al*. Detection of molecular targets on the surface of CD34+/CD38-- stem cells in various myeloid malignancies. Leuk Lymphoma 2006; 47:207-222.
72. Feller N, van der Pol MA, van Stijn A, *et al*. MRD parameters using immunophenotypic detection methods are highly reliable in predicting survival in acute myeloid leukaemia. Leukemia 2004; 18:1380-1390.
73. Gadhoum Z, Delaunay J, Maquarre E, *et al*. The effect of anti-CD44 monoclonal antibodies on differentiation and proliferation of human acute myeloid leukemia cells. Leuk Lymphoma 2004; 45:1501-1510.

74. Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. Nat Med 2006; 12:1167-1174.
75. Hauswirth AW, Florian S, Printz D, *et al*. Expression of the target receptor CD33 in CD34+/CD38-/CD123+ AML stem cells. Euro J Clin Invest 2007; 37:73-82.
76. Vercauteren S, Zapf R, Sutherland H. Primitive AML progenitors from most CD34 (+) patients lack CD33 expression but progenitors from many CD34(-) AML patients express CD33. Cytotherapy 2007; 9:194-204.
77. Jin L, Lee EM, Ramshaw HS, *et al*. Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells. Cell Stem Cell 2009; 5:31-42.
78. Cohen KA, Liu TF, Cline JM, Wagner JD, Hall PD, Frankel AE. Safety evaluation of DT388IL3, a diphtheria toxin/interleukin 3 fusion protein, in the cynomolgus monkey. Cancer Immunol , Immunother: CII 2005; 54:799-806.
79. Roberts AW. ASH Annual meeting. Abstract. 2008.
80. Nakayama M, Akiba H, Takeda K, *et al*. Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. Blood 2009; 113:3821-3830.
81. Kikushige Y, Akashi K. TIM-3 as a therapeutic target for malignant stem cells in acute myelogenous leukemia. Annals of the New York Academy of Sciences. Haematopoietic Stem Cells VIII2012; 17:0077-8923.
82. Wang Y, Liu Y, Malek SN, Zheng P, Liu Y. Targeting HIF1alpha eliminates cancer stem cells in hematological malignancies. Cell Stem Cell 2011; 8:399-411.
83. Marcela VM, Stephan AG, David LP, Carl HJ. Antibody-modified T cells: CARs take the front seat for hematologic malignancies. April 24, 2014; Blood: 123 (17).DOI: http://dx.doi.org/10.1182/blood-2013-11-492231.
84. Stephan AG, Michael K, David B, Richard A, David L.P, SusanR.R. Chimeric antigen receptor–modified T Cells for acute lymphoid leukemia. N Engl J Med 2013 DOI:10.1056/NEJMoa1215134. http://www.itmat.upenn.edu/docs/GrupppediALLNEJM2013.pdf
85. David LP, Bruce LL, Michael K, Adam B, Carl HJ. Chimeric antigen receptor–modified T cells in chronic lymphoid leukemia. N Engl J Med 2011; 365:725-733. doi: 10.1056/NEJMoa1103849.
86. Siegel R, Naishadham D, Jemal A. Cancer Statistics 2012 CA. Cancer Journal for Clinicians 2012; 62:10-29.
87. Du L, Rao G, Wang H, *et al*. CD44-positive cancer stem cells expressing cellular prion protein contribute to metastatic capacity in colorectal cancer. Cancer Res 2013; 73:2682-2694.
88. Fabian A, Barok M, Vereb G, Szollosi J. Die hard: are cancer stem cells the Bruce Willises of tumor biology? Cytometry Part A. The Journal of the International Society for Advancement of Cytometry 2009; 75:67-74.
89. Saigusa S, Tanaka K, Toiyama Y, *et al*. Correlation of CD133, OCT4, and SOX2 in rectal cancer and their association with distant recurrence after chemoradiotherapy. Ann Surgical Oncol 2009; 16:3488-3498.
90. Ricci-Vitiani L, Lombardi DG, Pilozzi E, *et al*. Identification and expansion of human colon-cancer-initiating cells. Nature 2007; 445:111-115.
91. Zhu L, Gibson P, Currle DS, *et al*. Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. Nature 2009; 457:603-607.
92. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 2007; 445:106-110.
93. Horst D, Kriegl L, Engel J, Kirchner T, Jung A. CD133 expression is an independent prognostic marker for low survival in colorectal cancer. British J Cancer 2008; 99:1285-1289.
94. Kojima M, Ishii G, Atsumi N, Nishizawa Y, Saito N, Ochiai A. CD133 expression in rectal cancer after preoperative chemoradiotherapy. Cancer Science 2010; 101:906-912.
95. Horst D, Scheel SK, Liebmann S, *et al*. The cancer stem cell marker CD133 has high prognostic impact but unknown functional relevance for the metastasis of human colon cancer. J Pathology 2009; 219:427-434.
96. Huang EH, Hynes MJ, Zhang T, *et al*. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. Cancer Research 2009; 69:3382-3389.
97. Du L, Wang H, He L, *et al*. CD44 is of functional importance for colorectal cancer stem cells. Clin Cancer Res 2008; 14:6751-6760.
98. Hochedlinger K, Yamada Y, Beard C, Jaenisch R. Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. Cell 2005; 121:465-477.
99. Wang XQ, Ongkeko WM, Chen L, *et al*. Octamer 4 (Oct4) mediates chemotherapeutic drug resistance in liver cancer cells through a potential Oct4-AKT-ATP-binding cassette G2 pathway. Hepatology 2010; 52:528-539.
100. Wen K, Fu Z, Wu X, Feng J, Chen W, Qian J. Oct-4 is required for an antiapoptotic behavior of chemoresistant colorectal cancer cells enriched for cancer stem cells: effects associated with STAT3/Survivin. Cancer Lett 2013; 333:56-65.
101. Kosinski C, Li VS, Chan AS, *et al*. Gene expression patterns of human colon tops and basal crypts and BMP antagonists as intestinal stem cell niche factors. Proc Natl Acad Sci USA 2007;104:15418-15423.
102. Fevr T, Robine S, Louvard D, Huelsken J. Wnt/beta-catenin is essential for intestinal homeostasis and maintenance of intestinal stem cells. Mol Cell Biol 2007; 27:7551-7559.
103. Nguyen NP, Almeida FS, Chi A, *et al*. Molecular biology of breast cancer stem cells: potential clinical applications. Cancer Treat Rev 2010; 36:485-491.
104. Merritt AJ, Potten CS, Watson AJ, *et al*. Differential expression of bcl-2 in intestinal epithelia. Correlation with attenuation of apoptosis in colonic crypts and the incidence of colonic neoplasia. J Cell Sci 1995; 108:2261-2271.

105. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA. Cancer Journal for Clinicians 2010; 60:277-300.
106. Kohler BA, Ward E, McCarthy BJ, *et al*. Annual report to the nation on the status of cancer, 1975-2007, featuring tumors of the brain and other nervous system. J Natl Cancer Inst 2011; 103:714-736.
107. Thun MJ, Hannan LM, Adams-Campbell LL, *et al*. Lung cancer occurrence in never-smokers: an analysis of 13 cohorts and 22 cancer registry studies. PLoS Medicine 2008; 5:e185.
108. Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 2003; 22:7265-7279.
109. Takebe N, Ivy SP. Controversies in cancer stem cells: targeting embryonic signaling pathways. Clinical Cancer Res 2010; 16:3106-3112.
110. Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. Cancer Res 2007; 67:4827-4833.
111. Garcia Campelo MR, Alonso Curbera G, Aparicio Gallego G, Grande Pulido E, Anton Aparicio LM. Stem cell and lung cancer development: blaming the Wnt, Hh and Notch signalling pathway. Clin Transl Oncol 2011; 13:77-83.
112. Bhardwaj G, Murdoch B, Wu D, *et al*. Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation. Nat Immunol 2001; 2:172-180.
113. Peacock CD, Wang Q, Gesell GS, *et al*. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proc Natl Acad Sci USA 2007; 104:4048-4053.
114. Rubin LL, de Sauvage FJ. Targeting the Hedgehog pathway in cancer. Nat Rev Drug discov 2006; 5:1026-1033.
115. Gaiano N, Fishell G. The role of notch in promoting glial and neural stem cell fates. Ann Rev of Neurosci 2002; 25:471-490.
116. Karanu FN, Murdoch B, Gallacher L, *et al*. The notch ligand jagged-1 represents a novel growth factor of human hematopoietic stem cells. Exp Med 2000; 192:1365-1372.
117. Radtke F, Raj K. The role of Notch in tumorigenesis: oncogene or tumour suppressor? Nat Rev Cancer 2003; 3:756-767.
118. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2006; 127:469-480.
119. Gat U, DasGupta R, Degenstein L, Fuchs E. De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 1998; 95:605-614.
120. Uematsu K, He B, You L, Xu Z, McCormick F, Jablons DM. Activation of the Wnt pathway in non small cell lung cancer: evidence of dishevelled overexpression. Oncogene 2003; 22:7218-7221.
121. Yin AH, Miraglia S, Zanjani ED, *et al*. AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 1997; 90:5002-5012.
122. Wu Y, Wu PY. CD133 as a marker for cancer stem cells: progresses and concerns. Stem Cells Dev 2009; 18:1127-1134.
123. Chen YC, Hsu HS, Chen YW, *et al*. Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. PloS One 2008; 3:e2637.
124. Eramo A, Lotti F, Sette G, *et al*. Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ 2008; 15:504-514.
125. Bertolini G, Roz L, Perego P, *et al*. Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. Proc Natl Acad Sci USA 2009; 106:16281-16286.
126. Leung EL, Fiscus RR, Tung JW, *et al*. Non-small cell lung cancer cells expressing CD44 are enriched for stem cell-like properties. PloS One 2010; 5:e14062.
127. Meng X, Li M, Wang X, Wang Y, Ma D. Both CD133+ and CD133- subpopulations of A549 and H446 cells contain cancer-initiating cells. Cancer Sci 2009; 100:1040-1046.
128. Miletti-Gonzalez KE, Chen S, Muthukumaran N, *et al*. The CD44 receptor interacts with P-glycoprotein to promote cell migration and invasion in cancer. Cancer Res 2005; 65:6660-6667.
129. Bankfalvi A, Terpe HJ, Breukelmann D, *et al*. Gains and losses of CD44 expression during breast carcinogenesis and tumour progression. Histopathology 1998; 33:107-116.
130. Shipitsin M, Campbell LL, Argani P, *et al*. Molecular definition of breast tumor heterogeneity. Cancer Cell 2007; 11:259-273.
131. Bourguignon LY, Gilad E, Peyrollier K. Heregulin-mediated ErbB2-ERK signaling activates hyaluronase synthases leading to CD44-dependent ovarian tumor cell growth and migration. J Biolog Chem 2007; 282:19426-19441.
132. Toole BP, Slomiany MG. Hyaluronan, CD44 and Emmprin: partners in cancer cell chemoresistance. Drug resistance updates: reviews and commentaries in antimicrobial and anticancer chemotherapy. Chemotherpy 2008; 11:110-121.
133. Taipale J, Chen JK, Cooper MK, *et al*. Effects of oncogenic mutations in smoothened and patched can be reversed by cyclopamine. Nature 2000; 406:1005-1009.
134. Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 2003; 422:313-317.
135. Tremblay MR, Lescarbeau A, Grogan MJ, *et al*. Discovery of a potent and orally active hedgehog pathway antagonist (IPI-926). J Med Chem 2009; 52:4400-4418.
136. Konishi J, Kawaguchi KS, Vo H, *et al*. Gamma-secretase inhibitor prevents Notch3 activation and reduces proliferation in human lung cancers. Cancer Res 2007; 67:8051-8057.
137. Haruki N, Kawaguchi KS, Eichenberger S, *et al*. Dominant-negative Notch3 receptor inhibits mitogen-activated protein kinase pathway and the growth of human lung cancers. Cancer Res 2005; 65:3555-3561.

138. Rizzo P, Osipo C, Foreman K, Golde T, Osborne B, Miele L. Rational targeting of Notch signaling in cancer. Oncogene 2008; 27:5124-5131.
139. Luu HH, Zhang R, Haydon RC, *et al*. Wnt/beta-catenin signaling pathway as a novel cancer drug target. Curr Cancer Drug Targets 2004; 4:653-671.
140. Haydon RC, Zhou L, He TC. Tyrosine kinase inhibitor STI-571: the new wonder drug of cancer therapy. Cancer Biol Ther 2004; 3:393-394.
141. He B, You L, Uematsu K, *et al* A monoclonal antibody againstWnt-1 induces apoptosis in human cancer cells. Neoplasia 2006; 1:7-14.
142. Smith LM, Nesterova A, Ryan MC, *et al*. CD133/prominin-1 is a potential therapeutic target for antibody-drug conjugates in hepatocellular and gastric cancers. Br J Cancer 2008; 99:100-109.

Original Article

Increased Risk of Hip Fracture in Diabetic Elderly

Shih-Wei Lai[1,2], Cheng-Li Lin[3,4], Kuan-Fu Liao[5,6]
[1]School of Medicine, China Medical University and [2]Department of Family Medicine, China Medical University Hospital, Taichung, Taiwan
[3]Department of Public Health, China Medical University and [4]Management Office for Health Data, China Medical University Hospital, Taichung, Taiwan
[5]Graduate Institute of Integrated Medicine, China Medical University and [6]Department of Internal Medicine, Taichung Tzu Chi General Hospital, Taichung, Taiwan

Kuwait Medical Journal 2015; 47 (2): 115 - 117

ABSTRACT

Objective: To investigate the relationship between diabetes mellitus (DM) and risk of hip fracture in older people in Taiwan
Design: Retrospective cohort study using the database for the period 1998 - 2010 from the Taiwan National Health Insurance Program
Setting: Taiwan National Health Insurance program
Subjects: There were 16,249 individuals aged 65 years or older with newly diagnosed DM as the diabetes group and 64,996 individuals without DM as the none-diabetes group.
Main Outcome Measures: The risk of hip fracture in both groups
Results: The diabetes group had a significantly higher incidence of hip fracture than the non-diabetes group (1080 Vs 859.6 per 100,000 person-years, incidence rate ratio 1.26, 95% CI 1.20, 1.31).
Conclusions: DM is associated with 1.26-fold increased risk of hip fracture in older people in Taiwan. Older people with DM should be closely followed to reduce the risk of hip fracture.

Keywords: diabetes mellitus, hip fracture, older people

INTRODUCTION

Hip fracture is a significant health challenge in older people worldwide. Extensive studies have demonstrated that diabetes mellitus (DM) is a risk factor for hip fracture, with 1.4 to 1.98-fold increased risk[1-3]. So far, only few clinical studies have focused on the association of DM and risk of hip fracture in older people in Taiwan[4]. Therefore, we conducted this cohort study to investigate this issue by utilizing the database for the period 2000 - 2010 from the Taiwan National Health Insurance Program. The details of insurance program can be found in previous studies[5,6].

SUBJECTS and METHODS

In this cohort study, there were 16,249 individuals aged 65 years or older with newly diagnosed DM as the diabetes group based on the International Classification of Diseases, 9th Revision-Clinical Modification (ICD-9 codes 250, 8357 male and 7892 female, mean age [standard deviation, SD] = 73.2 [6.2] years, mean follow-up period [SD] = 5.2 [3.6] years) and 64,996 individuals without DM as the non-diabetes group (33,428 male and 31,568 female, mean age [SD] = 72.5 [6.7] years, mean follow-up period [SD] = 5.5 [3.7] years), from the period 1998 to 2010. Both groups were well-matched as regards sex, age and the year of diagnosis of DM and were followed up for the incidence of hip fracture (ICD-9 codes 820), until hip fracture was diagnosed or until December 31, 2010. Individuals with hip fracture diagnosed before entering this cohort were excluded.

RESULTS

The DM group had a significantly higher incidence of hip fracture than the non-diabetes group (1080 Vs 859.6 per 100,000 person-years, IRR [incidence rate ratio] 1.26, 95% CI 1.20-1.31). With further stratification by sex, age and follow-up period, the incidence rates of hip fracture were all higher in the diabetes group than in the non-diabetes group, with statistical significance. Female diabetic individuals had a higher risk of hip fracture than male diabetic individuals (IRR 1.33 Vs 1.16). Diabetic individuals aged 75 – 84 years had the highest incidence than other sub-groups (incidence

Address correspondence to:
Kuan-Fu Liao, Department of Internal Medicine, Taichung Tzu Chi General Hospital, No.66, Sec. 1, Fongsing Road, Tanzi District, Taichung City, 427, Taiwan. Tel: 886-4-2205-2121, Fax: 886-4-2203-3986, E-mail: kuanfuliaog@gmail.com

Table 1: Incidence of hip fracture for diabetes and non-diabetes group in older people in Taiwan

Patient characteristics	Non-diabetes				Diabetes				Incidence rate ratio (95% CI) †
	N	Case	Person-years	Incidence rate	N	Case	Person-years	Incidence rate	
All	64996	3092	359703	859.6	16249	913	84538	1080	1.26(1.20, 1.31)
Sex									
Male	33428	1260	182425	690.7	8357	345	43211	798.4	1.16(1.08, 1.23)
Female	31568	1832	177279	1033.4	7892	568	41327	1374.4	1.33(1.25, 1.41)
Age group (years)									
65 - 74	42864	1359	261620	519.5	10716	481	61728	779.2	1.50(1.42, 1.58)
75 - 84	22132	1733	98083	1766.9	5533	432	22810	1893.9	1.08(1.00, 1.15)
Follow-up period									
< 5 years	13946	1822	240834	756.5	4001	524	57901	905	1.20(1.14, 1.26)
≥ 5 years	51050	1270	118870	1068.4	12248	389	26637	1460.4	1.37(1.28, 1.46)

Incidence rate: per 100,000 person-years
†Incidence rate ratio: diabetes Vs non-diabetes (95%CI)

rate =1893.9 per 100,000 person-years). The risk of hip fracture was higher among individuals with diabetes duration ≥ 5 years (IRR 1.37, 95% CI 1.28 - 1.46), as compared to individuals with diabetes duration < 5 years (IRR 1.20, 95% CI 1.14-1.26) (Table 1). The difference of cumulative incidence of hip fracture between the diabetes group and the non-diabetes group increased with follow-up period (Fig. 1).

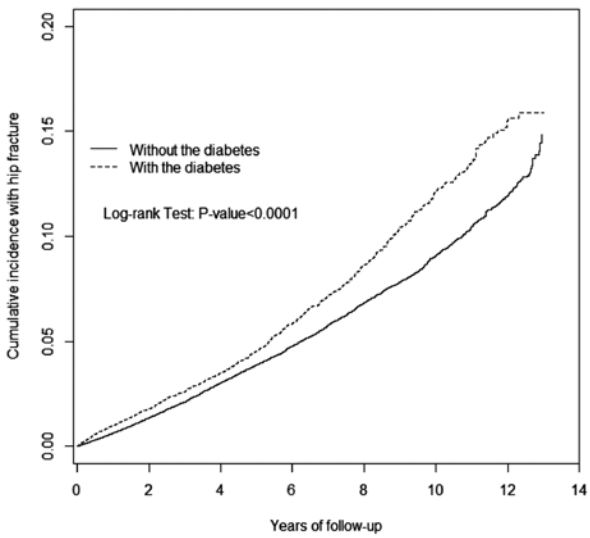

Fig. 1: Cumulative incidence of hip fracture for diabetes and non-diabetes group

DISCUSSION

To date, extensive evidence has shown that individuals with DM are at higher risk of hip fracture. A systematic review by Janghorbani *et al* has shown that the risk of hip fracture incidence is higher among diabetic patients (risk ratio = 1.7 - 6.3)[7]. In our study, the incidence rate ratio of hip fracture was 1.26 in diabetic individuals, as compared to those without diabetes. Despite confounders related to hip fracture not being adjusted, our findings were compatible with previous studies[1-3]. A cohort study by Koh *et al* in Singapore reported that there is a strong dose-response relationship between diabetic duration and risk of hip fracture[3]. Furthermore, we also found that the risk of hip fracture is 1.2-fold in individuals with diabetic duration < 5 years (95% CI 1.14-1.26) and up to 1.37-fold in individuals with diabetic duration ≥ 5 years (95% CI 1.28-1.46). That is, the longer the diabetic duration, the higher risk of hip fracture.

CONCLUSION

DM is associated with 1.26-fold increased risk of hip fracture in older people in Taiwan. Since hip fracture is a multi-factorial disorder, at least, older people with diabetes mellitus should be closely cared for due to high risk of hip fracture.

ACKNOWLEDGMENT

This study is supported in part by Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW104-TDU-B-212-113002), China Medical University Hospital, Academia Sinica Taiwan Biobank, Stroke Biosignature Project (BM104010092), NRPB Stroke Clinical Trial Consortium (MOST 103-2325-B-039 -006), Tseng-Lien Lin Foundation in Taichung in Taiwan, Taiwan Brain Disease Foundation in Taipei in Taiwan, and Katsuzo and Kiyo Aoshima Memorial Funds in Japan. These funding agencies did not influence the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest: The authors disclose no conflicts of interest.

REFERENCES

1. Ottenbacher KJ, Ostir GV, Peek MK, Goodwin JS, Markides KS. Diabetes mellitus as a risk factor for hip fracture in Mexican American older adults. J Gerontol A Biol Sci Med Sci 2002; 57:648-653.
2. Cortes-Sancho R, Perez-Castrillon JL, Martin-Escudero JC, Iglesias S, Alvarez-Manzanares P, Ramos R. Type 2 diabetes mellitus as a risk factor for hip fracture. J Am Geriatr Soc 2004; 52:1778-1779.
3. Koh WP, Wang R, Ang LW, Heng D, Yuan JM, Yu MC. Diabetes and risk of hip fracture in the Singapore Chinese Health Study. Diabetes Care 2010; 33:1766-1770.
4. Lu FP, Chan DC, Kuo HK, Wu SC. Sex differences in the impact of diabetes on the risk of geriatric conditions. Geriatr Gerontol Int 2013; 13:116-122.
5. Lai SW, Liao KF, Liao CC, Muo CH, Liu CS, Sung FC. Polypharmacy correlates with increased risk for hip fracture in the elderly: a population-based study. Medicine (Baltimore) 2010; 89:295-299.
6. Lai SW, Lin CH, Liao KF, Su LT, Sung FC, Lin CC. Association between polypharmacy and dementia in older people: a population-based case-control study in Taiwan. Geriatr Gerontol Int 2012; 12:491-498.
7. Janghorbani M, Van Dam RM, Willett WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol 2007; 166:495-505.

Original Article

A Bibliometric Study of Epidural Anesthesia: A 24-year Review

Mehdi Fathi[1], Marjan Joudi[2], Gholamreza Habibi[3], Sanam Javid[3], Amirhossein Mardani[3], Mehdi Aghasizadeh[4]
[1]Department of Anesthesia, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Department of Pediatric Surgery, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Farzan Clinical Research Institute, Tehran, Iran
[4]Department of Cardiology, Mashhad University of Medical Sciences, Mashhad, Iran

Kuwait Medical Journal 2015; 47 (2): 118 - 121

ABSTRACT

Objective: After introduction of epidural anesthesia (EA) in the early 20th century, it has become an essential technique in anesthesiology and many physicians consider it the gold standard analgesic technique for major surgeries. As EA has improved anesthesia methods dramatically and there is no previous bibliometric study in this field, we aimed to perform a scientometric analysis on this topic.
Design: Cross-sectional study
Setting: Mashad University of Medical Sciences, Iran
Subjects: Articles were retrieved from the Web of Science (ISI) from 1990 to 2013.
Intervention(s): The total number of published items was 4612; subsequent analysis was performed on results considering published items per year, country, funding agency, institution, journal, publication language, and author and subject area.
Main Outcome Measure: The results were analyzed considering published items per year, country, funding agency and institution, journal, publication language and author.
Results: Additionally, subject areas under which the articles were published were evaluated.
The primary search yielded 4612 publications; out of these 3200 (69.38%) were original articles. More than half of articles (2466) were published under "anesthesiology" subject. The USA was the leading country in producing articles under this topic and engaged highest collaboration rate with 64 collaborations.
Conclusion: It seems that there is an overall increase in total number of articles, citations and highly cited articles about EA during these two decades.

KEYWORDS: article, bibliometric, citation, EA

INTRODUCTION

Epidural anesthesia (EA) was introduced in early 20th century, about 50 years after the discovery of inhalation anesthesia. The first spinal analgesia was applied accidentally in 1885 by Leonard Corning on a dog[1]. Nowadays, this method is considered as an essential technique in anesthesiology[2], and many physicians consider EA as the gold standard analgesic technique for major surgeries. The reason is that this technique provides dynamic analgesia, allowing the patient to mobilize and resume normal activities unlimited by pain, attenuates the stress response to major surgery and reduces the incidence of postoperative complications such as pulmonary, thromboembolic and cardiac events[3-6]. Furthermore, EA can improve the quality of patient's recovery from major surgery and shorten length of hospital stay[7].

Nowadays, there is an increasing interest in scientometric studies for evaluating the research quality and productivity. Furthermore, it is possible to measure the impact of an article or a researcher on the scientific community by means of citation rating[8-11].

Considering the fact that EA has improved anesthesia methods dramatically and plays an important role in pain management strategies, and there are no previous studies in this field, we aimed to perform a scientometric analysis on EA.

MATERIAL AND METHODS

Data was retrieved from the Web of Science. All publications during the period of 1990 - 2013 were considered. The keyword "Epidural analgesia" was used to search articles published during this time interval. A total number of 4612 publications were

Address correspondence to:
Marjan Joudi, Department of Pediatric Surgery, Sheikh Pediatric Hospital, Mashhad University of Medical Sciences, Mashhad, Iran. P O Box: 13185-1678, Tehran, Iran. Tel: +985137284448, Fax: +9821 66423304, E-mail: mandala_110@yahoo.com

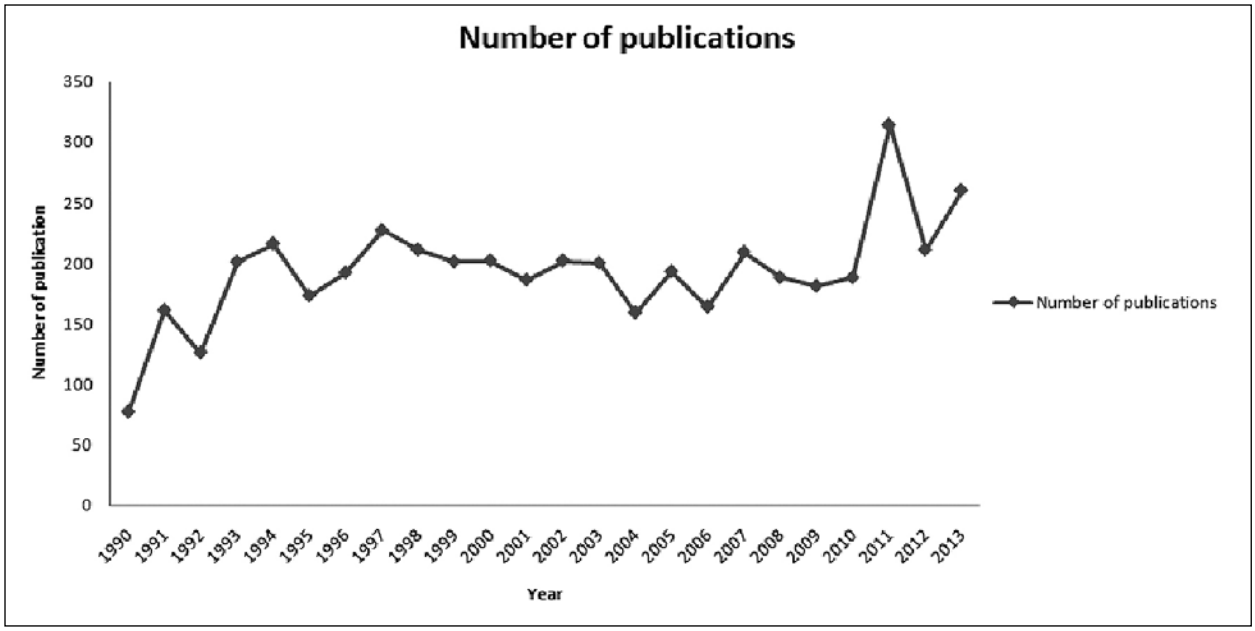

Fig. 1: Number of publications in each year from 1990 to 2013

obtained. Further analysis was performed on the obtained results considering published items per year, country, funding agency, institution, journal, publication language, and author and subject area.

Considering the fact that citation analyses provide a qualitative assessment of the data and are used as an indicator for research quality, all published items were evaluated considering the number of citations. Also, a citation analysis for highly cited publications was performed and citation per paper was calculated.

Furthermore, the collaboration between different countries was assessed by Intcoll and Pajek softwares. The collaborations were divided into two main groups (domestic and international) considering the method used by Kim[12]. Domestic collaborations (teamwork within a country) itself was divided into two subtypes; intra-institutional and extra–institutional collaboration considering the institutions involved in article production.

Statistical analysis

Data analysis was performed using descriptive statistics. Other analysis was done using "Web of Science" analysis tools".

RESULTS

The primary search with our keyword "Epidural analgesia" yielded 4612 publications, out of which, 3200 (69.38%)) were original articles and the rest were made up of proceeding papers (8.69%), letter to the editor (7.91%), review (5.55%) , meeting abstract (4.94%), editorial material (3.18%), notes (0.17%), corrections (0.10%) and correction addition (0.04%). Fig. 1 presents the number of publications in each year during the study period.

Total number of citations of the obtained articles was 58,862 times with average citations per item of 12.76. H-index for authors on this subject was 81. Additionally, 28 articles were cited more than 200 times, 64 were cited more than 100 times and 465 were not

Table 1: Percent distribution showing contributory role of first ten authors, countries, institutions and languages in publishing articles between 1990 and 2013 on epidural analgesia

Rank	Author	Percent	Country	Percent	Language	Percent	Institution	Percent
1	Sessler DI	1.62	USA	38.20	English	92.17	Harvard University	2.36
2	Sharrock NE	1.34	Germany	10.23	German	4.53	University of Calif San Francisco	2.05
3	Van Aken H	1.08	Japan	7.56	French	1.97	Cornell University	1.56
4	Secher NH	0.82	England	5.94	Spanish	0.52	Hosp Special Surg	1.45
5	Sculco TP	0.67	Canada	5.61	Portuguese	0.36	Texas University	1.25
6	Steven RA	0.58	France	3.90	Russian	0.23	University of Toronto	1.19
7	Liu SS	0.56	Turkey	3.16	Turkish	0.19	Virginia Mason Med Center	1.14
8	Salvatiea	0.56	Italy	2.62	-	-	Stanford University	1.04
9	Sato S	0.52	Sweden	2.53	-	-	University of Munster	0.86
10	Ramanathan S	0.45	Australia	2.05	-	-	University of Copenhagen	0.86

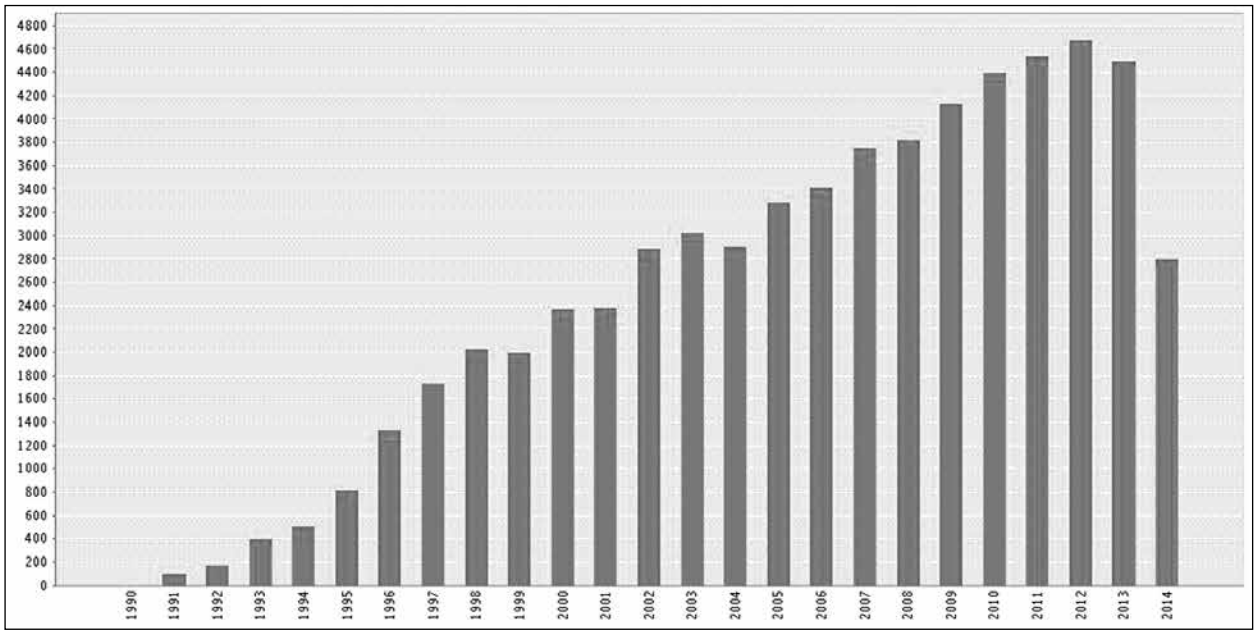

Fig. 2: Number of citations in each year from 1990 to 2013

cited. The USA and UK had the greatest contribution in publishing highly cited articles respectively. Fig. 2 demonstrates number of citations in each year during the study period.

"Anesthesia and Analgesia", "Anesthesiology" and "Canadian Journal of Anaesthesia - Journal Canadien D Anesthesie", "Regional Anesthesia" and "Acta Anaesthesiologica Scandinavica" ranked as first five journals for publishing highly cited articles.

Table 1 shows information about first ten authors, countries, institutions and languages of published articles. Sessler, with 75 publications (1.62%) was the most productive author in this field.

USA was the leading country in producing articles under this topic with 1762 (38.20%) publications. Germany, Japan, England and Canada also had great contributions by publishing more than two hundred articles.

Most of the publications were in English (92.17%), followed by articles in German and French, respectively.

Harvard University was the most prolific institution, publishing 109 (2.36%) articles related to this topic. National Heart Lung and Blood Institute and Natural Sciences and Engineering Research Council contributed a maximum number of articles supported by a special funding agency (each 3 (0.08 %)).

While more than half of the articles (2466) were published under "anesthesiology" subject, more than 1500 articles were also published under the subjects of "Surgery", "Obstetrics and Gynecology", "General Internal Medicine" and "Cardiovascular System Cardiology".

The analysis about collaborations revealed that from the total publications, 2074 (49.00%) had domestic collaboration and remaining (2158, 51.00%) had international collaboration. The domestic collaborations contributed to 39.74% of citations, while 60.26% of citations were international collaborations. Details are presented in Table 2.

Furthermore, the analysis revealed 202 collaborations between 72 countries. The highest collaboration rate was for USA with 64 collaborations. England, Canada, Germany and Switzerland ranked second to fifth positions considering the number of collaborations, respectively. USA had most of its collaborations with Argentina and Australia, respectively. Twelve countries had no collaborations like Jordan, Pakistan, and Albania. Fig. 3 shows the distribution of collaborations.

Table 2: Distribution of articles by collaboration types

Collaboration types 3827	No. of Articles	Number of citations	Average Number of citations
Internal-institutional collaborations	494	4272	8/64
External-institutional collaborations	1580	17311	10/95
International collaborations	2158	32714	15/15
Total	4232	54297	12/83

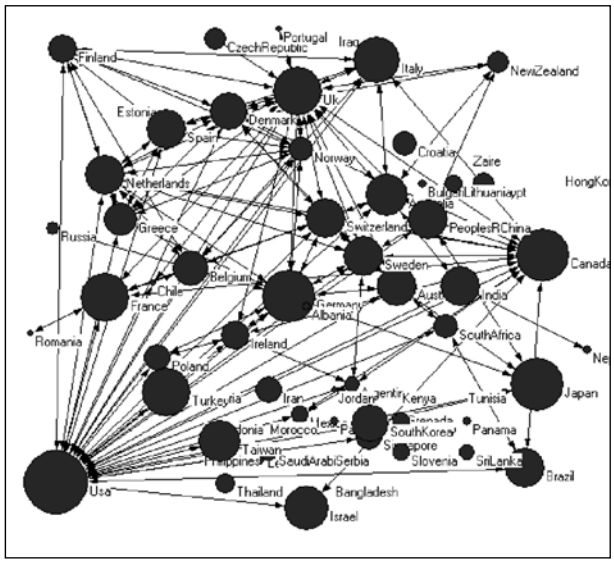

Fig. 3: Analysis of collaborations among different countries

DISCUSSION

After introduction of EA and its advantages, there was an increased interest in application of this method for surgeries. Our study revealed growing attention to EA according to the rise in the number of publications since 1990 that has also been accompanied with rise in the number of citations of these publications.

Consistent with our results, USA with contribution to more than 38% of the original articles about EA was the leading country in publishing articles. The results are the same as previously reported about USA contribution to the general anesthetic literature production, which is about 30 - 40% [13-14].

As expected, between institutions, Harvard University as the main contributing institution in the USA is outstanding by publishing 109 articles in this field of science.

In three out of the five leading countries including USA, Germany, Japan, England, and Canada in this field English is the official language and many countries with other languages publish their article in English. This is the reason for domination of English language in more than 90% of total articles published in this field.

Journals demonstrated different attribute toward article publication. The journal, 'Anesthesia and Analgesia' was at the first place for publishing articles related to EA. The journal 'Anesthesiology' and the "Canadian Journal of Anesthesia" ranked second and third, respectively. Considering the information above, it seems that most of the investigations were printed by anesthesia journals.

Limitations of this study include, using the Web of Science database as the only data source and not including other databases and selection of only one keyword and search term as other search terms might yield different results.

CONCLUSION

It seems that there is an overall increase in total number of articles, citations and highly cited articles about EA during the study period.

