ISSN 0125-152X
Volume 70, Number 1, January-February 2018

SMJ

Siriraj Medical Journal

ORIGINAL ARTICLE

1 Postoperative Pain Reduction After Additional Intraperitoneal Suction Following Laparoscopic Cholecystectomy: A Prospective Randomized Controlled Study
Akkaraphorn Deeprasertvit, et al.

6 A Simple and Practical Amniocentesis Model and the Procedure Success Rate After Training
Pharuhas Chanprapaph, et al.

12 Laparoscopic Bile Duct Exploration in Treating Difficult Common Bile Duct Stones in Sawanpracharak Hospital, A Tertiary Center in the Lower Northern Region of Thailand
Suppadech Tunrattanakul

17 Surgical Outcomes of Upper Tract Approach and Lower Tract Approach in Duplex Kidney: Single Center Experience
Soratree Saingarm, et al.

22 Performance of the Locally Made Disposable 10-gram Semmes-Weinstein Monofilament Compared to the Commercially Available Monofilament
Danu Prommin, et al.

28 PCR-Based Diagnosis of *Neoscytalidium dimidiatum* Infection Using Internal Transcribed Spacer 1 Region of Ribosomal DNA Primers
Charussri Leeyaphan, et al.

36 Mortality Rate and Predictive Factors for Invasive Fungal Rhinosinusitis: Experience in Siriraj Hospital
Premyot Ngaotepprutaram, et al.

44 The Teratogenic Effects of Dichlorvos on the Development of Chick Embryos
Jantima Roongruangchai, et al.

53 Is Non-Contrast Enhanced CT of Paranasal Sinus Adequate for the Diagnosis of Complicated Sinusitis?
Siri-on Tritrakarn, et al.

60 Correlation between Timed Barium Esophagogram and the Eckardt Stage in Achalasia
Waraporn Boonsomjint, et al.

66 Characteristics of Patients Admitted with Tubo-Ovarian Abscess and Surgical Treatment in Siriraj Hospital
Sayan Luangchaichaweng, et al.

70 HBA as a Potential Alternative to the TUNEL Assay for Assessment of DNA Integrity in Asthenozoospermic Spermatozoa
Pitak Laokirkkiat, et al.

CASE REPORT

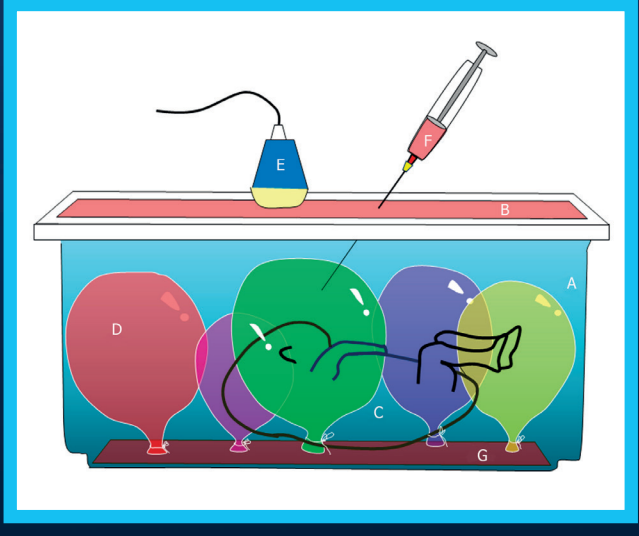
77 Numb Chin Syndrome: An Uncommon Presentation of Metastatic Non-Small Cell Lung Cancer Diagnosed by ^{18}F-FDG PET/CT Scan
Achiraya Teyateeti, et al.

81 Concomitant Primary and Secondary Syphilis: A Case Series from STD Clinic, Siriraj Hospital
Sukhum Jiamton, et al.

87 Outcomes of Expectant Management in HIV-Infected Pregnancy with Preterm Premature Rupture of Membranes at Less Than 34-Week Gestation: a Case Series
Nalat Sompagdee, et al

REVIEW ARTICLE

91 Secondary Acute Angle-Closure Attack: Diagnosis and Management
Sakaorat Petchyim



By Pharuhas Chanprapaph, et al.

Thai Association for Gastrointestinal Endoscopy

International Association of Surgeons Gastroenterologists & Oncologists Thailand Chapter

www.smj.si.mahidol.ac.th
E-mail: sijournal@mahidol.ac.th

SIRIRAJ MEDICAL JOURNAL

www.smj.si.mahidol.ac.th

First Editor: Ouay Ketusinh **Emeritus Editors:** Somchai Bovornkitti, Adulya Viriyavejakul, Sommai Toongsuwan, Nanta Maranetra, Niphon Poungvarin, Prasit Watanapa, Vithya Vathanophas, Pipop Jirapinyo, Sanya Sukpanichnant, Somboon Kunathikom

Executive Editor: Prasit Watanapa **Editorial Director:** Manee Rattanachaiyanont

Managing Editor: Gulapar Srisawasdi, Chenchit Chayachinda

Editor-in-Chief: Thawatchai Akaraviputh

Associate Editor: Varut Lohsiriwat, Prapat Wanitpongpan **Online Editor:** Puttinun Patpituck

International Editorial Board

Philip Board (Australian National University, Australia)
Richard J. Deckelbaum (Columbia University, USA)
Yozo Miyake (Aichi Medical University, Japan)
Yik Ying Teo (National University of Singapore, Singapore)
Harland Winter (Massachusetts General Hospital, USA)
Philip A. Brunell (State University of New York At Buffalo, USA)
Noritaka Isogai (Kinki University, Japan)
Yuji Murata (Aizenbashi Hospital, Japan)
Keiichi Akita (Tokyo Medical and Dental University Hospital, Japan)
Shuji Shimizu (Kyushu University Hospital, Japan)
David S. Sheps (University of Florida, USA)
Robin CN Williamson (Royal Postgraduate Medical School, UK)
Tai-Soon Yong (Yonsei University, Korea)
Anusak Yiengpruksawan (The Valley Robotic Institute, USA)
Stanlay James Rogers (University of California, San Francisco, USA)
Kyoichi Takaori (Kyoto University Hospital, Japan)
Tomohisa Uchida (Oita University, Japan)
Yoshiki Hirooka (Nagoya University Hospital, Japan)
Hidemi Goto (Nagoya University Graduate School of Medicine, Japan)
Kazuo Hara (Aichi Cancer Center Hospital, Japan)
Shomei Ryozawa (Saitama Medical University, Japan)
Christopher Khor (Singapore General Hospital, Singapore)
Yasushi Sano (Director of Gastrointestinal Center, Japan)
Mitsuhiro Kida (Kitasato University & Hospital, Japan)
Seigo Kitano (Oita University, Japan)
Ichizo Nishino (National Institute of Neuroscience NCNP, Japan)
Masakazu Yamamoto (Tokyo Women's Medical University, Japan)
Dong-Wan Seo (University of Ulsan College of Medicine, Korea)
George S. Baillie (University of Glasgow, UK)
G. Allen Finley (Delhousie University, Canada)
Sara Schwanke Khilji (Oregon Health & Science University, USA)
Matthew S. Dunne (Institute of Food, Nutrition, and Health, Switzerland)
Marianne Hokland (University of Aarhus, Denmark)
Marcela Hermoso Ramello (University of Chile, Chile)
Ciro Isidoro (University of Novara, Italy)
Moses Rodriguez (Mayo Clinic, USA)
Robert W. Mann (University of Hawaii, USA)
Wikrom Karnsakul (Johns Hopkins Children's Center, USA)
Frans Laurens Moll (University Medical Center Ultrecht, Netherlands)
James P. Dolan (Oregon Health & Science University, USA)
John Hunter (Oregon Health & Science University, USA)
Nima Rezaei (Tehran University of Medical Sciences, Iran)
Dennis J. Janisse (Subsidiary of DJO Global, USA)
Floker Meyer (Argonne National Laboratory, USA)
David Wayne Ussery (University of Arkansas for Medical Sciences, USA)
Intawat Nookaew (University of Arkansas for Medical Sciences, USA)
Victor Manuel Charoenrook de la Fuente (Centro de Oftalmologia Barraquer, Spain)
Karl Thomas Moritz (Swedish University of Agricultural Sciences, Sweden)

Editorial Board

Watchara Kasinrerk (Chiang Mai University, Thailand)
Rungroj Krittayaphong (Siriraj Hospital, Mahidol University, Thailand)
Wiroon Laupattrakasem (Khon Kaen University, Thailand)
Anuwat Pongkunakorn (Lampang Hospital, Thailand)
Nopporn Sittisombut (Chiang Mai University, Thailand)
Vasant Sumethkul (Ramathibodi Hospital, Mahidol University, Thailand)
Yuen Tanniradorm (Chulalongkorn University, Thailand)
Saranatra Waikakul (Siriraj Hospital, Mahidol University, Thailand)
Pa-thai Yenchitsomanus (Siriraj Hospital, Mahidol University, Thailand)
Surapol Issaragrisil (Siriraj Hospital, Mahidol University,Thailand)
Jaturat Kanpittaya (Khon Kaen University, Thailand)
Suneerat Kongsayreepong (Siriraj Hospital, Mahidol University, Thailand)
Pornchai O-Charoenrat (Siriraj Hospital, Mahidol University, Thailand)
Nopphol Pausawasdi (Siriraj Hospital, Mahidol University, Thailand)
Supakorn Rojananin (Siriraj Hospital, Mahidol University, Thailand)
Jarupim Soongswang (Siriraj Hospital, Mahidol University, Thailand)
Suttipong Wacharasindhu (Chulalongkorn University, Thailand)
Prapon Wilairat (Mahidol University, Thailand)
Pornprom Muangman (Siriraj Hospital, Mahidol University, Thailand)
Ampaiwan Chuansumrit (Ramathibodi Hospital, Mahidol University, Thailand)
Sayomporn Sirinavin (Ramathibodi Hospital, Mahidol University, Thailand)
Vitoon Chinswangwatanakul (Siriraj Hospital, Mahidol University, Thailand)

Statisticians: Saowalak Hunnangkul (Mahidol University, Thailand)
Medical Illustrator: Chananya Hokierti (Nopparat Rajathanee Hospital, Thailand)
Online Assistant: Surang Promsorn, Wilailuck Amornmontien, Hatairat Ruangsuwan **Editorial Office Secretary:** Amornrat Sangkaew

SIRIRAJ MEDICAL JOURNAL is published bimonthly, 6 issues a year (Jan-Feb, Mar-Apr, May-Jun, Jul-Aug, Sep-Oct and Nov-Dec) and distributed by the end of the last month of that issue.
SIRIRAJ MEDICAL JOURNAL is listed as a journal following the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (URM) by the International Committee of Medical Journal Editors (ICMJE) since 9 July 2010 [http://www.icmje.org/journals.html].

SIRIRAJ MEDICAL JOURNAL
www.smj.si.mahidol.ac.th

About the journal

The Siriraj Medical Journal (SMJ), published continuously since 1949, is an international, double-blinded, peer-reviewed, bimonthly journal. SMJ is the official journal of the Faculty of Medicine Siriraj Hospital, Mahidol University, which is the oldest medical school in Thailand. Siriraj Hospital is named after **Prince Siriraj Kakuttaphan**, a dying-young son of His Majesty the King Rama 5, who turned the history of medicine in Thailand from traditional medicine to modern medicine, and who also inspired his son, **Prince Mahidol**, to contribute and improve the public health system of Thailand for the benefits of mankind. SMJ is now available both in printed version and electronic version.

Aim and Scope

SMJ aims to promote scientific communication and innovation among researchers focusing on Medicine and Public Health.

Instructions for Authors

All manuscripts submitted to SMJ must not be previously unpublished or considered for publication elsewhere at any time during SMJ's review period. The editorial office will seek at least two experts who work in different institutions to consider the manuscript content for the peer-review process. The Editor-in- Chief will make the decision regarding acceptance of any manuscripts for publication by taking the opinions from at least one member of the editorial board and from the peer reviewers into the consideration.

Authors are encouraged to submit manuscripts via www.smj.si.mahidol.ac.th

For the text and tables, the manuscripts should be saved in a Microsoft word file. Page setup should be made on the size of A4 paper (210 x 297 mm). The font should be "Times New Roman" with the size of 12 pt. The top, bottom and side margins should be 25 mm. wide. All pages should be numbered consecutively together with the first author's name at the top right-hand corner, beginning with the title page. All digital files should be free of any virus or other malicious threats. No printout of the manuscripts is required. Any manuscript submission that fails to follow this instruction will be returned to the authors without review.

SMJ accepts the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (Seoul, 2008). **Thus, for any new research project involving human research subjects, including case report of more than 1 patient, starting from 1 March 2009, the authors need to submit a photocopy of a document of approval from Institutional Review Board (IRB) or Institutional Ethic Committee (IEC).** For any research project involving human research subjects starting before 1 March 2009 that does not have a document of approval from IRB or IEC, the authors need to convince the editorial board that the research does not violate any ethical concern on human research subjects. **In addition, for any new clinical trial starting from 1 March 2009, the authors need to register at the public clinical trial registry prior to the research conduct. The registration number should be placed at the end of the abstract in parentheses.**

All authors should approve the submission of the manuscript. The cover letter should indicate that the manuscript is submitted to SMJ only and it has never been published elsewhere in English or other languages. Authors should be aware of copyright violation, conflicts of interest, plagiarism, and other misconducts in biomedical research and publication. Written permissions from persons who provide personal communication or from those to be acknowledged in the manuscript are required in case the readers may infer their endorsement of such activities as technical assistance, provision of illustration etc. **Letter of Certification before Publishing Article(s) in Siriraj Medical Journal (SMJ)**

Types of manuscripts

Types of manuscripts subjected to peer review include original articles, review articles, case reports and letters to the Editor.