ACKNOWLEDGMENT

The authors would like to thank Farzan Institute for Research and Technology for technical assistance.
Source of Support: None

REFERENCES

1. Classical files, Survey of Anesthesiology.1960; 4:418.
2. Franco A, Diz JC. The history of the epidural block. Current Anaesth Crit Care 2000; 11:274-276.
3. Liu S, Carpenter RL, Neal JM. EA and analgesia. Anesthesiology 1995; 82:1474-1506.
4. Grass JA. The role of EA and analgesia in postoperative outcome. Anesthesiol Clin North America 2000; 18:407-428.
5. Park WY, Thompson JS, Lee KK. Effect of EA and analgesia on peri-operative outcome. Ann Surg 2001; 234:560-571.
6. Moraca RJ, Sheldon DG, Thirlby RC. The role of EA and analgesia in surgical practice. Ann Surg 2003; 238:663-673.
7. Nimmo SM. Benefit and outcome after epidural analgesia. Continuing Education in Anaesthesia, Critical Care & Pain 2004; 2:44-47. http://ceaccp.oxfordjournals.org/content/4/2/44.full
8. Gronert GA. Malignant hyperthermia. Anesthesiology 1980; 53:395-423.
9. Eger EI 2nd, Saidman LJ, Brandstater B. Minimum alveolar anesthetic concentration: a standard of anesthetic potency. Anesthesiology 1965; 26:756-763.
10. Watcha MF, White PF. Postoperative nausea and vomiting, its etiology, treatment, and prevention. Anesthesiology 1992; 77:162-184.
11. Baltussen A, Kindler CH. Citation classics in anesthetic journals. Anesth Analg 2004; 98:443-451.
12. Kim KW. Measuring international research collaboration of peripheral countries: taking the context into consideration. Scientometric 2006; 66:231-240.
13. Boldt J, Maleck W, Koetter KP. Which countries publish in important anesthesia and critical care journals? Anesth Analg 1999; 88:1175-1180.
14. Frame JD. National economic resources and the production of research in lesser developed countries. Social Studies of Science1979 ; 9:233-246.

Original Article

Knowledge, Attitude and Satisfaction of Health Care Providers Regarding Premarital Screening and Genetic Counseling Program in Jeddah

Nahla Khamis Ibrahim[1, 2], Bahaa Abalkhaeil[1], Jawaher Al Ahmadi[1], Hussein Al Bar[1], Waleed Milaat[1], Mahdi Qadi[1]
[1]Department of Family and Community Medicine, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
[2]Department of Epidemiology, High Institute of Public Health, Alexandria University, Alexandria, Egypt

Kuwait Medical Journal 2015; 47 (2): 122 - 127

ABSTRACT

Objective(s): To determine level of knowledge and attitudes of health care providers (HCP) regarding premarital screening and genetic counseling (PMSGC), to identify the predictors of high knowledge score and to verify their satisfaction with and recommendations for improving the program

Design: Cross-sectional study conducted during the January 2010 - January 2011 period

Setting: Outpatient clinics of three governmental hospitals in Jeddah

Subjects: Three hundred and forty-five HCP

Intervention(s): A self-administered questionnaire containing personal and socio-demographic data, 30 PMSGC knowledge items, and 14 attitude statements were used. HCP working in the program were asked about their satisfaction and recommendations for improvement.

Main Outcome Measure(s): Knowledge, attitudes and satisfaction of the HCP

Results: About one-half (51.6%) of the health care providers had satisfactory knowledge about PMSGC. After controlling for the confounding factors, the only predictor of satisfactory knowledge score was being a specialized provider (aOR = 2.86; 95% CI: 1.63 – 5.02). Regarding attitudes, almost all participants (99%) strongly agreed and agreed on the importance of the PMSGC program. Concerning satisfaction, half of HCP working in the program had excellent or very good scores for program confidentiality and higher percentages for counseling about discovered diseases.

Conclusions: HCP had good attitudes towards PMSGC program. However, there is some lack of knowledge. They recommended adding vaccinations, new screening and counseling to the current program. Formal training course(s) for HCP about the program were recommended.

KEY WORDS: genetic, health care providers, knowledge, premarital examinations

INTRODUCTION

Premarital programs become a focal point for both national and international public policy[1]. These programs offer the necessary guidance and foundation to help the young couples to evaluate their readiness to enter into a lifelong commitment. Premarital screening and genetic counseling (PMSGC) program can help in promoting health and improving the quality of life of young people after marriage[2]. Results of a meta-analysis of 23 pre-marital programs found that these programs are effective in immediate, short-term gains in interpersonal skills and in overall quality of the relationship[3].

The PMSGC program is important in the Gulf Region, especially in Saudi Arabia as the Saudi culture exhibits a strong preference for consanguineous marriage. The current average rate of this kind of marriage is 58% and it varies from 34 - 80% based on the geographic regions[4]. Probably because of this high rate of consanguineous marriage, Saudi Arabia has a high prevalence of hereditary hemoglobin disorders[5]. In response to these facts, the Saudi PMSGC was established by law in December 2003 and it came into operation in February 2004. The program began as a screening program for hemoglobinopathies, such as sickle cell anemia and thalassemias, in 2004. Viral

Address correspondence to:
Prof. Nahla Khamis Ragab Ibrahim, Department of Family and Community Medicine, Faculty of Medicine, King Abdulaziz University, Jeddah, KSA. P O Box 42806, 21551 Jeddah, Saudi Arabia. E-mail: nahlakhamis@yahoo.com (N.K.R. Ibrahim). http: www.kau.edu.sa/nibrahim, URL: http://nibrahim.kau.edu.sa/, http:// kau.edu.sa/ nibrahim (N.K.R. Ibrahim).

pathogen screening for human immunodeficiency virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) were added to the list in 2008[5-8].

Professionals in several disciplines are starting to appraise the potential creative benefits for premarital programs. As the demand for evaluation of premarital programs increases, the need for empirical evidence to establish components of effective outcomes are required[3].

Evaluation of the PMSGC program is essential for making decisions about the future of the program. Much has been written about the PMSGC program itself[5,6]. Some studies were conducted to assess knowledge and attitude of youth or consumers[7,9] of services in Saudi Arabia[8]. Scanty studies were done to assess health care providers' (HCP) knowledge and attitudes towards PMSGC program in Jeddah. So, such studies are urgently needed.

The objectives of present study were to assess the level of knowledge and attitudes of HCP regarding PMSGC program, to determine the predictors of high knowledge score, and to verify their satisfaction with and their recommendations for improving the program.

SUBJECTS AND METHODS

This cross-sectional study was done during the period January 2010 to January 2011.

Three Jeddah governmental hospitals were selected (two Ministry of Health (MOH) hospitals using the PMSGC program, and the King Abdulaziz University Hospital (KAUH)). The PMSGC services are provided for both Saudi or Non-Saudi couples.

Ethical statement: The study protocol was approved by the Institutional Review Board of the Faculty of Medicine, KAUH and conformed to the ethical standards of the Helsinki declaration. Administrative approvals were taken from both MoH and KAUH. An informed verbal consent was taken before enrollment.

A convenience sample method was used where all HCP working in the outpatient clinics (mainly well baby, hematology and genetic clinics, premarital counseling) of the three hospitals who were available on the day of interview were invited to participate in the study.

A self-administered questionnaire was used. Reliability of the questionnaire was assessed using Cranach's Alfa test and was found to be 85%. The questionnaire inquired about:

1. HCP personal and socio-demographic characteristics

2. Knowledge items: 30 Multiple Choice Questions (MCQ) with a single correct answer were asked. The questions inquired about the HCP's knowledge about the PMSGC program, investigations done, *etc.*

3. Attitudes: were graded on a 5-point Likert scale based on responses to 14 statements

4. Questions were only asked to the HCP working in the PMSGC program:-
   a) Satisfaction with the program: Questions were asked about the HCP satisfaction with the program regarding confidentiality, place of program, capacity of the place, counseling provided to all couples, counseling provided to couples with discovered hereditary or sexually transmitted diseases (STD). In addition, HCP were asked about their satisfaction with the program management, infrastructure, availability of equipment, computers, formats, *etc*.

   b) Recommendations for improving the program Statistical analysis**:** Analysis was done using SPSS version 21 (SPSS Inc, Chicago, Ill) and Epi-Info 3.5.1. Knowledge score was calculated for the 30 knowledge items. For each question, the incorrect and don't know answers were given a score of "0" while the correct response got a score of "1". Total knowledge score was calculated and ranged from 0 - 30. It was classified as: poor score: < 15, fair: 15 - < 20 and satisfactory: ≥ 20. Descriptive statistics were done as frequency, mean and standard deviation (M ± SD). Chi square test was used for comparison between categorical variables. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. For controlling confounding factors, a multivariate logistic regression analysis was done. It identified the predictors of high knowledge scores (fair and satisfactory) compared to poor score. Statistical significance was set at $p \leq 0.05$.

RESULTS

Three hundred eighty-four HCP were included in the study. Out of these, 345 (90% acceptance rate) returned the completed questionnaires. Their mean ± SD for age was 30.63 ± 9.1 years. Male HCP represented 35.9% of the sample while 18.6% of the selected health providers were working in the program (the study included 64 HCP out of a total of 100 providers working in Jeddah PMSGC program at the time of the study). Regarding their job description, specialists represented 24.1%, general practitioners and pharmacists amounted to 8.7%, while the rest (67.2%) were nurses, technicians, *etc.*

The mean ± SD of knowledge score of the HCP about PMSGC program was 18.9 ± 5.5 out of 30.

Results reveal that about one-half of providers (51.6 %) obtained a satisfactory knowledge score, while 25.5 % and 22.9 % got fair and poor scores, respectively.

Table 1 illustrates the relationship between the level of knowledge about PMSGC program and the studied variables. It is apparent from the table that HCP aged ≥ 30 years obtained significantly ($p < 0.05$) higher percentage of satisfactory knowledge score (57.1%) compared to those < 30 years (46.3%). On the other hand, there is no statistically significant difference ($p > 0.05$) between the levels of knowledge about PMSGC program and gender. HCP who had working experience of more than 15 years obtained significantly better knowledge score compared to those with lesser working experience ($X^2 = 4.97$, $p < 0.05$). Specialists got much better knowledge scores compared to the other jobs, with highly statistical significant difference ($X^2 = 19.08$, $p < 0.001$). Those who attended training course about PMSGC program and those who were working in the program or in the hospital using it had higher knowledge scores, although not statistically significant.

Regression analysis revealed that specialization (having post graduate degree) was the only predictor of obtaining satisfactory knowledge score after controlling for other confounders (aOR= 2.86; 95 % CI: 1.63 – 5.02).

Table 2 portrays the attitudes of HCP towards premarital program. It is obvious that almost all the participants (99.1%) strongly agreed and half of the HCP agreed, gave excellent and very good scores for program confidentiality and a similar percentage (49.2%) to the place of counseling in the hospital. Higher percentages of excellent and very good scores were given for counseling offered to couples who were found with hereditary diseases (62.7%) and to couples with STDs (57.8%). About two-thirds gave the similar grades for program management, availability of equipment, and computers. Analysis of results of the present study revealed that the majority of HCP recommended vaccinating all females with rubella vaccine (82.6%) and all couples with hepatitis B vaccines (91.3%).

The majority of the HCP recommended adding screening of other STDs (95.3%) and genetic diseases (91%). In addition, 87.2% of the HCP recommended adding counseling about family bonding. More than three- fourths of providers recommended adding counseling about psychiatric problems (78.4%), chronic diseases (75.8%) and fertility and reproduction (75.5%).

The importance of PMSGC. The vast majority of providers (95.4%) also strongly agreed and agreed that premarital program will contribute to reducing the prevalence of some genetic and STDs. On the other hand, more than 80% of providers strongly disagreed and disagreed that PMSGC program is against Islamic rules.

The level of satisfaction of HCP who were working in the PMSGC program is presented in Table 3. About

Table 1: Relationship between level of knowledge about PMSGC program and studied variables of health care providers in Jeddah

Variables	Poor Fair		Satisfactory		Total	X^2, (p-value)	OR , 95 % CI
	No	%	N	%			
Age (years)							
< 30	94	53.7	81	46.3	175	4.1	1.54
≥ 30	73	42.9	97	57.1	170	(0.04)	(1.008-2.35)
Sex							
Male	51	41.1	73	58.9	124	4.1	0.36
Female	116	52.2	105	47.5	221	(0.04)	(0.41-0.98)
Years of experience							
<15 years	141	51.5	133	48.5	274	4.97	1.84
≥ 15 years	26	36.6	45	63.4	71	(0.02)	(1.07-3.14)
Education							
Specialized	23	27.7	60	72.3	83	19.08	0.31(0.17-0.55)
G P or Pharmacists	15	50.0	15	50.0	30	(< 0.001)	0.80 (0.35-1.82)
Other	129	55.6	103	44.3	232		1 [RC]
Attending training course (s)							
Yes	35	44.9	43	55.1	78	0.5	0.83
No	132	49.4	153	50.6	267	(0.4)	(0.50-1.38)
Working in the program							
Yes	26	40.6	38	59.4	64	1.91	0.68
No	141	50.2	140	49.8	281	(0.1)	(0.39-1.18)
Working in the hospital provider program							
Yes	148	48.2	159	51.8	307	0.04	0.93
No	19	50.0	19	50.0	38	(0.8)	(0.47-1.83)
Total	167	48.4	178	51.6	345		

RC = Reference category, OR = odds ratio, CI = confidence interval

Table 2: Attitudes of health care providers in Jeddah towards premarital screening program

Attitude towards the program	Strongly agree		Agree		No opinion		Disagree		Strongly disagree	
	No.	%	No.	%	No.	%	No.	%	No.	%
PMSGC program is important	326	94.5	16	4.6	2	0.6	1	0.3	0	0.0
PMSGC program is against Islamic rules	17	4.9	12	3.5	33	9.6	99	28.7	184	53.3
Consanguinity may leads to hereditary diseases	170	49.3	149	43.2	12	3.5	9	2.5	5	1.4
PMSGC program will contribute to reduction in the prevalence of some genetic STDs	258	74.8	71	20.6	9	2.6	5	1.4	2	0.6
It is important to raise awareness about PMSGC program before marriage to reduce genetic STDs	299	86.7	40	11.6	4	1.2	1	0.3	1	0.3
Religious people should adopt the ideas of premarital program in their discussion	217	62.9	98	28.4	21	6.1	8	2.3	1	0.3
Person who do marriage contract should have the right to accept conducting marriage contract only if future couple did premarital screening	193	55.9	79	22.9	33	9.6	29	8.4	11	3.2
The law that obligate all future couples to conduct the PMSGC is important	229	66.4	95	27.5	12	3.5	7	2.0	2	0.6
No one should force any person to conduct genetic testing, but only encourage doing	99	28.7	89	25.8	14	4.1	77	22.3	66	19.1
In a case of discovery having or carrying STDs, marriage decision must be left to the choice of the couple	117	33.9	108	31.3	23	6.7	61	17.7	36	10.4
In the case of discovery having or carrying inherited disease, marriage decision must be left for freedom of couple	87	25.2	104	30.1	28	8.1	74	21.4	52	15.1
Test results that shows presence of genetic diseases should change marriage decision	111	32.2	100	29.0	81	23.5	35	10.1	18	5.2
It is important to apply a law that stop marriage upon discovery of a genetic disease	122	35.4	94	27.2	55	15.9	54	15.7	20	5.8
Premarital program breaks personal confidentiality	12	3.5	11	3.2	18	5.2	130	37.7	174	50.4

Table 3: Percentage of satisfaction of health care providers working in PMSGC program regarding the program

Satisfaction criteria	Excellent %	Very good %	Good %	Bad %	Very bad %
Place of PMC inside the hospital	27.9	31.1	23.0	8.2	9.8
Capacity of PMNSGC place	1.7	18.6	35.6	30.5	13.6
Degree of confidentiality	21.7	28.3	28.3	13.3	8.4
Doctor's dealing with the program	31.7	33.3	21.7	10.0	3.3
Number of providers in relation to consumers	18.3	30.0	31.7	15.0	5.0
Place for counseling	10.2	39.0	30.5	13.6	1.7
Counseling give to all consumers	22.0	44.1	18.6	13.6	1.7
Counseling given to those who have hereditary diseases	25.4	37.3	25.4	8.5	3.4
Counseling given to those who have infectious diseases	27.1	40.7	27.1	1.7	3.4
Laboratory diagnosis	20.3	35.6	33.9	8.5	1.7
Program management	27.1	40.7	20.3	6.8	5.1
Salaries	13.6	32.2	30.5	13.6	10.2
Availability of equipments	28.8	39.0	20.3	11.9	0.00
Availability of formats	25.4	30.5	27.1	11.9	5.1
Availability of computers	27.1	39.0	23.7	6.8	3.4

DISCUSSION

PMSGC is an extremely important program for detecting disorders and for reducing the incidence of offspring's diseases[10]. The current study is the first study that addresses the views of HCP working in Jeddah hospitals about the PMSGC program.

The WHO recommended numerous procedures to prevent genetic diseases like health education and the improvement of knowledge and attitude towards the control of hereditary genetic diseases[11]. In the current study, only about one-half of HCP obtained satisfactory knowledge score about PMSGC program, while 25.5% and 22.9% of them obtained fair and poor scores, respectively. The awareness level about the program needs improvement through organizing of educational campaigns[8]. Turkish physicians, in 2007, also thought that they didn't have sufficient knowledge about genetics or genetic counseling[12]. An earlier study conducted among physicians working at Mount Sinai Medical Center found that 71% of them

rated their knowledge of genetics and genetic testing as "fair" to "poor"[13].

Similarly, a recent study done in USA in 2013 illustrated that most of the internists (73.7%) from two academic medical centers in Columbia rated their genetics knowledge as very or somewhat poor[14]. Results of our previous study among female students at KAU revealed lower knowledge scores about PMSGC program before conducting an interventional educational campaign (80.9%, 12.5% and 6.6% obtained poor, fair and satisfactory scores , respectively)[8]. The lower scores reported from the previous Jeddah study compared to the current study may be attributed to the differences between the two target populations (the first one was among university students while the current one was done among HCP with higher knowledge levels).

The present study showed that the mean PMSGC knowledge score obtained by HCP was 18.9 ± 5.5 out of 30. These results coincide with another study done among family planning staff from Pennsylvania. They had a mean score of 4.45 out of 7 concerning some genetic questions[15].

HCP who received training on PMSGC had a higher percentage of satisfactory knowledge score compared to others. Our previous study among female university students in Jeddah revealed that there is marked improvement in student's knowledge after receiving PMSGC educational intervention[8]. Similarly, the WHO recommended that nations should work towards training primary health professionals to provide basic genetic counseling services. This training would help to increase their capacity and to address shortages of genetic counselors[4].

Regarding attitudes, results of the present study showed that almost all HCP (99.1%) strongly agreed or agreed on the importance of PMSGC program. The vast majority of them (98.3%) also agreed on importance of raising awareness about PMSGC before marriage and on that PMSGC will contribute to reduction of prevalence of some genetic and STDs (95.4%). These results coincide with our two previous studies among students and consumers of services in Jeddah[7,9]. These results also agree with a study done among 49 Pakistani doctors from Karachi; they showed positive attitudes towards provision of genetic counseling and the majority of them (98%) favored premarital screening for thalassemia[16]. Gillani, *et al* conducted another Pakistani study for assessing the attitudes of doctors, medical students, lawyers, parliament members and parents of thalassemic children towards genetic diagnosis. They found that premarital carrier screening was favored by 77% while 24% of the doctors favored making genetic screening mandatory[17].

Regarding satisfaction with the program, the current study showed that one-half of health care providers gave excellent and very good scores for program confidentiality. This percentage is lower than results obtained from a previous study conducted among consumers of the PMSGC program in Jeddah (80.0%)[7]. This discrepancy may be attributed to differences between both target populations and their evaluation of the program.

Premarital counseling provides an opportunity to intervene according to the identified risks. This intervention includes treatment, vaccination, counseling regarding behavior, genetic counseling, etc.[18]. HCP in the current study recommended adding vaccination of 'at risk' future spouse with some vaccines such as HBV vaccine. Another study was conducted in Turkey to monitor the cases identified as hepatitis B carriers and to vaccinate their prospective spouses with a rapid vaccination scheme during premarital tests. Results revealed that rapid vaccination program with HBV vaccine upon determination of Hepatitis B surface Antigen (HBsAg) positivity in premarital tests provides early protection[19]. This indicates a great need to apply the recommendation of health providers and to vaccinate the prospective spouses of discovered cases of HBV carriers with HBV vaccine within the activities of PMSGC program.

CONCLUSION

HCP in the current study have demonstrated positive attitudes towards PMSGC program. However, there is some lack of knowledge about the program. HCP recommended introduction of vaccinations such as Hepatitis B and rubella vaccine (for females) for those 'at risk'. They recommended additional screening for other genetic diseases and STDs and providing more counseling to current PMSGC program.

Educational programs on PMSGC for HCP in Jeddah are recommended to improve their knowledge. Availability of trained genetic and STDs counselors for providing adequate counseling for future couples (especially those with discovered problem) is highly recommended. Adding some vaccination such as HBV vaccine, other genetic and STDs screening is also suggested.

ACKNOWLEDGEMENT

Authors would like to thank all the fourth year medical students during the educational year 2010 for their contribution to the study. We also thank all the staff of the department of Family and Community Medicine. Special thanks to Dr. Nariman Hejazi, Dr. Jamil Bashawri, Dr. Hashim Feda, Dr. Rahela Iftikhar and Dr. Mervat Radi for their active participation in the study. The authors are grateful to the Jeddah

Directorate of Health Affairs, the heads of the selected hospitals, the dean of the Faculty of Medicine for their help and facilitation of this study. Special thanks to all the HCP participants for their agreement to participate and their kind contributions to this study.

Conflict of interest and funding: None

REFERENCES

1. M cGeorge CR, Carlson TS. Premarital Education: An assessment of program efficacy. Contemp Fam Ther 2006; 28:165-190.
2. Wang P, Wang X, Fang M, Vander Weele TJ. Factors influencing the decision to participate in medical premarital examinations in Hubei Province, Mid-China. BMC Public Health 2013; 13:217.
3. Carroll JS, Doherty WJ. Evaluating the effectiveness of premarital prevention programs: A meta-analytic review of outcome research. Family Relations 2003; 52:105-118.
4. Ballantyne A, Goold I, Pearn A. Medical genetic services in developing countries: the ethical, legal and social implications of genetic testing and screening. Geneva: WHO, 2006.
5. Memish ZA, Saeedi MY. Six-year outcome of the national premarital screening and genetic counseling program for sickle cell disease and beta-thalassemia in Saudi Arabia. Ann Saudi Med 2011; 31:229-235.
6. El-Hazmi MA. Pre-marital examination as a method of prevention from blood genetic disorders. Community views. Saudi Med J 2006; 27:1291-1295.
7. Ibrahim NK, Bashawri J, Al Bar H, *et al*. Premarital screening and genetic counseling program: knowledge, attitude, and satisfaction of attendees of governmental outpatient clinics in Jeddah. J Infect Public Health 2013; 6:41-54.
8. Ibrahim NK, Al-Bar H, Al-Fakeeh A, *et al*. An educational program about premarital screening for unmarried female students in King Abdul-Aziz University, Jeddah. J Infect Public Health 2011; 4:30-40. doi: 10.1016/j.jiph.2010.11.001.
9. Al-Aama JY, Al-Nabulsi BK, Alyousef MA, Asiri NA, Al-Blewi SM. Knowledge regarding the national premarital screening program among university students in western Saudi Arabia. Saudi Med J 2008; 29:1649-1653.
10. Abdel-Meguid N, Zaki MS, Hammad SA. Premarital genetic investigations: effect of genetic counselling. East Mediterr Health J 2000; 6:652-660.
11. Odelola JO, Adisa O, Akintaro OA. Knowledge of pre-marital genetic screening among students of Osun State Polyclinic in Nigeria. J Res Edu Soc 2011; 2:108-115.
12. Tomatir AG, Sorkun HC, Demirhan H, Akdağ B. Genetics and genetic counseling: practices and opinions of primary care physicians in Turkey. Genet Med 2007; 9:130-135.
13. Menasha JD, Schechter C, Willner J. Genetic testing: a physician's perspective. Mt Sinai J Med 2000; 67:144-151.
14. Klitzman R, Chung W, Marder K, *et al*. Attitudes and practices among internists concerning genetic testing. J Genet Couns 2013; 22:90-100.
15. Naylor EW. Genetic screening and genetic counseling: knowledge, attitudes, and practices in two groups of family planning professionals. Soc Biol 1975; 22:304-314.
16. Ashfaq M, Amanullah F, Ashfaq A, Ormond KE. The views of Pakistani doctors regarding Genetic Counseling Services - Is there a Future? J Genet Couns 2013; 22:721-32. doi: 10.1007/s10897-013-9578-2.
17. Gilani AI, Jadoon AS, Qaiser R, *et al*. Attitudes towards genetic diagnosis in Pakistan: a survey of medical and legal communities and parents of thalassemic children. In: Community Genet Switzerland: 2007 S. Karger AG, Basel. 2007:140-146.
18. Al-Arrayed SS, Hafadh N, Al-Serafi S. Premarital counseling: an experience from Bahrain. East Mediterr Health J 1997; 3:415-419.
19. Tosun S, Yuceturk M, Donmez AB, Gunduz T. Rapid immunization scheme for spouses of individuals estabilished as hepatitis B carriers during premarital tests. Clin Dev Immunol 2012; 2012:843134.

Original Article

Serum Uric Acid Levels are Elevated in Patients with Diastolic Dysfunction and Preserved Ejection Fraction

Ahmet Goktug Ertem[1], Mehmet Erat[2], Harun Kilic[3]
[1]Department of Cardiology, Ankara Penal Instution Campus State Hospital, Ankara, Turkey
[2]Department of Cardiology, Diskapi Yildirim Beyazit Education and Training Hospital, Ankara, Turkey
[3]Department of Cardiology, Sakarya University, School of Medicine, Sakarya, Turkey

Kuwait Medical Journal 2015; 47 (2): 128 - 132

ABSTRACT

ABSTRACT

Objectives: Elevated uric acid levels are associated with diastolic dysfunction in chronic heart failure patients. Uric acid is a marker of impaired oxidative metabolism and is correlated with endothelial function. In this study, we investigated whether uric acid levels correlate with the degree of left ventricular diastolic dysfunction (LVDD) in patients with normal ejection fraction.

Design: Prospective case-control study

Subjects and Methods: Uric acid levels were measured in 201 patients with normal ejection fraction. The study population was divided into two groups as controls and patients with LVVD. Patients with LVDD were divided into two groups according to dysfunction grade. (Normal, n = 64, Grade 1 LVDD, n = 74, Grade 2 LVDD, n = 63).

Interventions: For uric acid analysis, 5 ml of venous blood was drawn from each patient after at least 8 hours of fasting.

Main Outcome Measures: Serum uric acid levels were measured and LVDD was assessed by transthoracic echocardiography

Results: Uric acid levels were significantly lower in the normal diastolic function group than grade 1 LVDD and grade 2 LVDD groups respectively (4.28 ± 1.20 mg/dl, 8.17 ± 2.12 mg/dl, 9.52 ± 2.30 mg/dl, $p < 0.001$). Although Grade 2 LVDD patients had higher uric acid levels, there is no significant difference between grade 1 and grade 2 LVDD.

Conclusions: Our study demonstrated that uric acid level was significantly elevated in patients with diastolic dysfunction. Further studies are needed to assess whether inhibition of xanthine oxidase (XO) with allopurinol results in an improvement in diastolic dysfunction.

KEYWORDS: diastolic dysfunction, endothelial function, uric acid

INTRODUCTION

Some studies suggest that uric acid (UA) is potentially an independent risk factor for both cardiovascular disease and kidney disease[1-7]. Other studies have noted that the development of hypertension, obesity, kidney disease, and diabetes can be predicted by an elevated level of UA[5–12]. A strong relation between hyperuricemia and subsequent cardiovascular disease risk in unselected populations has been identified by epidemiological studies[13–15].

The mechanisms by which UA may engender organ damage is still incompletely understood, but there is increasing evidence that endothelial dysfunction is a fundamental mechanism whereby this substance may affect cardiovascular and renal function and structure[16].

Reduced bioavailability of nitric oxide (NO), which normally mediates local vasodilatation, inhibits platelet aggregation, and reduces local vascular inflammation, lead to endothelial dysfunction[17]. Cardiovascular disease can be predicted by endothelial dysfunction and it is central to the development and progression of atherosclerosis[18,19]. An inverse relationship between serum UA concentration and NO activity has been identified and therefore it is possible that UA directly influences endothelial dysfunction[20].

NO has been reported to exert significant effects on the relaxation phase of cardiac contraction, in

Address correspondence to:
Ahmet Goktug Ertem, MD, Sogutozu Konutları Sogutozu Mah, 2185. Sk 7/A No: 56 Cankaya, Ankara, Turkey. Tel: 00905323944334, Fax: 00903122540290, E-mail: agertem@hotmail.com

some cases even in the absence of changes in systolic function. NO selectively induce an earlier onset of isometric relaxation without affecting the rate of isometric tension development[21].

Previously, it was demonstrated that elevated UA levels are associated with diastolic dysfunction in chronic heart failure patients[22].

In this study, we investigated whether UA levels correlated with the degree of left ventricular diastolic dysfunction (LVDD) in patients with normal ejection fraction.

SUBJECTS AND METHODS

Study population

A total of 201 outpatient subjects with normal ejection fraction were studied between August 2012 and August 2013. Transthoracic echocardiography (Vivid 7, Vingmed Ultrasound, GE, Horten, Norway) was performed in the left lateral decubitus position. Pulse Wave (PW) Doppler mitral filling flow velocities (Em and Am waves) and the E / A ratio were calculated. Apical four chamber view of mitral annular tissue, Doppler and PW Doppler measurements taken with the Em and Am-wave velocities and mitral 'e' values were calculated for each patient. All measurements were made according to the recommendations of the American Echocardiography Society[23]. Normal diastolic function was described as E / A > 1, lateral e'> 8, grade 1 diastolic dysfunction was described as E / A < 1, lateral 'e' ≤ 8, grade 2 diastolic dysfunction was described as E / A > 1 and lateral e' ≤ 8. We also described normal ejection fraction (EF) as > 50%[23].

After echocardiography examinations, the study population was divided into two groups: control group with normal diastolic function and patient group with diastolic dysfunction. Patients with diastolic dysfunction were further divided into two groups according to dysfunction grade. (Normal, n = 64, Grade 1 LVDD, n = 74, Grade 2 LVDD, n = 63).

We excluded patients with gout, usage of XO (xanthine oxidase) inhibitors, atherosclerotic heart disease (acute coronary syndromes, and stable coronary heart disease), a history of smoking, cerebrovascular disease within the last three months, obstructive or non-obstructive hypertrophic cardiomyopathy, severe arrhythmias, uncorrected congenital heart disease, active perimyocarditis, usage of thiazide diuretics, chronic renal disease and diabetic nephropathy.

Serum blood urea nitrogen (BUN), serum creatinine and UA were measured in the routine laboratory by an automated technique using an auto analyzer after at least 8 hours of fasting. The blood plasma uric acid level between 3.6 mg/dl (~ 214 μmol/l) and 8.3 mg/dl (~ 494 μmol/l) (1 mg/dl = 59.48 μmol/l) was taken as the normal reference range for this study.

Statistical Methods

Data analysis was performed using SPSS for Windows 15.0 (Statistical Package for Social Science, SPSS inc., Chicago, IL, U.S.). Descriptive statistics were expressed as mean ± standard deviation. For the comparison of the averages of the two groups, we used t-test. We assessed the linear relationship between two variables with Pearson's (for normally distributed data) and Spearman's (for abnormally distributed data) correlation coefficient. For comparison of numerical data between groups, "one-way ANOVA" test and for multiple comparisons the "post-hoc Tukey's HSD" test were used. Finally, the relationship between clinical and demographical parameters (including serum UA) and diastolic dysfunction was analyzed by linear regression analysis. In the statistical analysis, $p < 0.05$ was considered statistically significant.

RESULTS

Clinical and echocardiographical parameters are shown in Table 1. The mean age of controls, grade 1 LVDD group and grade 2 LVDD group was 49.30 ±

Table 1: Clinical and echocardiographical characteristics of study patients

Clinical and echocardiographical variables	Normal (n : 64)	Grade 1 DD (n : 74)	Grade 2 DD (n : 63)	p-value
Age (years)	49.30 ± 10.99	64.22 ± 10.63	63.73 ± 10.51	< 0.001
Gender (female %)	48.9	52.2	55.3	0.898
Smoking (%)	26.7	25.8	27.3	0.864
BUN (mg/dl)	26.51 ± 8.88	27.56 ± 10.13	32.63 ± 13.71	0.004
Creatinine (mg/dl)	0.81 ± 0.19	0.85 ± 0.15	0.83 ± 0.17	0.359
Uric acid (mg/dl)	4.28 ± 1.20	8.17 ± 2.12	9.52 ± 2.30	< 0.001
Mitral E wave (m/s)	0.72 ± 0.16	0.57 ± 0.15	0.84 ± 0.21	< 0.001
Mitral A wave (m/s)	0.58 ± 0.12	0.87 ± 0.11	0.67 ± 0.18	< 0.001
LVEF (%)	65.29 ± 4.16	63.02 ± 3.61	62.45 ± 8.26	0.051
LA diameter (cm)	3.12 ± 0.36	3.46 ± 0.40	3.70 ± 0.56	< 0.001
Mitral annulus e' (cm/s)		5.94 ± 1.01	6.00 ± 1.20	0.758

BUN: blood urea nitrogen, DD: diastolic dysfunction, LA: left atrium, LVEF: left ventricle ejection fraction

10.99, 64.22 ± 10.63, 63.73 ± 10.51 years, respectively (p = 0.627). Comparison between controls and grade 1 LVDD (p = 0.140) and between controls and grade 2 LVDD (p = 0.273) between grade 1 LVDD and grade 2 LVDD. The overall p-value was < 0.001. UA levels were significantly lower in control subjects than grade 1 LVDD and grade 2 LVDD groups respectively (4.28 ± 1.20mg/dl, 8.17 ± 2.12 mg/dl, 9.52 ± 2.30 mg/dl, p < 0.001). Although grade 2 LVDD patients have higher UA levels, there was no significant difference between grade 1 LVDD and grade 2 LVDD (8.17 ± 2.12 mg/dl, 9. 52 ± 2.30 mg/dl, p = 0.511).

Ejection fraction of control subjects, grade 1 LVDD group and grade 2 LVDD group were 65.29 ± 4.16%, 63.02 ± 3.61%, 62.45 ± 8.26, respectively (p = 0.915 between controls and grade 1 LVDD; p = 0.269 between controls and grade 2 LVDD; p = 0.236 between grade 1 LVDD and grade 2 LVDD; overall p = 0.051). There were no significant differences between grade 1 LVDD group and grade 2 LVDD groups, in terms of mitral annulus e' (cm/s) (5.94 ± 1.01 vs. 6.00 ± 1.20, p = 0.758).

Table 2: Correlation analysis of uric acid and variables

Uric acid	Coeff. Corr.	p-value
Age	0.422**	< 0.001
Gender	- 0.049	0.409
Diastolic dysfunction	0.717**	< 0.001
Mitral A	0.263**	< 0.001
Mitral E	0.122	0.086
Mitral e'	- 0.004	0.961
Left atrium diameter	0.440**	< 0.001
Left ventricle ejection fraction	- 0.218**	0.002
Blood urea nitrogen	0.175*	0.013
Creatinine	0.178*	0.011

*Correlation is significant at the 0.05 level (p-value)
**Correlation is significant at the 0.01 level (p-value)

As shown in Table 2, serum uric acid levels correlated with age, LVDD grade, mitral A wave, left atrium (LA) diameter, LVEF, blood urea nitrogen (BUN) and creatinine (r = 0.422, p < 0.001; r = 0.717, p < 0.001; r = 0.263, p < 0.001; r = 0.440, p < 0.001; r = - 0.218, p = 0.002; r = 0.175, p = 0.013; r = 0.178, p= 0.011, respectively). After multivariate linear regression analysis, age and uric acid were independent variables for diastolic dysfunction (β = 0.314, p < 0.001 and β = 0.157, p = 0.039, respectively, Table 3).

Table 3: Multivariate linear regression analysis of the predictor factors for diastolic dysfunction

Variables	β	t	t
Age	0.314	4.323	4.323
Gender	- 0.076	- 1.052	- 1.052
Uric acid	0.157	2.081	2.081
BUN	- 0.117	- 1.311	- 1.311
Serum creatinine	0.124	1.238	1.238

BUN: blood urea nitrogen

DISCUSSION

Our study is the first study, which compared the relationship between UA levels and diastolic dysfunction grade. Previous studies evaluated relationship between UA levels and systolic heart failure or idiopathic cardiomyopathy[24].

High serum UA concentration as a marker of increased cardiovascular risk has been recognized as important for more than 50 years. The association between hyperuricemia and total cardiovascular mortality was statistically significant after multivariate adjustment[25,26].

UA is a pro-oxidant that can increase oxygen radicals in circulation, which may in turn promote the lipid oxidation, leading to vascular endothelial dysfunction, inflammation, NO production impairment, atherosclerosis, and thrombogenesis[16].

Since xanthine oxidase (XO) generates UA from xanthine, UA could reflect underlying XO activity. In addition, XO is also known to produce oxidants. Thus, the presence of XO-associated oxidants could be simply reflected by an elevated UA, which may be eventually responsible for the endothelial dysfunction[27]. The Framingham study showed that serum UA levels are an independent predictor of hypertension and progression to a higher BP stage. This may be related to activation of the renin-angiotensin system by UA[28].

In healthy humans, there is an inverse circadian relationship between serum UA levels and NO. It is supporting the hypothesis that UA impairs endothelial function[29].

Increased right and left atrial pressures among patients with ischemic heart disease or dilated cardiomyopathy in a small case series are some worse hemodynamic measures associated with hyperuricemia[30,31].

The major physio-pathological mechanism for diastolic heart failure in hyperuricemia might be the relationship between NO and UA[20]. In this present study, we demonstrated that UA levels were significantly increased in diastolic dysfunction group than control group. Furthermore, UA levels were increased in grade 2 diastolic heart failure group than grade 1 heart failure group, but there was no significant difference between both groups. The incidence of LVDD increases with age. Our diastolic dysfunction group had patients with older age than normal group. But after regression analysis, UA and age were independent factors for LVDD.

Effects of NO on left ventricle (LV) relaxation have been documented in the isolated ejecting buffer-perfused guinea pig heart, studied at constant preload, afterload and heart rate[32]. An effect of NO and / or cGMP on twitch relaxation and diastolic properties has also been reported in isolated cardiac myocytes. The

NO or cGMP-induced increase in myocyte diastolic length, in the absence of changes in diastolic Ca++ , has been suggested to be due to an acute reduction in active diastolic tone, and is analogous to the changes in LV diastolic pressure–volume relations observed in clinical studies[33,34].

Previously, it was demonstrated that elevated UA levels are associated with diastolic dysfunction in chronic heart failure patients[22]. They found no correlation between uric acid and left ventricular volumes, ejection fraction, or stroke volume. In a multivariate model, uric acid predicted diastolic dysfunction independently of renal function, diuretic dose, and left ventricular volumes.

There is an important distinction between UA as a coincidental or causal risk factor because, if UA is causal, treatment to lower serum UA concentrations may potentially reduce cardiovascular disease risk[35]. Various clinical studies have shown that endothelial function in patients with diabetes, patients with coronary artery disease, smokers, and, in particular, patients with CHF have been improved by XO inhibition[36]. The primary effect of allopurinol could be the improvement in endothelial function that has resulted in better myocardial perfusion, thereby improving LV function. Alternatively, the improvement in endothelial function could be secondary to a direct effect of allopurinol on LV function through improvement in myocardial energetic efficiency and oxygen consumption[37].

CONCLUSION

Our study demonstrated that UA level was significantly elevated in patients with diastolic dysfunction and normal ejection fraction. UA levels also correlated with age. The incidence of LVDD increases with age. Our diastolic dysfunction group had older patients than in the control group. But after regression analysis, UA and age were independent factors for LVDD. Further studies are necessary to see improvement of diastolic functions using UA lowering drugs.

Limitations of Study

This study is a single-center, observational, and non-randomized study. We also did not evaluate NO activity, which was directly affected by UA. Further studies are needed to compare uric acid and LVDD at similar ages.

ACKNOWLEDGEMENT

This study was presented in poster session at the 22nd European Meeting on Hypertension and Cardiovascular prevention London 26-29 April 2012, PP19, 134

Conflict of interest: None declared

REFERENCES

1. Niskanen LK, Laaksonen DE, Nyyssönen K, *et al*. Uric acid level as a risk factor for cardiovascular and all-cause mortality in middle-aged men: a prospective cohort study. Arch Intern Med 2004; 164:1546-1551.
2. Fang J, Alderman MH. Serum uric acid and cardiovascular mortality: the NHANES I epidemiologic follow-up study, 1971-1992. JAMA 2000; 283:2404-2410.
3. Alderman MH, Cohen H, Madhavan S, *et al*. Serum uric acid and cardiovascular events in successfully treated hypertensive patients. Hypertension 1999; 34:144-150.
4. Niskanen L, Laaksonen DE, Lindström J, *et al*. Serum uric acid as a harbinger of metabolic outcome in subjects with impaired glucose tolerance: the Finnish Diabetes Prevention Study. Diabetes Care 2006; 29:709-711.
5. Iseki K, Ikemiya Y, Inoue T, *et al*. Significance of hyperuricemia as a risk factor for developing ESRD in a screened cohort. Am J Kidney Dis 2004; 44:642-650.
6. Iseki K, Oshiro S, Tozawa M, *et al*. Significance of hyperuricemia on the early detection of renal failure in a cohort of screened subjects. Hypertens Res 2001; 24:691-697.
7. Tomita M, Mizuno S, Yamanaka H, *et al*. Does hyperuricemia affect mortality? A prospective cohort study of Japanese male workers. J Epidemiol 2000; 10:403-409.
8. Alper AB Jr, Chen W, Yau L, *et al*. Childhood uric acid predicts adult blood pressure: the Bogalusa Heart Study. Hypertension 2005; 45:34-38.
9. Dyer AR, Liu K, Walsh M, *et al*. Ten-year incidence of elevated blood pressure and its predictors: the CARDIA study. J Hum Hypertens 1999; 13:13-21.
10. Masuo K, Kawaguchi H, Mikami H, *et al*. Serum uric acid and plasma norepinephrine concentrations predict subsequent weight gain and blood pressure elevation. Hypertension 2003; 42:474-480.
11. Nakanishi N, Okamoto M, Yoshida H, *et al*. Serum uric acid and risk for development of hypertension and impaired fasting glucose or Type II diabetes in Japanese male office workers. Eur J Epidemiol 2003; 18:523-530.
12. Kang DH, Nakagawa T, Feng L, *et al*. A role for uric acid in the progression of renal disease. J Am Soc Nephrol 2002; 13:2888-2897.
13. Brand FN, McGee DL, Kannel WB, *et al*. Hyperuricemia as a risk factor of coronary heart disease: the Framingham study. Am J Epidemiol 1985; 121:11-18.
14. Levine W, Dyer AR, Shekelle RB, *et al*. Serum uric acid and 11.5 year mortality of middle-aged women findings of the Chicago Heart Association detection project in industry. J Clin Epidemiol 1989; 42:257-267.
15. Freedman DS, Williamson DF, Gunter EW, *et al*. Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I epidemiologic follow-up study. Am J Epidemiol 1995; 141:637-644.

16. Johnson RJ, Kang DH, Feig D, *et al*. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? Hypertension 2003; 41:1183-1190.
17. Rabelink TJ, Luscher TF. Endothelial nitric oxide synthase: host defense enzyme of the endothelium? Arterioscler Thromb Vasc Biol 2006; 26:267-271.
18. Landmesser U, Hornig B, Drexler H. Endothelial function: a critical determinant in atherosclerosis? Circulation 2004; 109:1127-1133.
19. Perticone F, Ceravolo R, Pujia A, *et al*. Prognostic significance of endothelial dysfunction in hypertensive patients. Circulation 2001; 104:191-196.
20. Maxwell AJ, Bruinsma KA. Uric acid is closely linked to vascular nitric oxide activity. Evidence for mechanism of association with cardiovascular disease. J Am Coll Cardiol 2001; 38:1850-1858.
21. Smith JA, Shah AM, Lewis MJ. Factors released from endothelium of the ferret and pig modulate myocardial contraction. J Physiol 1991; 439:1-14.
22. Cicoira M1, Zanolla L, Rossi A, *et al*. Elevated serum uric acid levels are associated with diastolic dysfunction in patients with dilated cardiomyopathy. Am Heart J 2002; 143:1107-1111.
23. Nagueh SF, Appleton CP, Gillebert TC, *et al*. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr 2009; 22:107–133.
24. Huang H, Huang B, Li Y, *et al*. Uric acid and risk of heart failure: a systematic review and meta-analysis. Eur J Heart Fail 2014; 16:15-24.
25. Gertler MM, Garn SM, Levy SA. Serum uric acid in relation to age and physique in health and in coronary heart disease. Ann Intern Med 1951; 34:1421-1431.
26. Chen JH, Chuang SY, Chen HJ, Yeh WT, Pan WH. Serum uric acid level as an independent risk factor for all-cause, cardiovascular, and ischemic stroke mortality: A Chinese cohort study. Arthritis Rheam 2009; 61:225-232.
27. George J, Carr E, Davies J, *et al*. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. Circulation 2006; 114:2508-2516.
28. Sundström J, Sullivan L, D'Agostino RB, *et al*. Relations of serum uric acid to longitudinal blood pressure tracking and hypertension incidence. Hypertension 2005; 45:28-33.
29. Kanabrocki EL, Third JL, Ryan MD, *et al*. Circadian relationship of serum uric acid and nitric oxide. JAMA 2000; 283:2240-2241.
30. Grocott-Mason R, Anning P, Evans H, *et al*. Modulation of left ventricular relaxation in isolated ejecting heart by endogenous nitric oxide. Am J Physiol 1994; 267:1804-1813.
31. Hoeper MM, Hohlfeld JM, Fabel H. Hyperuricaemia in patients with right or left heart failure. Eur Respir J 1999; 13:682-685.
32. Grocott-Mason R, Fort S, Lewis MJ, *et al*. Myocardial relaxant effect of exogenous nitric oxide in the isolated ejecting heart. Am J Physiol 1994; 266:1699-1705.
33. Shah AM, Spurgeon HA, Sollott SJ, *et al*. 8-Bromo-cGMP reduces the myofilament response to Ca 21 in intact cardiac myocytes. Circ Res 1994; 74:970-978.
34. Ito N, Bartunek J, Spitzer KW, *et al*. Effects of the nitric oxide donor sodium nitroprusside on intracellular pH and contraction in hypertrophied myocytes. Circulation 1997; 95:2303-2311.
35. Ross R. Atherosclerosis - an inflammatory disease. N Engl J Med 1999; 340:115-126.
36. Farquharson CAJ, Butler R, Hill A, *et al*. Allopurinol improves endothelial dysfunction in chronic heart failure. Circulation 2002; 106:221-226.
37. Cappola TP, Kass DA, Nelson GS, *et al*. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation 2001; 104:2407-2241.

Original Article

Perception of Medical Students Regarding Problem Based Learning

Ghadeer Al-Shaikh[1,2], Eman M Al Mussaed[2], Tahani N Altamimi[2,3], Hala Elmorshedy[2,4], Sadiqa Syed[2], Farida Habib[2]
[1]Department of Obstetrics and Gynecology, King Saud University / King Khalid University, Riyadh, KSA
[2]College of Medicine,Princess Nourah bint Abdulrahman University, Riyadh KSA
[3]Department of Family and Community Medicine, College of Medicine, University of Hail, KSA
[4]Department of Tropical Health, High Institute of Public Health, Alexandria, Egypt

Kuwait Medical Journal 2015; 47 (2): 133 - 138

ABSTRACT

Objectives: To assess medical students' perception about problem based learning (PBL)
Study Design: Descriptive cross-sectional survey
Setting: College of Medicine, Princess Nourah bint Abdulrahman University (PNU), Riyadh, KSA
Subjects: Second year medical students who attended fifteen or more PBL sessions
Intervention: Students were voluntarily invited to fill **a s**elf-administered questionnaire comprising 15 close-ended questions with a 5-point Likert scale responses
Main Outcome Measures: Student's perceptions and opinions
Results: Overall, students had a positive perception towards all the items; self-learning, critical thinking, integration of basic concepts into clinical science, identifying gaps in knowledge and improved problem solving skills. The mean value of all items was 19.77 ± 2.61 out of 25. Students who were satisfied with PBL were 59%. Almost 83% of students perceived that PBL stimulates critical thinking and 90.4% agreed that PBL integrates basic science with clinical knowledge. Although 73.1% of students found that PBL motivates self-learning, majority disagreed about increasing the frequency of PBL. Also, most of students were against increasing marks allocated for the assessment of PBL. Almost three quarters of students realized the value of PBL in improving communication skills and interpersonal relations. Students who thought that PBL is a preferable teaching tool for clinical concepts amounted to 69.2%.
Conclusion: Overall, students perceived PBL positively. While positive perception was maximum for integration of basic science into clinical knowledge and critical thinking, it was least for identification of knowledge gap and problem solving. Students also valued PBL in enhancing communication skills and promoting positive interpersonal relations. However, majority of students disagreed to have more frequent PBL sessions or to increase PBL exam marks.

Key Words: active learning, problem based learning, opinion, perception

INTRODUCTION

Problem-based learning (PBL) is an innovative strategy that changes the teaching context[1,2] from teacher-centered learning to student-centered, experiential and activity based learning. It gives students a chance to monitor their own learning and thus gain a degree of self-direction and independence in their studies. PBL signifies andragogy and constructivism which is an approach to knowledge that focuses on active role of learners[3]. Students activate their prior knowledge and build on existing conceptual knowledge framework, develop critical thinking, clinical reasoning and good communication skills[4]. Thus PBL is considered compatible with the modern theories of adult learning[5].

Effectiveness of PBL has forced medical institutions to adopt this system as an operating strategy in their curricula. Initially introduced as a case-based tool by McMaster University in mid-1970's, it was subsequently implemented by Maastricht University, Harvard Medical School[6] and followed by many Asian medical schools including Kingdom Saudi Arabia (KSA).

PBL may be used either as the mainstay of an entire curriculum or for delivery of individual courses, depending upon the needs and level of students. In practice, PBL is usually a part of an integrated curriculum using a system based approach to achieve learning outcomes in knowledge, skills and attitudes[7]. It is a teaching method that can be included in the

Address correspondenceto:
Dr. Ghadeer Al-Shaikh, MBBS, FRCSC, Associate Professor, Department of Obstetrics and Gynecology, King Saud University/King Khalid University Hospital, P O Box 7805, Riyadh, 11472, KSA, Tel: +966530044090, E-mail: ghadeer-alshaikh@hotmail.com

teacher's tool-kit along with other instructional strategies, rather than used as a sole educational strategy.

PBL has many advantages; it facilitates the acquisition of generic competencies and attitudes including team work, chairing a group, listening carefully to others, respect for colleagues' views, critical evaluation of literature and use of many resources of knowledge (journals, libraries, world wide web *etc)*. It encourages a deep approach to learning, promotes identification of relevant issues and prepares students for life-long learning in health care professions[8]. However after so many years of successful implementation of PBL as a gold standard in many medical schools all over the world, many challenges and drawbacks have been identified from literature and effectiveness of this method is being questioned[9].

The apparent disadvantages of PBL include: failure of students to develop an organized framework for their knowledge, inhibition of good teachers sharing their enthusiasm for their subject with students, teachers may not have skills to facilitate PBL sessions, lesser faculty acceptance of PBL, lower level of satisfaction among students in early years of program and lack of consistency in PBL classes[10, 11].

Students' feedback and input have registered many complaints, documented in different studies such as different levels of contents being discussed among different groups, diverse manners of facilitators conducting PBL sessions, achievements of same grades by hard working and average students, domination by good students in discussion sessions, lack of fairness in evaluations *etc*.[11]

College of Medicine at Princess Nourah bint Abdulrahman University, Riyadh (PNU), has implemented integrated hybrid PBL system from the first year of its inception. The aim of this study was to find out students perceptions and opinions regarding this tool of learning with which they were not familiar before and to assess students' satisfaction with their educational experience and accomplishments. Based on their responses, we could formulate important remedial measures in order to make it a useful educational tool.

SUBJECTS AND METHODS

Study design and study sample:

Cross-sectional study with convenient sampling from 2nd year medical students

The research question?

Does the implementation of PBL using clinical problems and scenarios increase knowledge and understanding of undergraduate medical students? A qualitative exploration of students' perception.

Study Setting

PNU started its Medical School in the year 2012 in collaboration with Medical College, King Saud University (KSU). A five-year curriculum is offered to the undergraduate medical students with first two years of basic sciences focusing on nine blocks, based on systems and divided into four semesters. The courses share full vertical and horizontal integration of basic science subjects: Anatomy, Physiology, and Biochemistry with Pathology, Microbiology, Pharmacology and few relevant topics of Community Medicine according to the themes of weeks.

Teaching and learning strategies include interactive lectures, tutorials, practical and demonstration, smart lab, history taking and PBL sessions. The design of PBL is based on real cases that demonstrates core learning objectives with integration of basic and clinical sciences knowledge. Brief clinical examination sessions were also introduced to students. Methods of assessment include a midterm exam and end of the block exam comprising multiple choice questions, OSPE and PBL formative and summative evaluation.

Faculty members were trained for conducting and constructing PBLs by a series of workshops. Students were also trained for developing group dynamics, team work, sharing information and presentations as well as communication skills. PBL sessions are conducted at two weeks intervals with students divided into six small groups of nine students each.

At the end of each block, students' feedback on instructional effectiveness, active self-learning, relevance and amount of knowledge learnt from cases and blocks is assessed.

Ethical consideration

Prior to the commencement of study, the proposal was submitted to Ethical Review Committee, Faculty of Medicine, PNU for approval. Verbal permission was taken from students.

The Questionnaire

A self-administered questionnaire consisting of fifteen questions related to important issues for evaluation of students' perception and opinions regarding effectiveness of PBL was employed. The questionnaire has been tested before for its applicability[12]. For this study it was translated by two experts and tested for face validity.

Responses to perception and opinion items were ordinal following a five-point Likert scale format, where one indicated 'strongly disagree' and five indicated 'strongly agree'. The higher the score the more likely the students considered PBL to be effective. The total perception score is 25 and the total opinion score is 30. The variables for perception included: stimulus for self-directed learning and critical thinking, integration

Table 1: Students' perception about problem based learning (PBL)

Item	Percent Response, N = 52					Mean Value
	SA	A	ND	DA	SDA	
1. Stimulates self-learning	23.1	50	23.1	3.8	0	3.92 ± 0.79
2. Stimulates critical thinking	34.6	48.1	17.3	0	0	4.17 ± 0.71
3. Integrates basic science with clinical knowledge	32.7	57.7	7.7	1.9	0	4.21 ± 0.67
4. Identifies knowledge gaps	17.3	42.3	34.6	1.9	3.8	3.67 ± 0.92
5. Enhances problem solving skills	19.2	46.2	28.8	5.8	0	3.79 ± 0.83
Satisfaction about PBL (items 1 - 4)	21.2	38.5	17.3	19.2	3.8	3.54 ± 1.15
Total mean value of perception	19.77 ± 2.61					

SA = strongly agree, A = agree, ND = not decided, DA = disagree, SDA = strongly disagree, PBL = problem based learning

of basic and clinical sciences knowledge, identification of gaps in knowledge and improvement in problem solving skills. The variables for opinion included; development of communication skills, team working skills, group consistency, and preference of PBL for clinical concepts, opinion about increasing marks and frequency of PBL.

Data Collection

Questionnaires were distributed among 54 students of second year who had attended more than fifteen PBL sessions. Students were assured that their participation is voluntary and their names were kept confidential.

Statistical analysis

Data were analyzed using SPSS version 20. Results of descriptive analysis were tabulated in the form of percentage, mean and standard deviation for each individual item. The overall perception and opinion scores were computed for all students. Satisfaction was measured on five-point Likert scale. In addition, students were dichotomized into satisfied and unsatisfied and perception and opinion scores were computed for each category. Student's t-test was applied to compare between the two categories and p-value of less than 0.05 was considered as statistically significant.

RESULTS

The study included 52 junior medical students, all enrolled in the 2nd year of Medical School, PNU, KSA. PBL is administered on biweekly basis and the total number of PBL sessions till the time of data collection was 15. Table 1, demonstrates student's perception about PBL. Overall, students had positive perception of all items, the mean value of all items was 19.77 ± 2.61. The minimum value was 3.67 ± 0.92 whereas only 59.6% of respondents perceived the benefit of PBL in identifying knowledge gaps, and 34.6% were not sure. The maximum mean values were; 4.17 ± 0.71 and 4.21 ± 0.67 for items number 2 & 3 respectively. 82.7% of students perceived that PBL stimulates critical thinking and 90.4% agreed that PBL integrates basic science with clinical knowledge. The mean value of self-learning was 3.92 ± 0.79, although 73.1% of students found that PBL motivates self-learning, yet 23.1% were not sure. As for the problem solving skills, 65.4% had positive perception, while 28.8% were not sure and 5.8% disagreed.

Table 2, summarizes student's opinion about PBL. The percentage of students who thought that PBL improves communication skills and interpersonal relations were 73% and 76.9% respectively. Those who disagreed that PBL improves communication skills and interpersonal relations were 11.5% and 9.6 % respectively.

Table 2: Students' opinions about PBL

Opinions	Percent Response, N = 52					Mean Value
	SA	A	ND	DA	SDA	
1. Improves communication skills	28.8	44.2	15.4	9.6	1.9	3.88 ± 1
2. Improves team working skills	19.2	57.7	13.5	5.8	3.8	3.83 ± 0.94
3. Group consistency	22	34	30	10	4*	3.6 ± 1.07
4. PBL is preferred for clinical concepts	36.5	32.7	11.5	15.4	3.8	3.83 ± 1.2
5. Individual assessment is preferred	81.3	---	----	----	18.8**	------
6. Increasing the marks for PBL	4	22	10	26	38*	2.28 ± 1.29
7. Increasing the frequency of PBL	1.9	13.5	7.7	32.7	44.2	1.96 ± 1.12
Total mean value of opinion	19.4 ± 3.24					

SA = strongly agree A = agree ND = not decided DA = disagree SDA = strongly disagree, PBL = problem based learning
*Total number of respondents were 50, **Total number of respondents were 48

Table 3: Mean score of perception and opinion according to the level of satisfaction

Satisfaction	Mean score	
	*Perception (Total score = 25)	**Opinion (Total score = 30)
Satisfied	21 ± 1.9	20.2 ± 2.6
Unsatisfied	17.9 ± 2.4	18.1 ± 3.7
T-test	5.2	2.3
P-value	0	0.02

* Dissatisfied = 21, Satisfied = 31 **Dissatisfied = 19, Satisfied = 29

Students who responded that PBL as a preferable teaching tool for clinical concepts amounted to 69.2%. 19.2% disagreed and 11.5 % were not sure. Regarding group consistency in PBL; only 54% of students agreed that the groups were consistent, whereas 30% and 14% were not sure and disagreed respectively. Individual assessment was preferred by the majority of students (81.3%). It is notable that more than 50% of students disagreed to increase the marks or frequency of PBL (64.6% and 76.9% respectively). The overall mean value of opinion items was not high (19.77 ± 2.61), with the minimum values for increasing marks and increasing frequency of PBL being 2.28 ± 1.29 &1.96 ± 1.12 respectively. The mean values for all other items were very close ranging from 3.60 ± 1.07 to 3.88 ± 1.00. Overall, 59.6% of students were satisfied with PBL and the mean value of satisfaction was 3.54 ± 1.15, while more than one fifth of students were not satisfied (24%) and 17.3% were indecisive. The differences in perception and opinion scores between satisfied and unsatisfied students were statistically significant (p-value < 0.05).

DISCUSSION

Several studies have considered PBL among the best educational strategies that empowers students in the health fields to develop higher cognitive, communication and research skills[3,5,8,13]. In KSA many medical schools continue to shift from the traditional lecture based learning (LBL) to PBL[14-16]. The preclinical phase at Faculty of Medicine, PNU is based on hybrid problem based learning aiming to equip our students with the teaching modalities that best enhance their medical practice skills during the clinical phase, stimulate self-directed learning and foster life-long learning. As a part of the ongoing educational monitoring, the present study was designed to assess students' perception and opinion about PBL.

Our study demonstrates that the overall students' perception was positive, majority of students admit that PBL helped them to apply basic science to explain clinical phenomena. Accordingly, 69% of students viewed this educational strategy as preferable for clinical concepts. It is worth noting that our PBL curricula are designed such that to integrate basic science to clinical scenarios of the PBL cases which are organized in parallel to different body systems[15,17,18)]. Most of the students also pointed out that PBL is helpful to sharpen their critical thinking and a good percentage of participants reported that PBL has stimulated self-learning. Tutor triggering questions and feedback on student assignments during the PBL sessions motivate student to build-up their knowledge through multidisciplinary educational resources. Thus, motivation is crucial to maintain self-directed learning. In addition, the positive competitive educational environment during the PBL sessions fosters student to search for knowledge from various resources, learn from each other, develop higher cognitive skills and develop the habit of questioning and gain self-confidence. Meanwhile in LBL, while education is teacher centered, information is fragmented and students are acting just as passive consumers[19-21].

The feedback of medical students, students from KSU and other regional and international health colleges highlighted the value of PBL system to foster self-directed learning and to integrate knowledge from several disciplines[6,15,12,22]. Moreover, students enrolled in PBL system achieved higher scores per topic as compared to students enrolled in traditional system for both single best multiple choice question exam and objective structured practical examination (OSPE) in a respiratory physiology course[19]. Similarly, the PBL positive effect was evident in a randomized multicenter study in pharmacology and in the National Board of Medical Examiners (part 2) clinical science examination[23,24]. Nevertheless, the conflicting results of studies claiming that PBL had no statistically significant differences on students' knowledge in comparison to traditional teaching strategies[25,26] might be attributed to differences in research methodology among different studies, the impact of culture on educational outcome and prior students' experience of the PBL system.

The influence of PBL on problem solving skills and identification of knowledge gaps scored the least of all items (just 65.4%, and 59.6% of students experienced a positive perception towards these items respectively). The observed low score for problem solving might be a reflection of the frequency at which PBL is implemented in our program which is only biweekly. In addition, the study encompassed only junior students who just finalized the first year of the preclinical phase. Contrary to this, in a study done at Faculty of Medicine, Qassim university which included students from year one through year five, 81.4% of participants pointed out that PBL system improved their problem solving skills[27].

Regarding communication skills and interpersonal relations, our study demonstrates that majority of students exhibited positive opinion. Certainly the structure of PBL sessions where only nine students comprise each group provides more chance for individual participation and active contribution. Also, while in such small groups, student feel more at ease to ask questions, postulate hypothesis and suggest necessary required information to justify their views. In spite of the positive perception and opinion of students about PBL in most of the addressed items, yet majority of them disagreed to increase either the frequency or the allocated marks of PBL. The underlying factors include, limited experience of our students of this system. They joined the medical school just one year ago and their background information in basic science is limited and their prior experience with PBL is nil. Resistance to change, work load, and the homogeneity of student group are all contributing factors for students to disagree on increasing the frequency and the marks of PBL. Similarly, the responses of students enrolled in integrated problem-based curriculum at King Saud Bin Abdul Aziz University (KSAU) were highly supportive. Yet, students comments included negative response as "huge amount to be learned and lots of self-directed learning"[14]. Adding to these factors, negative perception of PBL system includes uncertainty on accuracy of knowledge acquired due to language barrier and waste of time in the class[28].

Overall students' satisfaction about PBL is just acceptable. The limited experience with the PBL system might explain the degree of student satisfaction. Students' learning and teaching perspective is influenced by their previous experience and current instructional teaching modalities[27].

CONCLUSION

In conclusion, students' perception about PBL was variable. Positive perception was maximum for integration of basic science into clinical knowledge and critical thinking and least for identification of knowledge gap and problem solving skills. Most of participants valued the PBL system in enhancing communication skills and promoting positive interpersonal relations. However, majority of students disagreed to increase frequency or the exam marks of PBL. The negative perceptions and opinions provide useful information to further improve PBL sessions at Faculty of Medicine, PNU.

ACKNOWLEDGMENT

The authors would like to thank the College of Medicine Research Center, Deanship of Scientific Research at King Saud University in Saudi Arabia for their financial support to conduct this study.

REFERENCES

1. Dolmans DH, De Grave W, Wolfhagen IH, van der Vleuten CP. Problem-based learning: future challenges for educational practice and research. Med Educ 2005 July; 39:732-741.
2. Fish M, Moore S. Enquiry-based learning links psychology theory to practice. Br J Midwif 2005; 13:148-152.
3. Baerveldt C. Constructivism contested: implication of a genetic perspective in psychology. Integr Psychol Behav Sci, 2013; 47:156-166.
4. Barnett R. Knowing and becoming in the higher education curriculum. Studies in Higher Education 2009; 34:429-440.
5. Finucane PM, Johnson SM, Prideaux DJ. Problem-based learning: its rationale and efficacy. Med J Aust 1998; 168:445-448.
6. Milan LPB, Semer B, Rodrigues JM, Gianinin RJ. Traditional learning and problem-based learning: self-perception of preparedness for internship. Rev Assoc Med Bras 2012; 58:594-599.
7. Benner P, Sutphen M, Leonard V. Day L. Educating nurses: A call for radical transformation. 2009; San Francisco, CA:Jossey-Bass.
8. Wood DF. ABC of learning and teaching in medicine: Problem-based learning. BMJ 2003; 326:328-330.
9. Taylor D, Miflin B. Problem-based learning: where are we now? Medical Teacher 2008; 30:742-763.
10. Davis MH. AMEE Medical Education Guide No.15: Problem-based learning: a practical guide. Med Teach, 1999; 21:130-140.
11. Landeen J, Jewiss T, Vajoczki S, Vine M. Exploring consistency within a problem-based learning context: perception of students and faculty. Nurse Educ Pract, 2013; 13: 277 - 282
12. Habib F, Baig LA, Asad F. Opinion of medical students regarding problem based learning, J Pak Med Assoc 2006; 56:430-432.
13. Al Amodi AA. Problem-based learning sessions and undergraduate research: a medical student's perspective and experience. Perspect Med Educ 2014; 3:56-60.
14. Elzubeir MA. Teaching of the renal system in an integrated, problem-based curriculum. Saudi J Kidney Dis Transpl 2012; 23:93-98.
15. Azer SA, Hasanato R, Al-Nassar S, Somily A, AlSaadi MM. Introducing integrated laboratory classes in a PBL curriculum: impact on student's learning and satisfaction. BMC Med Educ 2013; 24:13-71. doi: 10.1186/1472-6920-13-71.
16. El-Naggar MM1, Ageely H, Salih MA, Dawoud H, Milaat WA. Developing an integrated organ / system curriculum with community-orientation for a new medical college in Jazan, Saudi Arabia. J Family Community Med 2007; 14:127-136.
17. Distlehorst LH, Dawson E, Robbs RS, Barrows HS. Problem-based learning outcomes: the glass half-full. Acad Med 2005; 80:294-299.
18. Azer SA. Introducing a problem-based learning program: 12 tips for success. Med Teach 2011; 33:808-813. doi: 10.3109/0142159X.2011.558137.

19. Meo SA. Evaluating learning among undergraduate medical students in schools with traditional and problem-based curricula. Adv Physiol Educ 2013; 37:249-253.
20. Matsuo O, Takahashi Y, Abe C, Tanaka K, Nakashima A, Morita H. Trial of integrated laboratory practice. Adv Physiol Educ 2011; 35:237-240. doi: 10.1152/advan.00047.2010.
21. Friedlander MJ, Andrews L, Armstrong EG, *et al*. What can medical education learn from the neurobiology of learning? Acad Med. 2011; 86:415-420. doi: 10.1097/ACM.0b013e31820dc197.
22. Koh GC, Khoo HE, Wong ML, Koh D. The effects of problem based learning during medical school on physician competency: a systematic review. CMAJ 2008; 178:34-41.
23. Burford HJ, Ingenito AJ, Williams PB. Development and evaluation of patient-oriented problem-solving materials in pharmacology. Acad Med 1990; 65:689-693.
24. Kaufman A1, Mennin S, Waterman R, *et al*. The New Mexico experiment: educational innovation and institutional change. Acad Med 1989; 64:285-294.
25. Miller SK. A comparison of student outcomes following problem-based learning instruction versus traditional lecture learning in a graduate pharmacology course. J Am Acad Nurse Pract 2003; 15:550-556.
26. Rideout E, England-Oxford V, Brown B. A comparison of problem-based and conventional curricula in nursing education. Adv Health Sci Educ Theory Pract 2002; 7:3-17.
27. Shamsan B, Syed AT. Evaluation of problem based learning course at college of medicine, Qassim University, Saudi Arabia. Int J Health Sci (Qassim) 2009; 3:249-258.
28. Huang R. Chinese international students' perceptions of the problem-based learning experience. Journal of Hospitality, Leisure, Sport and Tourism Education 2005; 4:36-43. ISSN: 1473-8376. DOI:10.3792/johlste.42.108

Original Article

Management of Pediatric Urinary Tract Infections in Kuwait: Current Practices and Practicality of New Guidelines

Entesar H Husain[1,2], Talal Al-Saleem[1], Yousef Marwan[1], Maha Al-Jalahma[1], Faisal Al-Kandari[1]
[1]Department of Pediatrics, Mubarak Al-Kabeer Hospital, Kuwait
[2]Department of Pediatrics, Faculty of Medicine, Kuwait University

Kuwait Medical Journal 2015; 47 (1): 139 - 143

ABSTRACT

Objective: To evaluate the practicality of applying the new international guidelines for urinary tract infection (UTI) management in children hospitalized with their first UTI in a tertiary care hospital in Kuwait
Design: Retrospective study
Setting: Pediatric wards at the Mubarak Al Kabeer Hospital; period of study: from June 2011 - May 2012
Subjects: Children up to 12 years of age with pyuria and at least 50,000 colonies per ml of a single uropathologic organism in an appropriately collected urine sample
Intervention: None
Main Outcome Measures: Incidence of vesico-ureteric reflux (VUR) in children with first UTI and normal renal ultrasound (RUS)
Results: One hundred and forty nine children were included. 53% were male. Recurrent UTI was present in 15% of cases. The most commonly isolated bacteria were: *E.coli* (69%), *Klebsiella pneumoniae* (11.4%) and *Pseudomonas aeruginosa* (5.4%). VUR was found in 7.5% children with first UTI and normal RUS and in 50% of children with abnormal RUS. In children with recurrent UTI, 23 % had VUR. Renal scarring was found in five children.
Conclusions: The finding of low VUR rate in children with first UTI and normal RUS makes changing the current practice for the diagnosis and management of UTI to recent guidelines safe and valuable.

KEY WORDS: antibiotic resistance, children, DMSA, ESBL, MCUG, vesicoureteral reflux

INTRODUCTION

Since the introduction of effective conjugate vaccines against *Haemophilus influenza* type b, and *Streptococcus pneumoniae*, the rate of bacteremia and meningitis as causes of fever in children have significantly dropped. As a result, urinary tract infection (UTI) has emerged as the most common cause of fever without a focus in children. UTI affects approximately 7 - 8% of girls and 2% of boys during the first eight years of life[1,2].

Previously published UTI guidelines namely the Royal College of Physicians (RCP) guidelines in 1991[3] and the American Academy of Pediatrics (AAP) guidelines in 1999[4] have emphasized the role of extensive imaging using renal ultrasound (RUS), micturating cystourethrogram (MCUG) and nuclear scan with dimercaptosuccinic acid (DMSA) with the first UTI episode in children less than two years of age. This extensive imaging was aimed at detecting vesicoureteral reflux (VUR) and renal scarring. Also, children with VUR of any grade were given antibiotic prophylaxis. These guidelines have been updated in the last two years. AAP now recommends no more imaging in children with first UTI if RUS was normal[5]. The United Kingdom (UK) National Institute for Health and Clinical Excellence (NICE) makes the same recommendation but also recommends MCUG in all infants less than six months presenting with atypical UTI[6]. Both recommend imaging in case of recurrent UTI.

At the Department of Pediatrics, Mubarak Al-Kabeer Hospital (MKH), UTI is managed according to the AAP guidelines of 1999. All admitted infants

Address correspondenceto:
Entesar H. Husain MD, FRCPC, Department of Pediatrics, Faculty of Medicine, Health Science Center, Kuwait University, P O Box 24923, Safat, Kuwait 13110. Tel & Fax: (965) 25338940, E-mail: ehusain@hsc.edu.kw, ehusain@live.com

with first UTI attack undergo RUS and MCUG. All children with suspected UTI are started empirically on intravenous cefotaxime until the urine culture results are available for proper antibiotic choice. On discharge from the hospital, children are started on antibiotic prophylaxis until their MCUG and continue on prophylaxis if there is vesico-ureteric reflux (VUR).

We conducted this study to evaluate the value of the currently applied guidelines in detecting VUR and renal scars in children with first UTI and if changing to the new guidelines will be safe and valuable. In addition, with the global increase in antibiotic resistance we want to identify if the currently empiric antibiotic used is suitable for the isolated bacteria.

SUBJECTS AND METHODS

We retrospectively reviewed the medical records of patients admitted to the pediatric wards at the Mubarak-Al-Kabeer hospital (MAKH) with the diagnosis of UTI from 1st June 2011 to 31st May 2012. We identified the admitted children by ICD code of 599.0. Children included were those who had both pyuria (white blood cells > 5 cells per high-power field) and a urine culture of 50,000 colonies per ml of a single uropathogenic organism in an appropriately-collected specimen of urine. We gathered the demographic data along with the clinical features of UTI for each patient. This included age, gender, symptoms, previous history of UTI, past medical history, urine culture results, organisms' and their antibiotic susceptibilities, days of antibiotic use, days of hospital stay and findings on imaging studies.

The study protocol was reviewed and ethically approved by the "Joint Ethics Committee of the Ministry of Health in Kuwait and the Faculty of Medicine in Kuwait University". The collected data were entered and analyzed using Statistical Package for Social Sciences SPSS (17.0). Frequencies and proportions were used to describe demographic, clinical and microbiological data. Chi-square analysis was done to study the association between the duration of hospitalization and age, fever duration and microorganism. A p-value of < 0.05 was considered as the cut-off value for statistical significance.

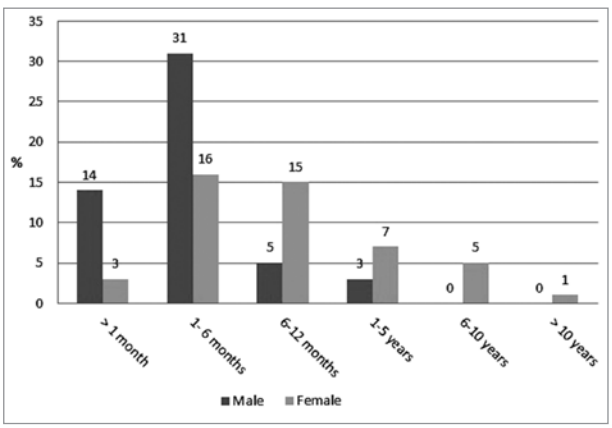

Fig. 1: Age and gender distribution of children admitted with UTI.

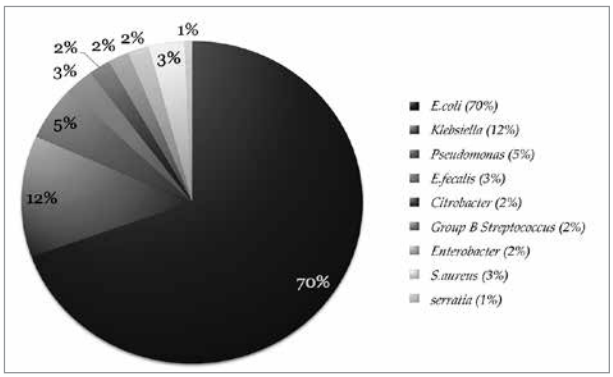

Fig. 2: Distribution of bacteria causing UTI in children

RESULTS

During the study period, 160 children were admitted with UTI. Only 149 fulfilled the study criteria and were subjected to further analysis, out of which 79 (53%) were boys. Out of the excluded patients, seven had mixed pathogens, two did not have pyuria and two had < 50,000 cfu/ml of bacteria. This was the first episode of UTI in 127 (85.2%) children. Eighty six (58%) children had UTI during the first six months of age. Fig, 1 shows the age and gender distribution of the children. Male children aged 0 - 6 months accounted for 31%, 69 (87%) out of which were not circumcised ($p < 0.001$). The top three presenting symptoms were: fever 129 (93.3%), vomiting 40 (26.8%), and poor feeding 29 (19.5%).