Original Articles

Original articles should be hypothesis-driven studies. Manuscripts should include an abstract, and appropriate experimental details and convincing results to support the conclusions. It must be written in English with no longer than 3,000 words for the text, excluding abstract, acknowledgments, references, and figure legends. The number of tables and figures/ illustrations should not exceeding 10 in total. The number for references is limited to 50. Manuscripts should contain sections, each of which starts on a new page: Title page, Abstract and keywords, Text (Introduction, Materials and methods, Results, and Discussion), References, Tables (with captions) and Figures (with legends).

Review Articles

Review Articles survey recent development in medicine and public health. The articles have a limit of 5000 words, excluding abstracts, references, tables and figures. The number of references is up to 100. The number of tables and illustrations should not exceed 8 in total. Systematic reviews (and meta-analyses whenever appropriate) are of interest. Manuscripts reporting systematic reviews and meta-analyses should comply with PRISMA statements (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

Case Reports

Case reports should contain no more than 1,500 words for the text, excluding abstract, acknowledgments, references, and legends of the figure. The number of tables and illustrations should not exceed 3 in total. Written permission to publish the case details or use a photograph of the unavoidably identifiable person must be obtained. Manuscripts should contain sections, each of which starts on a new page: Title, Abstract and keywords, Text (Introduction, Case presentation, and Conclusion), References, Tables (with captions) and Figures (with legends).

Letters to the Editor

Letters to the Editor are considered for publication, provided they do not contain material that has been submitted or published elsewhere. They do not contain abstracts or keywords; need not be divided into sections and should not contain figures. Only one table or illustration is permitted. An exception can be allowed by the Editor-in-Chief when appropriate. The text, excluding references, must not exceed 500 words. No more than 10 references are allowed. SMJ considers letters with commentaries and opinions on published papers of which topics may be useful and informative to the scientific community to be submitted.

Manuscript Format

This is strictly applied to the preparation of original articles. The structure includes a title page, abstract and keywords, text [introduction, materials and methods, results, discussion, acknowledgments, references used in Index Medicus (the Vancouver system)], tables with captions, and illustrations/ figures with legends.

Title page

The title page should give a concise title, the first and last names of all authors, their affiliations and the correspondence. The orders of contributors listed should be agreed amongst the investigators. Full contact details should be provided for the corresponding author.

Abstract and keywords

The abstract should be an structured paragraph of no more than 250 words: Introduction, Materials and Methods, Results and Conclusion. Three to ten keywords, which may appear in the title, should be given in alphabetical order below the abstract, each separated by a semicolon.

Introduction

This should give a short, clear account of the background and reasons for undertaking the study by reference only to the pertinent literature. It should not be a review of all literature in the field and do not repeat the Discussion here.

Materials and methods

This section should contain sufficient detail so that all experimental procedures can be repeated by others in conjunction with cited references. It may be divided into subheadings such as participants, intervention, randomization, and statistical analysis. Manuscripts should contain a statement to the effect that all human studies have been reviewed by a named institutional Ethics committee with relevant jurisdiction and that all participants had given, usually written, informed consent before their inclusion in the study. Clinical trials should be registered and the registration number should be stated. Details that might disclose the identity of the subjects under study should be omitted. Authors should also confirm that their work complies with the Code of Ethics of the World Medical Association

Results

The description of results should not reiterate data that appear in Tables and Figures and, likewise, the same data should not be displayed in both Tables and Figures. The Results section should be concise and follow a logical sequence.

Discussion

Do not repeat the Introduction, the Results and Figures and Tables. Discussion should (i) mention whether the results agree with or contradict previous findings, (ii) discuss the significance of the results against the background of existing knowledge, (iii) identify clearly those aspects that are novel, and (iv) state if the original hypothesis was confirmed. The final paragraph should highlight the main conclusion(s), and provide some indication of the direction future research should take.

Acknowledgements

These should be brief, and should include sources of financial support. Acknowledgements should be made only to those who have made a significant contribution to the study.

References

Authors are responsible for the accuracy of the references. These may include published articles as well as those in press. In the text of the manuscript, references to the literature should be numbered consecutively and indicated by a superscript. Each reference should be numbered individually and listed at the end of the manuscript; examples of citation format are given below. All authors should be listed for papers with up to six authors; for papers with more than six, the first six authors only should be quoted, followed by et al.

Examples of journal citation format

1. Akaraviputh T, Arunakul S, Lohsiriwat V, Iramaneerat C, Trakarnsanga A. Surgery for gastrointestinal malignant melanoma: experience from surgical training center. World J Gastroenterol 2010;16:745-8.
2. Lohsiriwat V, Lohsiriwat D, Boonnuch W, Chinswang watanakul V, Akaraviputh T, Riansuwan W, et al. Outcomes of sphincter-saving operation for rectal cancer without protective stoma and pelvic drain, and risk factors for anastomotic leakage. Dig Surg. 2008;25:191-7.
3. Geraud G, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. Headache. 2002;42 Suppl 2:S93-9.

Example of a book citation:

4. Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Example of an article in a book:

5. Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

Example of an article in an electronic book:

6. Akaraviputh T and Trakarnsanga A. Surgicla management of malignant melanoma of gastrointestinal tract. In: Murph M, editor. Melanoma in the clinic-Diagnosis, management and Complications of Malignancy. Available from: http://www.intechopen.com/articles/show/title/surgical-management-of-malignant-melanoma-of-gastrointestinal-tract

The names of journals cited should be abbreviated according to the 'International List of Periodical Title Word Abbreviations'. Citations of 'unpublished data' and e.g. 'S. A. Waksman, personal communication' should be written in the text parenthetically. Manuscripts that have been accepted for publication but are not yet published must be identified in the reference list as 'in press'.

Tables

Tables must supplement, not duplicate, the data in the text or Figures. Tables should consist of at least two columns; columns should always have headings. Tables should have a brief footnote that identifies all abbreviations used. The data in the Tables should be consistent with those cited in the relevant places in the text.

Figures

Figures must supplement, not duplicate, the data in the text or in Tables. Illustrations must clearly convey their message and be of high quality and sufficient size and clarity (especially lettering, arrows and data points) to be interpretable when reduced for publication. Figures should be given a title and numbered sequentially as Figure 1, Figure 2, etc. and cited (as Fig 1., Fig 2. etc.) sequentially in the text. Generate TIFF, EPS or JPG files. Keep all individual layers and place words, arrows and other symbols in one layer.

Written permission to use photograph of potentially identifiable person must be obtained from that person. The authors do not need to send the original written permission with the manuscript but they need to make a statement in the cover letter that they have already obtained the permission.

Summary checklist to submit a manuscript to SMJ

The submitted manuscript will be returned to the author(s) without review if its format is not correct or its content does not fit the scope of the journal. The summary checklist includes a cover letter, a manuscript in Microsoft word file (Abstract, Text, References, Acknowledgements and Tables with captions), a photocopy of a document of ethical approval if more than 1 subject were included, a photocopy showing the clinical trial registration and illustrations/figures in JPEG, TIFF or EPS.

Submission of a manuscript to SMJ

Please submit your manuscript online with **www.smj.si.mahidol.ac.th** or contact Ms. Amornrat Sangkaew (Editorial Office Secretary) **Address:** Siriraj Medical Journal, SiMR Bldg., 2nd Fl., Room No.207 Siriraj Hospital, Bangkok 10700, Thailand **Tel.** (662)419-2884
E-mail address: sijournal@mahidol.ac.th

Siriraj Medical Journal is the official publication organ of The IASGO Thailand and Faculty of Medicine Siriraj Hospital. The journal, the society and the Faculty are nonprofit organizations. The journal's expenses are covered through the Faculty of Medicine Siriraj Hospital's financial resources and advertising revenues. The journal implements the open access publication model. Throughout the publication process, neither the authors or readers charged a fee nor the journal or the Faculty demands any types of fees or charges.

Postoperative Pain Reduction After Additional Intraperitoneal Suction Following Laparoscopic Cholecystectomy: A Prospective Randomized Controlled Study

Akkaraphorn Deeprasertvit, M.D.*, Pichaya Deeprasertvit, M.D., Nisa Netcharussaeng, M.D.***

**Department of Surgery, Police General Hospital, **Department of Anesthesiology, Police General Hospital, Bangkok 10330, Thailand.*

ABSTRACT

Objective: To compare postoperative pain between conventional CO_2 releasing method and additional suction after laparoscopic cholecystectomy (LC) considering PCA Morphine consumption which makes pain measurement more accurate than pain score.

Methods: Eligible patients with indication for LC were randomly assigned preoperatively either to have the conventional CO_2 releasing method, or an additional 60 seconds of suction after LC. We collected demographic data, postoperative pain at 6, 12 and 24 hours, residual intraperitoneal pressure, operative time, intraoperative Morphine amount and Morphine PCA amount in 24 hours. Pain evaluation by PCA amount was designed to get more accurate primary outcomes.

Results: The patients were similarly distributed. Residual intraperitoneal pressure was statistically significantly different. Morphine PCA amount in both groups were not significantly different. Postoperative pain level at 6, 12 and 24 hours as secondary outcomes showed that suction group seemed to have slightly higher pain score at 6 and 12 hours but at 24 hours post LC, pain in suction group tended to be a bit lower than in non-suction group.

Conclusion: Additional CO_2 suction from this study does not reduce postoperative pain. However, with a larger study population, it might help us to consider results in the intervention group better.

Keywords: Post-laparoscopy pain syndrome; laparoscopic cholecystectomy; intra-abdominal pressure (Siriraj Med J 2018;70: 1-5)

INTRODUCTION

Cholecystectomy operation has been developed from open to laparoscopic surgery, which helps in reducing postoperative pain, returning faster to daily activities, and shortening recovery period.[1,2] Despite decreasing of postoperative pain, it is still an unwanted condition the patients receive after laparoscopic cholecystectomy (LC). Postoperative pain consists of incisional pain (parietal pain), deep intra-abdominal pain (visceral pain), and shoulder tip pain due to diaphragmatic irritation.[3,4] A post-laparoscopic pain syndrome is characterized by abdominal and shoulder tip pain for a few days after laparoscopic surgery.[5,6] Shoulder tip pain could be aggravated by mobilization, and it could be so severe that readmission is required. Most patients can tolerate the pain and get discharged from the hospital with oral analgesic agents.

The cause of the post-laparoscopy pain syndrome is thought to be residual carbon dioxide (CO_2) in the abdomen after completion of laparoscopy.[7] There have been some trials about relationship between residual intraperitoneal CO_2 and postoperative pain after LC. One

Correspondence to: Akkaraphorn Deeprasertvit
E-mail: deeprasertvit@gmail.com
Received 14 August 2017 Revised 25 October 2017 Accepted 1 November 2017
doi:10.14456/smj.2018.1

of those was a prospective randomized controlled trial, comparing patients receiving LC in 2 groups. First group had additional residual intraperitoneal CO_2 suction via flexible cannula. Second group released gas from trocar alone, as conventional LC procedures. Results showed significantly better outcome in 24-hour postoperative pain reduction in suction group than in non-suction group. However, there was no difference in hospital stay and recovery.[8]

However, there are not so many prospective randomized controlled studies of the relationship between residual intraperitoneal CO_2 and postoperative pain after LC. Moreover, pain score measurement in those studies were not quite accurate due to variable individual pain thresholds and subjective pain interpretation. Thus for pain measurement in this study, besides visual analog scale, we evaluated pain from analgesic consumption for better accuracy, using patient-controlled analgesia (PCA). This study aimed to compare intraperitoneal gas reducing methods and to choose it properly, which would result in postoperative pain reduction and faster recovery.

MATERIALS AND METHODS

Study Design and Participants

We did a single center, randomized, prospective, double blind (patients and investigator who collected data were blind) study in Police General Hospital, Bangkok, Thailand, from April 2015 to January 2016. LC was performed by 3 experienced surgeons in Police General Hospital, under general anesthesia. Eligible patients were randomly assigned preoperatively either to have the conventional CO_2 releasing method via trocar, or an additional 60 seconds of suction using laparoscopic suction device after LC.

We collected data including age, sex, BMI (kg/m^2), underlying diseases, ASA classification, diagnosis and indication for LC, postoperative pain by visual analog scale (0-10) at 6,12 and 24 hours, residual intraperitoneal pressure, operative time, intraoperative morphine amount, PCA Morphine consumption in 24 hours, and postoperative complications, such as peritonitis, surgical site infection and surgical wound bleeding.

Inclusion criteria were patients between 18 and 70 years of age with indications for LC, such as acute cholecystitis following TG13 diagnostic criteria and severity grading of acute cholecystitis[9], chronic cholecystitis, symptomatic gallstone or polyp, biliary pancreatitis, asymptomatic gallbladder polyp larger than 1 cm or gallstone larger than 2.5 cm. All patients with ASA status 1-3, who had no history of drug allergy, including paracetamol, narcotics, NSAIDs or sulfa. Patients who have been converted to open cholecystectomy, performance of an additional procedure, a surgical indication for postoperative intra-abdominal suction drainage, did not provide informed consent, or had contraindications for LC were excluded.

Randomization and masking

Randomization was done via a computer-generated permuted-block sequence, with a block size of 4. The treatment allocations were put in well-sealed, opaque envelopes and serially numbered. After cholecystectomy procedures, an envelope would be opened to identify intraperitoneal pressure reducing method. Each patient was blinded. Data collector was a doctor, who did not perform or assist the operation, who was not informed of intraperitoneal pressure reducing method intraoperatively.

Procedures

All patients had NPO at least 6 hours before the operation and received Celecoxib 400 mg, 1 capsule per oral on operating day. Prophylactic antibiotics were given 30 minutes before skin incision, which could be either Cefazolin 2 g IV or Ciprofloxacin 400 mg as alternative option, in case of penicillin or cephalosporin allergy. General anesthesia was established then Foley's catheter No.14 was inserted for intra-peritoneal pressure measurement after LC. Intraoperative morphine was titrated and adjusted following patient's vital signs.