The isolated bacteria are shown in Fig. 2. Three bacterial agents constituted 88% of all bacteria isolated. These bacteria were: *E.coli* 103 (69.1%), *Klebsiella pneumoniae* 18 (12.1%) and *Pseudomonas aeruginosa* 8 (5.4%). All children in the study were started empirically on cefotaxime and were switched to a narrower spectrum antibiotic according to the available susceptibilities. The antibiotic resistance

Table 1: Antibiotic resistance in percentage for the most common organisms and among the three common organisms

Antibiotic	E.coli % N = 103	Klebsiella % N = 17	Pseudomonas % N = 8	Resistance of 3 bacteria % N = 128
Ampicillin	55	59	100	59
Amox/clav	33	29	100	37
Amikacin	0	6	0	0.8
Cephalothin	48	6	100	45
Cefotaxime	28	6	100	30
Ceftazidime	22	0	0	17
Meropenem	0	0	0	0
Pip/Tazo	8	12	0	8
Trimethoprim	38	18	100	39
Nitrofurantoin	9	35	100	18

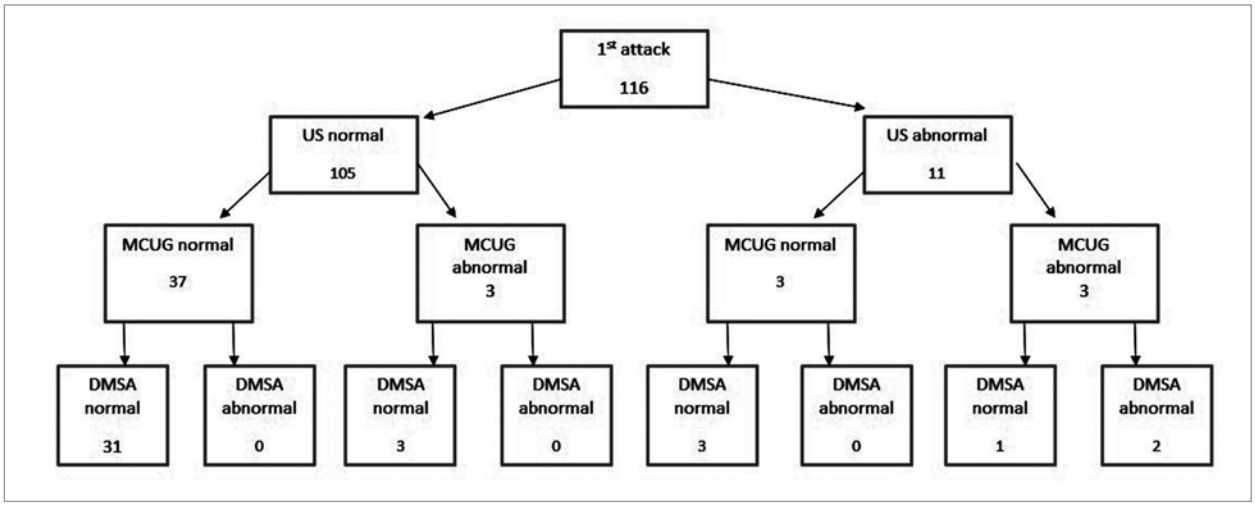

Fig. 3: Imaging results in children presenting with first UTI attack.
US = ultra sound, MUCG = micturating cystourethrogram, DMSA = dimercaptosuccinic acid

pattern for the previously listed bacteria is shown in Table 1. Duration of hospitalization was > 7 days in 82 children (55%). Prolonged hospitalization was associated with age < 3 months (p = 0.02) and duration of fever of more than 36 hours (p = 0.001) but no association was found with prolonged hospitalization and the isolated organism.

Renal ultrasound (RUS) was done for 136 (91%) children. MCUG was done for 59 (40%) of which 46 had the first attack of UTI and 13 had recurrent infection. DMSA scan was done for 50 (34%) patients out of which 40 had the first attack of UTI and 10 had recurrent infection. Only 6 / 46 (13%) children with first attack of UTI had VUR. In addition, only 3/40 (7.5%) children with first UTI episode and normal RUS were found to have VUR (Fig. 3). In those with abnormal RUS, VUR was found in 3/6 (50%) children (Fig. 3). In children with recurrent UTI, 3/13 (23%) had VUR (Fig. 4). There were three children who had normal RUS and still had VUR. Two of them had grade I VUR and the third had grade IV VUR.

Renal scarring was found in 5/50 (10%) children in our cohort, 3/40 (7.5%) with first UTI and 2/10 (20%) children with recurrent UTI. There were three children who had renal scarring with their first UTI, only one had normal RUS.

DISCUSSION

The overall rate of VUR in children with UTI (either first or recurrent attack) who had MCUG, in this study was 15% compared to the reported international rates of 25 - 40%[7,8]. This variation can be explained by the difference in the studied population in terms of genetic and racial back grounds as VUR is thought to be genetically linked[8]. In this study, we found that only 7.5% of children admitted with first UTI had VUR. This is less than previously reported (22%) in two hospitals in Kuwait in 2003[9]. We think this might be related to

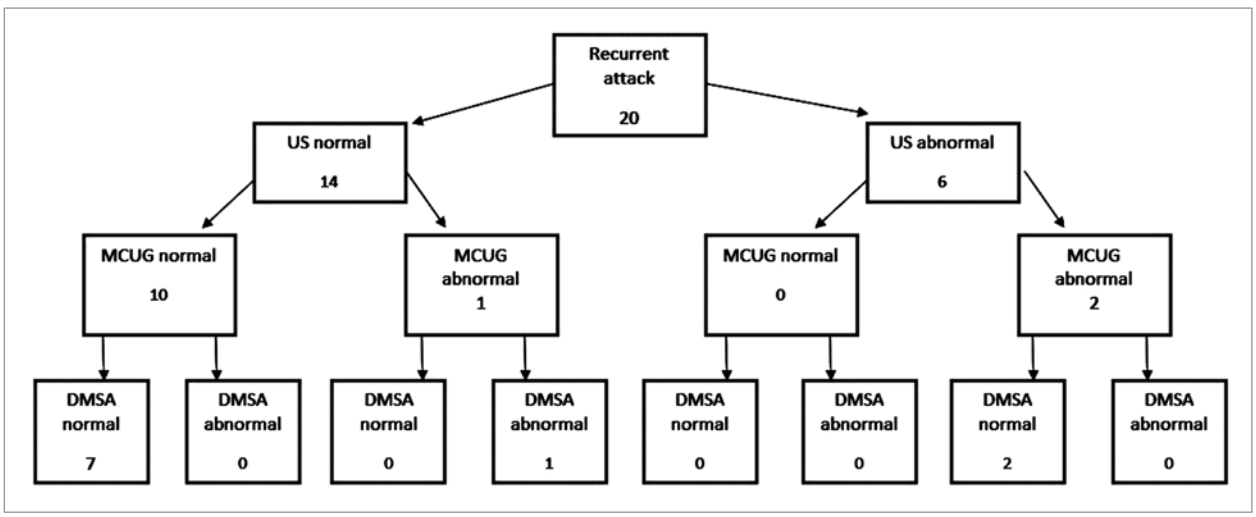

Fig. 4: Imaging results in children with recurrent UTI
US = ultra sound, MUCG = micturating cystourethrogram, DMSA = dimercaptosuccinic acid

the characteristics of patients included in the study as their cohort had 72% female patients while our cohort had 47% females. In another study in Kuwait conducted at Al-Jahra Hospital, VUR rate was 43%[10], but only 30% of their sample were children with first UTI episode.

The recent AAP and NICE guidelines recommend that children with first UTI and normal RUS, should not undergo MCUG and should not receive antibiotic prophylaxis[5,6]. We believe that this recommendation is applicable in Kuwait. In our study, children with first UTI and abnormal RUS were six times more likely to have VUR compared to children with normal RUS. None of patients with normal RUS had an abnormal DMSA which is the most important marker indicating long term complications.

The main uropathogens in our cohort were dominated by Gram negative bacteria namely; *E.coli*, *Klebsiella*, and *Pseudomonas*. These results differ from reports from Italy and Turkey where the gram positive *Enterococcus* spp. was one of the dominating organisms[11,12]. *Pseudomonas* spp. was responsible for 5.4% of the UTI's in our hospital, which will reflect on the empiric antibiotic used for managing UTI. It is worth noting that these organisms were not further analyzed in relation to gender, UTI recurrence, previous administration of antibiotics or an underlying renal abnormality.

Increased bacterial resistance to antibiotics is a global problem. We do not have the susceptibility pattern rates for the previous years to compare our data with. One of the most important findings in this study is the resistance to cefotaxime which is the empiric antibiotic used for UTI cases at MKH with a resistance rate of 28% for *E.coli* and 6% for *Klebsiela*. A similar rate of resistance was reported by Mohammad-Jafari from Iran[13], while higher rates of resistance for both organisms of 51% and 42% respectively were reported from Turkey[14]. Recently, resistance of Gram negative bacteria to third generation cephalosporin is on the rise due to production of extended spectrum β-lactamases (ESBL). In a retrospective study in Italy from 2007 to 2011, the authors noted an increase in UTI in pediatrics due to ESBL-producing organisms over the years of the study from 11.63 / 1000 patient-days to 27.48 / 1000 patient-days ($p = 0.05$)[15]. Tratselas *et al* found that clinical and microbiological outcomes, as well as formation of renal scars did not differ between groups of children with ESBL-producing UTI and non-ESBL[16]. Despite the findings of the latter study, AAP recommends knowing the local pattern of susceptibility of coliforms to antimicrobials before the initiation of empiric therapy[5]. In our study, two antibiotics with the least resistance against the three isolated uropathogens in our hospital were meropenem (0% resistance) and amikacin (0.8% resistance).

One limitation of this study, is that only 91% had RUS. The retrospective nature of this study and extraction of data from medical records might have resulted in loss of some data because of its unavailability. The other limitation is that we do not have the previous year's antibiotic susceptibility data to compare with the current status. We would be interested in results of studies conducted after the application of the new guidelines. We also think that continuous monitoring of the resistance pattern of the uropathogens is essential to guide future appropriate antibiotic use.

CONCLUSIONS

Our results clearly demonstrate that children with normal RUS will unlikely have VUR. Hence; the new practice guidelines for imaging should be adopted when managing children with UTI as these guidelines are safe and decrease exposure to unnecessary radiation and antibiotics. We have also documented the increased resistance of uropathogens to cephalosporins which makes cefotaxime an unsuitable choice for empiric therapy for UTI. Empiric antibiotic therapy for children admitted with UTI should be re-evaluated. Empiric use of aminoglycosides until the sensitivity pattern is available might be a reasonable choice.

ACKNOWLEDGMENT

Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. Hellström A, Hanson E, Hansson S, Hjälmås K, Jodal U. Association between urinary symptoms at 7 years old and previous urinary tract infection. Arch Dis Child 1991; 66:232-234.
2. Marild S, Jodal U. Incidence rate of first-time symptomatic urinary tract infection in children under 6 years of age. Acta Paediatr 1998; 87:549-552.
3. Royal College of Physicians: Guidelines for the management of acute urinary tract infection in childhood. Report of a working group of the Research Unit. JR Coll Physicians Lond 1991; 25:36-42.
4. American Academy of Pediatrics. Committee on Quality Improvement. Subcommittee on Urinary Tract Infection: Practice parameter: the diagnosis, treatment, and evaluation of the initial urinary tract infection in febrile infants and young children. Pediatrics 1999; 103:843-852.
5. Roberts KB, Subcommittee on urinary tract infection, Steering Committee on quality improvement and management: Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. Pediatrics 2011; 128:595-610.

6. National Institute for Health and Clinical Excellence (NICE). Urinary tract infection in children: diagnosis, treatment and long-term management. Clinical guideline 54; 2007. (Accessed April 30, 2013 at: http://guidance.nice.org.uk/CG054)
7. Cleper R, Krause I, Eisenstein B, Davidovits M. Prevalence of vesicoureteral reflux in neonatal urinary tract infection. Clin Pediatr 2004; 43:619-625.
8. Williams G, Fletcher JT, Alexander SI, Craig JC: Vesicoureteral Reflux. J Am Soc Nephrol 2008; 5:847-862.
9. Zaki M, Mutari GA, Badawi M, Ramadan D, Al deen Hanafy E. Vesicoureteric reflux in Kuwaiti children with first febrile urinary tract infection. Pediatr Nephrol 2003; 18:898-901.
10. Saleh SI, Tuhmaz MM, Sarkhouh MY, El-Ghawabi MA. Urinary tract infection in infants and children in Al-Jahra area, Kuwait: An overview. Kuwait Medical Journal 2003; 35: 31-35.
11. Pennesi M, L'erario I, Travan L, Ventura A. Managing children under 36 months of age with febrile urinary tract infection: a new approach. Pediatr Nephrol 2012; 27:611-615.
12. Catal F, Bavbek N, Bayrak O, *et al.* Antimicrobial resistance patterns of urinary tract pathogens and rationale for empirical therapy in Turkish children for the years 2000-2006. Int Urol Nephrol 2009; 41:953-957.
13. Mohammad-Jafari H, Saffar MJ, Nemate I, Saffar H, Khalilian AR. Increasing antibiotic resistance among uropathogens isolated during years 2006-2009: impact on the empirical management. Int Braz J Uro 2012; l38:25-32.
14. Yolbas I, Tekin R, Kelekci S, *et al***.** Community-acquired urinary tract infections in children: pathogens, antibiotic susceptibility and seasonal changes. Eur Rev Med Pharmacol Sci 2013; 17:971-976.
15. Giardino S, Bandettini R, Perotti M, *et al***.** Gram-negative urinary tract infections and increasing isolation of ESBL-producing or ceftazidime-resistant strains in children: results from a single-centre survey. Infez Med 2013; 21:29-33.
16. Tratselas A, Iosifidis E, Ioannidou M, *et al***.** Outcome of urinary tract infections caused by extended spectrum β-lactamase-producing Enterobacteriaceae in children. Pediatr Infect Dis J 2011; 30:707-710.

Case Report

Proximal Type of Epithelioid Sarcoma: A Rare Aggressive Tumor Presenting Simultaneously in Spine and Pelvis

Abhijeet B Kadam, Ashok K Rathod
Department of Orthopedic Surgery, Lokmanya Tilak Medical College and General Hospital, Sion, Mumbai, India

Kuwait Medical Journal 2015; 47 (2): 144 - 148

ABSTRACT

A 40-year-old female presented with bilateral lower limb weakness with bladder and bowel incontinence. MRI study revealed a destructive lesion involving the D7 vertebral body and a large tumor in the gluteal muscles invading the right iliac blade. A histological examination demonstrated a tumor comprising of rounded to ovoid pleomorphic epithelioid cells with marked cytological atypia. Tumor cells expressed CD 34, vimentin and focally pancytokeratin but were negative for CD31, EMA, SMA, WT1 and LCA. A D6-7 laminectomy with posterior decompression was done. Postoperatively, external beam radiotherapy was given. However, the patient deteriorated rapidly with no neurological improvement. Epitheiliod sarcomas and their recently described proximal variant, by virtue of being an exceedingly unusual tumor are often misdiagnosed or diagnosed late beyond the stage of salvage. This report highlights the histopathology and immunohistochemistry of this tumor and the differentials that need to be analyzed to correctly diagnose this entity.

KEY WORDS: CD31, CD34, epithelioid sarcoma, proximal variant

INTRODUCTION

The term epithelioid sarcoma (ES) was first described in 1970 by Enzinger, recognizing it as a distinct neoplastic entity simulating both a granuloma and a carcinoma[1]. Although there is still no consensus as to the line of cellular differentiation of this tumor, it has been suggested that ES is a tumor of primitive mesenchymal cells with fibroblastic and histiocytic differentiation. It is the most common primary sarcoma of the hand and wrist, prevalent in patients between 10 - 39 years of age with a male predominance[2,3]. Guillou in 1997 described a new variant of this tumor arising from axial locations such as the pelvis, perineum and genital tract and designated it as the "proximal type of epithelioid sarcoma" (PES). This proximal variant is characterized by a more aggressive behaviour and a histological picture showing predominance of large epithelioid cells with marked cytological atypia and intracytoplasmic hyaline inclusions, imparting a rhabdoid appearance to the tumor cells[4]. This variant has an immuno-phenotype similar to that of the classic ES.

CASE REPORT

In February 2011, a 40-year-old Muslim female housewife presented to us with a history of insidious onset and gradually progressing weakness in both lower limbs of one month duration. At presentation this culminated into an upper motor neuron type paraplegia with bladder and bowel involvement. On examination, the sensory level was at D9 - 10. There were no local signs of swelling, deformity or tenderness in the spine. Other associated complaints were significant weight loss and anorexia. The patient was a known diabetic on insulin therapy. There was also a history of total hysterectomy done seven years ago for an obstetric indication.

Radiographs of the dorso-lumbar spine revealed no abnormality. MRI study of the spine (Fig. 1 A - E) revealed altered signal intensity in the right half of the D7 vertebral body and its posterior elements. The lesion, predominantly in the posterior vertebral elements, measured 2.2 x 1 x 1 cm and was hyper-intense on STIR and hypo-intense on T1 weighted images. This lesion extended upto the epidural space

Address correspondence to:
Abhijeet B Kadam, MBBS, MS (Orthopedics), Dept. of Orthopedic Surgery, Lokmanya Tilak Medical College and General Hospital, Sion, Mumbai-400022, India. Tel: +91-9619684095, E-mail: abhijeetsr71@gmail.com

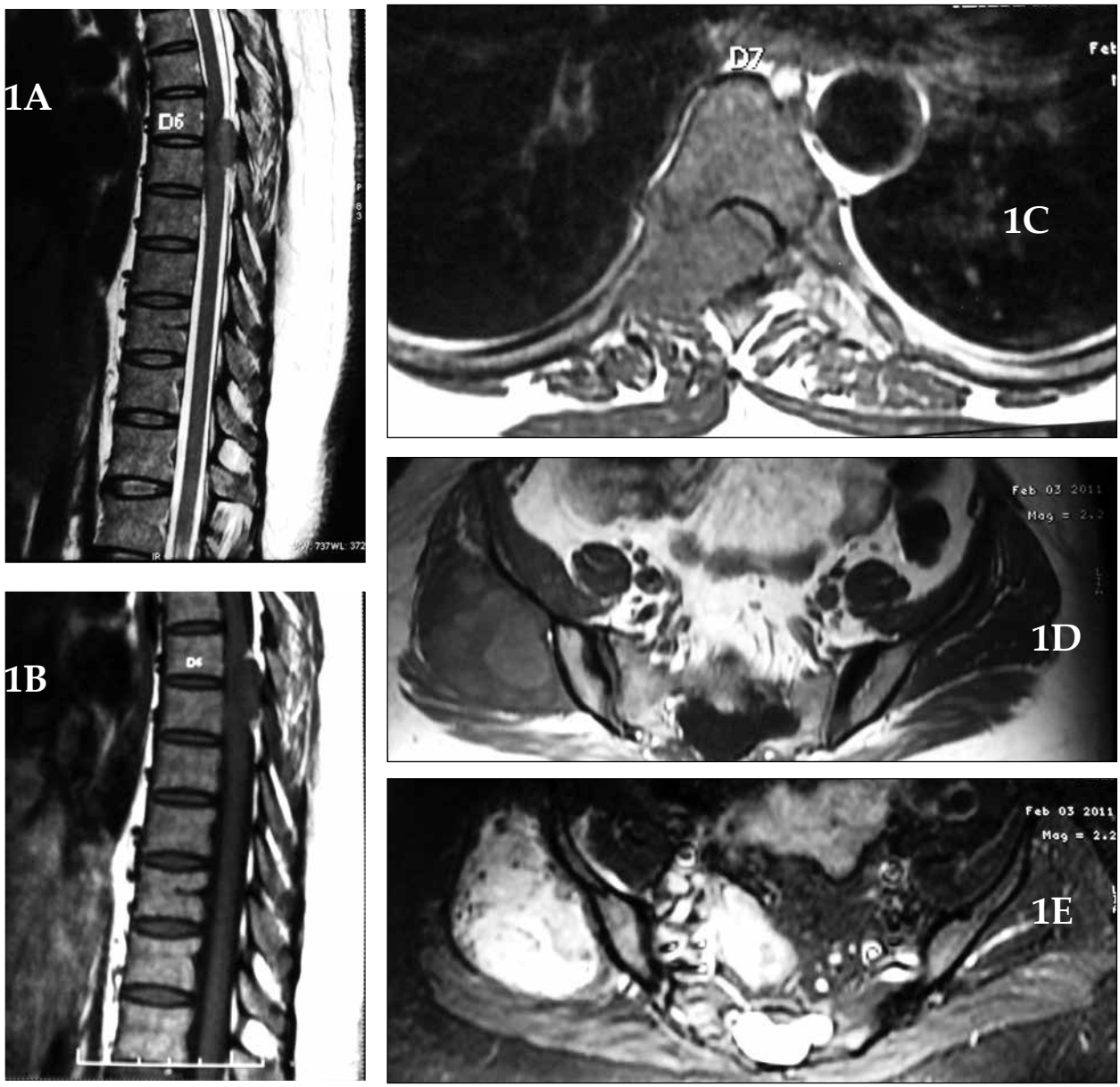

Fig. 1A - C: T2 and T1 weighted MRI images depicting the tumor involving the bodies and posterior elements of D6-7 vertebrae. **1D - E:** T2 and T1 weighted MRI images of the right pelvic location of tumor involving the glutei and extending medially across the ilium

compressing the right lateral aspect of spinal cord. Focal hyper-intense signals were seen in the spinal cord on T2 weighted images at this level. Screening through the pelvis revealed a large 1.4 x 5.8 x 8.0 cm sized tumor arising from the right iliac bone and invading the right glutei and the iliacus muscles. It was hyper-intense on STIR and hypo-intense on T1 weighted images.

Hematological investigations showed an elevated erythrocyte sedimentation rate (ESR, 60 mm at the end of 1hr). Hepatic and renal function tests were essentially within normal limits. Serum immunoglobulin levels were normal. Bence-Jones proteinuria was absent. Tumor marker assays of AFP, CEA, β-2 microglobulin, β-HCG, CA 15.3, CA 19.9, CA 125 were negative. Serology for antibodies against HIV, HbsAg and HCV antigens was negative. A chest radiograph obtained at time of presentation was also normal.

CT-guided biopsies from the involved D7 vertebra and right ilium were performed. Multiple tissue fragments were examined. Histopathology (Fig. 2 A - C) revealed spindle cells infiltrating collagenized tissue, with interspersed round to ovoid epithelioid cells showing nuclear pleomorphism. Large cells with vesicular nuclei, prominent nucleoli and abundant cytoplasm were also seen. These large cells showed occasional intra-cytoplasmic inclusions reminiscent of a 'rhabdoid' morphology. Focal areas of tumor necrosis were observed. Frequent mitoses were also noted. On

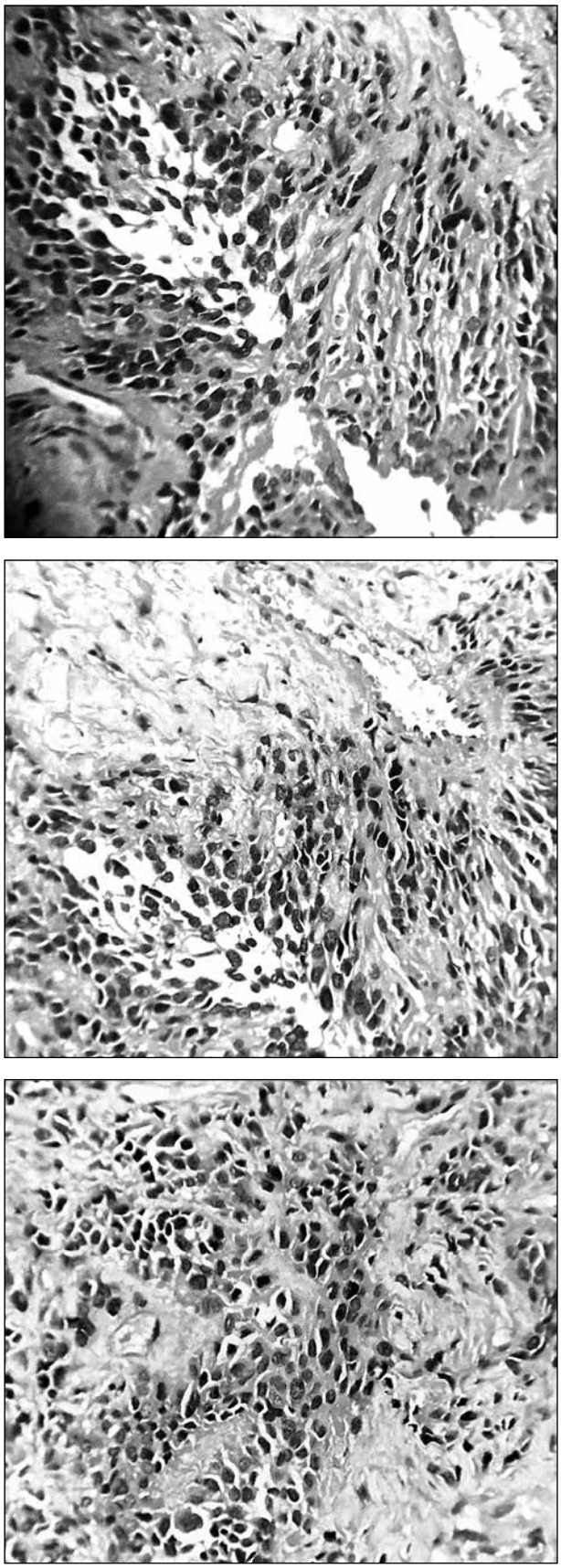

Fig. 2A - C: H & E staining of the biopsied tissue as viewed under 400 X magnification

immuno-histochemistry, the tumor cells expressed CD 34, vimentin and focal pancytokeratin positivity. They were immunonegative for CD31, WT1, EMA and SMA, LCA.

Considering the severe neurological deficit that the patient presented with, a D6 - 7 laminectomy with posterior decompression was done. Post-operatively external beam radiotherapy was administered. The patient showed no symptomatic improvement despite these measures. One month later, the patient expired at home (*i.e.*, within four months of onset of initial symptoms. Correlating immunohistochemistry findings with the histo-pathological picture and clinical presentation a diagnosis of a PES variant was made retrospectively.

DISCUSSION

ES, as a rare group of tumors, have been a curious pathological entity ever since their first description by Enzinger. The proximal type, first reported by Guillou in 1997 has frequently been confused with tumors such as synovial sarcoma, rhabdomyosarcoma, leiomyosarcoma, extraskeletal myxoid chondrosarcoma, undifferentiated carcinomas and epithelioid angiosarcoma.

Oval to polygonal cells arranged predominantly in cohesive sheet like pattern with interspersed large epithelioid cells with intra-cytoplasmic inclusions and prominent nucleoli resembling a rhabdoid morphology has been the classical description for the PES[4]. This was the picture seen in our case too. But a similar histopathological picture can also be seen in the above mentioned differentials.

Immuno-histochemistry marker studies were performed to differentiate between these entities. The characteristic immuno-profile of ES is the co-expression of keratin and vimentin[5]. Cytokeratin is expressed in over 75% cases using older immuno-histochemistry techniques, whereas in more recent series it has been reported in upto 94% of cases[5]. EMA is expressed in 50 - 96% of tumors but its pattern of reactivity demonstrates variability within the same lesion[5-7]. About 60% of cases have been shown to be positive for CD34 expression[5,7,8]

ES with a pseudovascular pattern may mimic epithelioid angiosarcomas, both of which are frequently positive for cytokeratin and CD34[9]. Also, many epithelioid angiosarcomas have a diffuse sheet-like growth pattern, and a vasoformative architecture is often not present. However, they have positive immunoreactivity for a sensitive and specific marker for endothelial differentiation, CD31[10].

Synovial sarcomas display an epithelial immunophenotype positive for both cytokeratin and EMA[11]. But, synovial sarcomas are consistently

negative for CD34 and usually show, at least focally, a biphasic pattern allowing correct diagnosis[10].

Epithelioid morphology can occasionally be seen in both rhabdomyosarcomas and leiomyosarcomas. It has also been reported that approximately 30% of leiomyosarcomas are immunoreactive for cytokeratin and EMA. However, ES are easily distinguished from these by the lack of a representative area, showing fascicles of elongated tumor cells with blunt-ended "cigar-shaped" atypical nuclei, and greater frequency of negativity for desmin and SMA[12].

High-grade large epithelioid cells and a rhabdoid phenotype observed in extraskeletal myxoid chondrosarcomas may cause confusion with ES[13]. The presence of the lobular architecture and typical appearance of cords and strands of eosinophilic chondroblasts embedded in a myxoid matrix readily distinguish extraskeletal myxoid chondrosarcoma from ES.

Distinction between PES and undifferentiated carcinoma also needs consideration. The occurrence of tumors in the subcutis or deep soft tissues without any connection with the overlying epidermis or cutaneous adnexa, the absence of histological features of squamous or glandular differentiation, and presence of CD34 reactivity favour the diagnosis of ES over undifferentiated carcinoma. The latter are negative for CD34 in most cases[10].

Another close differential diagnosis, which was entertained, was malignant extra-renal rhabdoid tumor (MERT). In terms of its biological behavior, MERT is quite aggressive and lethal. MERT is known to show inactivating mutations / deletions of both the alleles of tumor suppressor genes hSNF5 / IN11 on chromosome 22q11.2. Similar deletion has also been noted in PES[14]. On this basis, it has been proposed that PES might actually be representing a form of a "complex" rhabdoid tumor. However, MERT is an entity known to occur more commonly in young children.

Recently, deletion of the SMARCBI / INI1 gene with gene inactivation was reported in the proximal type of ES[14]. Immunohistochemical expression of INI1 was recently studied in 260 epithelioid malignant tumors, including 96 cases of ES, and showed a loss of expression in about 90% of distal and proximal ES cases[15]. Immunostaining for INI1 can be used to confirm the diagnosis of ES as a complement to epithelial markers and CD34[5]. However, in view of financial and infrastructural constraints, the above mentioned study could not be obtained in our case.

In this way, a diagnosis of PES was deduced in our case based on the presence of rhabdoid cells with an epithelioid morphology, exhibiting a polyphenotypic expression with mesenchymal markers (vimentin, CD34), along with an epithelial marker (CK). The importance of identifying this subtype lies in its aggressive behaviour, which was seen in our patient, who, despite adjuvant RT did not show improvement or regression of the tumor and expired within four months of the onset of symptoms.

Reported adverse prognostic features for epithelioid sarcoma in general include male gender, proximal / axial tumor location, large tumor size (> 5 cm), deep tumor location, high mitotic index, hemorrhage, necrosis and vascular invasion[3]. Moreover the presence of rhabdoid features is related to an aggressive behaviour, multimodal therapy resistance, and rapidly fatal outcome[16]. Our patient also had some of these associated poor prognostic factors. It would be worthwhile to identify more of such rare cases and their clinical outcomes.

Treatment of ES in general includes amputation or wide *en bloc* resection as well as radiation and chemotherapy. Unfortunately, PES is often resistant to multimodal therapy, and death from disease is seen more frequently compared to the classic form.

CONCLUSION

We conclude that PES are rare soft-tissue sarcomas of adults, with epithelioid features and a frequent rhabdoid phenotype. The importance of identifying this tumor relates to its aggressive behaviour and the possibility of improving survival with early diagnosis and initiation of appropriate therapy. A range of differentials needs to be kept in mind and ruled out on the basis of the clinical profile, morphology and a wide panel of relevant immuno-histochemistry markers.

REFERENCES

1. Enzinger FM. Epithelioid Sarcoma. A sarcoma simulating a granuloma or a carcinoma. Cancer 1970; 26:1029-1041.
2. Weiss S, Goldblum J. Enzinger and Weiss's Soft Tissue Tumors. 4th Edn. St Louis:Mosby; 2001.
3. Chase DR, Enzinger FM. Epithelioid sarcoma. Diagnosis, prognostic indicators, and treatment. Am J Surg Pathol 1985; 9:241-263.
4. Guillou L, Wadden C, Coindre JM, Krausz T, Fletcher CM. "Proximal-Type" epithelioid sarcoma, a distinctive aggressive neoplasm showing rhabdoid features. Am J Surg Pathol 1997; 21:130-146.
5. Miettinen M, Fanburg-Smith JC, Virolainen M, *et al*. Epithelioid sarcoma: An immunohistochemical analysis of 112 classical and variant cases and a discussion of the differential diagnosis. Hum Pathol 1999; 30:934-942.
6. Wick MR, Manivel JC. Epithelioid sarcoma and epithelioid hemangioendothelioma: an immunocytochemical and lectin-histochemical comparison. Virchows Arch A Pathol Anat Histopathol 1987; 410:309-316.

7. Daimaru Y, Hashimoto H, Tsuneyoshi M, Enjoji M. Epithelial profile of epithelioid sarcoma. An immunohistochemical analysis of eight cases. Cancer 1987; 59:134-141.
8. Arber DA, Kandalaft PL, Mehta P, Battifora H. Vimentin-negative epithelioid sarcoma. The value of an immunohistochemical panel that includes CD34. Am J Surg Pathol 1993; 17:302-307.
9. Meis-Kindblom JM, Kindblom LG. Angiosarcoma of soft tissue: a study of 80 cases. Am J Surg Pathol 1998; 22:683-697.
10. Miettinen M, Lindenmayeer E, Chaubal A. Endothelial cell markers CD31, CD34, and BNH9 antibody to H- and Y-antigens - evaluation of their specificity and sensitivity in the diagnosis of vascular tumors and comparison with von Willebrand factor. Mod Pathol 1994; 7:82-90.
11. Folpe AL, Schmidt RA, Chapman D, Gown AM. Poorly differentiated synovial sarcoma: immunohistochemical distinction from primitive neuroectodermal tumors and high grade malignant peripheral nerve sheath tumors. Am J Surg Pathol 1998; 22:647-682.
12. Iwata J, Fletcher CDM. Immunohistochemical detection of cytokeratin and epithelial membrane antigen in leiomyosarcoma: a systematic study of 100 cases. Pathol Int 2000; 50:7-14.
13. Lucas DR, Fletcher CDM, Adsay NV, Zalupski MM. High grade extraskeletal myxoid chondrosarcoma: a high-grade epithelioid malignancy. Histopathology 1999; 35:201-208.
14. Modena P, Lualdi E, Facchinetti F, Galli L, Texeira MR, Pilotti S, Sozzi G. SMARCB1 / INI1 tumor suppressor gene is frequently inactivated in epithelioid sarcomas. Cancer Res 2005, 65:4012-4019.
15. Hornick JL, Dal Cin P, Fletcher CDM. Loss of INI1 expression is characteristic of both conventional and proximal type epithelioid sarcoma. Mod Pathol 2007; 20:16.
16. Chase DR. Do “rhabdoid features” impart a poorer prognosis to proximal-type epithelioid sarcomas? Commentary. Adv Anat pathol 1997; 5:293-295.

Case Report

Uterine Leiomyoma with Peculiar Skeletal Muscle Like and Rhabdoid Cells - Case Discussion and Literature Review

Rajan Arora, Amany Abou-Bakr, Raeda Albannai
Department of Pathology, Farwaniya Hospital, Kuwait

Kuwait Medical Journal 2015; 47 (2): 149 - 152

ABSTRACT

Tumors with skeletal muscle differentiation (rhabdomyoma, rhabdomyosarcoma) and extra-renal rhabdoid tumors have been reported in uterus but cases of uterine leiomyoma with skeletal muscle like cells or cells resembling those of extra-renal rhabdoid tumors are rare. We describe clinical and pathological features of one such case of typical uterine leiomyoma in which histopathology showed presence of rounded, polygonal, and strap cells having abundant eosinophilic cytoplasm with round to oval nuclei, some of which were eccentric in position referred to as rhabdoid cells. Intra and extra-cytoplasmic hyaline globules were observed in these cells. However, no cross striations were seen. Immunohistochemistry confirmed their smooth muscle origin (positive for desmin and h-caldesmon while negative for cytokeratin, myogenin, myoglobin, and myo D1). We also review the pertinent literature and emphasize that presence of such cells may lead to problems in differential diagnosis. Their appropriate recognition is important so that overtly aggressive management of a benign tumor is avoided.

KEYWORDS: leiomyoma, skeletal muscle like cells, uterus

INTRODUCTION

Presence of skeletal muscle like cells or cells resembling those of extra-renal rhabdoid tumors is rarely reported in uterine leiomyomas[1-3], although tumors with true skeletal muscle differentiation (rhabdomyoma, rhabdomyosarcoma) and extra-renal rhabdoid tumors have been described in uterus[4-7]. The presence of these cells can cause diagnostic difficulties in separating them from epithelioid smooth muscle tumors, smooth muscle tumor of uncertain malignant potential, or tumors with skeletal muscle differentiation. We report clinical and pathological features of one such case which showed skeletal muscle like cells or rhabdoid cells, review the pertinent literature, and discuss the potential problems in differential diagnosis and its therapeutic implications.

CASE REPORT

Clinical findings: A 45-year-old lady presented to our gynecological OPD with complaint of bleeding per vagina for three years. An endometrial sampling was performed to know the etiology and she was diagnosed as simple hyperplasia on histopathology. Subsequently, she underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy.

Pathology: On gross examination an intramural leiomyoma measuring 3 cm in maximum diameter was identified with whitish whorled appearance. There was no hemorrhage or necrosis. Rest of the specimen was unremarkable except for two small simple cysts in right ovary. Microscopic examination confirmed the presence of simple hyperplasia of endometrium along with focal adenomyosis. The leiomyoma identified grossly showed rounded, polygonal or strap cells having abundant deeply eosinophilic cytoplasm (Fig. 1). They have round to oval and some eccentric nuclei with nuclear enlargement and prominent nucleoli. Intra and extra-cytoplasmic hyaline globules were observed in some cells (Fig. 2). No cross striations were seen. There was no significant mitosis, atypia, or necrosis. On immunostaining, these cells stained positively for vimentin, desmin, and h-caldesmon (Fig. 3), and were negative for cytokeratin, myogenin, and

Address correspondence to:
Dr. Rajan Arora, Senior Specialist, Department of Pathology, Farwaniya Hospital, P O Box 18373, Farwaniya 81004, Kuwait. Tel: +965-60486105, +965-66077283, E-mail : arorarajan73@rediffmail.com, drrajarora@yahoo.com

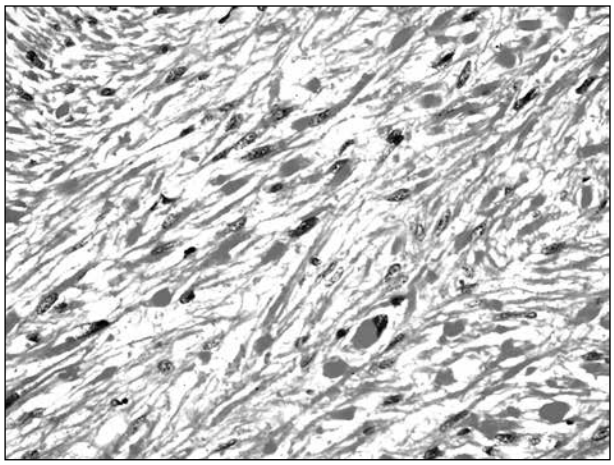

Fig 1: Photomicrograph showing strap shape cells with skeletal muscle like or rhabdoid appearance (H&E, X 400).