LC was started with 3 ports insertion, first one was 10-mm port at umbilicus, second one was 5-mm port 2 fingerbreadths below xiphoid, and third one was 5-mm port below right costal margin crossing anterior axillary line. Pneumoperitoneum was created with 12 mmHg pressure. Critical view of safety was achieved to identify Calot's triangle, then cystic duct and cystic artery were clamped with Hem-o-lok and cut.[10] After gallbladder was dissected from hepatic bed, an envelope was opened to choose intraperitoneal pressure reducing method, either conventional CO_2 reduction via trocar, or with additional suction for 60 seconds using laparoscopic suctioning device. When skin suture was done, intraperitoneal pressure measurement was performed by filling 50 ml of Normal Saline into urinary bladder via Foley's catheter connected to 50 cm approximately extension tube. Then Foley's catheter was clamped for 1 minute then released while holding extension tube upstraight. Pubic symphisis was considered as zero level baseline, and intraperitoneal pressure was measured in cmH_2O.[11,12]

All patients received PCA morphine as the postoperative pain management; morphine 1 mg/ml, no basal rate, PCA

dose 1 mg, lock out interval 6 minutes, 4-hour limited to 30 mg, from the time of recovery from anesthesia until 24 hours postoperatively, as well as 2 tablets of 500 mg Paracetamol orally around the clock every 6 hours. No additional pain control medication was administered. Diet was stepped up postoperatively as liquid, soft and regular diet on day 1, 2 and 3 respectively. Pain score at 6, 12 and 24 hours postoperatively and 24-hour morphine consumption data were collected. Patients were discharged from the hospital when regular diet was tolerable with no postoperative complications.

Outcomes

Primary endpoint was 24-hour morphine consumption. Secondary endpoints were postoperative pain score at 6, 12 and 24 hours, using visual analog scale (0-10), intraperitoneal pressure (mmHg), operative time (minutes), postoperative complications, such as peritonitis, surgical site infection and surgical wound bleeding.

Statistical analysis

In this study, a sample size of 60 (30 per group) was required to compare continuous data using mean±SD between two groups, with 80% power and $\alpha = 0.05$ two-sided likelihood ratio test. We used SD 2.7 as a reference from literature review of Celik's study in 2010.[1] Pain score difference more than 2 was considered as significant difference. Nevertheless, in case of dropping out of some of the patients in our study group, we enrolled 5% more of calculated sample size. Statistical analysis was done using Stata version 14. T-test was used to analyze our results as residual intraperitoneal pressure after LC, pain score, 24-hour morphine consumption, and operative time were continuous variables. Chi-square was used for postoperative complications.

RESULTS

There were 63 eligible patients for LC in the Police General Hospital, Bangkok Thailand, from April 2015 to January 2016. LC was performed by 3 experienced surgeons in the Police General Hospital. Three patients in our study were excluded while receiving protocol treatment due to conversion to open surgery. Thus there were 60 patients randomized into 2 groups; suction and non-suction (Fig 1). The demographic data of 60 patients was shown in the Table 1.

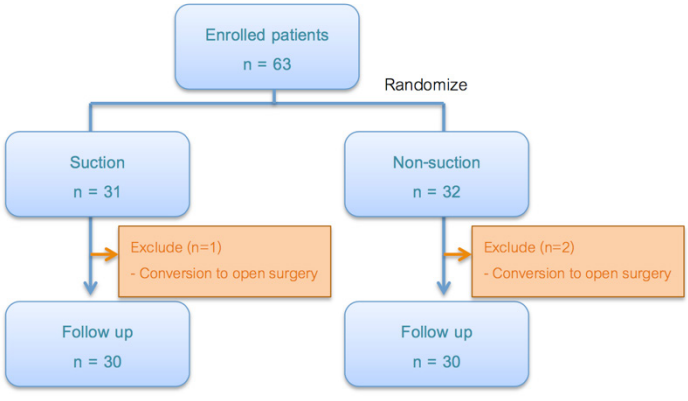


Fig 1. Consort diagram

TABLE 1. Demographic data

Characteristics	Suction (n=30)		Non-suction (n=30)		*p*-value
	n	%	n	%	
Gender					1.000
Female	20	66.7	20	66.7	
Male	10	33.3	10	33.3	
Age (years) (mean±SD)	47.6±13.5		50.2±11.9		0.428
BMI (kg/m^2) (mean±SD)	25.5±5.5		25.2±3.6		0.798
ASA					0.567
1	22	73.3	18	60.0	
2	6	20.0	8	26.7	
3	2	6.7	4	13.3	
Diagnosis					0.642
Cholecystitis	11	37.9	14	46.7	
Symptomatic gallstone	16	55.1	16	53.3	
Symptomatic gallstone polyp	1	3.5	0	0	
Biliary pancreatitis	1	3.5	0	0	

Most patients were female (66.7% and 66.7%) in both groups. Age ranged between 47.6±13.5 in suction group, and 50.2±11.9 in non-suction group respectively. BMI in suction group was 25.5±5.5 and 25.2±3.6 in non-suction group. ASA classifications were not different in both groups. Symptomatic gallstone was the diagnosis mostly found in both groups, with 16 patients each. According to the demographic data, patients in both study groups were not significantly different.

Residual intraperitoneal CO_2 suctioning was performed effectively with data showing significantly less intraperitoneal pressure in suction group ($p = 0.035$). 24-hour morphine consumption was 0.085±0.016 in suction group, and 0.104±0.019 mg/kg in non-suction group respectively (Table 2) with no statistical difference (p=0.464) as well as pain score at 6, 12 and 24 hours (Fig 2).

In this study, we collected data of intraoperative morphine use, which might affect postoperative morphine consumption and pain score. We found no significant difference between morphine consumption in both groups, which was 6.1±4.7 in suction group, and 7.1±4.4 mg/kg in non-suction group respectively ($p = 0.384$).

Mean operative time in suction group was 73.8±29.5 minutes, and 91.5±49.3 minutes in non-suction group, which took longer time in non-suction group than in suction group, without statistical difference ($p = 0.096$). Patients in both groups had no postoperative complications.

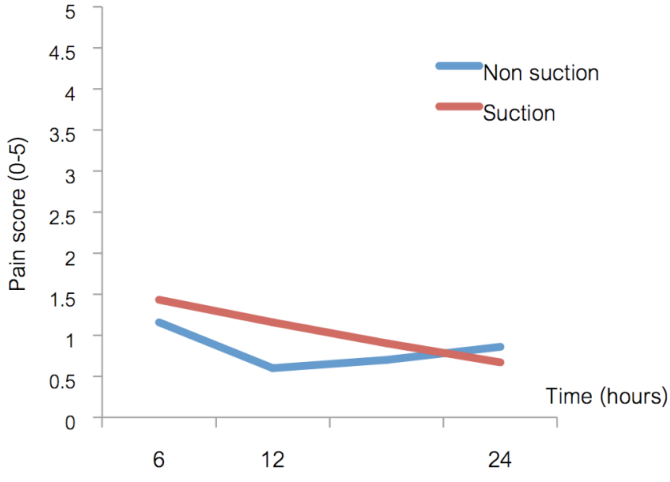


Fig 2. Postoperative pain score at 6, 12 and 24 hours

TABLE 2. Primary and secondary outcome.

	Suction (n=30)	Non-suction (n=30)	*p*-value
24-hr Morphine consumption (mg/kg)	0.085±0.016	0.104±0.019	0.464
Pain score			
6-hr Pain score	1.43±0.28	1.16±0.35	0.561
12-hr Pain score	1.16±0.31	0.60±0.24	0.156
24-hr Pain score	0.67±0.19	0.86±0.24	0.522
Intraoperative morphine (mg/kg)	6.1±4.7	7.1±4.4	0.384
Operative time (minutes)	73.8±29.5	91.5±49.3	0.096
Intraperitoneal pressure (mmHg)	4.1±2.1	5.7±3.5	0.035

DISCUSSION

Since the first LC by Prof. Dr. Erich Mühe in 1985[13], LC is nowadays considered as a gold standard surgery for cholelithiasis. Compared with open cholecystectomy (OC), LC causes less pain, shorter recovery, and shorter hospital stay. Nevertheless, the most common complaint after LC is postoperative pain.[14] Various causes of the pain are due to peritoneal stretching, diaphragmatic irritation by CO_2 or CO_2 absorption from the peritoneal cavity.[3,4,15] Previous studies of the relationship between residual intraperitoneal CO_2 and postoperative pain after LC are still inconclusive.

Joris et al., performed a prospective study on patients who underwent LC in order to investigate the time course of different pain components after LC. The results showed that postoperative pain mostly occurred within the first 24 hours.[16] Therefore we chose to evaluate visceral pain following this study. Pain level is caused by multiple factors, and pain score measurement in previous studies were not quite accurate due to variable individual pain thresholds and subjective pain interpretation. Besides using visual analog scale in our study, we measured pain level by analgesic consumption using patient-controlled analgesia (PCA) for finer accuracy.

Jorgensen et al., placed suprahepatic drain in all patients who underwent LC. Patients in treatment group received additional suction via suprahepatic drain which resulted in a dramatic decrease in the severity of shoulder tip pain and more favorable recovery than in control group.

The residual gas causes a loss of surface tension between visceral and parietal peritoneum especially at hepatic and diaphragmatic regions.[7] In the same way as a study of Sarvestani et al., residual pneumoperitoneum was calculated by the length of the diaphragmatic arc and height of the gas bubble below each hemi-diaphragm in 24-hour postoperative chest x-ray. The pneumoperitoneum was graded as absent, mild (1-5 mm), moderate (6-10 mm) and severe (>11 mm). The results showed significant lower pain score in absent and mild grade group than in moderate and severe group.[8]

In our study, the residual intraperitoneal CO_2 suction did not affect postoperative pain score. Moreover, intraperitoneal pressure was not related either to pain score or 24-hour morphine consumption, although it was a more accurate variable than pain score. This could be a result of different CO_2 reducing technique from other studies. Mean residual intraperitoneal pressure in our study, in suction group and non-suction group were 4.1±2.1 and 5.7±3.5 mmHg respectively. This was considered as very small amount compared with the results of previous studies of Lee et al., in which residual intraperitoneal pressure was left higher than 10 mmHg after manual decompression. Patients with high intraperitoneal pressure would have moderate pain[17], when in our study, both groups experienced only mild pain, which explained why there was no difference of postoperative morphine use.

Residual intraperitoneal CO_2 suction only took 1 minute, but operative time was longer in non-suction group with no statistically significant difference compared to suction group. We think it might be due to operation difficulty in some complicated cases in non-suction group, rather than suctioning itself.

The limitation in this study is that we did not collect data of the shoulder-tip pain which can occur after most laparoscopic surgery. Such pain could be attenuated by active suction of residual CO_2.[18,19] Further study is required to prove the effect of active suction of CO_2 and shoulder-tip pain.

In conclusion, residual intraperitoneal CO_2 suction could reduce intraperitoneal pressure effectively but did not decrease postoperative pain score or 24-hour morphine consumption.

REFERENCES

1. Celik AS, Frat N, Celebi F, Guzey D, Kaplan R, Birol S, et al. Laparoscopic cholecystectomy and postoperative pain: is it affected by intra-abdominal pressure? Surg Laparosc Endosc Percutan Tech. 2010;20:220-222.
2. Singla S, Mittal G, Raghav, Mittal RK. Pain management after laparoscopic cholecystectomy-a randomized prospective trial of low pressure and standard pressure pneumoperitoneum. J Clin Diagn Res. 2014;8(2):92-4.
3. Ekici Y, Bozbas H, Karakayali F, Salman E, Moray G, Karakayali H, et al. Effect of different intra-abdominal pressure levels on QT dispersion in patients undergoing laparoscopic cholecystectomy. Surg Endosc. 2009;23:2543-2549.
4. Bisgaard T, Kehlet H, Rosenberg J. Pain and convalescence after laparoscopic cholecystectomy. Eur J Surg. 2001;167:84-96.
5. Alexander JI, Hull MGR. Abdominal pain after laparoscopy: the value of a gas drain. Br J Obstet Gynaecol. 1987;94:267-269.
6. Chamberlain G. The recovery of gases insufflated at laparoscopy. Br J Obstet Gynaecol. 1984;1:367-370.
7. Jorgensen JO, Gillies RB, Hunt DR, Caplehorn JR, Lumley T. A simple and effective way to reduce postoperative pain after laparoscopic cholecystectomy. Aust N Z J Surg. 1995;65:466-469.
8. Sarvestani A, Zamiri M. Residual pneumoperitoneum volume and postlaparoscopic cholecystectomy pain. Anes Pain Med. 2014;4(4):e17366.
9. Yokoe M, Takada T, Strasberg SM, Solomkin JS, Mayumi T, Gomi H, et al. TG13 diagnostic criteria and severity grading of acute cholecystitis. J Hepatobiliary Pancreat Sci. 2013;20(1):35-46.
10. Strasberg SM, Brunt LM. Rationale and use of the critical view of safety in laparoscopic cholecystectomy. J Am Coll Surg. 2010;211(1):132-138.
11. Malbrain ML. Different techniques to measure intra-abdominal pressure (IAP): time for a critical re-appraisal. Intensive Care Med. 2004;30(3):357-371.
12. Kirkpatrick AW, Roberts DJ, De Waele J, Jaeschke R, Malbrain ML, De Keulenaer B, et al. Intra-abdominal hypertension and the abdominal compartment syndrome: updated consensus definitions and clinical practice guidelines from the World Society of the Abdominal Compartment Syndrome. Intensive Care Med. 2013;39:1190-1206.
13. Reynolds W. The first laparoscopic cholecystectomy. J Socie Laparoendosc Surg. 2001;5:89-94.
14. Bisgaard T, Klarskov B, Kristiansen VB, Callesen T, Schulze S, Kehlet H, et al. Multi-regional local anesthetic infiltration during laparoscopic cholecystectomy in patients receiving prophylactic multi-modal analgesia: a randomized, double-blinded, placebo-controlled study. Anesth Analg. 1999;89:1017-1024.
15. Wallace DH, Serpell MG, Basxter JN, O'Dwyer PJ. Randomized trial of different insufflation pressures for laparoscopic cholecystectomy. Br J Surg. 1997;84: 455-458.
16. Joris J, Thiry E, Paris P, Weerts J, Lamy M. Pain after laparoscopic cholecystectomy: characteristics effect and effect of intraperitoneal bupivacaine. Anesth Analg. 1995;81:379-384.
17. Lee JS, Kim EY, Lee SH, Han JH, Park SK, Na GH, et al. A simple method of reducing residual intraperitoneal carbon dioxide after laparoscopic cholecystectomy: a prospective, randomized, controlled study. J Laparoendosc Adv SurgTech A. 2014;24(8):563-566.
18. Esmat ME, Elsebae MM, Nasr MM, Elsebaie SB. Combined low pressure pneumoperitoneum and intraperitoneal infusion of normal saline for reducing shoulder tip pain following laparoscopic cholecystectomy. World J Surg. 2006;30:1969-1973.
19. Haghgoo A, Chaichian S, Ghahremani M, Nooriardebili S, Akbaian A, Moazzami B. The use of peritoneal suction drainage to reduce shoulder pain caused by gynecologic laparoscopy. Arch Iran Med. 2016;19(3):173-178.

A Simple and Practical Amniocentesis Model and the Procedure Success Rate After Training

Pharuhas Chanprapaph, M.D., M.Sc.*, Nantivadee Mamuang, M.D., Sanitra Anuwutnavin, M.D.***

**Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, **Charoenkrung Pracharak Hospital, Bangkok 10120, Thailand.*

ABSTRACT

Objective: To compare success rate of amniocentesis between model trained and non-model trained groups.

Methods: Siriraj amniocentesis simulator was practiced by 5 inexperienced obstetric residents. Forty aspirations of red solution from the targeted balloons using spinal needle number 20-G under the ultrasound guidance were performed. Thereafter, each trainee was allowed to perform the real amniocentesis under an expert supervision within a 5-minute period. Amniocentesis success rate of previous 5 non-model trained residents was retrospectively reviewed and considered as the control group.

Results: A comparison between each group was done to evaluate the success rate. There were 31 and 29 of amniocenteses performed by model trained and non-model trained groups, respectively. In comparison, the former had a non-significantly higher success rate (96.8% vs 82.2%; $p > 0.05$) with a significantly shorter operating time (34.27 ± 21.7 vs 73.38 ± 68.5 seconds; $p < 0.05$) than the latter. No obstetric adverse outcome was observed in both groups.

Conclusion: The amniocentesis-training program with model is beneficial for the beginners to develop their skill prior to the real practice, especially shorter operating time. There was a trend of higher success rate in the model-training group.

Keywords: Amniocentesis; success rate; training model; operative time (Siriraj Med J 2018;70: 6-11)

INTRODUCTION

Amniocentesis is a common invasive procedure performed during pregnancy for a variety of diagnostic and therapeutic indications. Amniocentesis for prenatal diagnosis is usually performed between the 15th and 20th week of gestation. Major complication associated with amniocentesis are rupture of membranes occurring 0.1-1.7 %.[1-3] Chorioamnionitis was present less than 1% whereas post procedural fetal loss was reported to be 0.6-1%.[4-7] Recently, post procedural abortion rate has been reported as low as 0.11% in one meta-analysis.[8] In the past, obstetric residents performed amniocentesis procedure using freehand technique under close supervision of the expert without prior model training. To achieve high success rate, the operators were required to skillfully and accurately insert a needle to the target and gently aspirate the amniotic fluid with ultrasound guidance. Therefore, we have innovated "Siriraj amniocentesis training model" to facilitate the beginners to practice and earn their operative proficiency without any harm to the patients. Thereafter, a one-week training program with 40-aspiration achievements was obligated prior to the actual amniocentesis. The main objective of this study was to compare the success rate and duration of amniocentesis performed between the model-trained group and previous non-model trained group.

MATERIALS AND METHODS

The research was conducted as a cross-sectional study at Maternal-Fetal Medicine Unit, Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University with an approval

Correspondence to: Pharuhas Chanprapaph
E-mail: pharuhasc@gmail.com
Received 9 June 2017 Revised 10 August 2017 Accepted 12 September 2017
doi:10.14456/smj.2018.2

of the institutional ethics committee (Si 260/2007). The medical records of pregnant women who underwent amniocentesis performed by ten 2nd year obstetrics and gynaecology residents between June 1, 2006 and May 30, 2007 were extensively reviewed. All participants (he or she) with average age of 25 years old had never before performed any invasive prenatal diagnosis. They were classified into non-model trained and model trained groups. Before the model invention, all apprentices in the past had to attend and assist the expert to perform amniocentesis for at least five procedures before they were subjectively evaluated and allowed to do the real procedure under supervision. The data of amniocentesis performed by five last non-model trained residents were retrospectively reviewed as the control group.

In the latter group, a 1-hour teaching session of amniocentesis simulator was prepared for each trainee, which consisted of five residents for 1 week. At the end of the session, their performances were systematically evaluated by one fetal medicine specialist (PC) using the assessment criteria of probe holding technique, needle control skill, target achievement, and operative time. The amniocentesis success rate performed by five model-trained residents were analyzed and compared to the first group.

All pregnant women had met the inclusion criteria of 1) singleton pregnancy 2) gestational age of 16-20 weeks 3) fulfilled the indication for amniocentesis such as advanced maternal age, familial history of abnormal chromosome, abnormal ultrasonographic finding of fetus and abnormal serum screening test results. Multifetal pregnancies or therapeutic amnioreduction were excluded from the study.

The Training Model and Practicing Program

Siriraj amniocentesis training model is designed by using the commercial household equipment consisting of a medium-sized (30 x 22 x 12 cm.) kitchen plastic food storage container with a waterproof lid. The center of the lid is removed in rectangular shape to make a plastic frame attached with a rubber sheet (17 x 25 cm.). This container is used to simulate intrauterine environment as the lid works as an anterior abdominal wall of a pregnant woman. Another rubber sheet is placed on the bottom of the container to prevent sonographic reverberation effect from the ultrasound probe. Then, the targets are prepared by using 5 or more elastic rubber balloons in different sizes filled with the red solution (few drops of Utaitip Thai herbal tint (Osotspa Company, Thailand) dissolved in 500 ml water). This red tint is made from numerous herbs namely Fang Yuan, Safflower, Saffron, Cinnamon, Jasmine and Lotus pollen. All balloons are finally tied to the rubber floor firmly to act as a target of needle aspiration. To make the sonographic visualization more realistic, a 20-week size of fetus dummy is placed alongside the balloons. It will help the beginner to develop needle control skill and make no harm to the fetus inside the uterine cavity. Finally, 8,000 ml of water is fully filled into the plastic container and the lid is perfectly sealed as shown in Fig. 1 and 2.

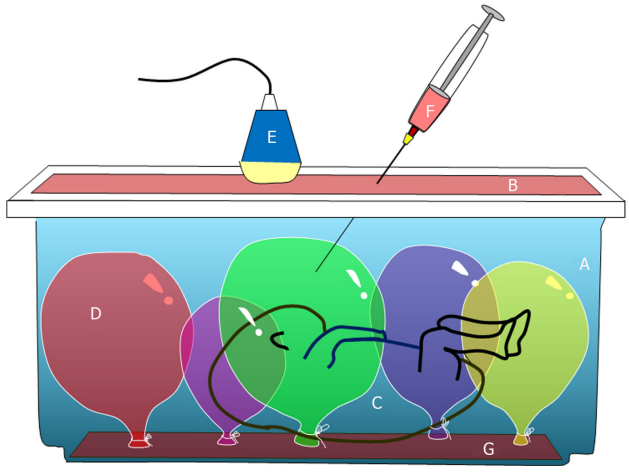


Fig 1. Siriraj amniocentesis training model diagram. A) Transparent plastic box (30 x 22 x 12 cm.). B) Cover lid with a rubber sheet in the middle. C) Fetal dummy (20-week size). D) Elastic balloons filled with red solution tied to the rubber floor. E) Transabdominal ultrasound probe. F) A 20-G spinal needle with a 10-ml. disposable syringe. G) Rubber sheet at the bottom.



Fig 2. Siriraj amniocentesis training model assembly. A) Top view. B) Close the lid. C) Side view. D) Top view of the model sealed with the lid.

During the one-week of practice, each trainee had to puncture the balloons by using a 20-gauge spinal needle under ultrasound guidance as shown in Fig 3. (Model Logiq 3, General Electric Company, Waukesha, U.S.A., and a convex transabdominal probe of 3-7 MHz). To accomplish this, one had to advance the needle towards the target correctly and aspirate the red solution from

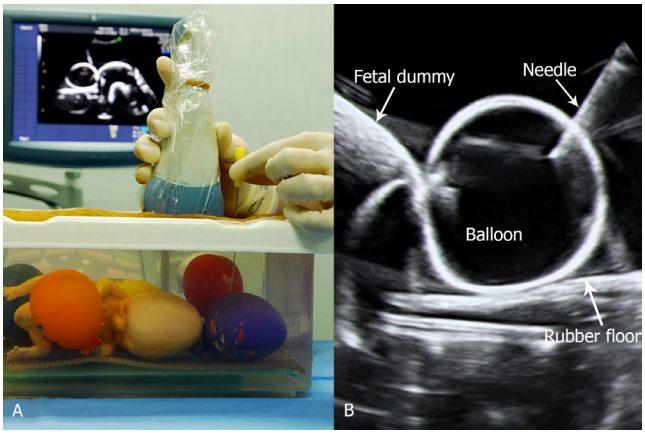


Fig 3. Amniocentesis training program. A) In training practice. B) Ultrasound findings.

the balloons for 40 successive times. At the end of the program, the trainees should be able to orientate transabdominal ultrasonographic probe appropriately and control needle efficiently. Thereafter, they will be allowed to perform the real amniocentesis under strict supervision.

In both groups, the amniocentesis was performed by using freehand technique under the expert supervision with transabdominal ultrasound guidance. A real time ultrasonographic scanner permitted confirmation of number, viability gestational age, location of placenta and the targeted amniotic fluid pocket. The puncture site was chosen based on the accessibility and quality of visualization. The procedure was carried out in an outpatient setting with sterile technique. The amniotic fluid of 20 ml. was aspirated using a 20-gauge spinal needle. Each trainee was limited to perform the procedure within 5 minutes per attempt. The duration was measured from the time the needle was inserted through the maternal skin until the aspiration was successful and the needle was withdrawn. Thereafter, the puncture site was observed for bleeding and the fetal heart rate was monitored for 30 seconds. The procedure was considered as failure if more than one attempt of uterine puncture occurred or the duration lasted longer than 5 minutes. In such case, the supervisor would take the responsibility to finish the procedure.

All relevant demographic data of pregnant patients as well as duration of procedures, placental site, route of procedure (transamniotic or transplacental), number of needle punctures, and related complication within 2 weeks after procedure were evaluated and compared between the two groups. Statistical analysis was performed by SPSS version 18.0 for windows (SPSS Inc., Chicago, IL, USA). Patient's demographic data were analyzed regarding mean, percentage, mean difference, standard deviation, and confidence interval. The Pearson Chi-square and Fisher's Exact test were used for the comparison between the groups. The independent sample *t*-test was used for comparing descriptive demographic data between the groups. *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Sixty medical records of pregnant women who underwent amniocentesis were eligible for this study. Thirty one cases were performed by model trained group and the remaining were done without. The data showed no difference of demographic data between both groups. The maternal age ranged from 23 to 46 years with gestational age of 18 weeks (Table 1).

TABLE 1. Demographic data in both groups were presented in mean ± standard deviation.

Characteristic data	Model-trained group (Range or %)	Non-model trained group (Range or %)
Age (years)	37.32 ± 3.439 (26-46)	36.76 ± 4.09 (23-43)
Body mass index (kg/m^2)	22.69 ± 3.36 (17.78-32.47)	24.08 ± 2.85 (18.90-29.62)
Gestational age (weeks)	18.71 ± 1.189 (16-21)	18.48 ± 0.95 (16-21)
Amniocentesis indications		
Advanced maternal age	28 (90.3%)	27 (91.7%)
Previous abnormal chromosome child	2 (6.5%)	2 (6.9%)
Others	1 (3.2%)	0 (0)

The most common indication for amniocentesis was advanced maternal age followed by previous abnormal chromosome child. The number of posterior placenta was slightly higher than the anterior placenta and transamniotic approach was the major route (83.3%) in both groups (Table 2).

Regarding the success rate, there was a trend towards the model trained group than that of the non-model trained group although the results were not significantly different (96.8% vs 82.2%; $p > 0.05$, Fisher's exact test). The mean duration of procedure in model trained group was 40 seconds shorter than that of non-model trained group with statistical significance ($p < 0.05$) (Table 3). Among six failure procedures, five of them were from the non-model trained group. The most common cause of failure was from needle disorientation. Neither amniotic fluid leakage nor fetal death was observed within 2 weeks after procedure.

TABLE 2. Data of amniocentesis procedure.

Characteristic data	Model-trained group (n=31)	Non-model trained group (n=29)
Placental site		
Anterior	14 (45%)	12 (41.4%)
Posterior	16 (51.6%)	17 (58.6%)
Lateral	1 (3.2%)	0
Puncture approach		
Transamniotic	28 (90.3%)	22 (75.9%)
Transplacental	3 (9.7%)	7 (24.1%)

TABLE 3. Comparison of the success rate and duration of amniocentesis procedure between model trained and non-model trained groups.