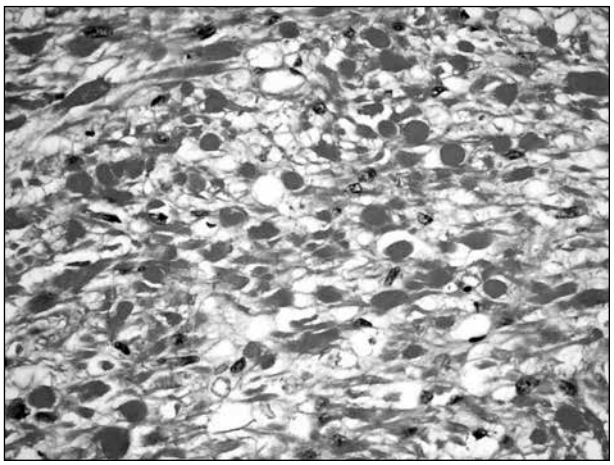

Fig 2: Photomicrograph showing intra and extra cytoplasmic hyaline globules (H&E, X 400).

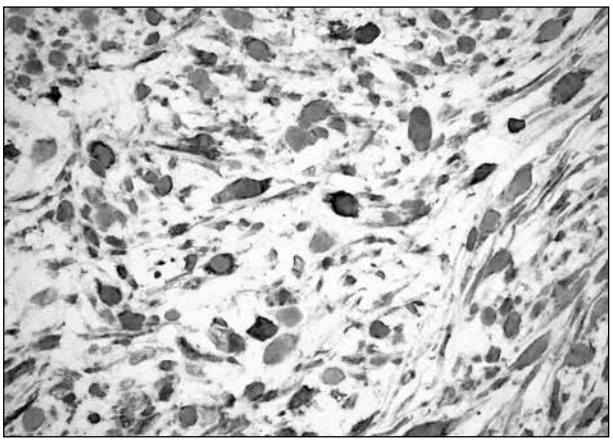

Fig 3: Photomicrograph demonstrating strong positive immunostaining for desmin (DAB, X 400).

myo D1 proving them to be of smooth muscle origin. A diagnosis of leiomyoma with skeletal muscle like or rhabdoid cells was rendered. The patient is doing well after one and half year of follow-up.

DISCUSSION

Skeletal muscle like cells or cells resembling those of extra-renal rhabdoid tumors are rarely seen in uterine leiomyomas. They have distinctive appearance on light microscopy and are referred to as "rhabdoid cells". They show round, polygonal, or strap shape with abundant deeply eosinophilic cytoplasm and may contain intra / extra cytoplasmic globules / inclusions having fibrillar or occasionally hyaline appearance. They do not display cross striations and have eccentric round to oval nuclei with vesicular to coarse chromatin and conspicuous nucleoli. Two out of the three tumors studied by Watanabe *et al*[8] in 2003 contained these cells like the present case including intense immunoreactivity for vimentin and desmin. However, h-caldesmon was focal rather than diffuse, and cytokeratin stained a few cells in one case. They interpreted them as exhibiting immature smooth muscle differentiation mimicking smooth muscle cells of the fetal uterus and were designated leiomyoblastoma. In another report[9] on eight atypical (bizarre) leiomyomas, the authors described these cells. Only one of them was examined ultra-structurally and an association of rhabdoid cells with atypical bizarre leiomyomas was suggested. Since all of their cases were obtained from consultive files of difficult cases, they did not review a series of typical leiomyomas. Our case reveals the occurrence of rhabdoid cells in a typical leiomyoma. Recently an analysis of 10 typical and two atypical leiomyomas describe the occurrence of these cells highlighting the presence of intra-cytoplasmic inclusion bodies. Two of their cases showed these bodies in more than 80% of tumor cells[10].

Since filamentous components of the smooth muscle cells of leiomyomas and myometrium comprise myofilaments and intermediate filaments and usually show positive reaction for SMA, desmin, and vimentin, patho-genetically the inclusion bodies found in the present and previously reported cases may be related to abnormal aggregates of actin or intermediate filaments, with a disordered equilibrium between synthesis and turnover of filament protein. In the study of Dundr *et al*[10], two types of inclusions were found by electron microscopy. One of them was characterized by a filamentous structure with various proportions of intermediate and actin filaments. These inclusions were eosinophilic, PAS negative, and at least at the periphery actin, desmin, and h-caldesmon positive. Another type of inclusions was composed of dense granular material without apparent fibrillar structure. These inclusions were basophilic, or unstained on H&E, were PAS positive and showed negative immunohistochemical staining. They suggest that the dense granular bodies may represent

progression of the aggregation of filaments leading to loss of the filamentous structure, or they could be related to globules named Thanatosomes related to cell injury and apoptosis[11,12].

The primary differential diagnosis of tumors with rhabdoid cells is tumors with skeletal muscle differentiation. We found two previous case reports of leiomyomas with skeletal muscle cells in the literature, and both documented convincing histologic, immunohistochemical, and ultrastructural evidence of true skeletal muscle differentiation[2,3]. However, bona fide skeletal muscle differentiation in uterine tumors is most commonly observed as a rhabdomyosarcomatous component in a malignant mixed mullerian tumor or mullerian adenosarcoma. Pure uterine rhabdomyosarcomas occur but are most commonly of cervical origin[4,13]. These can be distinguished from present case morphologically (presence of pleomorphism, mitosis, and cross striations) and immunohistochemically (Myo D1, myogenin positive). Rhabdomyoma is another differential diagnosis, but they occur exclusively in lower female genital tract.

Pure malignant rhabdoid tumors of uterus are rare [6,7,14] and this diagnosis is one of exclusion as rhabdoid phenotype is reported in endometrial stromal sarcoma, epithelioid smooth muscle tumors, leiomyosarcoma, and malignant mixed mullerian tumor[15-18]. Although our case revealed clearly benign features and has background population of typical leiomyoma cells, the rounded cells seen were similar to extra-renal rhabdoid tumors. Extra-renal rhabdoid tumors exhibit hyaline eosinophilic globular inclusions, vesicular nuclei, central prominent nucleoli, and are positive for vimentin and cytokeratin. In our case, these cells were positive for vimentin, and smooth muscle markers, but not cytokeratin. That the rhabdoid cells in our case demonstrated evidence of smooth muscle differentiation support that these cells likely represent variant expression of the smooth muscle phenotype and would be expected to have the benign outcome of the leiomyomas.

In cases where the rhabdoid morphology is diffuse and the immunophenotype is equivocal, molecular analysis of the hSNF5 / INI1 gene or immunostaining for the INI1 product may assist in revealing the correct diagnosis[19].

Other neoplasms in the differential diagnosis include epithelioid smooth muscle tumors and smooth muscle tumors of uncertain or low malignant potential. The cells of epithelioid smooth muscle tumors characteristically are devoid of rhabdoid phenotype and have granular eosinophilic or clear cytoplasm and central nuclei in contrast to more fibrillary cytoplasm and eccentrically placed nuclei. In addition epithelioid smooth muscle tumors often are immunoreactive for cytokeratin, and negative for smooth muscle markers. Absence of high mitosis and true necrosis separate the case described from smooth muscle tumors of low malignant potential.

CONCLUSION

Presence of peculiar skeletal muscle like cells or cells resembling extra-renal rhabdoid tumor in uterine leiomyoma represent smooth muscle cells with an unusual phenotype. This entity must be kept in mind and needs to be distinguished from uterine tumors with rhabdomyomatous or rhabdosarcomatous differentiation or extra-renal rhabdoid tumors since such cells can cause diagnostic dilemma leading to erroneous diagnosis and overtly aggressive management.

REFERENCES

1. Hendrickson MR, Kemson RL. Surgical pathology of the uterine corpus. In: Bennington JL, ed. Major problems in pathology series. Vol 12. Philadelphia: WB Saunders; 1980.
2. MartinReay DG, Christ ML, Lapata RE. Uterine leiomyoma with skeletal muscle differentiation. Report of a case. Am J Clin Pathol 1991; 96:344-347.
3. Fornelli A, Paquinelli G, EusebiV. Leiomyoma of the uterus showing skeletal muscle differentiation: a case report. Hum Pathol 1999; 30:356-359.
4. Jaworski RC, Rrncoret RH, Moir DH. Pleomorphic rhabdomyosarcoma of the uterus. Case report with a review of the literature. Br J Obstet Gynaecol 1984; 91:1269-1273.
5. Jacques SM, Lawrence WD, Malviya VK. Case report. Mixed embryonal rhabdomyosarcoma and fetal rhabdomyoma. Gynecol Oncol 1993; 48:272-276.
6. Cho KR, Rosenshein NB, Epstein JI. Malignant rhabdoid tumor of the uterus. Int J Gynecol Pathol 1989; 8:381-387.
7. Hsueh S, Chang TC. Malignant rhabdoid tumor of the uterine corpus. Gynecol Oncol 1996; 61:142-146.
8. Watanabe K, Ogura G, Suzuki T. Leiomyoblastoma of the uterus: an immunohistochemical and electron microscopic study of distinctive tumours with immature smooth muscle cell differentiation mimicking fetal uterine myocytes. Histopathology 2003; 42:379-386.
9. Parker RL, Young RH, Clement PB. Skeletal muscle-like and rhabdoid cells in uterine leiomyomas. Int J Gynecol Pathol 2005; 24:319-325.
10. Dundr P, Povýsil C, Tvrdík D, Mára M. Uterine leiomyomas with inclusion bodies: an immunohistochemical and ultrastructural analysis of 12 cases. Pathol Res Pract 2007; 203:145-151.

11. Hes O, Benakova K, Vanecek T, Sima R, Michal M. Clear cell type of renal cell carcinoma with numerous hyaline globules: a diagnostic pitfall. Pathol Int 2005; 55:150-154.
12. Papadimitriou JC, Drachenberg CB, Brenner DS, Newkirk C, Trump BF, Silverberg SG. "Thanatosomes": a unifying morphogenetic concept for tumor hyaline globules related to apoptosis. Hum Pathol 2000; 31:1455-1465.
13. McClugge WG, Lioe TF, McClelland HR, Lamki H. Rhabdomyosarcoma of the uterus: report of two cases including one of the spindle cell variant. Int J Gynecol Cancer 2002; 12:128-132.
14. Cattani MG, Viale G, Santini D, Martinelli GN. Malignant rhabdoid tumor of the uterus: an immunohistochemical and ultrastructural study. Vichows Arch A Pathol Anat Histopathol 1992; 420:459-462.
15. Fitko R, Brainer J, Schink JC, August CZ. Endometrial stromal sarcoma with rhabdoid differentiation [letter]. Int J Gynecol Pathol 1990; 9:379-381.
16. Prayson RA, Goldblum JR, Hart WR. Epithelioid smooth muscle tumors of the uterus. A clinicopathologic study of 18 patients. Am J Surg Pathol 1997; 21:383-391.
17. Levine PH, Mittal K. Rhabdoid epithelioid leiomyosarcoma of the uterine corpus. Int J Surg Pathol 2002; 10:231-236.
18. Mount SL, Lee KR, Taatjes DJ. Carcinosarcoma (malignant mixed mullerian tumor) of the uterus with a rhabdoid tumor component. An immunohistochemical, ultrastructural, and immunoelectron microscopic case study. Am J Clin Pathol 1995; 103:235-239.
19. Hoot AC, Russo P, Judkins AR, Perlman EJ, Biegel JA. Immunohistochemical analysis of hSNF5/INI1 distinguish renal and extra-renal malignant rhabdoid tumors from other pediatric soft tissue tumors. Am J Surg Pathol 2004; 28:1485-1491.

Case Report

Magnet Ingestion: A Case Report and Review of Literature

Sunil Kumar[1], Vipul Gupta[1], Wasmi Al Fadli[1]
Department of Pediatric Surgery, Ibn Sina Hospital, Kuwait

Kuwait Medical Journal 2015; 47 (2): 153 - 154

ABSTRACT

Foreign-body ingestion is relatively common in the pediatric population and most objects pass through the gastrointestinal tract with minimal complications. Swallowing more than one magnet is not uncommon worldwide and it frequently leads to serious consequences. We report a case of a 4-year-old boy who had accidentally swallowed two magnets which were passed spontaneously without any sequelae.

KEYWORDS: Magnetic ingestion, multiple magnets

INTRODUCTION

Foreign body ingestion in children is a common condition as they experiment throughout their development. Magnetic object ingestion is, however, very rare, but does occur and it is very important that the hazards of ingesting magnetic bodies are well known to the physician. In isolation, a single magnet is typically innocuous and is expected to behave much like other foreign bodies, however, several reports in literature proclaim the danger in children whenever more than one swallowed magnet travels beyond the stomach[1]. When the magnets attract each other they might hold the intestinal wall in between them resulting in ischemia, pressure necrosis, perforation, fistula formation and / or intestinal obstruction[2]. Herein, we report a case of ingestion of two magnets in a four-year-old boy who was fortunate enough to have passed them spontaneously without complications. Management of children with magnetic ingestion is discussed to familiarize clinicians who are dealing with such cases.

CASE REPORT

A four-year-old male child presented to our emergency room with non-bilious vomiting and epigastric pain four hours after accidentally ingesting two magnets half an hour apart. He was hemodynamically stable and had soft abdomen without any tenderness or guarding. Abdominal radiograph revealed two oval shaped radio-opaque foreign bodies stuck to each other (Fig.1). Parents had witnessed him playing with a set of oval shaped magnets before he developed symptoms. He was taken for upper gastrointestinal endoscopy in emergency when two magnets were seen in the duodenum. While an attempt was made to remove them endoscopically the magnets got dislodged and were pushed distally. The child was kept under close observation because of the fear of perforation and obstruction. He remained stable without any more symptoms and had soft abdomen on repeated clinical examinations. He was allowed to have normal diet and was monitored closely for any signs of bowel perforation or obstruction. Parents were counseled and consent was obtained for emergency surgery at any time. A progressive movement of the magnets was observed on serial X-rays and on 3rd day after ingestion he spontaneously passed two identical, oval shaped, smooth magnets each one measuring 4.0 x 1.5 x 1.5 cm (Fig. 2). The child was discharged on full oral feeds with instructions to report back if he had any pain, fever or vomiting. Upper gastrointestinal contrast follow-through study done after two weeks ruled out any stricture or fistula between the bowel loops. He was symptom free on his follow-up visit after six months.

DISCUSSION

Foreign body ingestion is a common clinical problem in pediatrics with 80% of cases involving children between the ages of six months and three years[3]. In 80 - 90% of cases, spontaneous passage through the gastrointestinal tract occurs once the

Address correspondence to:
Dr Sunil Kumar, Department of Pediatric Surgery, Ibn Sina Hospital, P O Box 13115, Safat-13042, Kuwait. Tel: 00965- 2434794, Fax: 24834864
Mobile 00965-66018599, E-mail: sunilyadav90@hotmail.com

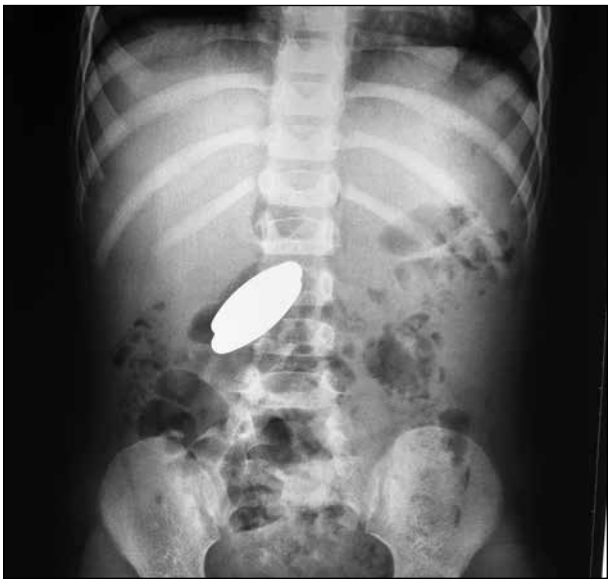

Fig. 1: Abdominal radiograph showing two radioaque foreign bodies (stuck to each other) in upper abdomen

Fig. 2: Two identical oval shaped, smooth magnets passed spontaneously without any sequelae

foreign body has entered the small bowel, so that surgical intervention is not usually necessary[4]. Upper gastrointestinal foreign bodies are amenable to retrieval by endoscopy[5] or Foley balloon catheter extraction[6]. The ingestion of only one magnet does not cause a problem but when more than one or multiple magnets are ingested, the individual magnets tend to interact through the bowel wall leading to pressure necrosis of intestinal wall. A review of the published literature revealed previous cases of children aged 2 - 3 years in whom obstruction, perforation, fistula formation, and adhesions occurred after multiple magnetic foreign body ingestion[3,6]. When a case of magnet ingestion is encountered, one must differentiate between ingestion of a single magnet or multiple magnets by taking a thorough history and obtaining adequate radiographic images. Ingestion of a single magnet can be managed in a similar way as in ingestion of other foreign bodies, with a trial of conservative management, expecting uneventful passage through the gastrointestinal tract but when more than one magnet has been ingested; endoscopic removal must be performed without delay unless the magnets have travelled beyond pylorus[7]. Once magnets have passed the pylorus, some authors prefer prompt surgical intervention even if the patient is asymptomatic[3]. On the other hand, others think that close observation must be made, and only if there are signs of complications or unchanged location of ingested magnets on serial plain X-ray (showing two magnets stuck together), would surgical intervention be necessary[8]. Our patient had no signs of perforation or obstruction and position of the magnets changed on serial abdominal X-rays. Therefore, no surgical intervention was necessary.

Clinicians who care for children should be aware of the risks associated with multiple magnet ingestion. If magnets stay in the same location shown by repeated X-rays, surgical intervention should not be delayed.

REFERENCES

1. Oestreich AE. Danger of multiple magnets beyond the stomach in children. J Natl Med Assoc 2006; 98:277-279.
2. Dutta S, Barzin A. Multiple magnet ingestion as a source of severe gastrointestinal complications requiring surgical intervention. Arch Pediatr Adolesc Med 2008; 162:123-125.
3. Lee SK, Beck N, Kim H. Mischievous magnets: unexpected health hazard in children. J Pediatr Surg 1996; 31:1694-1695.
4. Laurance Hill J, Voigt RW. Foreign bodies. In: Ashcraft KW, ed. Pediatric surgery, 3rd edn. Philadelphia: WB Saunders Company, 2000:146-152.
5. Hachimi-Idrissi S, Corne S, Vandenplas Y. Management of ingested foreign bodies in childhood: our experience and review of the literature. Eur J Emerg Med 1998; 5:319-323.
6. Morrow SE, Bicker SW, Kennedy AP, Snyder CL, Sharp RJ, Ashcraft KW. Balloon extraction of esophageal foreign bodies in children. J Pediatr Surg 1998; 33:266-270.
7. Hebra A, Tagge EP. Esophagoscopy and esophageal foreign bodies. In: Ziegler MM, Azizkhan RG, Weber TR, eds. Operative Pediatric Surgery. New York: Mc Graw-Hill, 2003; 331-339.
8. Chung JH, Kim JS, Song YT. Small bowel complication caused by magnetic foreign body ingestion of children: two case reports. J Pediatr Surg 2003; 38:1548-1550.

Case Report

A Lateral Cervical Cyst as the Initial Presentation of an Occult Papillary Thyroid Carcinoma: A Case Report

Tzu-Hang Chi[1, 2], Kun-Wei Hsieh[1], Shang-Tao Chien[3]
[1]Department of Otolaryngology, Kaohsiung Armed Forces General Hospital, Kaohsiung, Taiwan
[2]Department of Otolaryngology, Taoyuan Armed Forces General Hospital, Taoyuan, Taiwan
[3]Department of Pathology, Kaohsiung Armed Forces General Hospital, Kaohsiung, Taiwan

Kuwait Medical Journal 2015; 47 (2): 155 - 157

ABSTRACT

Papillary carcinoma accounts for 85% to 90% of all malignant thyroid tumors. Papillary thyroid carcinoma commonly extends to the lymphatic system; metastatic lymph nodes usually present as a solid mass in the anterior or lateral aspect of the neck. Cervical cysts are usually considered benign lesions; branchial cleft cysts are the most common type of cyst identified at the lateral cervical region of the neck. A lateral cervical cyst as the initial manifestation of an occult thyroid carcinoma is rare. Aside from a thorough physical examination of the head and neck, ultrasound, computed tomography and fine needle aspiration cytology should be performed to determine the diagnosis for a lateral cervical cyst. Excisional biopsy should be performed, if the fine needle aspiration cytology is negative. We report a rare case of lateral cervical cyst, as the initial presentation of an occult papillary thyroid carcinoma.

KEYWORDS: excisional biopsy, fine needle aspiration cytology, papillary carcinoma, ultrasound

INTRODUCTION

In general, most of the cervical neck cysts of patients younger than 40 years of age are benign lesions and most commonly identified as branchial cleft cysts. Malignant lateral cervical neck cysts metastasized from squamous cell carcinoma of Waldeyer' s ring are less common, but should be included in the differential diagnosis of lateral cervical neck cysts. An occult thyroid carcinoma initially presenting as a lateral cervical cyst is rare[1]. We report a case of a 34-year-old man with a lateral cervical neck cyst that presented as the initial manifestation of an occult papillary thyroid carcinoma.

CASE REPORT

A 34-year-old man who had been relatively healthy and without significant systemic disease and a negative family history presented to the otolaryngology outpatient department with a slow-growing painless right cervical mass noted for the past six months. The patients denied habitual cigarette smoking and alcohol consumption.

The initial physical examination showed one palpable 4.0 x 3.0 cm mass deep in the right sternocleidomastoid muscle. The neck mass was smooth, mobile, non-tender, and there was no additional lymphadenopathy or palpable mass over the thyroid gland. The nose, ears, oral cavity, pharynx and larynx were within normal limits. The computed tomography of the nasopharynx to the neck showed one 3.5 x 2.5 cm, cystic-like hypodense lesion under the right sternocleidomastoid muscle and next to the carotid sheath (Fig. 1). Fine needle aspiration cytology of the cyst was performed and the report was negative for a malignancy. Based on the physical, radiological, cytological examinations and consideration of the patient's history, a tentative diagnosis of a branchial cleft cyst was made.

An excisional biopsy under general anesthesia was performed. The cystic-like tumor was completely removed and no tract connecting the tumor to the pharynx or hyoid bone was identified. On gross examination of the specimen, it had a thin wall with dark-brown serous fluid contents. Microscopic

Address correspondence to:
Kun-Wei Hsieh, Department of Otolaryngology, Kaohsiung Armed Forces General Hospital 2, Chung Cheng 1st Road, Kaohsiung, 802, Taiwan, Republic of China. Tel: +886-7-7494965, Fax: +886-7-7495175, E-mail: cooljackychi@yam.com

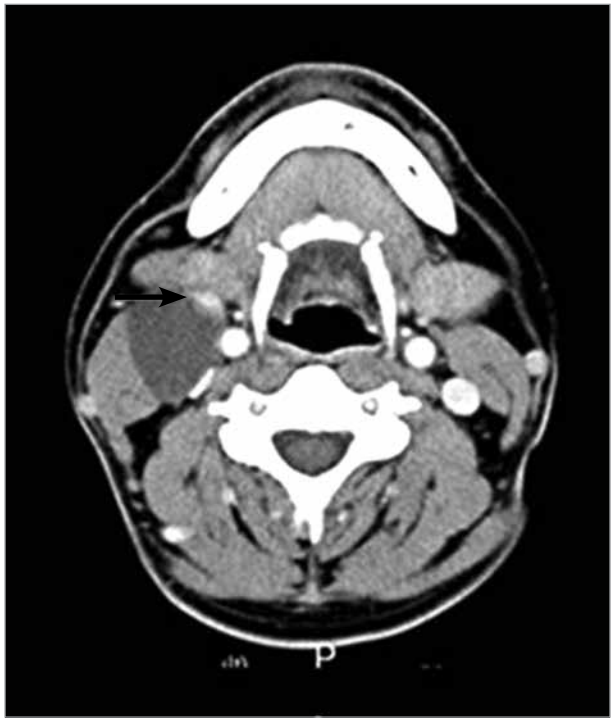

Fig. 1: Computed tomography of the nasopharynx to neck showing one hypodense lesion with intra-lesional enhancing elements of about 3.5 x 2.5 cm under the right sternocleidomastoid muscle and next to the carotid sheath

examination revealed a metastatic papillary type thyroid carcinoma (Fig. 2). After the operation, a thyroid ultrasound was performed which showed an ill-defined solid hypoechoic nodule about 9.8 x 7.5 mm in the right lobe of the thyroid gland (Fig. 3). A total thyroidectomy was performed with a right-sided functional neck dissection. Microscopic examination confirmed a unifocal papillary carcinoma measuring 0.7 x 0.7 x 0.7 cm in the right lobe of the thyroid gland and metastatic papillary thyroid carcinoma in two paratracheal lymph nodes. The patient recovered with no complications. The patient was given radioactive iodine (I^{131}) ablation followed by thyroid replacement therapy, and follow-up examinations for one year. The patient has had no evidence of recurrence or any other additional problems.

DISCUSSION

Lateral cervical cysts are usually considered benign lesions and are found most commonly in patients younger than 40 years of age. Among these lateral cervical cysts, branchial cleft cysts are the most common. Compared with benign lateral cervical cysts, malignant lateral cervical cysts as the initial presenting sign of squamous cell carcinoma of Waldeyer' s ring is less common. Without further diagnostic evaluation, the common benign lateral cervical cyst, branchial cleft cyst and malignant lateral cervical cyst metastasized

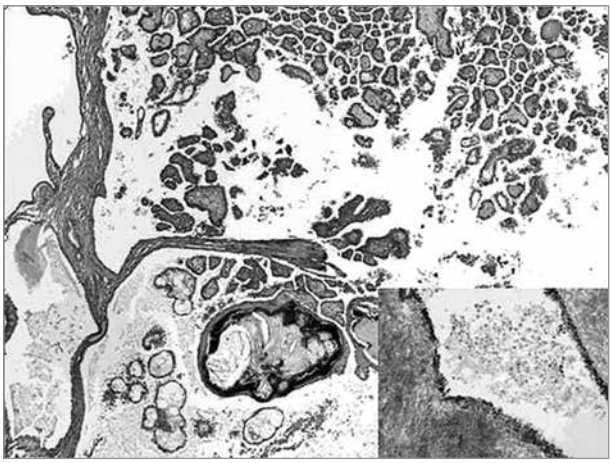

Fig. 2: Histological examination of the tumor reveals a significant papillary growth pattern. (Hematoxylin and eosin stain x 40). Inlet: Immunostaining of lining cells for thyroid transcription factor-1 (TTF-1) was positive (Hematoxylin and eosin stain x 200).

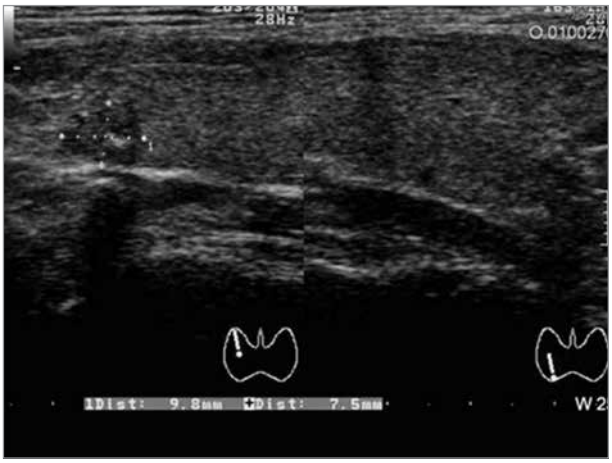

Fig. 3: Thyroid ultrasound shows an ill-defined solid hypoechoic nodule about 9.8 x 7.5 mm in the right lobe of the thyroid gland

from occult squamous cell carcinoma of Waldeyer' s ring cannot be differentiated.

Papillary carcinoma is the most common type of all malignant thyroid tumors. Metastasis of papillary thyroid carcinoma occurs due to lymphatic spread. The incidence of metastatic cervical lymph nodes with occult papillary thyroid carcinoma is around 30%[2]. Metastatic cervical lymph nodes commonly manifest as solid masses in the lateral cervical region; however, the appearance of a lateral cervical cyst as the initial presentation is rare[3]. This phenomenon could be explained by central liquefaction and the resultant cystic degeneration inside a lymph node from metastasis of a papillary thyroid carcinoma [4].

The treatment and prognosis of a malignant lateral cervical cyst from an occult metastasized papillary thyroid carcinoma are different than for a benign lateral cervical cyst. A delay in the correct diagnosis can increase the mortality rate. For a one-year delay

in the diagnosis of a papillary thyroid carcinoma, the mortality rate increases two to three fold[5].

The initial approach to a patient with a neck mass should include a comprehensive history as well as a careful head and neck physical examination. In addition, ultrasound and computed tomography examinations are important to distinguish between benign and malignant cervical cysts. For the ultrasound images, a metastatic lateral cervical cyst from an occult papillary thyroid carcinoma may have a thickened outer wall with septae; however, these cysts may look like branchial cleft cysts[6]. Furthermore, a metastatic lateral cervical cyst from an occult papillary thyroid carcinoma may present with intracystic enhancement elements with computed tomography.

Fine needle aspiration cytology is useful for the diagnosis of cervical mass. The sensitivity rate for a solid cervical mass is about 90% to 100%; however, the false negative rate of cervical cysts is as high as 50%[7]. Prior reports suggest that aspiration fluid with a dark brown color may be characteristic of metastatic papillary thyroid carcinoma[8]. Excisional biopsy should be performed even with negative fine needle aspiration cytology. Frozen-section analysis of the excised specimen can confirm a malignancy even when ultrasound or computed tomography cannot rule it out. If the result of frozen-section analysis of the cyst reveals a metastatic papillary thyroid carcinoma, thyroidectomy and neck dissection should be performed immediately[9].

CONCLUSION

In summary, although a malignant lateral cervical cyst metastasized from an occult papillary thyroid carcinoma is rare, it should always be included in the differential diagnosis of a lateral cervical neck cyst. A thorough history and physical examination are important strategies for the initial assessment; fine needle aspiration cytology of the lateral cervical cyst is also essential. In addition, an excisional biopsy should be performed, if fine needle aspiration cytology is negative. Frozen-section examination during the operation should be considered, if the clinical malignancy cannot be completely ruled out.

REFERENCES

1. Huseyin Seven, Arzu Gurkan, Ugur Cinar, Cetin Vural, Suat Turgut. Incidence of occult thyroid carcinoma metastases in lateral cervical cysts. Am J Otolaryngol 2004; 25:11-17.
2. Hubert JP, Kiernan PD, Beahrs OH, Mcconahey WM, Woolner LB. Occult papillary carcinoma of the thyroid. Arch Surg 1980; 115:394-398.
3. Takayuki. Nakagawa, Tadayoshi Takashima, Kenta Tomiyama. Differential diagnosis of a lateral cyst and solitary cystic lymph node metastasis of occult thyroid papillary carcinoma. J Laryngol Otol 2001; 115:240-242.
4. Sidhu S, Lioe TF, Clement B. Thyroid papillary carcinoma in lateral neck cyst: Missed primary tumour or ectopic thyroid carcinoma within a branchial cyst? J Laryngol Otol 2000; 114:716-718.
5. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. Am J Med 1994; 97:418-428.
6. Wunderbaldinger P, Harisinghani MG, Hahn PF, *et al*. Cystic lymph node metastases in papillary thyroid carcinoma. Am J Roentgenol 2002; 178:693-697.
7. Christine G. Gourin, Jonas T. Johnson. Incidence of unsuspected metastases in lateral cervical cysts. Laryngoscope 2000; 110:1637-1641.
8. Mcdermott D, Watters GDM. Metastatic papillary thyroid carcinoma presenting as a typical branchial cyst. J Laryngol Otol 1996; 110:490-492.
9. Rabinov CS, Ward PH, Pucheck T. Evolution and evaluation of lateral cystic neck masses containing thyroid tissue: Lateral aberrant thyroid revisited. Am J Otolaryngol 1996; 17:1215.

Case Report

Postmenopausal Ovarian Hyperthecosis

Sundus AlDuaiJ[1], Suha Abdulsalam[2], Khulood Al Asfore[2]
[1]Department of Endocrinology, Mubarak Al Kabeer Hospital, Kuwait
[2]Department of Medicine, Mubarak Al Kabeer Hospital, Kuwait

Kuwait Medical Journal 2015; 47 (2): 158 - 160

ABSTRACT

Ovarian hyperthecosis has variable clinical importance. It can cause hyperandrogenism, particulary in premenopausal women, and may be a rare cause of androgenic alopecia in postmenopausal women. The physiological level of androgens secreted by ovarian stromal cells is greatly increased with hyperplastic or neoplastic transformation leading to possible clinical consequences. We report a case of postmenopausal ovarian hyperthecosis.

KEY WORDS: hyperandrogenism, postmenopausal

INTRODUCTION

The term hyperthecosis refers to the presence of nests of luteinized theca cells in the ovarian stroma due to differentiation of the ovarian interstitial cells into steroidogenically active luteinized stromal cells.

The nests or islands of luteinized theca cells are scattered throughout the stroma of the ovary, rather than being confined to areas around cystic follicles as in the polycystic ovary syndrome. The result is greater production of androgens. It is not clear why hyperthecosis occur. Bilateral ovarian stromal hyperthecosis occasionally causes virilization in premenopausal women[1]. However, a previous review article found only two previously reported cases of stromal hyperthecosis in postmenopausal women[2].

CASE REPORT

A 54-year-old Kuwaiti female, reaching menopause at the age of 50 years with no significant postmenopausal symptoms, was first seen in endocrine clinic with three years history of hirsutism and frontal hair loss. She was known to have type 2 diabetes and hypertension, had menarche at age 13 and described that she had oligomenorrhea since menarche and infertility, but had two successful pregnancies after clomiphene treatment. On October 2010, she had partial gastrectomy due to stomach cancer followed by chemotherapy and currently, the patient is in remission.

Physical examination revealed temporal and anterior baldness and increase facial hair on her sideburns and chins. She was overweight with body mass index of 28 kg/m^2. Examination of the chest, heart, abdomen and pelvis were otherwise normal.

Initial investigation showed normal full blood count, lipid profile, renal and liver function tests. Her fasting blood sugar was 9.1 mmol/l. Hormonal profile revealed raised serum total testosterone 401 nmol/l (0.3 - 3.0), with sex hormone binding globulin 15 nmol/l (20 - 118), dehydroepiandrosterone sulfate 7.4 nmol/l (0.9 - 11.7) and androstenedione 22.4 nmol/l (1.6 - 9.4). The gonadotrophins which are luteinizing hormones and follicular stimulating hormone (FSH) were in the postmenopausal range, whereas the serum estradiol level was slightly elevated for postmenopausal state (340 pmol/l). Thyroid function tests were normal.

The results of further diagnostic tests were as follows: morning serum cortisol after overnight dexamethasone suppression test (1 mg) was normally suppressed (20 nmol/l). Short synacthen test (250 micrograms cortisone intravenously) was done to exclude the diagnosis of late onset congenital adrenal hyperplasia (Table 1).

A significant decrease in dehydroepiandrosterone (1.6 nmol/l), but only partial suppression of testosterone (2.8 nmo/l) followed low dose dexamethasone therapy (0.5 mg 4 times a day for 5 days). Ultrasound examination of adrenals and transvaginal ultrasound

Address correspondence to:
Sundus AL Duaij, Department of Endocrinology, Mubarak Al Kabeer Hospital, Kuwait. Tel: +965-99734486 (M), dr-sundus1@hotmail.com

Table 1: Short synacthen test (250 micrograms cortisone intravenous) to exclude the diagnosis of late onset congenital adrenal hyperplasia

Time	Cortisol nmol/l	17 hydroxyprogesterone nmol/l
0	323	1.6
30	683	14
60	763	14

of ovaries were normal. Magnetic resonance image of abdomen and pelvis was normal.

In view of these clinical and biochemical observations, a diagnosis of ovarian hyperthecosis (severe variant of polycystic ovary syndrome) was made.

The patient subsequently received spironolactone and metformin treatment. At the same time, she started laser therapy with improvement in her hirsutsim.

DISCUSSION

We describe a case of postmenopausal hyperthecosis, a severe variant of polycystic ovary syndrome who presented with hirsutism and responded well to antiandrogen therapy.

Ovarian hyperthecosis is characterized by significantly increased stromal tissue with luteinized theca cells[3]. It is an uncommon disorder with androgen overproduction. Ovarian hyperthecosis shares many clinical features with polycystic ovary syndrome including hirsutism, acne and menstrual irregularities. However, it tends to be more likely associated with virilisation[4]. Most women with ovarian hyperthecosis are obese, with long standing history of hirsutism. The hirsutism is usually severe and most of the women shave daily. Many also have clitoral enlargement, temporal balding, deepening of the voice and a male habitus. Most have amenorrhea and the remaining have irregular anovulatory cycles. Acanthosis nigrican is suggestive of severe insulin resistance.

Despite marked hyperandrogenism as in our case, mainly testosterone is elevated in most cases and postmenopausal ovarian hyperthecosis generally presents as a non-neoplastic, functional disorder, which result from abnormal regulation of ovarian steroidogenesis[5].

Unlike polycystic ovary syndrome, which occurs only during the reproductive years, hyperthecosis of the ovaries can occur in postmenopausal women. Severe hirsutism and virilization in postmenopausal women are more often due to ovarian hyperthecosis than virilizating ovarian tumor[6], which is an ovarian tumor made up of hormone secreting cells due to excessive male hormone (androgen) production. Previous reports suggest that ovarian hyperthecosis occurs mainly in association with insulin resistance that manifest with central obesity, hypertension, hyperlipidemia, hyperinsulinemia and type 2 diabetes or impaired glucose tolerance[7,8], as was the case in our patient .