Group	Cases	Failure	Success rate	P-value (<0.05)	Mean Duration (sec)	Range (sec)	P-value (<0.05)
Model trained	31	1	96.8%	0.098	34.27	15-130	0.006
Non-model trained	29	5	82.8%		73.38	17-300	

DISCUSSION

Amniocentesis is a common and useful invasive technique performed during second trimester of pregnancy for a variety of diagnostic and therapeutic indications. With the use of assisted reproductive technology in advanced age women, the high incidence of multifetal gestations has also dramatically increased. Regarding the first-trimester fetal aneuploidy screening including cell-free DNA test, its high performance has a large impact to the decreasing number of total invasive procedures especially amniocentesis.[9,10] This procedure is sometimes challenging and may lead to adverse fetal complications. In the past, post procedural fetal loss rate in singleton pregnancy and uncomplicated twin pregnancies were reported to be 0.6-1%[4-7] and 0.96%,[11] respectively. To gain experience, the beginner required practice on many human subjects. Initially, the learning curve of each performer was obtained slowly under the stressful supervision. As a result, the procedure related complications and adverse outcome may increase. Therefore, preclinical training with simulation model is essential and encouraging for all beginners to minimize the fetal risk.[12-15] As a result, post amniocentesis fetal loss rate has recently been reported as low as 0.11% in one meta-analysis.[8] In the future, prenatal invasive test like amniocentesis will only be preserved for the authorized operators who can complete model-training course.

Obviously, the model helped the trainee to be familiar with probe orientation and needle control shortly after training. Our data showed the favorable outcome in

the model trained group with the higher success rate and the shorter procedure duration compared to the non-model trained group. Maher JE, et al reported an amniocentesis stimulator for training since 1998.[16] However, to construct the model is a time consuming process in which gelatin mixture must be prepared and preserved in the refrigerator 8 to 12 hours before the training session. Recently, several amniocentesis-training models have been proposed to simulate the real procedure and facilitate the beginner to develop operative skill with impressive outcome.[12-14] Some of the simulators require fresh meat including chicken breast, beef heart, pig's uterus and embryo to imitate subcutaneous fat, uterus and fetus.[12,17] In practice, these raw materials are costly and hard to preserve and may generate undesirable odor.

Therefore, we have designed our own simple but practical amniocentesis instructional model for a one-week training course. Aforementioned, it is constructed from safe and available household materials which costs lower than 30 US dollars. The whole unit is convenient to be prepared and assembled within 20 minutes. It is also very easy to clean up and preserve the model without any decomposing materials. The only consumable part of this model is the targeted balloons which need to be replaced after training. By changing the size and the position of balloons, the angle between the needle and probe can be adjusted to improve the skill. The balloon may be replaced by disposable examination gloves or condoms if necessary.[18] The red solution allows the operator to self-evaluate the success of the aspiration and it could be omitted or be replaced by any other color solution.

The disadvantage of our model is not to offer real tissue resistance sensation during puncture. However, it allows the learners to develop the most essential skill of keeping the needle track inside the ultrasound beam towards the targeted balloon. Additionally, the use of this model can make both operators and patients overcome anxiety during the clinical practice. The most common cause of amniocentesis failure in this study resulted from needle disorientation found mainly in the non-model trained group. However, no serious complication was observed in both groups.

Since then, every obstetrics and gynecologist resident in the 2nd year of training in our department has to attend one-hour teaching session with the expert. They have to complete the amniocentesis model-training course with 40 successful balloon aspirations before being allowed to perform the real amniocentesis under strict supervision. According to our annual report, there were at least 1/3 of the total amniocenteses which were performed by 2nd year residents per year. Our study of 3,307 patients from 5-year data collection revealed that total pregnancy loss within 4 weeks of amniocentesis was only 0.2% (95% confidence interval, 0.1%-0.4%) which was lower than previous reports and comparable with the recent meta-analysis review.[8] This finding obviously represented the high-quality performance of our institute and also reflected the impact of the amniocentesis skill of the operators acquired by the model training program.

In summary, our data shows that the amniocentesis-training program with model is beneficial for the beginners to develop their skill prior to the real practice, especially shorter operating time. There is a trend of higher success rate in the model-training group.

ACKNOWLEDGMENTS

We would like to thank you Dr. Chulaluk Komoltri from the Division of Clinical Epidemiology for her valuable advice and statistical analysis. Our gratitude also extends to Ms. Prakong Chuenwattana and Mrs. Sommai Viboonchat from Maternal-Fetal Medicine Unit, Department of Obstetrics and Gynaecology Siriraj Hospital for their assistance in amniocentesis training model set up.

Disclosures of Potential Conflicts of Interests

All authors in this research have no potential conflict of interest.

REFERENCES

1. Philip J, Silver RK, Wilson RD, Thom EA, Zachary JM, Mohide P, et al. Late first-trimester invasive prenatal diagnosis: results of an international randomized trial. Obstet Gynecol 2004 ; 103:1164-73.
2. Borgida AF, Mills AA, Feldman DM, Rodis JF, Egan JF. Outcome of pregnancies complicated by ruptured membranes after genetic amniocentesis. Am J Obstet Gynecol 2000; 183: 937-9.
3. Randomised trial to assess safety and fetal outcome of early and midtrimester amniocentesis. The Canadian Early and Mid-trimester Amniocentesis Trial (CEMAT) Group. Lancet 1998; 351: 242-7.
4. Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. Cochrane Database Syst Rev 2003: CD003252.
5. Kong CW, Leung TN, Leung TY, Chan LW, Sahota DS, Fung TY, et al. Risk factors for procedure-related fetal losses after mid-trimester genetic amniocentesis. Prenat Diagn 2006; 26: 925-30.
6. Reid KP, Gurrin LC, Dickinson JE, Newnham JP, Phillips JM. Pregnancy loss rates following second trimester genetic amniocentesis. Aust N Z J Obstet Gynaecol 1999; 39: 281-5.
7. Workman MR, Philpott-Howard J. Risk of fetal infection from invasive procedures. Hosp Infect 1997; 35: 169-74.

8. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015; 45: 16-26.
9. Ekelund CK, Jorgensen FS, Petersen OB, Sundberg K, Tabor A, Danish Fetal Medicine Research G. Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study. BMJ 2008; 337: a2547.
10. Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2015; 45: 249-66.
11. Kan AS, Lee CP, Leung KY, Chan BC, Tang MH, Chan VH. Outcome of twin pregnancies after amniocentesis. J Obstet Gynaecol Res 2012; 38: 376-82.
12. Cordier AG, Fuchs F, Tassin M, Saada J, Letourneau A, Brisset S, et al. Teaching invasive prenatal procedures: effectiveness of two simple simulators in training. Prenat Diagn 2016; 36: 905-10.
13. Nizard J, Duyme M, Ville Y. Teaching ultrasound-guided invasive procedures in fetal medicine: learning curves with and without an electronic guidance system. Ultrasound Obstet Gynecol 2002; 19: 274-7.
14. Pittini R, Oepkes D, Macrury K, Reznick R, Beyene J, Windrim R. Teaching invasive perinatal procedures: assessment of a high fidelity simulator-based curriculum. Ultrasound Obstet Gynecol 2002; 19: 478-83.
15. Smith JF, Jr., Bergmann M, Gildersleeve R, Allen R. A simple model for learning stereotactic skills in ultrasound-guided amniocentesis. Obstet Gynecol 1998; 92: 303-5.
16. Maher JE, Kleinman GE, Lile W, Tolaymat L, Steele D, Bernard J. The construction and utility of an amniocentesis trainer. Am J Obstet Gynecol 1998; 179: 1225-7.
17. Zubair I, Marcotte MP, Weinstein L, Brost BC. A novel amniocentesis model for learning stereotactic skills. Am J Obstet Gynecol 2006; 194: 846-8.
18. Karasahin E, Alanbay I, Ercan M, Yenen MC, Dede M, Baser I. Simple, cheap, practical and efficient amniocentesis training model made with materials found in every obstetrics clinic. Prenat Diagn 2009; 29: 1069-70.

Laparoscopic Bile Duct Exploration in Treating Difficult Common Bile Duct Stones in Sawanpracharak Hospital, A Tertiary Center in the Lower Northern Region of Thailand

Suppadech Tunrattanakul, M.D.

Department of Surgery, Sawanpracharak Hospital, Nakornsawan 60000, Thailand.

ABSTRACT

Objective: To study the effectiveness of laparoscopic bile duct exploration in treating patients with difficult common bile duct stones (CBD stones) in Sawanpracharak Hospital, a single tertiary hospital.

Methods: Retrospective data collection of patients with difficult common bile duct stones treated with laparoscopic bile duct exploration (LBDE) from September 2015 to December 2016.

Results: Thirteen patients were included in the study (nine males, six females) ranging from 26 to 92 years of age (Mean: 67.3 ± 23.3). Operative time and hospital stay averaged 169 minutes (95% CI: 144.3 - 194.9) and 7.6 days (95% CI: 4.8 - 10.4) respectively. All bile duct stones were successfully cleared. There were no mortalities and one patient (8.3 %, 95% CI: 2.1 - 38.5) suffered from a superficial surgical site infection. Conversion to open surgery was required in three cases (20.0 %, 95% CI: 4.3 - 48.1), due to Mirizzi's syndrome.

Conclusion: In patients with difficult common bile duct stones, laparoscopic bile duct exploration appears to be a safe and effective form of treatment. However, due to the variety of difficult common bile duct stones cases, the open surgery conversion rate and duration of treatment times were both relatively high.

Keywords: Laparoscopic bile duct exploration (LBDE); difficult common bile duct stones (Siriraj Med J 2018;70: 12-16)

INTRODUCTION

Common bile duct stones (CBD stones) or choledocholithiasis develops in about 10-20% of patients with gallstones.[1] Endoscopic management is accepted as a mainstay intervention.[1,2] However, in approximately 10 -15% of CBD stones endoscopic management may prove to be challenging, and in some studies these are commonly referred to as "difficult CBD stones".[3,4] The primary characteristics of difficult CBD stones include the presence of a large number of stones (>10), a large stone (a diameter of >15 mm), unusual morphology, the stone being in a difficult location (e.g. intra hepatic, cystic duct, proximal to stricture), or the presence of Mirrizi's syndrome.[3]

Other contributing factors may include difficulty of accessing the bile duct (such as periampullary diverticulum), post-gastrectomy Billroth type II anatomy or Roux-en -Y-gastrojejunostomy.[3] In such cases, challenging endoscopic techniques and/or more sophisticated endoscopic equipment are required.[3,4] Subsequent endoscopic sessions may also be required in order to clear all stones and stone fragments, and in some cases, additional surgical procedures may be required.[3-5]

Sawanpracharak Hospital is a tertiary hospital located in the lower northern region of Thailand. Endoscopic treatment is the mainstay management of CBD stones, although the ability to deal with difficult CBD stones cases endoscopically remains limited and restricted by the lack of more specialized endoscopic equipment. Therefore, laparoscopic bile duct exploration (LBDE) has been

Correspondence to: Suppadech Tunruttanakul
E-mail: sdtaaa@yahoo.com
Received 26 July 2017 Revised 26 September 2017 Accepted 3 October 2017
doi:10.14456/smj.2018.3

utilized as an alternative option. It's efficacy has been supported by medical evidence[6,7] and it has been practiced in some centers, as the mainstay management of CBD stones.[5,8,9] Studies have shown that in many aspects it is comparable to endoscopic treatment. Benefits include a shorter treatment period and the possibility of removing the gallbladder in the same session.[6,7,10] It follows then, that in locations, where specialized endoscopic equipment is lacking, but general laparoscopic equipment is readily available, LBDE appears to be a plausible solution for difficult CBD stones. The aim of this study was to explore the safety and effectiveness of LBDE treatment for patients affected by difficult CBD stones.

MATERIALS AND METHODS

The target population were patients with difficult CBD stones, who were treated with LBDE in Sawanpracharak Hospital from September 2015 to December 2016. The methods that were utilized to detect the presence of CBD stones in the bile duct were endoscopic retrograde cholangiography (ERC), magnetic resonance cholangiopancreatography (MRCP) or ultrasonography (US).

Classification criteria for classifying the stones as "difficult CBD stones" included if stone's diameter was ≥ 15mm, if the numbers of stones was more than 10, if the CBD stone(s) could not be completely cleared in one session of endoscopic treatment (endoprothesis- stent inserted during sessions), and finally if the bile duct could not be accessed after one or two attempts.

The data collected included sex, age, classification of difficult CBD stones as described above, bile duct diameter (as acquired from ERC and MRCP), duration of operation in minutes, if the case was converted to open surgery (inclusing reason), usage of T tube and/or drain, length of hospital stay and any complications.

Data Collection and Statistical Analysis

Data was retrospectively acquired from our database utilizing endoscopic data collection software (Endosmart, Kainatic engineering Co., Ltd., Thailand) and hospital records.

Data was expressed using mean ± standard deviation or percentage concurrently with a 95% confidence interval.

This study was approved by the Sawanpracharak Hospital Medical Ethics Committee (Certification number: 14/ 2560).

RESULTS

There were a total of fifteen patients included. Characteristics of the patients were summarized in Table 1 concurrently with the respective- definition of difficult CBD stones. Most of the LBDE results indicated that the stones were too large to be cleared by general endoscopic equipment. All except two patients, were diagnosed as having difficult CBD stones by ERC and preliminarily treated with biliary stent insertion. In respect of the two patients, accessing of bile duct failed in both cases due to excessively redundant duodenal folds resulting in failure to identify the ampulla's opening. MCRP and ultrasound for CBD stone detection was then utilized for investigation and confirmation in these cases.

There was one case where three attempts of ERC and stone removal were performed, before resorting to LBDE. In three cases, during the LBDE session, open surgery was deemed necessary, due to the detection of Mirizzi's syndrome, cholecysto-biliary fistula type (Corlette Classification type II[11]) and Csendes classification of at least type III since it involved more than two thirds of common bile duct diameter.[12] In only one case, was the pathology suspected during ERC as it was indicated by impacted stones in the cystic duct junction area. In another two cases, ERC only revealed the presence of numerous stones filling the proximal part of the bile duct which narrowed distally.

The results of LBDE were displayed in Table 2 and all the patients' data was summarized in Table 3. On average, the patients were 67.3 ± 23.3 years of age, ranging from 26 to 92 years of age. The patients were mostly male (60 %). The average duration of operation was 169 (± 39.9) minutes or approximately three hours. The conversion rate to open surgery was 20 % (three cases). All three patients subsequently had their bile ducts reconstructed with Roux-en-Y hepaticojejunostomy.

All but three patients treated with LBDE were discharged after approximately one week. One of the three LBDE patients with an extended stay in the hospital suffered a superficial surgical site infection.

T-tubes were inserted in two patients because of an inability to pass a choledochoscope into the duodenum (uncertain of bile duct stricture). Nevertheless, T-tube cholangiograms were done and showed no stricture and no retained stones in both cases. The tubes were then subsequently withdrawn.

Tube drains were inserted in four patients because of contaminated operative field. Three patients with tube drains were hospitalized for more than a week and one of them contracted an *Escherichia Coli* wound infection.

All the bile duct stones in the fifteen patients were successfully cleared during the surgical session and no further endoscopic treatment was required. There was no mortality. Morbidity rate was 8.3% (one case). Although quite high proportionally, it only had to be treated by local wound drainage and intravenous antibiotics.

TABLE 1. Characteristic of patients, endoscopic findings and number of session.

No	Sex	Age	Definition of difficult CBD stone	CBD size (cm)	ERCP session before surgery
1	Male	81	Number 2-3, Size 1.5 -2 cm	2	1
2R	Male	40	Number 2, Size 1 and 2 cm, cystic duct junction impaction	2	1
3	Female	86	Number 1, Size 1.5 cm	1.5	1
4	Male	92	Number 4-5, Size 1 - 2 cm	1.8	1
5	Male	74	Number 1, Size 1.5 cm	1.5	1
6	Female	87	Failed endoscopi bile duct cannulation*	1.2	1
7	Male	82	Number 2-3, Size 1 - 1.5 cm	1.5	1
8	Female	38	Number >5, Size largest 1.5 cm	1.5	3
9R	Male	26	Number 3-4, Size largest 1.5, Narrowed distal bile duct	1.5	1
10	Female	84	Failed endoscopi bile duct cannulation†	1.8	1
11R	Female	45	Number >5, Size 1 - 1.2	1.2	1
12	Male	46	Number 1, Size 1.5 cm, abnormal ampullar location	1.5	1
13	Male	50	Number 1, Size 1.5, Narrowed distal bile duct	1.8	1
14	Female	92	Number >5, Size 1 - 1.5 cm	2	1
15	Male	86	Number >5, Size 1 - 1.5 cm	1.8	1

* CBD stones was confirmed by MRCP, † CBD stones was confirmed by US, R Mirizzi syndrome was later diagnosed

TABLE 2. Results of laparoscopic bile duct exploration.

No	Sex	Age	Operative time (Minutes)	Hospital stay (Days)	Complication (Yes/No)	Conversion (Yes/No)	T tube (Yes/No)	Drain (Yes/No)
1	M	81	125	7	No	No	No	No
2*	M	40	-	-	-	Yes	-	-
3	F	86	180	16	Yes	No	No	Yes
4	M	92	210	11	No	No	No	Yes
5	M	74	180	16	No	No	Yes	Yes
6	F	87	110	5	No	No	No	No
7	M	82	165	6	No	No	No	No
8	F	38	240	4	No	No	No	No
9*	M	26	-	-	-	Yes	-	-
10	F	84	210	7	No	No	No	No
11*	F	45	-	-	-	Yes	-	-
12	M	46	180	4	No	No	Yes	No
13	M	50	170	5	No	No	No	No
14	F	92	115	3	No	No	No	No
15	M	86	150	7	No	No	No	Yes

* Converted to open surgery subjects.
Abbreviations: M = Male, F = Female

TABLE 3. Summary of the results of laparoscopic bile duct exploration.

	Mean (± SD)	95% Confidence Interval
Age (Years)	67.3 (± 23.3)	54.4 - 80.2
Sex (Male): N (%)	9 (60.0)	32.3 - 83.7
Operative time (Minutes)	169 (± 39.9)	144.3 - 194.9
Hospital stay (Days)	7.6 (± 4.4)	4.8 - 10.4
Conversion: N (%)	3 (20.0)	4.3 - 48.1
Complication: N (%)	1 (8.3)	2.1 - 38.5
Drain: N (%)	4 (33.3)	9.9 - 65.1
T tube: N (%)	2 (16.7)	2.1 - 48.4

* In total 12 patients, converted to open surgery subjects excluded

DISCUSSION

CBD stone treatment has been changing and advancing for many decades. In the past, the common approach was bile duct exploration during open surgery. Not only could this procedure clear CBD stones but it could also remove gallstones in the same operation. The results were also excellent, with very low morbidity and mortality.[13] Later, laparoscopic cholecystectomy (LC) was introduced and proved to set a new standard of surgical management.[14] With greater technological advances in the field of endoscopy, endoscopic treatment has also been accepted and proven as a complementary procedure[1,2] These two procedures, when combined, are not only highly effective, but also on par with the current trend of minimally invasive procedures. Today, surgeons have gained greater experience in laparoscopic surgery while concurrently witnessing the introduction of more sophisticated laparoscopic equipment. Thus, laparoscopic bile duct exploration has now returned as an attractive option for dealing with patients with CBD stones, with the additional benefit of being able to remove the gallbladder in the same session.

Studies, including systematic review and meta-analysis, have shown the advantages of LBDE over endoscopic (combined with LC) being a single-stage approach, requiring a shorter hospital stay and incurring lower medical costs, with comparable efficacy regarding stone clearance. It is also safe with no difference in morbidity, mortality and incidence of retained stones.[6,7,10] However, although the term "single stage approach" is appealing, studies revealed that the single stage approaches incurred high costs of investigation, such as MRCP or CT to confirm the existence of CBD stones before surgery.[5, 8]

LBDE also required a prolonged learning curve for acquisition of the requisite laparoscopic skills.[5] Furthermore, in a situation where the stones are detected in a small bile duct, the trans-cystic approach, which requires more sophisticated laparoscopic instruments and/or a small sized choledochoscope, appears to be more suitable than the trans-ductal approach.[15] Nevertheless, considering the divergent views and opinions in medical reviews and textbooks, there is to date no "best" approach for dealing with CBD stones. The approach should therefore depend on the situation and resources available in each individual institute, such as whether specialists and /or facilities are available or not.[5,13]

In the context of Sawanpracharak Hospital, there are adequate facilities readily available to treat CBD stones endoscopically, although if the stones are classified as difficult[3], the endoscopic capability is limited. Laparoscopic instruments are readily available, though there is a lack of some instrumentation (e.g. no miniature choledochoscope to retrieve CBD stones in small sized bile duct). For these reasons, within the context of this situation, endoscopic treatment appears to be a suitable choice to treat non-difficult CBD stones especially in small sized bile duct whereas LBDE, trans-ductal approach, should be the preferred alternative in cases of difficult CBD stones.

From our results, LBDE appears to be both safe and effective. There was only one minor (and treatable) complication without necessitating further invasive intervention and no mortality resulted. LBDE can also be performed with minimal risk for elderly patients who comprised the majority of the target sample in our study. Most patients could return home in about one week. For all cases, stones were cleared in only one session of surgery without further treatment.

There was a high incidence of patients with Mirizzi's type II syndrome (Csendes classification type III or greater[12]). ERC failed to detect the likelihood of this

condition in two out of three patients. In other studies, difficulties were reported in diagnosing this syndrome preoperatively, and for ERC detection rate was around 55 to 90%.[16] Even if combined with MRCP, detection rate would still be only 68.5%.[17] In one of our patients, this condition was suspected from ERC and computer tomography was done, but this also failed to confirm the presence of this pathology. The high rate of conversion might primarily be due to the fact that only extreme cases were selected for LBDE. Coupled with the difficulty of preoperative diagnosis, physicians who are willing to adopt this approach should be prepared to deal with potential challenges.

Not only was there a high rate of conversion, but most of the gallbladders in this group of patients appear to have been difficult. Although this could not be displayed as a variable, it is reflected in the operation times. The length of the surgeries, at nearly three hours on average, is a likely indicator of the level of difficulty involved. Close to half of the duration of the procedures were dedicated to dealing with the gallbladders.

With regard to T tubes and tube drains, a systematic review reported against the routine usage of T tubes.[18] In this study, T tubes were inserted in only two cases. However, most of the subjects had already undergone endoscopic sphincterotomy during ERC. A positive result without T tube insertion might not be possible in general cases. Although all four cases with tube drain required longer hospital stays, this author suggests that intra-operative pathology rather than the tube itself was the operative cause for their prolonged stay.

Admittedly, there are some limitations in this study. Firstly, regarding, methodology, the study was only a small, observational study in the absence of a more sizable group, which was reflected in the wide 95% confidence interval. Secondly, the study was focused on a single hospital with a potentially unique availability of resources. Lastly, the medium-long term results are not yet available.

CONCLUSION

LBDE with trans-ductal approach appears to be both a safe and effective management for patients with difficult CBD stones. It should be noted that this subgroup of patients represented extreme cases. Accordingly, physicians who are willing to adopt this approach should be aware of and anticipate problems arising from both difficult gallbladder and difficult bile duct pathology entailing reconstruction. In order to establish and confirm treatment efficacy, a further and larger scale study is required.

REFERENCES

1. Freitas ML, Bell RL, Duffy AJ. Choledocholithiasis: evolving standards for diagnosis and management. World J Gastroenterol. 2006;12(20):3162-7.
2. Carr-Locke DL, Cotton PB. Biliary tract and pancreas. Br Med Bull. 1986;42(3):257-64.
3. Trikudanathan G, Navaneethan U, Parsi MA. Endoscopic management of difficult common bile duct stones. World J Gastroenterol. 2013;19(2):165-73.
4. Stefanidis G, Christodoulou C, Manolakopoulos S, Chuttani R. Endoscopic extraction of large common bile duct stones: A review article. World J Gastrointest Endosc. 2012;4(5): 167-79.
5. Williams EJ, Green J, Beckingham I, Parks R, Martin D, Lombard M, et al. Guidelines on the management of common bile duct stones (CBDS). Gut. 2008;57(7):1004-21.
6. Hu KC, Wang HY, Chang WH, Chu CH, Lin SC, Liu CJ, et al. Clinical presentations of patients from different age cohorts with biliary tract stone diseases. J Gastroenterol Hepatol. 2014;29(8):1614-9.
7. Dasari BV, Tan CJ, Gurusamy KS, Martin DJ, Kirk G, McKie L, et al. Surgical versus endoscopic treatment of bile duct stones. Cochrane Database Syst Rev. 2013;(12):CD003327.
8. Lee HM, Min SK, Lee HK. Long-term results of laparoscopic common bile duct exploration by choledochotomy for choledocholithiasis: 15-year experience from a single center. Ann Surg Treat Res. 2014;86(1):1-6.
9. Chan DS, Jain PA, Khalifa A, Hughes R, Baker AL. Laparoscopic common bile duct exploration. Br J Surg. 2014;101(11):1448-52.
10. Zhu HY, Xu M, Shen HJ, Yang C, Li F, Li KW, et al. A meta-analysis of single-stage versus two-stage management for concomitant gallstones and common bile duct stones. Clin Res Hepatol Gastroenterol. 2015;39(5):584-93.
11. Corlette MB, Bismuth H. Biliobiliary fistula. A trap in the surgery of cholelithiasis. Arch Surg. 1975;110(4):377-83.
12. Csendes A, Diaz JC, Burdiles P, Maluenda F, Nava O. Mirizzi syndrome and cholecystobiliary fistula: a unifying classification. Br J Surg. 1989;76(11):1139-43.
13. K. Papalezova BC. Stones in the bile duct: clinical features and open surgical approaches and techniques. In: W.R. JarnaginJ BW, BüchlerW.C, ChapmanM.I. D'Angelica., ed. Blumgart's Surgery of the Liver, Pancreas and Biliary Tract 1. 5th ed: Elsevier; 2012.p. 532-49.e2.
14. Soper NJ, Stockmann PT, Dunnegan DL, Ashley SW. Laparoscopic cholecystectomy. The new 'gold standard'? Arch Surg. 1992; 127(8):917-21.
15. Puhalla H, Flint N, O'Rourke N. Surgery for common bile duct stones--a lost surgical skill; still worthwhile in the minimally invasive century? Langenbecks Arch Surg. 2015;400(1):119-27.
16. Beltran MA. Mirizzi syndrome: history, current knowledge and proposal of a simplified classification. World J Gastroenterol. 2012;18(34):4639-50.
17. Lledo JB, Barber SM, Ibanez JC, Torregrosa AG, Lopez-Andujar R. Update on the diagnosis and treatment of mirizzi syndrome in laparoscopic era: our experience in 7 years. Surg Laparosc Endosc Percutan Tech. 2014;24(6):495-501.
18. Gurusamy KS, Koti R, Davidson BR. T-tube drainage versus primary closure after laparoscopic common bile duct exploration. Cochrane Database Syst Rev. 2013;(6):CD005641.

Surgical Outcomes of Upper Tract Approach and Lower Tract Approach in Duplex Kidney: Single Center Experience

Soratree Saingarm, M.D.*, Phichaya Sujijantararat, M.D.*, Bansithi Chaiyaprasithi, M.D.*, Thawatchai Mankongsrisuk, M.D.*, Santosh Shrestha, M.D., Kittipong Phinthusophon, M.D.***

**Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, ** Department of Surgery, Western Regional Hospital, Pokhara, Nepal.*

ABSTRACT

Objective: We reviewed the result of upper and lower tract approaches in duplex collecting systems at Siriraj Hospital between January 2011 and January 2017, focusing on reoperation rate and postoperative urinary tract infection.
Methods: We analyzed the medical records of 57 patients with duplex kidneys, ectopic ureter and ureterocele. 45 patients were treated with open surgical therapy including upper tract approach which consisted of upper pole nephrectomy and ureteropyelostomy, and lower tract approach which was common sheath reimplantation. Information included age at operation, gender, ectopic ureter and ureterocele location, vesicoureteral reflux (VUR), hydrohephrosis status and presenting symptoms.
Results: Upper tract approach was performed in 23 patients, 3 patients were reoperated because of persistent ureterocele in 2 cases and VUR in 1 case (13.6%, p = 0.233). One was lost to follow-up. Among 22 patients who underwent lower tract approach, no patient required second operation with antibiotic prophylaxis.
Conclusion: In the absence of ureterocele and VUR, upper tract approach surgery appears to be successful treatment of duplex kidneys. When compared with upper tract approach, the lower tract approach surgery is more effective for open surgical therapy in duplex kidneys if sample size increases.