Imaging is required to rule out ovarian neoplasm and to measure endometrial thickness, as there is an association between ovarian hyperthecosis and endometrial cancer[9]. As described in a previous study, it is likely to be related to increased androgen production by the luteinized theca cells, which then serve as precursors for estrogen production[[0-12]. Sonographic features of ovarian hyperthecosis are variable. The most frequent finding is a normal ovary, as was the case in our patient. A small percentage of affected ovaries may have co-existing morphological features of polycystic ovary syndrome and infrequently a small solid mass may be seen[13]. Treatment of hyperthecosis should include weight reduction, insulin sensitizing agents such as metformin and thiazolidinedies. However, hirsutism can be controlled by antiandrogen therapy that includes spironolactone and gonadotropin releasing hormone agonists. If patient is in the reproductive age, oral contraceptive pills can be used or clomiphene, if she seek fertility.

CONCLUSION

Although ovarian hyperthecosis is a recognized cause of premenopausal virilization, it should also be included as an unusual cause of postmenopausal virilization. The demonstration that this syndrome can develop in the postmenopausal period, as in our patient, strongly suggest that it is a distinct entity and not a late stage of polycystic ovary syndrome, as has been suggested.

REFERENECES

1. Goldman JM, Kapadia LJ. Virilization in postmenopausal women due to ovarian stromal hyperthecosis .Postgrad Med J 1991; 67:304-306.
2. Van Heyningen C, MacFarlane IA, Diver MJ, Muronda C, Tuffnell D. Virilization due to ovarian hyperthecosis in a postmenopausal women. Gynecol Endocrinol 1988; 2:331-338.
3. Rajput R, Bhansali A, Singh A. Ovarian hyperthecosis and response to antiandrogens: An uncommon presentation of a common disorder. J Obstet Gynaecol 2008; 28:249-250.
4. Ashaweah K, Aghilla MM, Randeva HS. Androgenic alopecia in postmenopausal ovarian hyperthecosis. J Obstet Gynaecol 2011; 31:351-352.
5. Krug E, Berga SL. Postmenopausal hyperthecosis functional dysregulation of androgenesis in climacteric ovary. Obstet Gynecol 2002; 99:893-897.

6. Efstathiadou Z, Tsatsoulis A. Long-term remission of ovarian hyperandrogenism after short-term treatment with a gonadotropin-releasing hormone agonist. Fertil steril 2001; 75:59-62.
7. Nagamani M, Dinh T, Kelver ME. Hyperinsulinemia in hyperthecosis of the ovaries. Am J Obstet Gynecol 1996; 154:384-387.
8. Barth JH, Jenkins M, Belchtz PE. Ovarian hyperthecosis, diabetes and hirsutes in postmenopausal women. Clin Endocrinol 1997; 46:123-128.
9. Clement PB, Kurman RJ. Non-neoplastic lesion of the ovary. Blaustein's Pathology of the Female Genital Tract 2002; 5:675-728.
10. Sasono H, Fukunaga M, Silverberg SG. Hyperthecosis of the ovary. Int J Gynecol Pathol 1989; 96:311-320.
11. Ronnett BM, Zaino RJ, Ellenson LH, Kurman RJ. Endometrial cancer. In : Kurman RJ, Editor. Blaustein's Pathology of the Female Genital Tract 5th ed. New York: Springer 2002; 5:501-559.
12. Jongen VH, Hollema H, van der Zee AG, Santema JG, Heineman MJ. Ovarian stromal hyperplasia and ovarian vein steroid levels in relation to endometroid endometrial cancer. BJOG 2003; 110:690-695.
13. Brown DL, Henrichsen TL, Clayton AC, *et al*. Ovarian stromal hyerthecosis: Sonographic features and histological association. J Ultrasound Med 2009; 28:587-593.

Case Report

Dysplasia Epiphysealis Hemimelica with Bony and Soft-Tissue Abnormalities: A Case Report and Review of the Literature

Li-Feng Qin[1], Han Fang[2], Dan Peng[1]
[1]Department of Orthopedics, Second Xiang-Ya Hospital, Central South University, Changsha, China
[2]Department of Cardiovascular Disease, Xiang-Ya Hospital, Central South University, Changsha, China

Kuwait Medical Journal 2015; 47 (2): 161 - 165

ABSTRACT

We report a case of a 3-year-old boy with dysplasia epiphysealis hemimelica (DEH) who presented with a hard painless swelling on the medial side of his left ankle joint. Plain X-rays, CT and MRI showed a bony tumor and associated soft-tissue abnormalities. At age one year, he had been diagnosed with an osteochondroma and underwent incomplete surgical excision at a local hospital. Due to joint impingement and restricted movement, we chose to completely excise the tumor. Pathologically, the specimen showed evidence of an osteochondroma. From its clinical, imaging, and pathological features and reports in the medical literature, a diagnosis of DEH was established. DEH is a rare variant of osteochondroma, resulting in many patients being incorrectly diagnosed or diagnosed with osteochondroma alone. It is important to differentiate low-grade malignant tumor or osteochondroma-like parosteal osteosarcoma from DEH of the talus, especially when accompanied by soft tissue abnormalities. Surgical treatment is mandatory in patients with symptoms such as pain, joint impingement and deformation. Incomplete excision of an articular lesion may lead to recurrence and additional surgery, making it less effective than complete excision.

KEY WORDS: dysplasia epiphysealis hemimelica (DEH), osteochondroma, soft-tissue abnormalities, talus, Trevor's disease

INTRODUCTION

Dysplasia epiphysealis hemimelica (DEH), or Trevor's disease, is a rare skeletal developmental disorder characterized by osteocartilaginous overgrowth involving single or multiple epiphyses on one side of the body, especially in children[1-3]. DEH can be classified into ''localized'', affecting a single bone; ''classical'', affecting more than one area in a single lower extremity; or ''generalized'', involving an entire lower limb from the pelvis to the foot[4]. These lesions can also be classified as juxta-articular (*i.e.*, adjacent to the articular surface) or articular (*i.e.*, directly involving the joint surface forms)[5], or as intra or extra-articular variety[6].

We report a case of a 3-year-old boy with DEH, who presented with a recurring hard painless swelling of his left ankle, with MRI showing associated soft-tissue abnormalities. At age one year, he had been diagnosed with an osteochondroma at a local hospital and underwent partial excision. However, it recurred after two years.

CASE REPORT

A 3-year-old boy presented at Xiang-Ya Second Hospital with a painless swelling and gradually enlarging deformation on the medial aspect of his left ankle. Two years earlier, at age one year, he had presented with deformation and abnormal gait, for which he underwent incomplete excision at a local hospital (Fig. 1A). Pathologic findings showed an osteochondroma.

Physical examination showed a 4-cm-long surgical scar on the medial side of his left ankle, along with a 2 X 3 cm swelling on the posterio-medial side. The mobility of the ankle was reduced, with medial and lateral rotations of 10° - 0° - 15° and dorsiflexion and

Address correspondence to:
Dr Dan Peng, Department of Orthopedics, Xiang-Ya Second Hospital, Central South University, 139# Ren-min road, Changsha 410010, Hunan, P R China. Tel: 86-731- 5295827, Fax: 86-731-5295827, E-mail: xyeypdyz@163.com

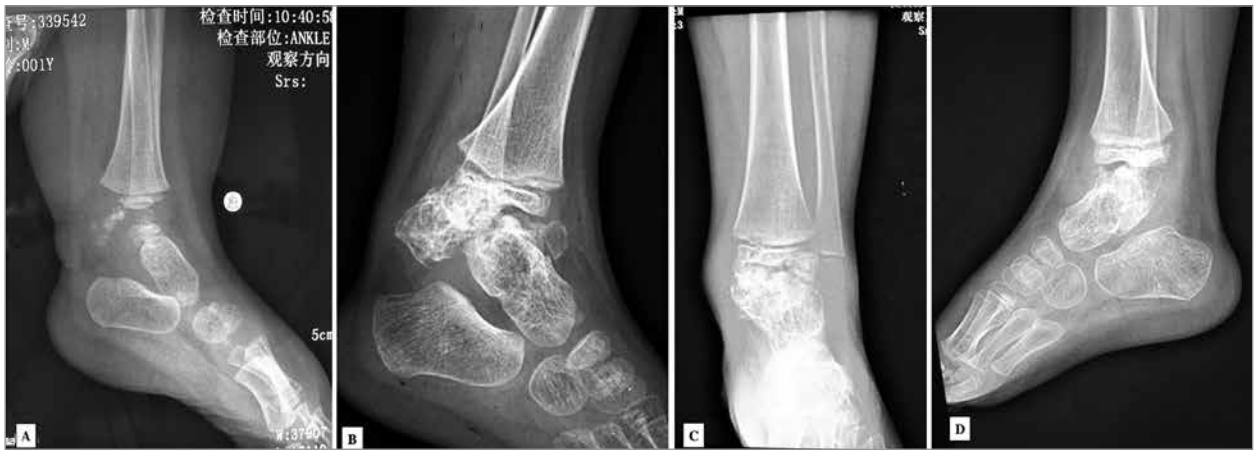

Fig. 1: Clinical course in our patient. (A) At one year of age, this patient had undergone incomplete excision at a local hospital. (B - C) Plain X-rays of his left ankle at three years of age, showing a bony tumor on the posterior-medial side of the distal epiphysis of the tibia and talus, with an irregular and thickened structure and swelling of the surrounding soft tissue. (D) X-ray six months after complete excision, showing no recurrence of tumor, although the local joint space of the talus-ankle articular had become narrower.

plantar flexion of 10°- 0°- 20°. There was no evidence of wasting and no discrepancy between the lengths of his lower limbs.

A plain X-ray showed a bony tumor on the posterio-medial side of the distal epiphysis of his left tibia and talus, with swelling of surrounding soft tissues (Fig. 1, B - C). Computerized tomography (CT) showed an irregular, expanding bony tumor, with osteo-epiphysis of the left distal tibia and the left medial and posterior ankle. The tumor was of heterogeneous density, including cystic low density and high-density spotted state focus (Fig. 2A). Three-dimensional reconstructed images showed that his talus-ankle articular surface was ossified and the local joint space was narrowed, with osteoporosis and a cystic euphotic zone in the left talus (Fig. 2B - C). These findings suggested tumor recurrence in the medial epiphysis of the left tibia and talus; moreover, the presence of soft tissue lesions suggested a low-grade malignant tumor.

MRI showed a bony tumor on the medial side of the distal epiphysis of the tibia and talus (Fig. 3). T1-weighted MRI showed a mass of low to intermediate signal intensity, whereas T2-weighted MRI showed a mass of intermediate to high signal intensity. Swelling of the surrounding soft tissues, increased effusion, and heterogeneous signals in the left calcaneus and talus were observed, again suggesting tumor recurrence in the medial epiphysis of the left tibia and talus, although epiphyseal dysplasia of the left tibia could not be ruled out. Plain X-rays of the left knee and

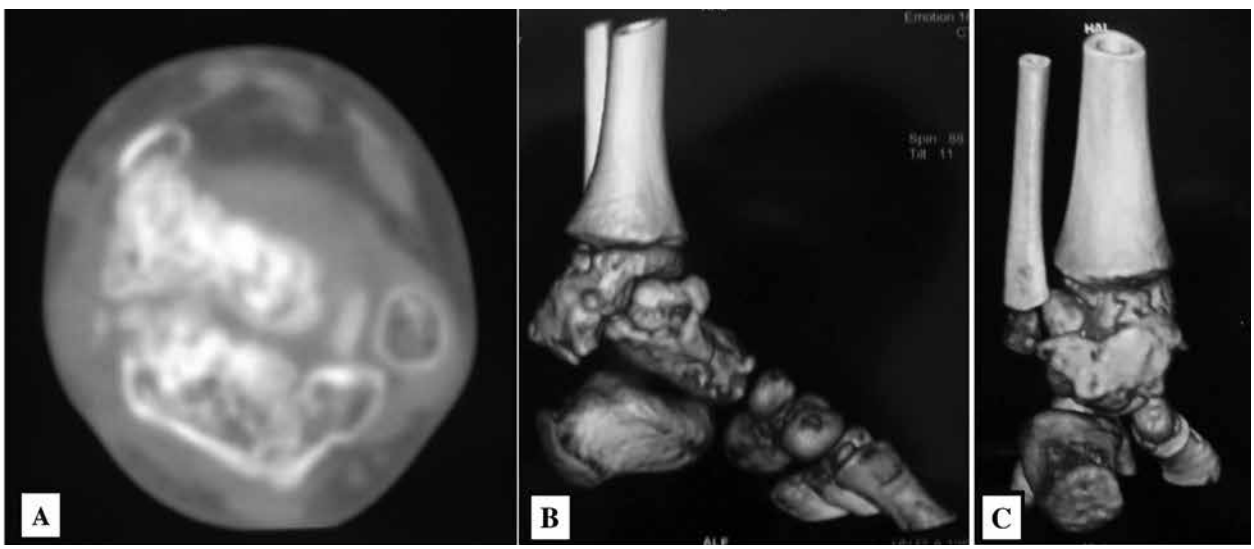

Fig. 2: CT findings in our patient, showing (**A)** an irregular and expansional bony tumor (osteoepiphysis in the left distal tibia, left media and posterior ankle) with heterogeneous density (cystic low density and high-density spotted state focus). The talus-ankle articular surface had ossified and the local joint space had narrowed. Swelling of local soft tissue was observed, as well as osteoporosis and a cystic euphotic zone in the left talus. (B, C) Three-dimensional reconstructed images are shown.

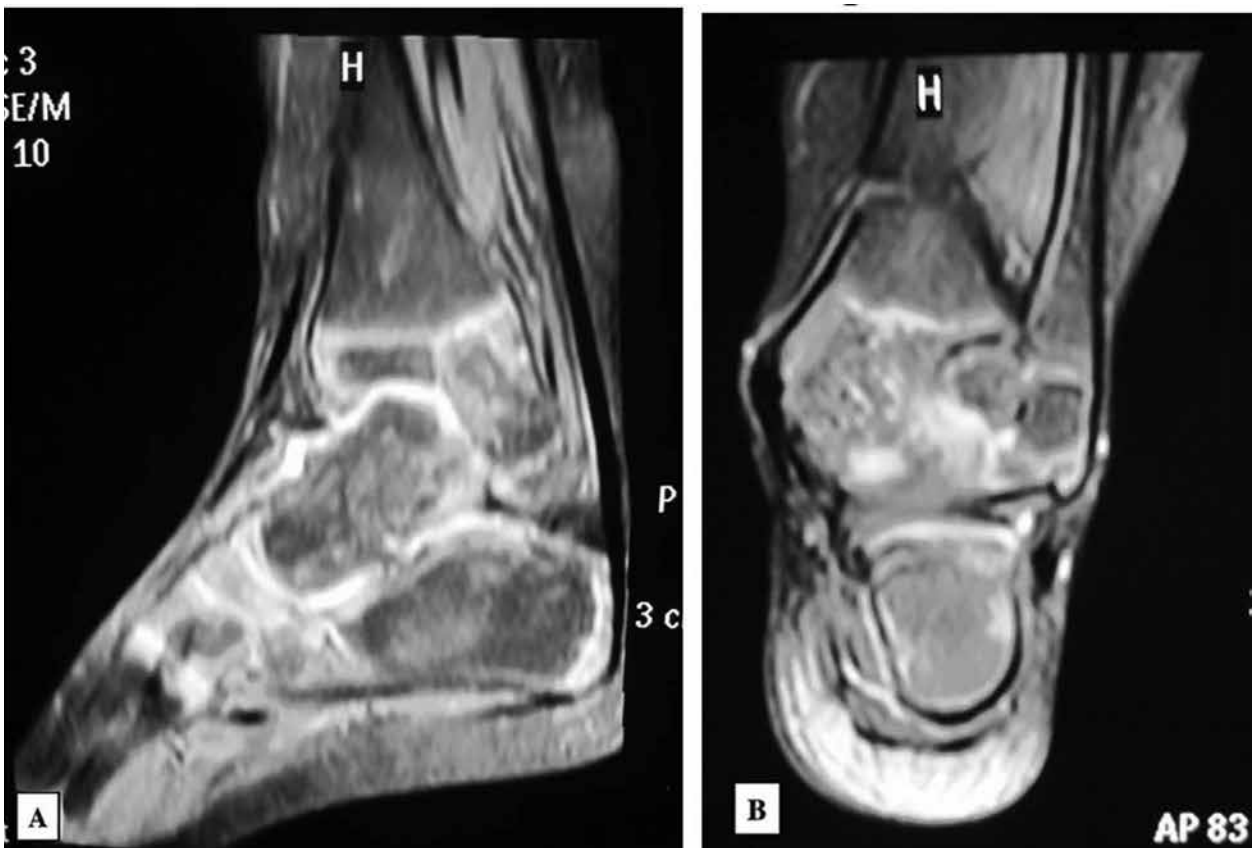

Fig. 3: MRI findings in our patient, showing **(A - B)** a bony tumor measuring 2 x 3 cm on the medial side of the distal epiphysis of the tibia and talus. T2- weighted MRI showed intermediate to high signal intensity, with swelling of the surrounding soft tissues, increased effusion, and a heterogeneous signal in the left calcaneus and talus.

right ankle and knee showed no abnormalities. Blood biochemistry showed elevated concentrations of alkaline phosphatase (290.51 μ/l; normal range: 30.0-110.0 μ/l) and acid phosphatase (7.3 μ/l; normal range: 0.0-5.55 μ/l), while other tests, including routine blood tests, electrolytes, erythrocyte sedimentation rate and C-reactive protein concentrations were all within normal range.

Because this patient presented with painless swelling, joint deformity and limited range of motion, we performed surgery. The medial and posterior aspects of the epiphysis of the left distal tibia were

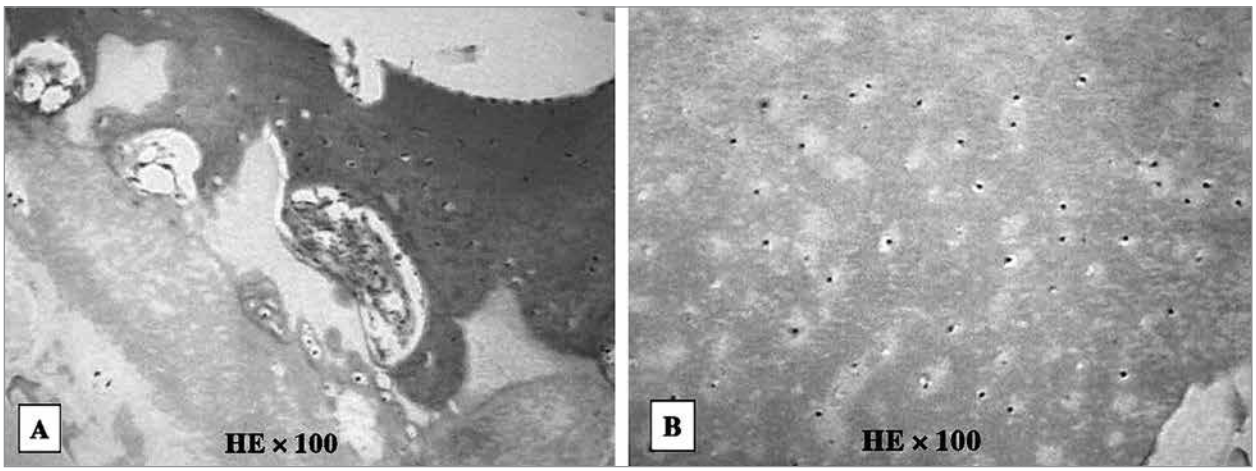

Fig. 4: Histopathological examination, showing a benign osteochondroma, composed of cortical and medullay bone with overlying hyaline cartilage

completely excised, along with the overgrowth of the posterior talus. Histopathological examination of the specimen revealed a benign osteochondroma, composed of cortical and medullary bone with overlying hyaline cartilage (Fig. 4). The patient was followed-up for six months, during which he showed no recurrence of pain or deformity and no limitations in ankle movements (Fig. 1D).

DISCUSSION

DEH is a rare skeletal developmental disorder, usually diagnosed at age 2 - 14 years, but rarely in adults[7]. Its reported incidence is 1:1,000,000[6], but it may be much higher[5], since many patients may be improperly diagnosed, including the diagnosis of an osteochondroma[8-10]. DEH is two-fold more common in men than in women[11]. Most lesions affect one side of the joint, with a 2:1 medial-to-lateral ratio. In two-thirds of patients, more than one epiphysis is affected[11]. DEH usually involves a single lower extremity, especially around the knee and ankle. Although asymptomatic in most patients, DEH may cause mechanical symptoms, including swelling, reduced mobility, stiffness, articular deformity, limb length discrepancy, and early secondary osteoarthritis, depending on size and location.

Imaging, by X-ray, CT and MRI, has a major role in the diagnosis of DEH. Radiographically, DEH presents with asymmetrical overgrowth on one side of an epiphysis, with irregular or premature calcifications. Following a diagnosis of DEH, a skeletal survey should be performed to determine whether there are multiple locations, using methods such as scintigraphy and whole-body MR imaging[12].

Bony and associated soft tissue abnormalities are extremely rare in DEH[13,14]. Despite the imaging features of DEH, it must be differentiated from low-grade malignant tumors and osteochondroma-like parosteal osteosarcomas when the talus is involved, especially when associated with soft tissue abnormalities. The CT and MRI findings in our patient suggested tumor recurrence, and the presence of soft tissue lesions suggested the possibility of a low-grade malignant tumor.

Pathologically and histologically, DEH lesions are similar to benign solitary osteochondromas[1]. The lesion may be a pedunculated mass with a cartilaginous cap or an enlarged irregularity of the articular surface. Gene expression assays, including EXT1 and EXT2, are all within normal ranges in DEH, but are lower in osteochondromas due to a mutation[15,16]. Because these tests are costly, clinical and radiological findings are important diagnostic tools[17]. Based on the clinical, imaging and pathological features of the specimen and reports in the medical literature, a diagnosis of DEH was established.

Treatment of DEH ranges from simple observation to surgical excision, depending on the location and extent of involvement. In the absence of articular symptoms, simple observation is recommended[5]. Patients who exhibit symptoms such as pain, joint deformity, or limited range of motion should be treated surgically. Although excising a juxta-articular lesion generally yields excellent results, excising an intra-articular lesion may lead to early secondary osteoarthritis, which may then require arthrodesis. Excision of lesions directly involving the joint surface, with articular localization, is generally not recommended unless the lesion becomes a loose body. The risks of recurrence until the epiphyses are closed suggest the need for continuous monitoring[10]. Little is known about the results of incomplete excision of articular DEH. Although two patients who underwent incomplete excision without additional surgery were reported to be doing well, with no evidence of local recurrence or physical problems and slow resolution and disappearance of the residual DEH masses[14], others have shown poor clinical outcomes, including local recurrence[5]. DEH lesions increase in size until skeletal maturity, without malignant transformation[5,10].

CONCLUSION

DEH is a rare variant of osteochondroma, resulting in an incorrect diagnosis in many patients. It is important to differentiate low-grade malignant tumors and osteochondroma-like parosteal osteosarcomas from DEH of the talus, especially when accompanied by soft tissue abnormalities. Surgical treatment is mandatory in patients with symptoms such as pain, joint impingement and deformation. Incomplete excision of an articular lesion may lead to recurrence and additional surgery.

REFERENCES

1. Fairbank TJ. Dysplasia epiphysialis hemimelica (tarso-epiphysial aclasis). J Bone Joint Surg Br 1956; 38:237-257.
2. Mouchet AA, Belot J. Tarsomegaly. J Radiol Electrol 1926; 10:289-293.
3. Trevor D. Tarso-epiphysial aclasis: a congenital error of epiphysial development. J Bone Joint Surg Br 1950; 32B:204-213.
4. Azouz EM, Slomic AM, Marton D, Rigault P, Finidori G. The variable manifestations of dysplasia epiphysealis hemimelica. Pediatr Radiol 1985; 15:44-49.

5. Kuo RS, Bellemore MC, Monsell FP, Frawley K, Kozlowski K. Dysplasia epiphysealis hemimelica: clinical features and management. J Pediatr Orthop 1998; 18:543-548.
6. Acquaviva A, Municchi G, Marconcini S, *et al*. Dysplasia epiphysealis hemimelica in a young girl: role of MRI in the diagnosis and follow-up. Joint Bone Spine 2005; 72:183-186.
7. Freihaut RB, O'Keane JC, Stephens MM. Dysplasia epiphysealis hemimelica with associated osteochondral lesion of the talus: a case report and review of the literature. Foot Ankle Int 2007; 28:727-730.
8. Joshi D, Kumar N, Singh D, Lal Y, Singh AK. Osteochondroma of the talus in a male adolescent. J Am Podiatr Med Assoc 2005; 95:494-496.
9. Keser S, Bayar A. Osteochondroma of the talar neck: a rare cause of callosity of the foot dorsum. J Am Podiatr Med Assoc 2005; 95:295-297.
10. Azzoni R. Dysplasia epiphysealis hemimelica of the talus. J Orthop Traumatol 2009; 10:43-46.
11. Rosero VM, Kiss S, Terebessy T, Köllö K, Szöke G. Dysplasia epiphysealis hemimelica (Trevor's disease): 7 of our own cases and a review of the literature. Acta Orthop 2007; 78:856-861.
12. Volders D, Vandevenne JE, Van de Casseye W. Trevor's disease and whole-body MRI. Eur J Radiol 2011; 79:363-364.
13. Peduto AJ, Frawley KJ, Bellemore MC, Kuo RS, Foster SL, Onikul E. MR imaging of dysplasia epiphysealis hemimelica: bony and soft-tissue abnormalities. AJR Am J Roentgenol 1999; 172:819-823.
14. Bahk WJ, Lee HY, Kang YK, Park JM, Chun KA. Chung YG. Dysplasia epiphysealis hemimelica: radiographic and magnetic resonance imaging features and clinical outcome of complete and incomplete resection. Skeletal Radiol 2010; 39:85-90.
15. Glick R, Khaldi L, Ptaszynski K, Steiner GC. Dysplasia epiphysealis hemimelica (Trevor disease): a rare developmental disorder of bone mimicking osteochondroma of long bones. Hum Pathol 2007; 38:1265-1272.
16. Fletcher C, Unni K, Mertens F. Pathology and genetics of tumour of soft tissue and bone. In: World Health Organization classification of tumours. Fletcher C, Unni K, Mertens F, editors. Lyon: International Agency for Research on Cancer Press; 2002: 229-230.
17. Gokkus K, Aydin AT, Uyan A, Cengiz M. Dysplasia epiphysealis hemimelica of the ankle joint: a case report. J Orthop Surg 2011; 19:254-256.

Case Report

Unexplained Peritonitis due to *Neisseria Gonorrhoeae* Secondary to a Sterile Tubo-ovarian Abscess

Wadha Alfouzan[1,2], Rita Dhar[1*]
[1]Microbiology Unit, Department of Laboratories, MoH Farwania Hospital, Kuwait
[2]Department of Microbiology, Health Sciences Center, Faculty of Medicine, Kuwait University, Jabriya, Kuwait

Kuwait Medical Journal 2015; 47 (2): 166 - 167

ABSTRACT

A 43-year-old woman presented to the surgical emergency department with acute abdominal pain associated with nausea, vomiting, constipation, fever and dysuria. Exploratory laparotomy was performed based on clinical diagnosis of acute appendicitis. Post-laparotomy, the diagnosis of pelvic inflammatory disease (PID), left tubo-ovarian abscess and peritonitis due to *Neisseria gonorrhoeae* was made. The patient responded well to antibiotics and surgical management but was lost to follow-up.

KEY WORDS: *neisseria gonorrhoeae*, pelvic inflammatory disease, peritonitis, sexually transmitted disease, tubo-ovarian abscess

INTRODUCTION

Neisseria gonorrhoeae is the etiological agent of gonorrhea, a sexually transmitted disease. In females, the infection involves endocervix giving rise to increased or altered vaginal discharge in 50% of the patients while the other 50% cases remain asymptomatic. If left untreated, gonorrhea can lead to serious complications, such as, pelvic inflammatory disease (PID). Bacteremia due to *N. gonorrhoeae* causes disseminated gonococcal infection, which may result in arthritis, endocarditis, skin infections and meningitis, whereas peritonitis remains a rare complication.

CASE REPORT

A-43-year-old multigravida woman, an expatriate from India working as a servant in a Kuwaiti household, presented to the surgical emergency in August, 2008 with acute abdominal pain of one day duration. The pain was localized in the right iliac fossa and associated with nausea, vomiting, constipation, fever and dysuria. Signs and symptoms of genital infection were absent. She was diabetic and on hypoglycemic treatment for the past five years and other than that, her past medical history was unremarkable. No history of genital infection or any surgical procedure could be elicited. Although being away from her spouse, she denied indulging in extramarital relationship while working in Kuwait for more than one year. On physical examination, the patient appeared to be in pain. Her pulse rate was 120/min, temperature of 39 ^{0}C, respiratory rate of 24 / min and blood pressure was 90 / 60 mmHg. Abdominal examination revealed generalized tenderness and voluntary guarding localized to the right iliac fossa. There was no organomegaly or shifting dullness and bowel sounds were audible. Chest examination was normal.

Initial laboratory work up showed elevated white blood cell count of 20.9 x 10^9/l (neutrophils 92.4%, lymphocytes 5.6%), hemoglobin concentration 13.5 g/l, C-reactive protein 265 mg/l (normal range < 8.0 mg/l), blood glucose 21.5 mmol/l, serum creatinine 79 μmol/l, potassium 3.6 mmol/l, sodum 133 mmol/l and normal liver function tests. Urinalysis was significant for the presence of glucose, ketones and nitrates but negative for leukocytes.

In view of the patient's history and laboratory results, a presumptive diagnosis of acute appendicitis was made and an exploratory laparotomy was performed approximately 20 hours after admission. Surgical exploration revealed a normal appendix, pyogenic pelvic inflammation and inflammatory reaction involving small intestines and in the vicinity of appendix. Although the uterus appeared normal,

Address correspondence to:
Dr Rita Dhar, MD, Department of Microbiology, Maternity Hospital, Sabah Area, Kuwait. P O Box 7719, 64008 Fahaheel, Kuwait. Tel: 965 -24842100 ext. 4055 (work), 965 - 99108193 (Mob), E-mail: ritadhar50@hotmail.com

left-sided tubo-ovarian abscess (TOA) was detected, which was drained and the pus was sent for culture. After proper abdominal toilet, a Malecot catheter was left in place for post-operative abdominal drainage. Antibiotic therapy with cefuroxime, clindamycin and metronidazole was continued and the patient was transferred to ICU in view of septicemia, respiratory failure, electrolyte imbalance and uncontrolled diabetes with ketoacidosis.

Microbiology

The culture of pus from TOA as well as blood and urine failed to grow any organisms. However, the culture of draining fluid through Malecot catheter yielded scanty but pure growth of pale grey colonies on 5% sheep blood agar, chocolate agar and Brucella agar. The isolate was identified as *N. gonorrhoeae* by Gram-stained smear findings, positive oxidase and negative DNase tests and by API-NH (bioMerieux, France); profile number 1001. The identification was confirmed by a commercial DNA assay (BD Probe Tec™ ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assays, Becton Dickinson, Sparks, MD, USA). Following this information, culture of endocervix was attempted but failed to isolate *N. gonorrhoeae*. Ideally, we would have preferred to repeat amplified DNA assay on the endocervical sample as well, but it was inadvertently missed out.

DISCUSSION

Ascending infection through lower genital tract is considered to be the most acceptable theory in the causation of PID, which may lead to the development of a TOA. In almost 99% cases, acute PID is consequent to ascending infection, whereas in < 1% of cases, other routes such as transperitoneal, hematogenous or lymphatic spread are suggested[1]. Although *N. gonorrhoeae* is considered one of the common causes of lower genital tract infections and associated PID, its role in the formation of TOA has not been well-substantiated. In many articles in the literature, it has been pointed out that the etiology of PID complicated by TOA is usually polymicrobial involving both aerobic and anerobic microbial flora[2]. Interestingly, *N. gonorrhoeae* has not been found to be among the spectrum of microorganisms isolated from TOA[2,3]. In our case, the culture of the pus from TOA failed to grow any microbes. Arguably, pre-operative antibiotics could have resulted in sterile pus although persistence of microorganisms is more likely expected because of low redox potential in pus, poor penetration of antimicrobials, high levels of antibiotic degrading enzymes and impaired phagocytosis by polymorphonuclear leukocytes found within abscesses. Complications like peritonitis due to *N. gonorroeae* remains a rarity with only a few reports published in the literature[4-6]. In a case report published in 2009[4], *N. gonorrhoeae* was identified by nucleic acid amplification and not by culture, which is considered the gold standard. The striking similarity that emerges from these cases including ours is, that all patients were women aged over 30 years, who presented with acute abdomen and the final diagnosis was possible after surgical intervention followed by isolation of *N. gonorrhoeae* from purulent exudates in the peritoneal cavity.

CONCLUSION

In women the possibility of sexually transmitted diseases (STD) and their complications such as acute abdomen should be kept in mind. The surgeons should be aware of this entity and should ask for gynecological advice. Specific microbiological diagnosis is important in guiding the appropriate therapy

ACKNOWLEDGMENT

We wish to thank Dr. Ambly Kumaran for her contribution by sharing with us the operative findings mentioned in the manuscript.

REFERENCES

1. Canas AM, Holloran-Schwartz B, Myles T. Tuboovarian abscess 12 years after total abdominal hysterectomy. Obstet Gynecol 2004; 104:1039-1041.
2. Cohen CR, Gravelle L, Symekher S, Waiyaki P, Stamm WE, Kiehlbauch JA. Etiology of persistent tubo-ovarian abscess in Nairobi, Kenya. Infect Dis Obstet Gynecol 2003; 11:45-51.
3. Landers DV, Sweet RL. Tubo-ovarian abscess: contemporary approach and management. Rev Infect Dis 1983; 5:876-884.
4. Wilmore SMS, Reynolds CJ. Gonococcal peritonitis diagnosed post laparotomy in a 38-year-old woman: a case report. Cases Journal 2009; 2:8080-8082.
5. Akahane T, Kawakami Y, Oana K. A case of bacterial peritonitis caused by *Neisseria gonorrhoeae*. Kansenshogaku Zasshi 2001; 75:894-897.
6. Weeks AG, Entman SS. Gonococcal peritonitis after tubal ligation. A case report. J Reprod Med 1991; 36:683-684.

Selected Abstracts of Articles Published Elsewhere by Authors in Kuwait

Kuwait Medical Journal 2015; 47 (2): 168 - 171

Spinal Segmental Myoclonus as an Unusual Presentation of Multiple Sclerosis

Alroughani RA[1,2], Ahmed SF[3,4], Khan RA[5], Al-Hashel JY[6,7]
[1]Department of Medicine, Division of Neurology, Amiri Hospital, Arabian Gulf Street, Sharq, 13041, Kuwait. E-mail: alroughani@gmail.com
[2]Department of Medicine, Neurology Clinic, Dasman Diabetes Institute, Kuwait, Kuwait. E-mail: alroughani@gmail.com
[3]Department of Neurology, Ibn Sina Hospital, Kuwait, Kuwait. E-mail: samerelshayb@hotmail.com.
[4]Department of Neurology and Psychiatry, Faculty of Medicine, Al-Minia University, Minya, Egypt. E-mail: samerelshayb@hotmail.com
[5]Department of Neurology, Ibn Sina Hospital, Kuwait, Kuwait. E-mail: rkhan1948@gmail.com
[6]Department of Neurology, Ibn Sina Hospital, Kuwait, Kuwait. E-mail: jasemkumsa@hotmail.com
[7]Department of Medicine, Kuwait University, Kuwait, Kuwait. E-mail: jasemkumsa@hotmail.com

BMC Neurol. 2015; 15(1):15. doi: 10.1186/s12883-015-0271-y

Background: Unusual presentations of multiple sclerosis (MS) at onset may post a diagnostic dilemma to the treating neurologists. Spinal myoclonus is rare in MS and may lead to perform extensive investigations to rule out other etiologies affecting the spinal cord.
Case Presentation: We described a 31-year-old male who presented with involuntary brief jerky movements of the left shoulder and arm with significant wasting of shoulder muscles. In retrospect, the patient had a progressive right leg weakness one year prior to his presentation. Needle electromyography confirmed the presence of rhythmic irregular burst discharges in motor units of muscles expanding from the third to the sixth cervical region with normal nerve conduction parameters. There was no evidence of cortically generated myoclonic jerks using time-locked electroencephalogram. Magnetic Resonance Imaging of the brain and cervical cord along with the presence of oligoclonal bands in cerebral spinal fluid confirmed the diagnosis of MS. Based on the history and progressive clinical features, a diagnosis of primary progressive MS was established.
Conclusion: Spinal myoclonus can be the presenting manifestation of MS in association with demyelinating plaques in the root exit zones of the spinal cord. Spinal myoclonus may pose a diagnostic challenge when it presented at the disease onset and especially in patients with progressive course at onset. Our patient represents the first reported primary progressive MS case in the literature with spinal myoclonus presentation.

Effect of Epigallocatechin Gallate on Uncoupling Protein 2 in Acute Liver Injury

Jamal MH[1], Ali H[2], Dashti A[2], Al-Abbad J[1], Dashti H[1], Mathew C[2], Al-Ali W[3], Asfar S[1]
[1]Department of Surgery, Faculty of Medicine, Health Sciences Center, Kuwait University Kuwait
[2]Department of Medical Laboratory Sciences (MLS), Faculty of Allied Health Sciences, Health Sciences Center, Kuwait University Kuwait
[3]Department of Pathology, Faculty of Medicine, Health Sciences Center, Kuwait University Kuwait

Int J Clin Exp Pathol 2015; 8(1):649-54. eCollection 2015

Background: The aim of this study was to investigate the effect of epigallocatechin gallate (EGCG) on uncoupling protein 2 regulation in an acute liver injury-animal model.