Keywords: Duplex kidney; double collecting system; upper tract approach; lower tract approach; ureterocele; ectopic ureter (Siriraj Med J 2018;70: 17-21)

INTRODUCTION

Patients with duplex collecting system may have wide range of presentation i.e. ectopic ureter, ectopic ureterocele, obstruction or reflux as well as moiety function. Due to these the treatment modalities and approaches are still controversial.

Treatment may be endoscopic or open and the approach can be upper tract approach consisting of upper pole nephrectomy or ureteropyelostomy depending upon the moiety function. Lower tract approach consists of common sheath reimplantation and ureterocele excision.[1,5]

Many reports on endoscopic management showed about 70-80% of ureteroceles can be successfully treated which supported the endoscopic treatment on the grounds of less aggressive choice.[5,7,10]

Since the advantage of endoscopic treatment for intravesical ureterocele patient was already well defined[10], we tried to refine our results to find the concept for best practice in duplex patients comparing upper tract and lower tract approach.

MATERIALS AND METHODS

After approval of the Institutional Ethics Committee (Si 450/2017), we reviewed the medical records of 57 patients with duplex kidneys, ectopic ureter and ureterocele

Correspondence to: Kittipong Phinthusophon
E-mail: kittamm@hotmail.com
Received 6 September 2017 Revised 17 October 2017 Accepted 18 October 2017
doi:10.14456/smj.2018.4

who underwent operation at Siriraj Hospital between January 2011 and January 2017.

Information included gender, ectopic ureter and ureterocele location, age at operation, vesicoureteral reflux (VUR), hydronephrosis status, initial serum creatinine and presenting symptoms. Preoperative diagnostic studies included ultrasonography, voiding cystourethrography (VCUG), intravenous pyelography (IVP) or computed tomography urography (CTU) or magnetic resonance urography (MRU), 99m-Tc-dimercaptosuccinic acid (DMSA) or renal scintigraphy and examination under anesthesia (EUA). Primary outcome was the requirement for secondary procedure as a definitive treatment. At the follow up visit we assessed urinary tract infection (UTI), incontinence, ultrasonography, VCUG and renal scintigraphy.

RESULTS

We analysed 57 patients with ectopic ureters or ectopic ureteroceles in our study. Twelve patients were excluded from the study which consisted of 9 patients with endoscopic treatment, 1 patient with chronic kidney disease (CKD) at initial presentation, 1 patient with supernumerary kidneys and one patient with single system ureterocele. Forty-five patients were treated with open surgical therapy including upper tract approach which consisted of upper pole nephrectomy and ureteropyelostomy and lower tract approach which was common sheath reimplantation and excision of ureterocele. There were 23 upper tract and 22 lower tract procedure. The median follow up was 33 months (range 7-60 months).

From the demographic data, there were no statistically difference in age, gender, initial serum creatinine, complete or incomplete duplication and side comparing between both groups. The patients mostly presented with UTI with no statistically difference between both groups. (Tables 1-3)

The patient in upper tract approach group presented with ectopic ureter 6 cases, ectopic ureterocele 14 cases and incomplete duplex 3 cases. Upper pole nephrectomy was performed in 18 renal units and ureteropyelostomy was performed in 5 renal units. From the lower tract approach group, the patient presented with ectopic ureter 18 cases, ectopic ureterocele 3 cases, incomplete duplex 1 case. Open common sheath reimplantation was used in 16 renal units, ureterocele excision with common sheath reimplantation was used in 3 renal units and laparoscopic pneumovesicum was used in 3 renal units.

According to the primary outcome in our study, secondary procedures were needed in 3 cases out of 22 in upper tract approach whereas, there was no secondary procedure in lower tract approach. Among the 3 patients that required secondary operation in upper tract approach, 2 had persistent ureterocele and one

TABLE 1. Demographic data of the patients with duplex kidney.

	Lower Tract Approach (n = 22)	Upper Tract Approach (n = 23)	P-value
Age (years)	4.45 (1.5-22)	2.67 (0.58-36)	0.134
Sex			0.372
Female	17 (77.3%)	15 (65.2%)	
Male	5 (22.7%)	8 (34.8%)	
Side of duplex kidney			0.842
Left	13 (59.1%)	13 (56.5%)	
Right	6 (27.3%)	5 (21.7%)	
Bilateral	3 (13.6%)	5 (21.7%)	
Serum creatinine	0.4 (0.2-0.9)	0.4 (0.1-0.8)	0.916
Ipsilateral duplex system			0.608
Complete duplex	21 (95.5%)	20 (87.0%)	
Incomplete duplex	1 (4.5%)	3 (13.0%)	

TABLE 2. Presenting symptoms of the patients with duplex kidney.

	Lower Tract Approach (n = 22)	Upper Tract Approach (n = 23)	P-value
Symptoms			
UTI	20 (90.9%)	19 (82.6%)	0.665
Incontinence	9 (40.9%)	2 (8.7%)	0.012
Flank pain	2 (9.1%)	0 (0%)	0.233
Prenatal US	2 (9.1%)	3 (13.0%)	1
Incidental finding	0 (0%)	3 (13%)	0.233
LUTS	1 (4.5%)	4 (17.4%)	0.346
Asymptomatic pyuria	1 (4.5%)	4 (17.4%)	0.346

Abbreviations: urinary tract infection = UTI, ultrasound = US, lower urinary tract symptoms = LUTs

TABLE 3. Indication for surgery in the patients with duplex kidney.

	Lower Tract Approach (n = 22)	Upper Tract Approach (n = 23)	P-value
Indication for surgery			
Breakthrough UTI	13 (59.1%)	11 (47.8%)	0.449
Severe UTI	5 (22.7%)	5 (21.7%)	1
Poor renal function	3 (13.6%)	10 (43.5%)	0.027
Increased cortical scar	1 (4.5%)	2 (8.7%)	1
Symptomatic	9 (40.9%)	4 (17.4%)	0.082

Abbreviation: urinary tract infection = UTI

had VUR. All underwent lower tract procedures. The rate of secondary procedure was higher in upper tract approach, but there was no statistically significant (P = 0.233) difference in our study. There was no statistical difference of perioperative complication in both groups. Four patients each in upper tract and lower approach had postoperative fever which was managed conservatively. There was no wound infection, incontinence or other perioperative complications in our study. (Table 4)

In the follow up period after upper pole nephrectomy, ureterocele was decompressed well (11/14 cases, 78.6%). Ureteropyelostomy also had satisfactory outcome. All 5 cases had improvement of hydronephrosis from ultrasonographic or renogram findings. In the follow up of lower tract approach including pnemovesicoscopic common sheath reimplantation, we found improvement of hydronephrosis in all cases except 2 patients who had persistent hydronephrosis and no progression during the follow up period. All of them could eventually discontinue prophylactic antibiotic.

DISCUSSION

The goals in management of ureteroceles and ectopic ureters are renal function preservation, UTI prevention, and incontinence correction. The treatment options are sometimes controversial and depend on surgeon's preference. There are many factors that we have to concern including renal function of each moiety, opening of ectopic ureters and type of ureteroceles, and presentation of VUR.

TABLE 4. Post operative complications in the patients with duplex kidney.

	Lower Tract Appriach (n = 22)	Upper Tract Approach (n = 23)	P-value
Re-operation	0 (0%)	3 (13.6%)	0.233
Perioperative complications			1
No complication	18 (81.8%)	19 (82.6%)	37
Fever with UTI	4 (18.2%)	4 (17.4%)	8
Hospital stay	11 (5-25)	7 (5-25)	0.001
Urinary incontinence	0	0	0
Follow up time	31.17 (8.18 - 60.39)	33.24 (7.03-70.24)	(p=0.963)

Abbreviation: urinary tract infection = UTI

There are many choices of treatment which can be categorized into endoscopic and surgical approach techniques which can be performed in two approaches. First, upper tract approach which is upper pole nephrectomy and ureteropyelostomy. The second, lower tract approach which are reimplantation and excision of ureterocele.

Nowadays endoscopic ureterocele incision already has its own specific usage especially in intervesical ureterocele[3,6], but surgical approach for upper tract and lower tract corrections still have to be defined in their own suitable cases. According to our study lower tract approach delivers excellent outcome with no secondary procedure, low perioperative complication rate, no incontinence, no UTI during the follow up period which is the same as previous studies.[12,13] Also this procedure also corrects high grade reflux with high success rate.[11] However, it still has disadvantage of longer hospital stay and extensive reconstructive procedure.[9]

We performed 3 cases of pnemovesicoscopic common sheath reimplantation in complete renal duplication where both ureteral openings were intravesical and close to each other. The technique is feasible to perform, but takes a longer operative time. There was no complication in all 3 cases and antibiotic prophylaxis could be discontinued. There has been no literature which has reported this operation in duplex cases.

Upper tract approach including upper pole nephrectomy and ureteropyelostomy are less extensive procedures and have high rate of ureterocele decompression in our study (78.6%). The result was related to retrospective study of Husman D, et al in which 17 of 72 patients with ectopic ureterocele were treated with endoscopic decompression after partial nephrectomy (24%).[4] As in J Gomes et al's report, decompression of ureterocele was detected in all cases.[6] The disadvantage of this approach is incidence of secondary procedure that is caused by secondary VUR or residual ureteral stump infection.[2,8] In our study there were 3 cases (3/23 cases, 13.04%) that needed secondary procedure in upper tract approach which was comparable to previous studies 12%[1] and 18%.[14] The important technique to reduce this complication is upper ureteral removal as distal as possible.

In comparison to lower tract approach, upper tract approach is easier to perform and provides good outcome especially in the circumstance that reflux is absent, but pediatric urologist should be familiar with both approaches. The main reason is lower tract approach provide the excellent result for duplex kidneys with VUR regardless of reflux grading and it is also the definitive operation after failure of upper tract approach.

CONCLUSION

In the absence of ureterocele and VUR, upper tract approach surgery appears to be successful treatment of duplex kidneys. When compared with upper tract approach, the lower tract approach surgery is more effective for open surgical therapy in duplex kidneys if sample size increases.

However, there were limitations in our study. The retrospective nature of this study had some potential bias such as operation selection and small sample size. The time of follow up was also not enough to evaluate subsequent complications such as hypertension.

Conflict of interest

All authors have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

REFERENCES

1. Yong SL, Yoon SH, Myungjoon K, Hyun JJ, MiJung L, Young JI, Sang WH. Factors associated with complications of the ureteral stump after proximal ureteroureterostomy. J Urol 2012; 188: 1890-4.
2. Pearce R, Subramaniam R. Partial nephroureterectomy in a duplex system in children: the need for additional bladder procedures. Pediatr Surg Int 2011; 27: 1323-6.
3. Douglas EC. Management of the neonatal ureterocele. Current urology reports 2001; 2: 102-5.
4. Husmann D, Strand B, Ewalt D, Michele C, Steve K, Terry A. Management of ectopic ureterocele associated with renal duplication: a comparison of partial nephrectomy and endoscopic decompression. J Urol 1999; 162: 1406-9 .
5. Shimada K, Matsumoto F, Matsui F. Surgical treatment for ureterocele with special reference to lower urinary tract reconstruction. Urol Int 2007;14:1063-7.
6. Gomes J, Mendes M, Castro R, Reis A. Current role of simplified upper tract approach in the surgical treatment of ectopic ureteroceles: a single centre's experience. Eur Urol 2002;41: 323-7.
7. Shekarriz B, Upadhyay J, Fleming P, Gonzalez R, Barthold JS. Long-term outcome based on the initial surgical approach to ureterocele. J Urol 1999;162:1072-6.
8. Choi H, Oh SJ. The management of children with complete ureteric duplication: selective use of uretero-ureterostomy as a primary and salvage procedure. BJUI 2000;86:508-12.
9. Yong SL, Young JI , Sang HS, Bascuna RT, Ji YH , Sang WH. Complications after common sheath reimplantation in pediatric patients with complicated duplex system. Urology 2015;85: 457-62.
10. Sander JC, Bilgutay AN, Stanasel I, Chester JK, Janzen N, Gonzales WT, et al. Outcome of endoscopic incision for the treatment of ureterocele in children at a single institution. J Urol 2015;193:662-7.
11. Ellsworth PI, Lim DJ, Walker RD, Stevens PS, Barraza MA, Mesrobian HG. Common sheath reimplantation yields excellent results in the treatment of vesicoureteral reflux in duplicated collecting systems. J Urol 1996;155:1407-9.
12. Christopher DG, Bradley PK, Cheng EY, Kropp KA . Primary lower urinary tract reconstruction for nonfunctioning renal moieties associated with obstructing ureteroceles. J Urol 2005;173:198-201.
13. El GA, Miranda J, Truong T, Monfort G. Ectopic ureter with complete ureteric duplication: conservative surgical management. J Pediatr Surg 1996; 31:467-72.
14. Denes FT, Danilovic A , Srougi M. Outcome of Laparoscopic Upper-Pole Nephrectomy in Children with Duplex Systems. J Endourol March 2007; 21:162-8.