Methods: Twenty seven male Wistar rats were divided into three groups: control group (n = 9), TAA group (n = 9): acute liver injury was induced by the intraperitoneal injection of thioacetamide (200 mg/kg) and EGCG/TAA (n = 9 rats): Epigallocatechin gallate was given two weeks prior to the induction of acute liver injury by thioacetamide. The levels of uncoupling protein 2, CRP, TNF-α and interleukins (IL) 6 and 18 were analyzed in the liver using PCR analysis.
Results: Q-PCR analysis showed that the genetic expression of UCP2, TNF-α and CRP in the EGCG/TAA group was the least in comparison to other groups ($P \leq 0.005$). The IL-6 and IL-18 were upregulated after induction of acute liver injury, but this upregulation was significantly less in the group that received epigallocatechin gallate (EGCG/TAA) compared to the TAA group. In addition, histological examination showed a reduction in hepatocyte injury in EGCG/TAA compared to the TAA group.
Conclusion: Epigallocatechin gallate administration prior to induction of acute liver injury down-regulates uncoupling protein 2 expression and reduces IL-6, IL-18, TNF-α and CRP.

A Novel PKD1 Variant Demonstrates a Disease-Modifying Role in Trans with a Truncating PKD1 Mutation in Patients with Autosomal Dominant Polycystic Kidney Disease

Ali H[1], Hussain N[2], Naim M[3], Zayed M[4], Al-Mulla F[5], Kehinde EO[6], Seaburg LM[7], Sundsbak JL[8], Harris PC[9]
[1]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Health Sciences Center, Kuwait University, Jabriya, Kuwait. E-mail: hamad.ali@hsc.edu.kw
[2]Division of Nephrology, Mubarak Al-Kabeer Hospital, Ministry of Health, Jabriya, Kuwait. E-mail: nhussainku@yahoo.com
[3]Division of Nephrology, Mubarak Al-Kabeer Hospital, Ministry of Health, Jabriya, Kuwait. E-mail: medhat_naim@hotmail.com
[4]Department of Radio diagnosis, Mubarak Al-Kabeer Hospital, Ministry of Health, Jabriya, Kuwait. E-mail: moh.zayed81@gmail.com
[5]Department of Pathology, Faculty of Medicine, Health Sciences Center, Kuwait University, Jabriya, Kuwait. E-mail: Fahd@al-mulla.org
[6]Department of Surgery, Division of Urology, Faculty of Medicine, Health Sciences Center, Kuwait University, Jabriya, Kuwait. E-mail: ekehinde@hotmail.com
[7]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, USA. E-mail: Seaburg.Lauren@mayo.edu
[8]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, USA. E-mail: Sundsbak.Jamie@mayo.edu
[9]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, USA. E-mail: Harris.Peter@mayo.edu

BMC Nephrol 2015; 16:26. doi: 10.1186/s12882-015-0015-7

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common form of Polycystic Kidney Disease (PKD) and occurs at a frequency of 1/800 to 1/1000 affecting all ethnic groups worldwide. ADPKD shows significant intrafamilial phenotypic variability in the rate of disease progression and extra-renal manifestations, which suggests the involvement of heritable modifier genes. Here we show that the PKD1 gene can act as a disease causing and a disease modifier gene in ADPKD patients.
Methods: Clinical evaluation of a family with ADPKD was performed to diagnose and assess disease progression in each individual. PKD1 was genotyped in each individual by targeted sequencing.
Results: Targeted screening analysis showed that the patients with ADPKD in the family had the PKD1: p.Q2243X nonsense mutation. A more severe disease phenotype, in terms of estimated Glomerular Filtration Rate (eGFR) and total kidney volume, was observed in two patients where in addition to the mutation, they carried a novel PKD1 variant (p.H1769Y). Other patients from the same family carrying only the (p.Q2243X) mutation showed milder disease manifestations.
Conclusion: ADPKD shows significant intrafamilial phenotypic variability that is generally attributed to other modifier genes. In this rare case, we have shown that a variant at PKD1, in trans with the PKD1 mutation, can also act as a modifier gene in ADPKD patients. Understanding the molecular mechanism through which the gene exerts its disease modifying role may aid our understanding of the pathogenesis of ADPKD.

Molecular Basis for the Effects of Zinc Deficiency on Spermatogenesis: An Experimental Study in the Sprague-dawley Rat Model

Omu AE[1], Al-Azemi MK[1], Al-Maghrebi M[2], Mathew CT[2], Omu FE[3], Kehinde EO[4], Anim JT[5], Oriowo MA[6], Memon A[7]
[1]Department of Obstetrics and Gynaecology, Faculty of Medicine, Kuwait University, Kuwait
[2]Department of Anatomy (Electron Microscopy Unit), Faculty of Medicine, Kuwait University, Kuwait
[3]College of Nursing, PAAET, Ministry of Health, Kuwait
[4]Department of Surgery, Faculty of Medicine, Kuwait University, Kuwait
[5]Department of Pathology, Faculty of Medicine, Kuwait University, Kuwait
[6]Department of Pharmacology and Toxicology, Faculty of Medicine, Kuwait University, Kuwait
[7]Division of Primary Care and Public Health, Brighton and Sussex Medical School, Falmer, Sussex, BN1 9PX, United Kingdom

Indian J Urol 2015; 31(1):57-64. doi: 10.4103/0970-1591.139570

Introduction: The objective of this study is to investigate the molecular mechanisms underlying the effects of zinc deficiency on spermatogenesis in the Sprague-Dawley (SD) rat.
Materials And Methods: Three groups of eight adult male SD rats were maintained for 4 weeks on a normal diet as control, zinc deficient diet and zinc deficient diet with zinc supplementation of 28 mg zinc/kg body weight respectively. Using standard techniques, the following parameters were compared between the three groups of experimental animals at the end of 4 weeks: (a) Serum zinc, magnesium (Mg), copper (Cu), selenium (Se) and cadmium (Cd), (b) serum sex hormones, malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPX), (c) interleukin-4 (IL-4), tumor necrosis factor-alpha (TNF-α), Bcl-2, Bax and caspase-3 expression in the testes, (d) assessment of apoptosis of testicular cells using electron microscopy and (e) testicular volume and histology using the orchidometer and Johnsen score, respectively.
Results: The zinc deficient group showed a reduction of testicular volume, serum concentrations of Zn, Cu, Se, Mg, SOD, GPX, IL-4, Bcl-2 and testosterone ($P < 0.05$), as well as increased levels of serum Cd, MDA and tissue TNF-α, Bax, caspase-3 and apoptosis of the germ cells ($P < 0.05$) compared with control and zinc supplementation groups.
Conclusion: Zinc deficiency is associated with impaired spermatogenesis because of reduced testosterone production, increased oxidative stress and apoptosis. These findings suggest that zinc has a role in male reproduction.

Clinical Predictors of Disease Progression in Multiple Sclerosis Patients with Relapsing Onset in a Nation-Wide Cohort

Alroughani RA[1], Akhtar S, Ahmed SF, Al-Hashel JY
[1]Division of Neurology, Amiri Hospital, Sharq, Kuwait

Int J Neurosci. 2014 Nov 11. [Epub ahead of print]

Background: Predicting disease progression over time is challenging despite the available literature data. Aim: To assess whether baseline clinical variables of MS patients would predict the conversion to progressive phase of the disease.
Materials & Methods: Utilizing the national MS registry, patients who had relapsing onsets and had confirmed EDSS score at baseline and follow-up visits were included. Primary progressive MS and CIS patients were excluded. Clinical variables (gender, age at onset, disease duration, number of relapses, EDSS score) were collected. The end point was conversion to secondary progressive MS. Chi Square and multivariable logistic regression were used to determine the influence of clinical variables on disease progression.
Results: Data of 803 MS patients with relapsing onset were analyzed. Eighty five (10.6%) patients reached the end point. The risk of disease progression was significantly higher in men ($p = 0.015$), in patients who

developed MS ≥ 40 years of age (p = 0.041) and who had ≥ 3 relapses during their disease course (p < 0.001). Spinal cord presentation at onset was predictive of progression (aOR = 2.01; p = 0.06) while optic neuritis at onset was associated with lower risk of progression (aOR = 0.30; p = 0.03). EDSS score at first visit did not influence disease progression when tested at 2 different cutoffs (EDSS < 4 vs. ≥ 4 and EDSS < 6 vs. ≥ 6) using multivariable logistic regression analysis (p = 0.960 and p = 0.866), respectively.
Conclusion: Men and patients who presented at age 40 yeas or beyond had increased risk of MS progression. Spinal cord symptoms at onset and 3 or more relapses were predictive of progression.

Evaluation of Neurotrophic Tyrosine Receptor Kinase 2 (NTRK2) as a Positional Candidate Gene for Variation in Estimated Glomerular Filtration Rate (eGFR) in Mexican American Participants of San Antonio Family Heart Study

Thameem F[1,2], Voruganti VS[3,4], Blangero J[5], Comuzzie AG[6], Abboud HE[7,8]
[1]Division of Nephrology, Department of Medicine, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX, 78229, USA. E-mail: thameem@uthscsa.edu
[2]Department of Biochemistry, Faculty of Medicine, Kuwait University, Safat, 13110, Kuwait. E-mail: thameem@uthscsa.edu
[3]Department of Nutrition, University of North Carolina at Chapel Hill, Kannapolis, NC, 28081, USA. E-mail: saroja@unc.edu
[4]UNC Nutrition Research Institute, University of North Carolina at Chapel Hill, Kannapolis, NC, 28081, USA. E-mail: saroja@unc.edu
[5]Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX, 78227, USA. E-mail: john@txbiomedgenetics.org
[6]Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX, 78227, USA. E-mail: tony@txbiomedgenetics.org
[7]Division of Nephrology, Department of Medicine, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX, 78229, USA. E-mail: abboud@uthscsa.edu.
[8]South Texas Veterans Healthcare System, San Antonio, TX, 78229, USA. E-mail: abboud@uthscsa.edu

J Biomed Sci. 2015; 25;22(1):23

Background: The estimated glomerular filtration rate (eGFR) is a well-known measure of kidney function and is commonly used for the diagnosis and management of patients with chronic kidney disease. The inter-individual variation in eGFR has significant genetic component. However, the identification of underlying genetic susceptibility variants has been challenging. In an attempt to identify and characterize susceptibility genetic variant(s) we previously identified the strongest evidence for linkage of eGFR occurring on chromosome 9q21 in the Mexican American participants of San Antonio Family Heart Study (SAFHS). The objective of the present study was to examine whether the common genetic variants in Neurotrophic Tyrosine Receptor Kinase 2 (NTRK2), a positional candidate gene on 9q21, contribute to variation in eGFR.
Results: Twelve tagging single nucleotide polymorphisms (SNPs) across the NTRK2 gene region were selected (r2 ≥ 0.80, minor allele frequency of ≥ 0.05) from the Hapmap database. SNPs were genotyped by TaqMan assay in the 848 Mexican American subjects participated in the SAFHS. Association analysis between the genotypes and eGFR (estimated by the Modification of Diet in Renal Disease equation) were performed by measured genotype approach as implemented in the program SOLAR. Of the 12 common genetic variants examined, the rs1036915 (located in 3'UTR) and rs1187274 (located in intron-14), present in perfect linkage disequilibrium, exhibited an association (P = 0.017) with eGFR after accounting for the effects of age, sex, diabetes, diabetes duration, systolic blood pressure and blood pressure medication. The carriers of minor allele of rs1036915 (G; 38%) had increased eGFR (104 ± 25 ml/min/1.73 m2) in comparison to the carriers of major allele A (98 ± 25 ml/min/1.73 m2).
Conclusion: Together, our results suggest for the first time that the genetic variants in NTRK2 may regulate eGFR.

Forthcoming Conferences and Meetings

Compiled and edited by
Babichan K Chandy

Kuwait Medical Journal 2015; 47 (2): 172 - 182

2015 International Society on **Thrombosis & Haemostasis** (ISTH) Congress
Jun 20 - 25, 2015
Canada / Ontario / Toronto
Contact: ISTH Headquarters
Phone: 919-929-3807; Fax: 919-929-3935
Email: headquarters@isth.org

14th World Congress in **Fetal Medicine**
Jun 21 - 25, 2015
Greece / Crete
Contact: The Fetal Medicine Foundation
Phone: 011-44-20-7034-3070; Fax: 011-44-20-7034-3071
Email: fmfeducation@fetalmedicine.com

16th International **Coeliac Disease** Symposium
Jun 21 - 24, 2015
Czech Republic / Prague
Contact: Congress Secretariat, Guarant International
Phone: 011-420-284-001-444; Fax: 011-420-284-001-448
Email: icds2015@guarant.cz

Frontiers of **Cell Signaling**
Jun 21 - 24, 2015
China / Shanghai
Contact: Abcam plc
Phone: 647-799-3007; Fax: 647-799-3014

2015 Paris Live Interventional **Neuroradiology & Neurosurgery** Course
Jun 22 – 24, 2015
France / Paris
Contact: Europa Digital & Publishing

17th Milan **Breast Cancer** Conference
Jun 23 – 26, 2015
Italy / Milan
Contact: Organizing Secretariat, MZ Congressi srl
Phone: 011-39-2-6680-2323; Fax: 011-39-2-668-6699
Email: mbcc@ieo.it

2015 Dublin **Pathology**: 8th Joint Meeting of the British Division of the IAP and The Pathological Society
Jun 23 – 25, 2015
Ireland / Dublin
Contact: Pathological Society of Great Britain & Ireland
Phone: 011-44-20-7872-5750 / 5751
Email: admin@pathsoc.org

2015 International Scientific Conference on **Probiotics & Prebiotics**
Jun 23 – 25, 2015
Hungary / Budapest
Contact: Organising Secretariat, PAMIDA International Ltd.
Phone: 011-421-91-719-4367
Email: info@probiotic-conference.net

2nd Annual **Microbiology & Infectious Diseases** Asia Congress
Jun 23 – 24, 2015
Singapore / Singapore
Contact: Steph Punfield, Marketing Executive, Oxford Global
Phone: 011-44-18-6524-8455
Email: s.punfield@oxfordglobal.co.uk

Basic Practical Skills in **Obstetrics & Gynaecology**
Jun 23 – 24, 2015
United Kingdom / Preston
Contact: Royal College of Obstetricians & Gynaecologists
Phone: 011-44-20-7772-6200; Fax: 011-44-20-7723-0575

11th International Congress on Complications during **Cardiovascular Intervention**: Management & Prevention
Jun 24 - 26, 2015
Switzerland / Lausanne
Contact: Congress Secretariat, E&E PCO
Phone: 011-43-1-867-4944 ext. 0
Fax: 011-43-1-867-4944 ext. 9
Email: office@ee-pco.com

11th International Symposium on **Endovascular Therapeutics**
Jun 24 - 27, 2015
Spain / Barcelona
Contact: SITE Secretariat
Phone: 011-34-93-505-2503; Fax: 011-34-93-488-3703
Email: secretariat@sitesymposium.org

2015 International Society for **Stem Cell Research** (ISSCR) Annual Meeting
Jun 24 - 27, 2015
Sweden / Stockholm
Contact: ISSCR Headquarters
Phone: 224-592-5700; Fax: 224-365-0004
Email: isscr@isscr.org

29th Computer Assisted **Radiology & Surgery** (CARS) International Congress & Exhibition
Jun 24 - 27, 2015
Spain / Barcelona
Contact: Mrs. Franziska Schweikert, Conference Manager
Phone: 011-49-77-4292-2434
Fax: 011-49-77-4292-2438
Email: office@cars-int.org

61st American Society for **Artificial Internal Organs** (ASAIO) Annual Conference
Jun 24 - 27, 2015
United States / Illinois / Chicago
Contact: ASAIO
Phone: 561-999-8969; Fax: 561-999-8972
Email: info@asaio.com

14th International Congress on **Pediatric Pulmonology**
Jun 25 - 28, 2015
Poland / Krakow
Contact: Anne Flore Bidart, MD, Secretariat
Phone: 011-33-4-9703-8597; Fax: 011-33-4-9703-8598
Email: CIPP@CIPP-MEETING.ORG

5th Annual **Oncologic** Imaging Course (OIC)
Jun 25 - 27, 2015
Croatia / Dubrovnik
Contact: Ana Krasevac, Ms, OIC Office
Phone: 011-385-1-455-0335
Fax: 011-385-1-455-0242
Email: office@oncoic.org

90th Annual Meeting of the American Society of **Parasitologists** (ASP)
Jun 25 - 28, 2015
United States / Nebraska / Omaha
Contact: Allen Press, Customer Service Team, ASP
Email: asp@allenpress.com

GALEN Foundation Course: **Neuroradiology**
Jun 25 - 27, 2015
United States / Florida / St. Petersburg
Contact: European School of Radiology
Phone: 011-43-1-533-4064
Fax: 011-43-1-533-4064 ext. 447
Email: communications@myESR.org

Internal Medicine for Primary Care: **Endo/Obesity/ Vasc/Gastro**
Jun 25 - 28, 2015
United States / Alberta / Lake Louise
Contact: Medical Education Resources, Inc.
Phone: 800-421-3756 or 303-798-9682
Fax: 303-798-5731
Email: info@mer.org

15th International Symposium on **Viral Hepatitis & Liver Disease**
Jun 26 - 28, 2015
Germany / Berlin
Contact: Juliane McCarty, MCI Deutschland GmbH/ MCI Berlin Office
Phone: 011-49-30-204-590
Email: isvhld@mci-group.com

19th Annual **Hypertension, Diabetes & Dslypidemia** Conference
Jun 26 - 28, 2015
United States / South Carolina / Charleston
Contact: Walter Ejnes, President, Continuing Education Company Inc
Phone: 800-327-4502; Fax: 516-539-3555
Email: barbara@cmemeeting.org

3rd Parekh Mid Year Indo US **Foot & Ankle** Course
Jun 26 - 27, 2015
India / Panaji
Contact: Healthway Hospital
Phone: 011-91-832-222-4966
Email: indousgoa@gmail.com

Breast Ultrasound with the Masters
Jun 26 - 28, 2015
Singapore / Singapore
Contact: International Institute for Continuing Medical Education
Phone: 205-467-0290
Email: IICMEMAIL@gmail.com

Cannabinoids for the Treatment of **Epilepsy**
Jun 26, 2015
United States / New York / New York
Contact: Maria Mercado, Continuing Medical Education, NYU Langone Medical Center
Phone: 212-263-5295; Fax: 212-263-5293
Email: maria.mercado@nyumc.org

Geriatric Medicine for Primary Care
Jun 26 - 28, 2015
United States / Nevada / Las Vegas
Contact: Medical Education Resources, Inc.
Phone: 800-421-3756 or 303-798-9682
Fax: 303-798-5731
Email: info@mer.org

New & Emerging Therapies for **Colorectal Cancer**
Jun 26, 2015
United States / Pennsylvania / Hershey
Contact: Continuing Education, Penn State Hershey
Phone: 800-243-1455
Email: ContinuingEd@hmc.psu.edu

7th International Conference on **Children's Bone Health**
Jun 27 - 30, 2015
Austria / Salzburg
Contact: Janet Crompton, Conference Organiser, European Calcified Tissue Society
Phone: 011-44-14-5354-9929; Fax: 011-44-14-5354-8919
Email: iccbh@ectsoc.org

Dermatologic Procedures
Jun 27 - 28, 2015
United States / Nevada / Las Vegas
Contact: Heather Osborne, Education Coordinator, National Procedures Institute
Phone: 866-674-2631 or 512-870-8051; Fax: 512-329-0442
Email: heather@npinstitute.com

Joint Exam & Injections
Jun 27 - 28, 2015
United States / Nevada / Las Vegas
Contact: Heather Osborne, Education Coordinator, National Procedures Institute
Phone: 866-674-2631 or 512-870-8051; Fax: 512-329-0442
Email: heather@npinstitute.com

4th Annual Global **Healthcare** Conference
Jun 29 - 30, 2015
Singapore / Singapore
Contact: Conference Secretariat, Global Science & Technology Forum
Phone: 011-65-6327-0166; Fax: 011-65-6327-0162
Email: secretariat@globalhc-conf.org

Dermatology for Primary Care
Jun 29 - Jul 1, 2015
United States / Arizona / Sedona
Contact: Leslie Burk, MCE Conferences Inc.
Phone: 888-533-9031; Fax: 858-777-5588
Email: info@mceconferences.com

Hypertension – State of the Art in 2015
Jun 29, 2015
United Kingdom / London
Contact: Conferences Team, Royal College of Physicians of London
Phone: 011-44-20-3075-2389; Fax: 011-44-20-7487-5218
Email: conferences@rcplondon.ac.uk

Psychological & Neuropsychological Impact of Paediatric & TYA Cancer on Patients & Their Families
Jun 29, 2015
United Kingdom / London
Contact: Education and Conference Centre, The Royal Marsden NHS Foundation Trust
Phone: 011-44-20-7808-2924; Fax: 011-44-20-7808-2334
Email: conferencecentre@rmh.nhs.uk

UK Training in Emergency **Airway Management** Course
Jun 29 - 30, 2015
United Kingdom / Birmingham, UK
Contact: Meetings and Events, Royal College of Anaesthetists
Phone: 011-44-20-7092-1670, Fax: 011-44-20-7092-1730
Email: events@rcoa.ac.uk

3rd **Rare Diseases** Summer School
Jul 1 - 3, 2015
Switzerland / Zurich
Contact: Elisabetta Vannoni, University of Zurich
Email: elisabetta.vannoni@kispi.uzh.ch

ENT Imaging with the Masters
Jul 1 - 4, 2015
Singapore / Singapore
Contact: International Institute for Continuing Medical Education
Phone: 205-467-0290

Preceptorship Course in **Head & Neck** Cancer
Jul 1 - 3, 2015
Italy / Milan
Contact: Debora Urbinelli, Meridiano Congress International
Email: debora.urbinelli@meridiano.it

Targeted Treatments for **Haematological Cancers**
Jul 1, 2015
United Kingdom / London
Contact: Education and Conference Centre, The Royal Marsden NHS Foundation Trust
Phone: 011-44-20-7808-2921, Fax: 011-44-20-7808-2334
Email: conferencecentre@rmh.nhs.uk

Cancer Update Course - Belfast
Jul 2, 2015
United Kingdom / Belfast
Contact: GP Update
Phone: 011-44-11-8960-7077
Email: mail@gp-update.co.uk

Endocrinology Update
Jul 2, 2015
United Kingdom / London
Contact: Conferences Team, Royal College of Physicians of London
Phone: 011-44-20-3075-2389, Fax: 011-44-20-7487-5218
Email: conferences@rcplondon.ac.uk

Infection in **Cardiac** Surgery
Jul 2 - 3, 2015
United Kingdom / Windsor (UK)
Contact: European Association for Cardio-Thoracic Surgery
Phone: 011-44-17-5383-2166; Fax: 011-44-17-5362-0407

2015 Asia Pacific Conference on **Cardiometabolic Diseases** Management
Jul 4 - 5, 2015
India / Mumbai
Contact: Debora Urbinelli, Congress Coordinator, Meridiano Congress International
Phone: 011-39-6-8859-5232; Fax: 011-39-6-8859-5234
Email: debora.urbinelli@meridiano.it

11th Summer Academy of **Dermatopathology**
Jul 6 - 10, 2015
Austria / Graz
Contact: Dr. Lorenzo Cerroni, International Society of Dermatopathology
Phone: 011-43-316-385-2423; Fax: 011-43-316-385-14957
Email: lorenzo.cerroni@medunigraz.at

18th International Confederation for **Plastic, Reconstructive & Aesthetic Surgery** World Congress
Jul 6 - 10, 2015
Austria / Vienna
Contact: Mr. Nikos Antonopoulos, Congress Secretariat, ZITA Congress & Travel S.A.
Phone: 011-30-211-100-1782; Fax: 011-30-210-664-2116
Email: N.AN@ZITA-CONGRESS.GR

2015 Advanced Instructional Course on **Arthroplasty & Arthroscopy**
Jul 6 - 10, 2015
Netherlands / Utrecht
Contact: Arthroscopy & Arthroplasty 2015
Phone: 011-31-30-276-9174; Fax: 011-31-30-276-9251
Email: info@shoulder-elbow-knee.nl

2015 **Physiology**
Jul 6 - 8, 2015
United Kingdom / Cardiff
Contact: Sarah Bundock, The Physiological Society
Phone: 011-44-20-7993-0457
Email: sbundock@physoc.org

Burns Management
Jul 7, 2015
Australia / Angaston
Contact: South Australian Postgraduate Medical Education Association
Phone: 011-61-8-8274-6060; Fax: 011-61-8-8274-6000
Email: admin@sapmea.asn.au

Preceptorship in **Multiple Sclerosis** - Canada
Jul 7 - 8, 2015
Canada / British Columbia / Vancouver
Contact: Concetta Di Palma, Meridiano Congress International
Email: concetta.dipalma@meridiano.it

2015 Vaccines for **Enteric Diseases**
Jul 8 - 10, 2015
United Kingdom / Edinburgh
Contact: Caroline Sumner, Events Director, Meetings Management
Phone: 011-44-14-8342-7770; Fax: 011-44-14-8342-8516
Email: csumner@meetingsmgmt.u-net.com

21st International **Liver Transplantation** Society (ILTS) Annual International Congress
Jul 8 - 11, 2015
United States / Illinois / Chicago
Contact: ILTS
Phone: 856-439-0500; Fax: 856-439-0525
Email: ilts@ilts.org

22nd International Meeting on Advanced **Spine Techniques**
Jul 8 - 11, 2015
Malaysia / Kuala Lumpur
Contact: Ann Shay, Meetings Manager, Scoliosis Research Society
Phone: 414-289-9107; Fax: 414-276-3349
Email: ashay@srs.org

2nd International **Sun Protection & Anti-Ageing Skin Care** Conference Asia
Jul 8 - 9, 2015
Singapore / Singapore
Contact: Julia Thatcher, Summit Events Ltd
Phone: 011-44-20-7828-2278
Email: julia.thatcher@summit-events.com

Soft Tissue Sarcoma: A Clinical Update
Jul 8, 2015
United Kingdom / London
Contact: Education and Conference Centre, The Royal Marsden NHS Foundation Trust
Phone: 011-44-20-7808-2921; Fax: 011-44-20-7808-2334
Email: conferencecentre@rmh.nhs.uk

Head & Neck Preceptorship: Focus on Comprehensive Management
Jul 9 - 10, 2015
France / Nice
Contact: Titty Alvino, Congress Coordinator, Meridiano Congress International
Phone: 011-39-6-8859-5310; Fax: 011-39-6-8859-5234
Email: titty.alvino@meridiano.it

Laparoscopic Colorectal Cadaver Course
Jul 9 - 10, 2015
United Kingdom / NEWCASTLE UPON TYNE upon Tyne Surgery
Contact: Lorraine Waugh, NEWCASTLE UPON TYNE Surgical Training Centre
Phone: 011-44-19-1223-1264; Fax: 011-44-19-1223-7248
Email: lorraine.waugh@nuth.nhs.uk

Seizures, Spells & Shakes: **Neurology** for the Non-Neurologist
Jul 9 - 11, 2015
United States / South Carolina / Kiawah Island
Contact: Continuing Medical Education, Georgia Regents University
Phone: 706-721-2329; Fax: 706-721-4642
Email: coned@gru.edu

Advanced **Aortic & Mitral Valve Reconstructive Surgery**
Jul 10 - 11, 2015
United Kingdom / Windsor (UK)
Contact: European Association for Cardio-Thoracic Surgery
Phone: 011-44-17-5383-2166; Fax: 011-44-17-5362-0407

Advanced Course in **Acute Hand Injuries** in Adults
Jul 10, 2015
United Kingdom / Derby
Contact: Stefania Wigelsworth, Postgraduate Administrator, Pulvertaft Hand Centre
Phone: 011-44-13-3278-7490
Email: stefania.wigelsworth@nhs.net

19th International Conference on **Prenatal Diagnosis & Therapy**
Jul 12 - 15, 2015
United States / District of Columbia / Washington
Contact: International Society for Prenatal Diagnosis
Phone: 434-979-4773; Fax: 434-977-1856
Email: info@ispdhome.org

Basic Practical Skills in **Obstetrics & Gynaecology**
Jul 12 - 13, 2015
India / New Delhi
Contact: Royal College of Obstetricians & Gynaecologists
Phone: 011-44-20-7772-6200; Fax: 011-44-20-7723-0575

World **Nutraceutical Conference** and Expo
Jul 13 - 15, 2015
United States / Pennsylvania / Philadelphia
Contact: Jon Stewart, Mr, OMICS Group Inc.
Phone: 888-843-8169
Email: nutraceuticals.conference@omicsonline.net

2015 NRG **Oncology** Jul Meeting
Jul 15 - 19, 2015
United States / Colorado / Denver
Contact: NRG Oncology
Phone: 267-519-6630

2015 Primary Care: **Neurology** Update British Isles Cruise
Jul 15 - 27, 2015
Netherlands / Amsterdam
Contact: Continuing Education, Inc
Phone: 800-422-0711
Email: registrar@continuingeducation.net

2015 New Zealand **Orthopaedic Spine** Society Meeting
Jul 16 - 19, 2015
Fiji / Natadola
Contact: Tanya Turchie, Conference Manager, New Zealand Orthopaedic Association
Phone: 011-64-4-913-9899
Email: tanya@nzoa.org.nz

Expert Witness: Excellence in **Report Writing**
Jul 16, 2015
United Kingdom / London
Contact: Royal College of Obstetricians & Gynaecologists
Phone: 011-44-20-7772-6200; Fax: 011-44-20-7723-0575

Family Medicine Update for Primary Care
Jul 17 - 19, 2015
Canada / Quebec / Quebec City
Contact: Leslie Burk, MCE Conferences Inc.
Phone: 888-533-9031; Fax: 858-777-5588
Email: info@mceconferences.com

Sclerotherapy
Jul 17, 2015
United States / Maryland / Baltimore
Contact: Heather Osborne, Education Coordinator, National Procedures Institute
Phone: 866-674-2631 or 512-870-8051; Fax: 512-329-0442
Email: heather@npinstitute.com

Anti-Aging Medicine: Advances in Hormone Replacement
Jul 18 - 19, 2015
United States / Maryland / Baltimore
Contact: Heather Osborne, Education Coordinator, National Procedures Institute
Phone: 866-674-2631 or 512-870-8051; Fax: 512-329-0442
Email: heather@npinstitute.com

Musculoskeletal Ultrasound
Jul 18 - 19, 2015
United States / Maryland / Baltimore
Contact: Heather Osborne, Education Coordinator, National Procedures Institute
Phone: 866-674-2631 or 512-870-8051; Fax: 512-329-0442
Email: heather@npinstitute.com

Primary Care: **Allergy & Immunology** Western Mediterranean Cruise
Jul 19 - 26, 2015
Spain / Barcelona
Contact: Continuing Education, Registrar, Continuing Education, Inc
Phone: 800-422-0711
Email: registrar@continuingeducation.net

2015 Advanced Hospital **Epidemiology** Course
Jul 20 - 24, 2015
Australia / Palm Cove
Contact: Conference Secretariat, ASHM Conference & Events Division
Phone: 011-61-2-8204-0770; Fax: 011-61-2-8204-0779
Email: meeting@asid.net.au

Cycling CME **Rocky Mountains**
Jul 21 - 25, 2015
United States / Colorado / Vail
Contact: Terri Reeder, Administrator, Cycling CME
Phone: 970-243-0230
Email: terri@cyclingcme.com

2015 Preceptorship in **Metastatic Colorectal Cancer**
Jul 22 - 24, 2015
United Kingdom / Liverpool
Contact: David H. Slangen, Congress Coordinator, Meridiano Congress International
Phone: 011-39-6-8859-5250; Fax: 011-39-6-8859-5234
Email: david.slangen@meridiano.it

Advanced **Dysrhythmia** Boot Camp
Jul 22 - 24, 2015
United States / Texas / Houston
Contact: Jerry W. Jones, MD FACEP, CEO, Medicus of Houston
Phone: 713-931-5423; Fax: 888-308-7807
Email: jwjmd@medicusofhouston.com

Comprehensive **Colposcopy**
Jul 22 - 25, 2015
United States / California / San Diego
Contact: American Society for Colposcopy & Cervical Pathology
Phone: 301-733-3640; Fax: 240-575-9880

Adult & Pediatric **Infectious Diseases** for Primary Care
Jul 24 - 26, 2015
United States / South Carolina / Myrtle Beach
Contact: Leslie Burk, MCE Conferences, MCE Conferences Inc.
Phone: 888-533-9031; Fax: 858-777-5588
Email: info@mceconferences.com

2015 Best Evidence **ENT**
Jul 25 - 28, 2015
United States / Wisconsin / Kohler
Contact: Diann Fiscus, Program Manager, Medical College of Wisconsin
Phone: 414-805-5609; Fax: 414-805-7936
Email: dfiscus@mcw.edu

20th World Congress on **Heart Disease**
Jul 25 - 27, 2015
Canada / British Columbia / Vancouver
Contact: Cardiology Online, Inc.
Phone: 310-657-8777; Fax: 310-659-4781
Email: klimedco@ucla.edu

6th International **Stress & Behavior** Society Regional Stress & Behavior Neuroscience & Biological Psychiatry Conference
Jul 25 - 26, 2015
Japan / Kobe
Contact: NA Nutsa, Conference Secretary, International Stress and Behavior Society
Phone: 240-899-9571
Email: isbs.congress@gmail.com

2015 International **Spine Intervention** Society (ISIS) Annual Meeting
Jul 28 - Aug 1, 2015
United States / Nevada / Las Vegas
Contact: ISIS
Phone: 415-457-4747; Fax: 415-457-3495
Email: registration@spinalinjection.org

4th Munich International Summer Academy of Practical **Dermatology**
Jul 28 - Aug 1, 2015
Germany / Munich
Contact: Bridget Barbieri, Project Manager, Interplan Fortbildungswoche GmbH
Phone: 011-49-89-5482-34773
Email: b.barbieri@interplan.de

2015 Hands-On **Transvaginal Pelvic Ultrasound Imaging & Doppler**
Jul 31, 2015
United States / Texas / Dallas
Contact: Amy Donaldson, Registrar, Keith Mauney & Associates Ultrasound Training Institutes
Phone: 800-845-3484 or 972-353-3200; Fax: 817-577-4250
Email: info@kmaultrasound.com

9th International Master Course on **Aging Skin** (IMCAS) Asia
Jul 31 - Aug 2, 2015
Indonesia / Bali
Contact: IMCAS
Email: contact@imcas.com

9th Annual Update in **Liver and Gastrointestnal Diseases**: Hot Topics for the Clinical Practice
Aug 1 - 2, 2015
United States / Florida / Clearwater Beach
Contact: Continuing Medical Education, University of Florida College of Medicine
Phone: 352-733-0064; Fax: 352-733-0007
Email: cme-mail@ufl.edu

Clinical Topics in **Anesthesia** - Banff
Aug 3 - 7, 2015
Canada / Alberta / Banff
Contact: Coleen Hilliard, Meeting Coordinator, Northwest Anesthesia Seminars
Phone: 509-547-7065; Fax: 509-547-1265
Email: coleen@nwas.com

World Congress on **Breast Cancer**
Aug 3 - 5, 2015
United Kingdom / Birmingham, UK
Contact: Paul Hederson, Dr., Omics
Phone: 650-268-9744; Fax: 650-618-1414
Email: breastcancer@conferenceseries.net

Medical CBT: Ten-Minute **Cognitive Behaviour Therapy Techniques** for Real Doctors
Aug 5 - 7, 2015
Canada / British Columbia / Whistler
Contact: Greg Dubord, MD, CME Director, CBT Canada
Phone: 877-466-8228
Email: registrar@cbt.ca

2015 Infectious Diseases Society for **Obstetrics & Gynecology** (IDSOG)
Aug 6 - 8, 2015
United States / Oregon / Portland
Contact: IDSOG
Phone: 312-676-3928
Email: idsog@idsog.org

Modern **ENT** Imaging in Istanbul
Aug 6 - 9, 2015
Turkey / Istanbul
Contact: International Institute for Continuing Medical Education
Phone: 205-467-0290
Email: IICMEMAIL@gmail.com

Transplant Ethics: Dilemmas & Discussions
Aug 7 - 8, 2015
United States / Minnesota / Minneapolis
Contact: Mayo School of Continuous Professional Development
Phone: 800-323-2688
Email: cme@mayo.edu

2015 **Ophthalmology** Update
Aug 8, 2015
United States / Wyoming / Jackson
Contact: Brea Cole, University of Mississippi Medical Center
Email: cbcole@umc.edu

Basic Practical Skills in **Obstetrics & Gynaecology**
Aug 8 - 9, 2015
United Kingdom / Surrey, UK
Contact: Royal College of Obstetricians & Gynaecologists
Phone: 011-44-20-7772-6200; Fax: 011-44-20-7723-0575

Osteoporosis: Essentials for Clinicians
Aug 8 - 9, 2015
United States / Connecticut / Hartford
Contact: International Society for Clinical Densitometry
Phone: 860-259-1000, Fax: 860-259-1030
Email: iscd@iscd.org

Industrial Biotechnology Congress
Aug 10 - 12, 2015
United Kingdom / Birmingham, UK
Contact: Jasmine Margulis, Programme Coordinator, Omics International Company
Phone: 650-268-9744
Email: industrialbio@conferenceseries.com

2015 Mayo Clinic Updates in **Urology**
Aug 14 - 15, 2015
United States / Minnesota / Rochester (MN)
Contact: Mayo School of Continuous Professional Development
Phone: 800-323-2688
Email: cme@mayo.edu

Burns Management
Aug 14, 2015
Australia / Clare
Contact: South Australian Postgraduate Medical Education Association
Phone: 011-61-8-8274-6060; Fax: 011-61-8-8274-6000
Email: admin@sapmea.asn.au

Neurology & Psychiatry for Primary Care
Aug 14 - 16, 2015
Bermuda / Southampton, Bermuda
Contact: Leslie Burk, MCE Conferences
Phone: 888-533-9031; Fax: 858-777-5588
Email: info@mceconferences.com

Emergency Medicine Greek Isles Cruise
Aug 16 - 23, 2015
Italy / Venice
Contact: Continuing Education, Continuing Education, Inc
Phone: 800-422-0711
Email: registrar@continuingeducation.net

2015 Molecular & Cellular Basis of **Breast Cancer** Risk & Prevention
Aug 17 - 20, 2015
Australia / Cairns
Contact: Matthew Kirkby, Zing Conferences CIC
Phone: 011-44-12-2375-0020
Email: matthew.kirkby@zingconferences.com

3rd Annual International Conference on Advances in **Medical Research**
Aug 17 - 18, 2015
Singapore / Singapore
Contact: Conference Secretariat, Global Science & Technology Forum
Phone: 011-65-6327-0166; Fax: 011-65-6327-0162
Email: secretariat@medicalconf-ed.org

Gamma Knife® Perfexion™ Radiosurgery Training Course
Aug 17 - 21, 2015
United States / Ohio / Cleveland
Contact: Martha Tobin, Cleveland Clinic Florida
Phone: 216-445-3449
Email: tobinm@ccf.org

2015 Asia Pacific **Cancer** Conference
Aug 20 - 22, 2015
Indonesia / Bali
Contact: Pusti, Project Officer, Pharma Pro Int.
Phone: 011-62-21-6386-9502; Fax: 011-62-21-6386-9503
Email: apcc2015@pharma-pro.com

2015 **Pathology** Summer School for Medical Students
Aug 21 - 22, 2015
United Kingdom / Oxford
Contact: Michelle Merrett, Royal College of Pathologists
Email: michelle.merrett@rcpath.org

31st International Conference on **Pharmacoepidemiology & Therapeutic Risk** Management
Aug 22 - 26, 2015
United States / Massachusetts / Boston
Contact: International Society for Pharmacoepidemiology
Phone: 301-718-6500; Fax: 301-656-0989,
Email: ISPE@paimgmt.com

3rd International Society for **Gynecologic Endoscopy (**ISGE) African Conference
Aug 23 - 26, 2015
Kenya / Nairobi
Contact: Stefano Betocchi, Secretary Office, ISGE
Phone: 011-39-345-777-9818
Email: secretariat@isge.org

46th World Congress of **Surgery**
Aug 23 - 27, 2015
Thailand / Bangkok
Contact: Congress Secretariat, CDM-Thailand
Phone: 011-66-2-965-8909; Fax: 011-66-2-965-8919
Email: wcs2015@cdmthailand.com

2015 Global Forum on **Research & Innovation** for Health
Aug 24 - 27, 2015
Philippines / Manila
Contact: COHRED Events Team, The Council on Health Research for Development (COHRED)
Phone: 011-63-2-837-7534
Email: secretariat@forum2015.org

2015 International Symposium on **Auditory and Audiological** Research (ISAAR)
Aug 26 - 28, 2015
Denmark / Nyborg
Contact: ISAAR
Email: isaar@isaar.eu

22nd Budapest **Nephrology** School
Aug 26 - 31, 2015
Hungary / Budapest
Contact: László Rosivall M.D., PhD. DSc., International Nephrology Research & Training Center, Semmelweis University
Phone: 011-36-1-2100-100; Fax: 011-36-1-2100-100
Email: rosivall.laszlo@med.semmelweis-univ.hu

2015 Wyoming Society of **Otolaryngology, Head & Neck Surgery** (WSOHNS) Conference
Aug 27 - 28, 2015
United States / Wyoming / Jackson
Contact: Mary Beth Robertson, WSOHNS Conference Coordinator, WSOHNS
Phone: 801-369-3583
Email: mbr@wsohns.org

7th European **Plastic Surgery** Research Council Meeting
Aug 27 - 30, 2015
Germany / Hamburg
Contact: Sandra Gottschalg, Congress Management,
Phone: 011-49-36-4131-16350
Fax: 011-49-36-4131-16243

Selected Topics in **Infectious Diseases & Rheumatology** for the PCP Baltic Cruise
Aug 28 - Sep 6, 2015
Denmark / Copenhagen
Contact: Continuing Education, Continuing Education, Inc
Phone: 800-422-0711
Email: registrar@continuingeducation.net

10th Asia Pacific **Burn** Congress in conjunction with ISBI Course
Aug 29 - 31, 2015
Indonesia / Bali
Contact: Jessica, Project Officer, PT Pharma Pro Int.
Phone: 011-62-21-6386-9502; Fax: 011-62-21-6386-9503
Email: apbc2015@pharma-pro.com

Anti-Aging Medicine: Advances in Hormone Replacement
Aug 29 - 30, 2015
United States / Illinois / Chicago
Contact: Heather Osborne, Education Coordinator, National Procedures Institute
Phone: 866-674-2631 or 512-870-8051; Fax: 512-329-0442
Email: heather@npinstitute.com

Osteoporosis: Essentials for Clinicians
Aug 29 - 30, 2015
United States / Missouri / St. Louis
Contact: International Society for Clinical Densitometry
Phone: 860-259-1000; Fax: 860-259-1030
Email: iscd@iscd.org

Podiatric Medicine at Kent State University
Aug 29 - Sep 5, 2015
United Kingdom / Southampton
Contact: Continuing Education, Continuing Education, Inc
Phone: 800-422-0711

13th World Conference: The **Esophagiome**
Aug 31 - Sep 3, 2015
Monaco / Monaco-Ville
Contact: Michele Liegeon, World Organization for Specialized Studies on Diseases of the Esophagus (OESO)
Phone: 011-33-1-5537-9015
Email: michele.liegeon@oeso.org

5th Conference of International Union Against **Tuberculosis & Lung Disease** Asia Pacific Region
Aug 31 - Sep 2, 2015
Australia / Sydney
Contact: Terri Growcott, Conference Manager
Phone: 011-61-2-9254-5000; Fax: 011-61-2-9251-3552
Email: info@aprunion2015.com

5th International Symposium on **Critical Bleeding**
Aug 31 - Sep 1, 2015
Denmark / Copenhagen
Contact: Pär I Johansson, Professor, Congress Chair, The Meeting Planners
Phone: 011-45-5060-7420
Email: br@meetingplaners.dk

2015 International Meeting on **Respiratory Pathogens**
Sep 2 - 4, 2015
Singapore / Singapore
Contact: Stella Chee, Executive Conference Administrator
Phone: 011-65-6379-5260; Fax: 011-65-6475-2077
Email: admin@acedaytons-direct.com

2015 **Skin Vaccination** Summit
Sep 2 - 4, 2015
Switzerland / Lausanne
Contact: Caroline Sumner, Events Director, Meetings Management
Phone: 011-44-14-8342-7770; Fax: 011-44-14-8342-8516
Email: csumner@meetingsmgmt.u-net.com

Workshop on **Neurobiology of the Epilepsies**
Sep 2 - 6, 2015
Turkey / Istanbul
Contact: Headquarters Staff, International League Against Epilepsy
Phone: 860-586-7547; Fax: 860-586-7550

2nd World Congress on **NeuroTherapeutics**: Dilemmas, Debates, Discussion
Sep 3 - 6, 2015
Czech Republic / Prague
Contact: Secretariat, CongressMed Ltd
Phone: 011-972-73-706-6954
Email: dddn@congressmed.com

7th International Conference on **Ocular Infections**
Sep 3 - 4, 2015
Spain / Barcelona
Contact: Janine Koeries, Conference Secretariat, Paragon Group
Phone: 011-41-2253-3094
Email: secretariat@ocularinfections.com

7th World Conference on **Ovulation Induction**
Sep 3 - 5, 2015
Italy / Bologna
Contact: Organizing Secretariat, Gynepro Educational
Phone: 011-39-51-22-3260; Fax: 011-39-51-22-2101
Email: segreteria@gynepro.it

Cancer Diagnosis & Therapy Congress
Sep 3 - 4, 2015
United Kingdom / London
Contact: Rajesh K Guru, Conference Producer, MnM Conferences
Phone: 011-91-20-6075-6011
Email: rajesh.guru@mnmconferences.com

2015 International **Liver Cancer** Association (ILCA) Conference
Sep 4 - 6, 2015
France / Paris
Contact: ILCA Office
Email: info@ilca-online.org

6th **EuCornea** Congress
Sep 4 - 5, 2015
Spain / Barcelona
Contact: European Society of Ocular & Surface Disease Specialists
Phone: 011-353-1-288-3674; Fax: 011-353-1-209-1112
Email: eucornea@eucornea.org

16th World Conference on **Lung Cancer**
Sep 6 - 10, 2015
United States / Colorado / Denver
Contact: Conference Secretariat, International Conference Services
Phone: 604-681-2153; Fax: 604-681-1049
Email: wclc2013@icsevents.com

17th Asia Pacific League of Associations for **Rheumatology** Congress
Sep 6 - 9, 2015
India / Chennai
Contact: Coralie Deguerville, APM, Kenes Asia
Phone: 011-65-6292-4710; Fax: 011-65-6292-4721
Email: aplar2015@kenes.com

25th World Congress of **Lymphology**
Sep 7 - 12, 2015
United States / California / San Francisco
Contact: Congress Secretariat, Meeting Achievements
Phone: 219-465-1115; Fax: 219-548-8619
Email: lymphology2015@meetingachievements.com

Heart & Lung Transplantation
Sep 7 - 8, 2015
United Kingdom / Windsor (UK)
Contact: European Association for Cardio-Thoracic Surgery
Phone: 011-44-17-5383-2166; Fax: 011-44-17-5362-0407

Hepatobiliary Malignancy Workshop
Sep 7 - 8, 2015
Austria / Vienna
Contact: Ana Galan, Education Coordinator
Phone: 011-32-2-775-0243
Email: ana.galan@essoweb.org

Medical Retina
Sep 7 - 11, 2015
Switzerland / Lugano
Contact: European School for Advanced Studies in Ophthalmology
Phone: 011-41-91-921-1154; Fax: 011-41-91-921-1154

45th International Society of **Psychoneuroendocrinology** (ISPNE) Annual Conference
Sep 8 - 11, 2015
United Kingdom / Edinburgh
Contact: Nicolas Rohleder, Ph.D., Secretary General, ISPNE
Email: rohleder@brandeis.edu

2nd International Surgical Aspects of **Cardiopulmonary Transplantation** Course
Sep 9, 2015
United Kingdom / NEWCASTLE UPON TYNE upon Tyne
Contact: Anna Burley, Aesculap Academy
Email: anna.burley@aesculap-academy.com

2015 Faculty of **Neuropsychiatry** Annual Conference
Sep 10 - 11, 2015
United Kingdom / London
Contact: Royal College of Psychiatrists
Phone: 011-44-20-3701-2622 / 2611; Fax: 011-44-20-3701-2761
Email: rbrake@rcpsych.ac.uk

2015 **Viral Hepatitis** Congress
Sep 10 - 12, 2015
Germany / Frankfurt
Contact: Sophie Hoad, Senior Account Director, KnowledgePoint360
Phone: 011-44-16-2566-4392; Fax: 011-44-16-2566-4391
Email: hep@ashfieldhealthcare.com

3rd International **Oncology** Conference
Sep 10 - 11, 2015
United Arab Emirates / Abu Dhabi
Contact: Afsal Ahmad, Mena Conference
Phone: 011-971-2-491-9888
Email: afsal.ahmad@menaconf.com

Basic **Surgical Skills** (Edinburgh)
Sep 10 - 11, 2015
United Kingdom / Edinburgh
Contact: Linda Perttula, Royal College of Surgeons of Edinburgh
Phone: 011-44-13-1527-1565
Email: l.perttula@rcsed.ac.uk

Deep Brain Stimulation for Movement Disorders - Grenoble
Sep 10 - 11, 2015
France / Grenoble
Contact: International Secretariat, International Parkinson & Movement Disorder Society
Phone: 414-276-2145; Fax: 414-276-3349
Email: info@movementdisorders.org

International Conference on **Acute Myeloid Leukemia**: Molecular & Translational - Advances in the Biology & Treatment
Sep 10 - 12, 2015
Hungary / Budapest
Contact: Nicolas Jaillard, Conference Coordinator, European School of Haematology
Phone: 011-33-1-5727-6833; Fax: 011-33-1-5727-6838
Email: nicolas.jaillard@univ-paris-diderot.fr

Thoracic Pathology
Sep 11 - 13, 2015
United States / New York / New York
Contact: Continuing Medical Education, Memorial Sloan Kettering Cancer Center
Phone: 646-227-2025; Fax: 212-557-0773
Email: brodheap@mskcc.org

2015 **Acute and Chronic Leukemias**
Sep 12, 2015
United States / Arizona / Scottsdale
Contact: Mayo School of Continuous Professional Development
Phone: 480-301-4580
Email: mca.cme@mayo.edu

15th European Society for **Biomedical Research on Alcoholism** Congress
Sep 13 - 16, 2015
Spain / Valencia
Contact: Technical Secretariat, Viajes El Corte Ingles, S.A.
Phone: 011-34-963-107-189; Fax: 011-34-963-411-046
Email: esbra2015@viajeseci.es

17th Congress of the European Society for **Organ Transplantation**
Sep 13 - 16, 2015
Belgium / Brussels
Contact: Congress Administrative Secretariat, Aim Group International Vienna
Phone: 011-43-1-402-7755; Fax: 011-43-1-402-7731
Email: esot2015@aimgroup.eu

2015 **Fertility** Society of Australia Annual Meeting
Sep 13 - 16, 2015
Australia / Canberra
Contact: Waldron Smith Management
Phone: 011-61-3-9645-6359; Fax: 011-61-3-9645-6322
Email: kimo@wsm.com.au

2015 International Society for **Hemodialysis** Congress
Sep 13 - 16, 2015
Malaysia / Kuala Lumpur
Contact: Shu Shan, Conference Secretariat, Console Communications Sdn Bhd
Phone: 011-603-2162-0566; Fax: 011-603-2161-6560
Email: ishd2015@console.com.my

2015 **PCR** London Valves
Sep 13 - 15, 2015
Germany / Berlin
Contact: Julie Albagnac, Europa Organisation
Phone: 011-33-5-3445-2645
Email: pcrlondonvalves@europa-organisation.com

2015 World **STI & HIV** Congress
Sep 13 - 16, 2015
Australia / Brisbane
Contact: Conference Secretariat
Phone: 011-61-2-8204-0770; Fax: 011-61-2-8204-0779
Email: info@worldsti2015.com

11th North East International **Flap** Course
Sep 14 - 17, 2015
United Kingdom / newcastle Upon Tyne upon Tyne
Contact: Secretary to Mr Matt Erdmann, North East Flap Course
Phone: 011-44-19-1333-6989; Fax: 011-44-19-1333-2337
Email: matt.erdmann@cddft.nhs.uk

3rd International Conference on **Bioprocess & Engineering**
Sep 14 - 15, 2015
United States / Maryland / Baltimore
Contact: Suzana Joan, bioprocess, Omics International
Phone: 702-508-5200
Email: bioprocess@conferenceseries.com

4th International Conference on **Nephrology & Therapeutics**
Sep 14 - 16, 2015
United States / Maryland / Baltimore
Contact: James Crawler, 4th International Conference on Nephrology & Therapeutics,OMICS
Phone: 650-268-9744
Email: jamescrawler123@gmail.com

Basic Practical Skills in **Obstetrics & Gynaecology**
Sep 14 - 15, 2015
United Kingdom / London
Contact: Royal College of Obstetricians & Gynaecologists
Phone: 011-44-20-7092-1670; Fax: 011-44-20-7723-0575

Chronic Pain Management for the Family Physician
Sep 14, 2015
Canada / Alberta / Calgary
Contact: Sylvia Vespa, Cumming School of Medicine, University of Calgary
Phone: 403-909-9095
Email: sylvia.vespa@albertahealthservices.ca

International Conference on Targeting **Diabetes & Novel Therapeutics**
Sep 14 - 16, 2015
United States / Nevada / Las Vegas
Contact: Hailey Watson, Event Manager, OMICS International
Phone: 650-268-9744; Fax: 650-618-1414
Email: diabeticmedications@omicsgroup.com

Medical Ethics
Sep 14 - 18, 2015
United Kingdom / London
Contact: Centre for Continuing Professional Development, Imperial College London
Phone: 011-44-20-7594-6881; Fax: 011-44-20-7594-6883
Email: cpd@imperial.ac.uk

MRI of the Joints
Sep 14 - 18, 2015
France / Paris
Contact: Walter Rijsselaere, Erasmus Course on Magnetic Resonance Imaging
Phone: 011-32-2-477-5322; Fax: 011-32-2-477-5362
Email: walter.rijsselaere@uzbrussel.be

1st International Conference on **Paediatric Acquired Brain Injury**
Sep 16 - 18, 2015
United Kingdom / Liverpool
Contact: Colleen LoGrande, International Brain Injury Association
Fax: 703-960-6603
Email: clogrande@internationalbrain.org

2015 Faculty of **Child & Adolescent Psychiatry** Annual Conference
Sep 16 - 18, 2015
United Kingdom / Brighton
Contact: *Royal* College of Psychiatrists
Phone: 011-44-20-3701-2604
Email: csimms@rcpsych.ac.uk

Advanced **Airway Workshop**
Sep 16, 2015
United Kingdom / London
Contact: Meetings and Events, Royal College of Anaesthetists
Phone: 011-44-20-7092-1670; Fax: 011-44-20-7092-1730
Email: events@rcoa.ac.uk

WHO-Facts Sheet

1. Measles
2. Leishmaniasis
3. Adolescent Pregnancy
4. Immunization Coverage

Compiled and edited by
Babichan K Chandy

Kuwait Medical Journal 2015; 47 (2): 183 - 188

1. MEASLES

Overview

Measles is a highly contagious, serious disease caused by a virus. In 1980, before widespread vaccination, measles caused an estimated 2.6 million deaths each year.

The disease remains one of the leading causes of death among young children globally, despite the availability of a safe and effective vaccine. Approximately 145,700 people died from measles in 2013 – mostly children under the age of five.

Measles is caused by a virus in the paramyxovirus family and it is normally passed through direct contact and through the air. The virus infects the mucous membranes, then spreads throughout the body. Measles is a human disease and is not known to occur in animals.

Accelerated immunization activities have had a major impact on reducing measles deaths. During 2000-2013, measles vaccination prevented an estimated 15.6 million deaths. Global measles deaths have decreased by 75% from an estimated 544, 200 in 2000 to 145, 700 in 2013.

KEY FACTS

- Measles is one of the leading causes of death among young children even though a safe and cost-effective vaccine is available.
- In 2013, there were 145, 700 measles deaths globally – about 400 deaths every day or 16 deaths every hour.
- Measles vaccination resulted in a 75% drop in measles deaths between 2000 and 2013 worldwide.
- In 2013, about 84% of the world's children received one dose of measles vaccine by their first birthday through routine health services – up from 73% in 2000.
- During 2000-2013, measles vaccination prevented an estimated 15.6 million deaths making measles vaccine one of the best buys in public health.

Signs and symptoms

The first sign of measles is usually a high fever, which begins about 10 to 12 days after exposure to the virus, and lasts 4 - 7 days. A runny nose, a cough, red and watery eyes, and small white spots inside the cheeks can develop in the initial stage. After several days, a rash erupts, usually on the face and upper neck. Over about three days, the rash spreads, eventually reaching the hands and feet. The rash lasts for 5 to 6 days, and then fades. On average, the rash occurs 14 days after exposure to the virus (within a range of 7 - 18 days).

Most measles-related deaths are caused by complications associated with the disease. Complications are more common in children under the age of five, or adults over the age of 20. The most serious complications include blindness, encephalitis (an infection that causes brain swelling), severe diarrhoea and related dehydration, ear infections, or severe respiratory infections such as pneumonia. Severe measles is more likely among poorly nourished young children, especially those with insufficient vitamin A, or whose immune systems have been weakened by HIV/AIDS or other diseases.

In populations with high levels of malnutrition and a lack of adequate health care, up to 10% of measles cases result in death. Women infected while pregnant are also at risk of severe complications and the pregnancy may end in miscarriage or preterm delivery. People who recover from measles are immune for the rest of their lives.

Address correspondence to:
Office of the Spokesperson, WHO, Geneva. Tel.: (+41 22) 791 2599; Fax (+41 22) 791 4858; Email: inf@who.int; Web site: http://www.who.int/

Who is at risk?

Unvaccinated young children are at highest risk of measles and its complications, including death. Unvaccinated pregnant women are also at risk. Any non-immune person (who has not been vaccinated or was vaccinated but did not develop immunity) can become infected.

Measles is still common in many developing countries – particularly in parts of Africa and Asia. The overwhelming majority (more than 95%) of measles deaths occur in countries with low per capita incomes and weak health infrastructures.

Measles outbreaks can be particularly deadly in countries experiencing or recovering from a natural disaster or conflict. Damage to health infrastructure and health services interrupts routine immunization, and overcrowding in residential camps greatly increases the risk of infection.

Transmission

The highly contagious virus is spread by coughing and sneezing, close personal contact or direct contact with infected nasal or throat secretions.

The virus remains active and contagious in the air or on infected surfaces for up to 2 hours. It can be transmitted by an infected person from 4 days prior to the onset of the rash to 4 days after the rash erupts.

Measles outbreaks can result in epidemics that cause many deaths, especially among young, malnourished children. In countries where measles has been largely eliminated, cases imported from other countries remain an important source of infection.

Treatment

No specific antiviral treatment exists for measles virus.

Severe complications from measles can be avoided through supportive care that ensures good nutrition, adequate fluid intake and treatment of dehydration with WHO-recommended oral rehydration solution. This solution replaces fluids and other essential elements that are lost through diarrhoea or vomiting. Antibiotics should be prescribed to treat eye and ear infections, and pneumonia.

All children in developing countries diagnosed with measles should receive two doses of vitamin A supplements, given 24 hours apart. This treatment restores low vitamin A levels during measles that occur even in well-nourished children and can help prevent eye damage and blindness. Vitamin A supplements have been shown to reduce the number of deaths from measles by 50%.

Prevention

Routine measles vaccination for children, combined with mass immunization campaigns in countries with high case and death rates, are key public health strategies to reduce global measles deaths. The measles vaccine has been in use for 50 years. It is safe, effective and inexpensive. It costs approximately one US dollar to immunize a child against measles.

The measles vaccine is often incorporated with rubella and/or mumps vaccines in countries where these illnesses are problems. It is equally effective in the single or combined form. Adding rubella to measles vaccine increases the cost only slightly, and allows for shared delivery and administration costs.

In 2013, about 84% of the world's children received 1 dose of measles vaccine by their first birthday through routine health services – up from 73% in 2000. Two doses of the vaccine are recommended to ensure immunity and prevent outbreaks, as about 15% of vaccinated children fail to develop immunity from the first dose.

2. LEISHMANIASIS

Overview

Leishmaniasis is caused by a protozoa parasite from over 20 Leishmania species and is transmitted to humans by the bite of infected female phlebotomine sandflies. Over 90 sandfly species are known to transmit Leishmania parasites.

KEY FACTS

- There are 3 main forms of leishmaniases – visceral (often known as kala-azar and the most serious form of the disease), cutaneous (the most common), and mucocutaneous.
- Leishmaniasis is caused by the protozoan Leishmania parasites which are transmitted by the bite of infected sandflies.
- The disease affects some of the poorest people on the planet, and is associated with malnutrition, population displacement, poor housing, a weak immune system and lack of resources.
- Leishmaniasis is linked to environmental changes such as deforestation, building of dams, irrigation schemes and urbanization.
- An estimated 1.3 million new cases and 20, 000 to 30 000 deaths occur annually.
- Only a small fraction of those infected by Leishmania parasites will eventually develop the disease.

There are 3 main forms of the disease:

1. Visceral leishmaniasis (VL also known as kala-azar) is fatal if left untreated. It is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia. It is highly endemic in the Indian subcontinent and in East Africa. An estimated 200,000 to 400,000 new cases

of VL occur worldwide each year. Over 90% of new cases occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan.

2. Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis and causes skin lesions, mainly ulcers, on exposed parts of the body, leaving life-long scars and serious disability. About 95% of CL cases occur in the Americas, the Mediterranean basin, the Middle East and Central Asia. Over two thirds of new CL cases occur in 6 countries: Afghanistan, Algeria, Brazil, Colombia, Iran (Islamic Republic of) and the Syrian Arab Republic. An estimated 0.7 million to 1.3 million new cases occur worldwide annually.
3. Mucocutaneous leishmaniasis leads to partial or total destruction of mucous membranes of the nose, mouth and throat. Almost 90% of mucocutaneous leishmaniasis cases occurs in the Plurinational State of Bolivia, Brazil and Peru.

Transmission

Leishmania parasites are transmitted through the bites of infected female phlebotomine sandflies. The epidemiology of leishmaniasis depends on the characteristics of the parasite species, the local ecological characteristics of the transmission sites, current and past exposure of the human population to the parasite and human behaviour. Some 70 animal species, including humans, have been found as natural reservoir hosts of Leishmania parasites.

Mediterranean basin

In the Mediterranean basin, visceral leishmaniasis is the main form of the disease. It occurs in rural areas, in villages in mountainous regions and also in some periurban areas, where Leishmania parasites live on dogs and other animals.

South-East Asia

In South-East Asia, visceral leishmaniasis is the main form of the disease. Transmission generally occurs in rural areas below 600 m above sea level, with a heavy annual rainfall, with a mean humidity above 70%, a temperature range of 15–38 °C, abundant vegetation, subsoil water and alluvial soil. The disease is most common in agricultural villages where houses are frequently constructed with mud walls and earthen floors, and cattle and other livestock live close to humans.

East Africa

In East Africa, there are frequent outbreaks of visceral leishmaniasis in the northern acacia–balanite savanna and the southern savanna and forest areas where sandflies live around termite mounds. Cutaneous leishmaniasis occurs in the highlands of Ethiopia and other places in East Africa, where increased human–fly contact occurs in villages built on rock hills or river banks, which are the natural habitat of hyraxes.

Afro-Eurasia

In Afro-Eurasia, cutaneous leishmaniasis is the main form of the disease. Agricultural projects and irrigation schemes can increase the prevalence of cutaneous leishmaniasis as people who have no immunity to the disease move in to work on the projects. Large outbreaks in densely populated cities also occur, especially during war and large-scale population migration. The parasites causing cutaneous leishmaniasis live mainly on humans or rodents.

Americas

Kala-azar in the Americas is very similar to that found in the Mediterranean basin. The habit of keeping dogs and other domestic animals inside the house is thought to promote human infection. The epidemiology of CL in the Americas is complex, with variations in transmission cycles, reservoir hosts, sandfly vectors, clinical manifestations and response to therapy, and multiple circulating Leishmania species in the same geographical area.

Post-kala-azar dermal leishmaniasis (PKDL)

PKDL is a sequel of visceral leishmaniasis that appears as macular, papular or nodular rash usually on face, upper arms, trunks and other parts of the body. It occurs mainly in East Africa and on the Indian subcontinent, where up to 50% and 5 – 10% of patients with kala-azar, respectively, develop the condition. It usually appears 6 months to one or more years after kala-azar has apparently been cured, but can occur earlier. People with PKDL are considered to be a potential source of kala-azar infection.

Leishmania-HIV co-infection

Leishmania-HIV co-infected people have high chance of developing the full-blown clinical disease, and high relapse and mortality rates. Antiretroviral treatment reduces the development of the disease, delays relapses and increases the survival of the co-infected patients. High leishmania-HIV co-infection rates are reported from Brazil, Ethiopia and the state of Bihar in India.

Major risk factors

Socioeconomic conditions: Poverty increases the risk for leishmaniasis. Poor housing and domestic sanitary conditions (e.g. lack of waste management, open sewerage) may increase sandfly breeding and resting sites, as well as their access to humans. Sandflies are attracted to crowded housing as these provide a good source of blood-meals. Human behaviour, such

as sleeping outside or on the ground, may increase risk. The use of insecticide-treated bednets reduces risk.

Malnutrition: Diets lacking protein-energy, iron, vitamin A and zinc increase the risk that an infection will progress to kala-azar.

Population mobility: Epidemics of both main forms of leishmaniasis are often associated with migration and the movement of non-immune people into areas with existing transmission cycles. Occupational exposure as well as widespread deforestation remain important factors. For example, people settling in areas that used to be forests may be moving near sandflies' habitat. This can lead to a rapid increase in cases.

Environmental changes: Environmental changes that can affect the incidence of leishmaniasis include urbanization, domestication of the transmission cycle and the incursion of agricultural farms and settlements into forested areas.

Climate change: Leishmaniasis is climate-sensitive, and strongly affected by changes in rainfall, temperature and humidity. Global warming and land degradation together affect the epidemiology of leishmaniasis in a number of ways:

- changes in temperature, rainfall and humidity can have strong effects on vectors and reservoir hosts by altering their distribution and influencing their survival and population sizes;
- small fluctuations in temperature can have a profound effect on the developmental cycle of Leishmania promastigotes in sandflies, allowing transmission of the parasite in areas not previously endemic for the disease;
- drought, famine and flood resulting from climate change can lead to massive displacement and migration of people to areas with transmission of leishmaniasis, and poor nutrition could compromise their immunity.

Diagnosis and treatment

In visceral leishmaniasis, diagnosis is made by combining clinical signs with parasitological, or serological tests (rapid diagnostic tests and others). In cutaneous and mucocutaneous leishmaniasis serological tests have limited value. In cutaneous leishmaniasis, clinical manifestation with parasitological tests confirms the diagnosis.

The treatment of leishmaniasis depends on several factors including type of disease, parasite species and geographic location. Leishmaniasis is a treatable and curable disease. All patients diagnosed as visceral leishmaniasis require prompt and complete treatment. Detailed information on treatment of the various forms of the disease by geographic location is available in the WHO technical report series 949 on the control of leishmaniasis.

Prevention and control

Prevention and control of leishmaniasis require a combination of intervention strategies because transmission occurs in a complex biological system involving the human host, parasite, sandfly vector and in some causes an animal reservoir host. Key strategies include:

- Early diagnosis and effective case management reduces the prevalence of the disease and prevents disabilities and death. Currently there are highly effective and safe anti-leishmanial medicines particularly for VL and access to these medicines has significantly improved.
- Vector control helps to reduce or interrupt transmission of disease by controlling sandflies, especially in domestic conditions. Control methods include insecticide spray, use of insecticide–treated nets, environmental management and personal protection.
- Effective disease surveillance is important. Early detection and treatment of cases helps reduce transmission and helps monitor the spread and burden of disease.
- Control of reservoir hosts is complex and should be tailored to the local situation.
- Social mobilization and strengthening partnerships – mobilization and education of the community with effective behavioural change interventions with locally tailored communication strategies. Partnership and collaboration with various stakeholders and other vector-borne disease control programmes is critical.

3. ADOLESCENT PREGNANCY

Contexts

For some adolescents, pregnancy and childbirth are planned and wanted, but for many they are not. Adolescent pregnancies are more likely in poor, uneducated and rural communities. In some countries, becoming pregnant outside marriage is not uncommon. By contrast, some girls may face social pressure to marry and, once married, to have children. More than 30% of girls in low- and middle-income countries marry before they are 18; around 14%, before they are 15.

Some girls do not know how to avoid getting pregnant: sex education is lacking in many countries. They may feel too inhibited or ashamed to seek contraception services; contraceptives may be too

expensive or not widely or legally available. Even when contraceptives are widely available, sexually active adolescent girls are less likely to use them than adults. Girls may be unable to refuse unwanted sex or resist coerced sex, which tends to be unprotected.

KEY FACTS

- About 16 million girls aged 15 to 19 and some 1 million girls under 15 give birth every year—most in low- and middle-income countries.
- Complications during pregnancy and childbirth are the second cause of death for 15 - 19 year-old girls globally.
- Every year, some 3 million girls aged 15 to 19 undergo unsafe abortions.
- Babies born to adolescent mothers face a substantially higher risk of dying than those born to women aged 20 to 24.

Birth rates

There has been a marked, although uneven, decrease in the birth rates among adolescent girls since 1990, but some 11% of all births worldwide are still to girls aged 15 to 19 years old. The vast majority of these births (95%) occur in low- and middle-income countries.

The 2014 World Health Statistics indicate that the average global birth rate among 15 to 19 year olds is 49 per 1000 girls. Country rates range from one to 299 births per 1000 girls, with the highest rates in sub-Saharan Africa.

Adolescent pregnancy remains a major contributor to maternal and child mortality, and to the cycle of ill-health and poverty.

Health effects

Pregnancy and childbirth complications are the second cause of death among 15 to 19 year olds globally. However, there have been significant drops in the number of deaths in all regions since 2000, most notably in South-East Asia where mortality rates fell from 21 to 9 per 100 000 girls. Some 3 million unsafe abortions among girls aged 15 to 19 take place each year, contributing to maternal deaths and to lasting health problems.

Early childbearing increases the risks for both mothers and their newborns. In low- and middle-income countries, babies born to mothers under 20 years of age face a 50% higher risk of being still born or dying in the first few weeks versus those born to mothers aged 20 - 29. The younger the mother, the greater the risk to the baby. Newborns born to adolescent mothers are also more likely to have low birth weight, with the risk of long-term effects.

Economic and social consequences

Adolescent pregnancy can also have negative social and economic effects on girls, their families and communities. Many girls who become pregnant have to drop out of school. A girl with little or no education has fewer skills and opportunities to find a job. This can also have an economic cost with a country losing out on the annual income a young woman would have earned over her lifetime, if she had not had an early pregnancy.

WHO response

WHO published guidelines in 2011 with the UN Population Fund (UNFPA) on preventing early pregnancies and reducing poor reproductive outcomes. These made recommendations for action that countries could take, with six main objectives:

1. reducing marriage before the age of 18;
2. creating understanding and support to reduce pregnancy before the age of 20;
3. increasing the use of contraception by adolescents at risk of unintended pregnancy;
4. reducing coerced sex among adolescents;
5. reducing unsafe abortion among adolescents;
6. increasing use of skilled antenatal, childbirth and postnatal care among adolescents.

4. IMMUNIZATION COVERAGE

Overview

Immunization averts an estimated 2 - 3 million deaths every year from diphtheria, tetanus, pertussis (whooping cough), and measles. Global vaccination coverage—the proportion of the world's children who receive recommended vaccines—has remained steady for the past few years.

During 2013, about 84% (112 million) of infants worldwide received 3 doses of diphtheria-tetanus-pertussis (DTP3) vaccine, protecting them against infectious diseases that can cause serious illness and disability or be fatal. By 2013, 129 countries had reached at least 90% coverage of DTP3 vaccine.

KEY FACTS

- Immunization prevents illness, disability and death from vaccine-preventable diseases including cervical cancer, diphtheria, hepatitis B, measles, mumps, pertussis, pneumonia, polio, rotavirus diarrhoea, rubella and tetanus.
- Global vaccination coverage is holding steady.
- Immunization currently averts an estimated 2 - 3 million deaths every year.
- But an estimated 21.8 million infants worldwide are still missing out on basic vaccines.

Global immunization coverage 2013

Haemophilus influenzae type b (Hib) causes

meningitis and pneumonia. Hib vaccine had been introduced in 189 countries by the end of 2013. Global coverage with 3 doses of Hib vaccine is estimated at 52%. There is great variation between regions. In the Americas, coverage is estimated at 90%, while it is only 18% and 27% in the Western Pacific and South-East Asia Regions respectively.

Hepatitis B is a viral infection that attacks the liver. Hepatitis B vaccine for infants had been introduced nationwide in 183 countries by the end of 2013. Global coverage with 3 doses of hepatitis B vaccine is estimated at 81% and is as high as 92% in the Western Pacific.

Human papillomavirus - the most common viral infection of the reproductive tract—can cause cervical cancer, and other types of cancer and genital warts in both men and women. Human papillomavirus vaccine was introduced in 55 countries by the end of 2013.

Measles is a highly contagious disease caused by a virus, which usually results in a high fever and rash, and can lead to blindness, encephalitis or death. By the end of 2013, 84% of children had received 1 dose of measles vaccine by their second birthday, and 148 countries had included a second dose as part of routine immunization.

Meningitis A is an infection that can cause severe brain damage and is often deadly. By the end of 2013—3 years after its introduction—more than 150 million people in African countries affected by the disease had been vaccinated with MenAfriVac, a vaccine developed by WHO and PATH.

Mumps is a highly contagious virus that causes painful swelling at the side of the face under the ears (the parotid glands), fever, headache and muscle aches. It can lead to viral meningitis. Mumps vaccine had been introduced nationwide in 120 countries by the end of 2013.

Pneumococcal diseases include pneumonia, meningitis and febrile bacteraemia, as well as otitis media, sinusitis and bronchitis. Pneumococcal vaccine had been introduced in 103 countries by the end of 2013, and global coverage was estimated at 25%.

Polio is a highly infectious viral disease that can cause irreversible paralysis. In 2013, 84% of infants around the world received 3 doses of polio vaccine. Targeted for global eradication, polio has been stopped in all countries save 3: Afghanistan, Nigeria and Pakistan. Polio-free countries have been infected by imported virus, and all countries—especially those experiencing conflict and instability—remain at risk until polio is fully eradicated. Rotaviruses are the most common cause of severe diarrhoeal disease in young children throughout the world. Rotavirus vaccine was introduced in 52 countries by the end of 2013, and global coverage was estimated at 14%.

Rubella is a viral disease which is usually mild in children, but infection during early pregnancy may cause fetal death or congenital rubella syndrome, which can lead to defects of the brain, heart, eyes and ears. Rubella vaccine was introduced nationwide in 137 countries by the end of 2013.

Tetanus is caused by a bacterium which grows in the absence of oxygen, e.g. in dirty wounds or in the umbilical cord, if it is not kept clean. It produces a toxin which can cause serious complications or death. The vaccine to prevent maternal and neonatal tetanus had been introduced in 103 countries by the end of 2013. An estimated 82% of newborns were protected through immunization. Maternal and neonatal tetanus persist as public health problems in 25 countries, mainly in Africa and Asia.

Yellow fever is an acute viral haemorrhagic disease transmitted by infected mosquitoes. As of 2013, yellow fever vaccine had been introduced in routine infant immunization programmes in 35 of the 44 countries and territories at risk for yellow fever in Africa and the Americas and coverage was estimated at 41%.

Key challenges

Despite improvements in global vaccine coverage during the past decade, there continue to be regional and local disparities resulting from:

- limited resources;
- competing health priorities;
- poor management of health systems; and
- inadequate monitoring and supervision.

In 2013, an estimated 21.8 million infants worldwide were not reached with routine immunization services, of whom nearly half live in three countries: India, Nigeria and Pakistan.

Priority needs to be given to strengthening routine vaccination globally, especially in the countries that are home to the highest number of unvaccinated children. Particular efforts are needed to reach the underserved, especially those in remote areas, in deprived urban settings, in fragile states and strife-torn regions.

WHO response

The Global Vaccine Action Plan: The Global Vaccine Action Plan (GVAP) is a roadmap to prevent millions of deaths through more equitable access to vaccines. Countries are aiming to achieve vaccination coverage of ≥ 90% nationally and ≥ 80% in every district by 2020. While the GVAP should accelerate control of all vaccine-preventable diseases, polio eradication is set as the first milestone. It also aims to spur research and development for the next generation of vaccines.