Performance of the Locally Made Disposable 10-gram Semmes-Weinstein Monofilament Compared to the Commercially Available Monofilament

Danu Prommin, Ph.D.*, Pasu Sirisalee, Ph.D.*, Parinya Junhunee, B.Sc.*, Bongkoch Praewpipat, B.PO.*, Navaporn Chadchavalpanichaya, M.D., Atchara Suwannakin, M.A.**, Gulapar Srisawasdi, M.D.****

**National Metal and Materials Technology Center, National Science and Technology Development Agency, Pathumthani 12120, **Department of Rehabilitation, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: The aim of this study was to find a technique to make a disposable monofilament which is accurate and affordable for Thailand context. A prototype of local made monofilament was developed and tested with the calibrated universal machine test to evaluate its performance compared with the commercial monofilament. Effect of the uncontrolled humidity condition on the performance of the commercial monofilament was also studied.

Methods: Review of literatures showed no published specifications of the monofilament. The investigators set the concepts that materials must be locally available and affordable. Also the unit cost must be affordable for primary care units in Thailand. Monofilament's fabrication technique was developed and the accuracy test of monofilament force was performed and compared with the commercially available monofilament.

Results: The specification of locally made disposable 10-gram Semmes-Weinstein monofilament was identified. The force of the monofilaments developed was in an acceptable range for a standard clinical practice and comparable to the commercially available monofilament. From the study, the effect of Thailand's humidity conditions can deteriorate the performance of the monofilament. The investigators decided to use a disposable monofilament one to ensure its hygiene and accuracy.

Conclusion: The local made disposable 10-gram Semmes-Weinstein monofilament was developed. The material is available and affordable for Thailand context. The accuracy of the monofilament's force was measured by a calibrated testing apparatus. Availability, durability, and cost are important issues to be concerned for medical tools that have been recommended to use worldwide. The result from this study could be applied to other countries that have similar conditions as Thailand.

Keywords: Monofilament; disposable; specification; humidity (Siriraj Med J 2018;70: 22-27)

INTRODUCTION

Diabetes Mellitus is one of the top ten causes reducing the disability adjusted life years (DALYS) of Thai population.[1] The National Health Examination Survey (NHES) conducted in 2009 found an overall diabetes prevalence of 7.5% in Thailand and 31% of them were undiagnosed.[2] According to International Diabetes Federation (http://www.idf.org/membership/wp/thailand), there were more than 4 million people with diabetes in Thailand in 2015. Diabetes can lead to several complications e.g. Retinopathy, Nephropathy, and Neuropathy. Peripheral neuropathy is a complication in approximately 50% of patients with diabetes, and up to 50% of patients with peripheral neuropathy may not have symptoms.[3] Neuropathy usually leads to loss of protective sensation at foot, chronic and infected wounds at foot and finally foot amputation. The rate of foot amputation of people with diabetes is up to 13.7 per 1,000 people

Correspondence to: Pasu Sirisalee
E-mail: pasus@mtec.or.th
Received 15 May 2017 Revised 25 October 2017 Accepted 30 October 2017
doi:10.14456/smj.2018.5

per year, which is 10-times higher than healthy people.[4] Thus, a routine diagnosis foot protective sensation is crucial. Various methods may be employed to test for neuropathy such as temperature differentiation, pin-prick test, vibration perception threshold and the 10-g monofilament.[5] One of the widely used foot diagnoses for foot protective sensation is using Semmes-Weinstein monofilament or 10-g monofilament. It has become popular because it is easy to use, reliable and lower cost than other methods. The criteria of the monofilament test to determine the loss of foot protective sensation is using the monofilament no. 5.07. If the person does not respond to the test by monofilament no. 5.07, the person is considered to have lost their foot protective sensation. However, the genuine Semmes-Weinstein monofilament has to be imported and is unaffordable by most public hospitals in Thailand. Currently, public healthcare practitioners have to use home-made monofilament or free monofilament from pharmaceutical companies. The quality and reliability of these monofilaments are sometimes questionable. The monofilament no. 5.07 must accurately apply 10 grams force to the patient's foot to diagnose the patient's foot protective sensation. However, Booth and Young found that only 70% of commercial Semmes–Weinstein monofilaments they have tested were accurate (buckling force within +/- 1 g of 10 g).[6] There are many factors that affect the performance of the monofilament including the environmental condition. The durability of the monofilament is also sceptical after repeated usage. Yong et al., reported a reduction in average buckling force by 0.8 g and 1.2 g after 200 and 500 cycles of compression respectively.[7] Therefore, we decided to develop the disposable monofilament to avoid the aforesaid problems. Our disposable monofilament is made of locally available materials in order to be accessible and affordable. The prototype of locally made monofilament was tested with the calibrated universal machine test to evaluate its performance compared to the commercial monofilament. Effect of the uncontrolled humidity condition on the performance of the commercial monofilament was also studied.

MATERIALS AND METHODS

A set of forty commercial monofilaments (two packages with twenty monofilaments a package) was put in indoor humidity condition (uncontrolled humidity condition), while another set of forty of the same commercial monofilaments was put in controlled condition with fixed humidity at 45%. A set of forty prototype monofilaments was also prepared from a roll of nylon fishing line 0.5 mm in diameter, cut into 35 mm lengths measured from a fixed point by fixing a stamp paper clip to the end, and then put it in controlled condition with fixed humidity at 45%. With a universal testing machine (Instron 5943, Norwood, MA, USA) at polymer physics and rheology laboratory, National Metals and Materials Technology Center, 10 N load cell was used to measure compressive forces on all monofilaments. Load pattern began with 1 mm/s with 10 mm downward loading, 3 mm/s with 10 mm upward to original point of unloading, and 2 seconds of holding, respectively, and then repeated those three back and forth for 16 times. Testing time was 2 days.

For statistical analysis, statistic t-test was used to check, whether in this case, the humidity condition affected the average forces of 16 consecutive compressions by comparing their averages and assuming homogeneity of variance between groups. Therefore, the null hypothesis (H0), was that difference between two average forces of 16 consecutive compressions, was zero and the alternative hypothesis (H1) was that difference between two average forces of 16 consecutive compressions was not zero. However, to check if disposable monofilament, was is accurate and affordable, the commercial and prototype monofilaments were compared. Before using statistic t-test to test the difference between two average forces, statistic F-test of difference of two variance between groups of samples had to be tested. Therefore, the null hypothesis (H0) was that standard deviation of force of commercial monofilaments equalled standard deviation of force of prototype monofilaments and the alternative hypothesis (H1) was that standard deviation force of commercial did not equal standard deviation force of prototype monofilaments. If the result of H1 was rejected, the commercial and prototype monofilaments could be compared and then concluded which one was better using statistic t-test.

RESULTS

First of all there was a failed test on a commercial monofilaments in controlled condition with fixed humidity at 45%, then the sample size was reduced to 39 in this condition. The average forces of 16 consecutive compressions of commercial monofilaments put in indoor humidity condition and controlled condition with fixed humidity at 45% were 8.29 and 9.23 grams, respectively. The standard deviation forces of 16 consecutive compressions of commercial monofilaments put in indoor humidity condition and controlled condition with fixed humidity at 45% were 0.22 and 0.20 grams, respectively. Figs 2 and 3 were testing results of the commercial monofilaments with different humidity conditions. They were comparable patterns for both graphs, which were very high forces at

first compression and then decreased over time. Their average forces were different by about a gram. Therefore, statistic t-test was used to check whether their averages were different or not by assuming the same variance of those groups. The p-value of statistic t-test was zero, which came from t-value of 21.262 and degrees of freedom of 77. The null hypotheses were rejected which meant their averages were different. Therefore, the forces of commercial monofilaments put in indoor humidity condition was lower as shown in Table 1.

The average and standard deviation forces of 16 consecutive compressions of prototype monofilaments put in controlled condition with fixed humidity at 45% were 9.19 and 0.37 grams, respectively. As shown in Fig 4,

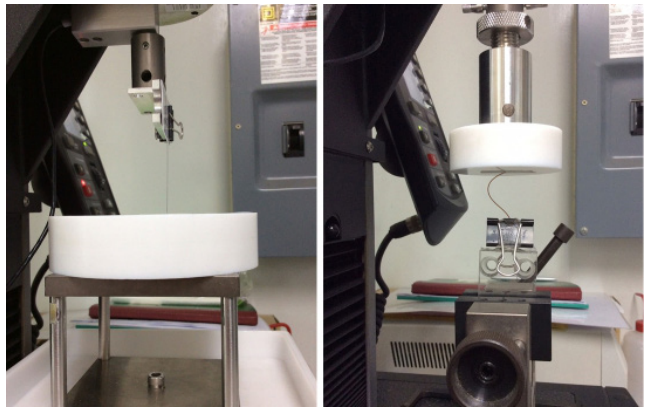


Fig 1. Commercial monofilament (left) and prototype monofilament (right) on a universal testing machine for compression test to simulate sensation testing on the patient's foot.

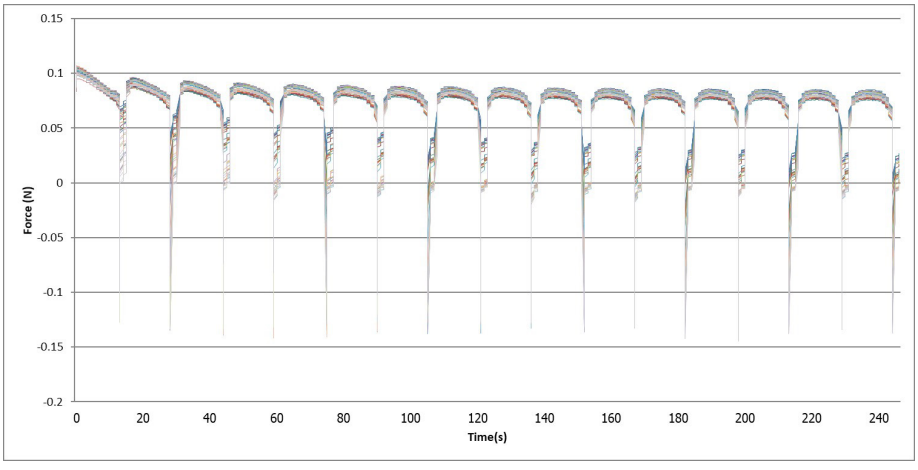


Fig 2. Forces of 16 consecutive loading, unloading, and resting pattern of commercial monofilaments put in indoor humidity condition.

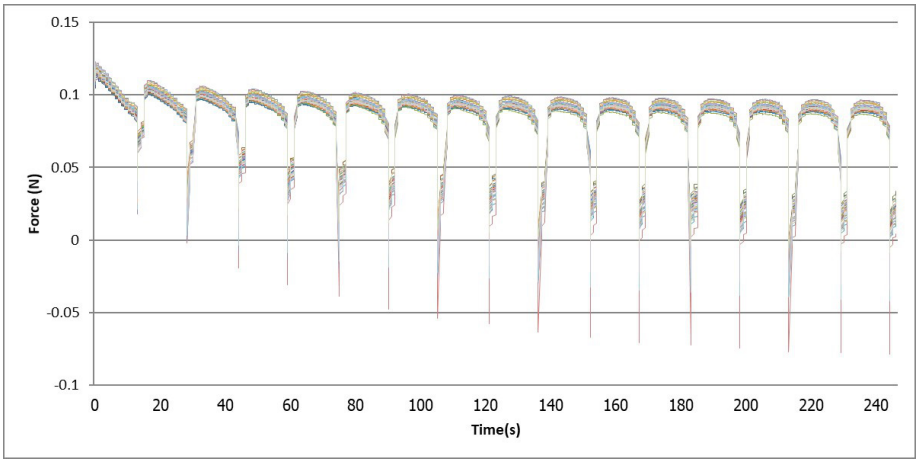


Fig 3. Forces of 16 consecutive loading, unloading, and resting pattern of commercial monofilaments put in controlled condition with fixed humidity at 45%.

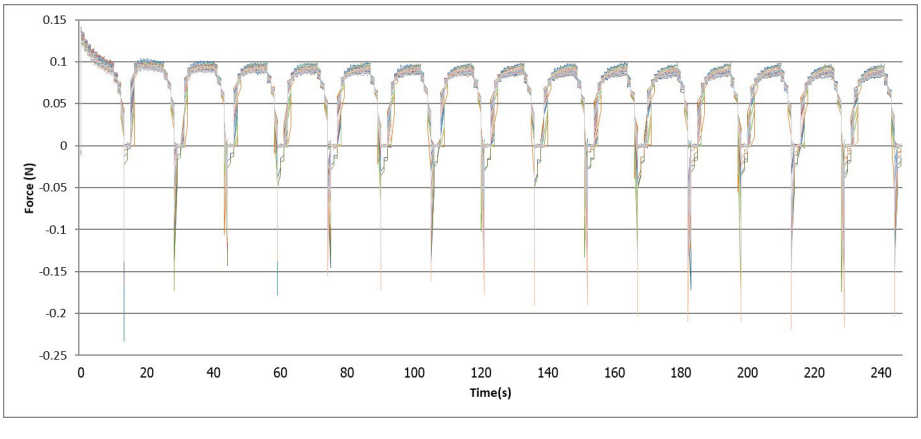


Fig 4. Forces of 16 consecutive loading, unloading, and resting pattern of prototype monofilaments put in controlled condition with fixed humidity at 45%.

they were also comparable patterns compared with commercial ones, which were very high forces at first compression and then decreased over time. However, they were different groups, thus two variances had to be checked. The p-value of statistic F-test was 0.0001, which came from F-value for a left-tailed test of 0.2840 and both degrees of freedom of 39 and 38 for commercial and prototype monofilaments, respectively. Thus, the null hypotheses were rejected. Therefore, average difference between commercial and prototype could not be compared because the two variances were different as shown in Table 2. Even though both the average peak forces could not statistically be compared. as shown in Fig 5, the average peak forces of commercial monofilaments and prototype in controlled condition with fixed humidity at 45% after 16 consecutive compressions were slightly higher than the average peak force of commercial monofilaments in indoor humidity condition in Fig 5.

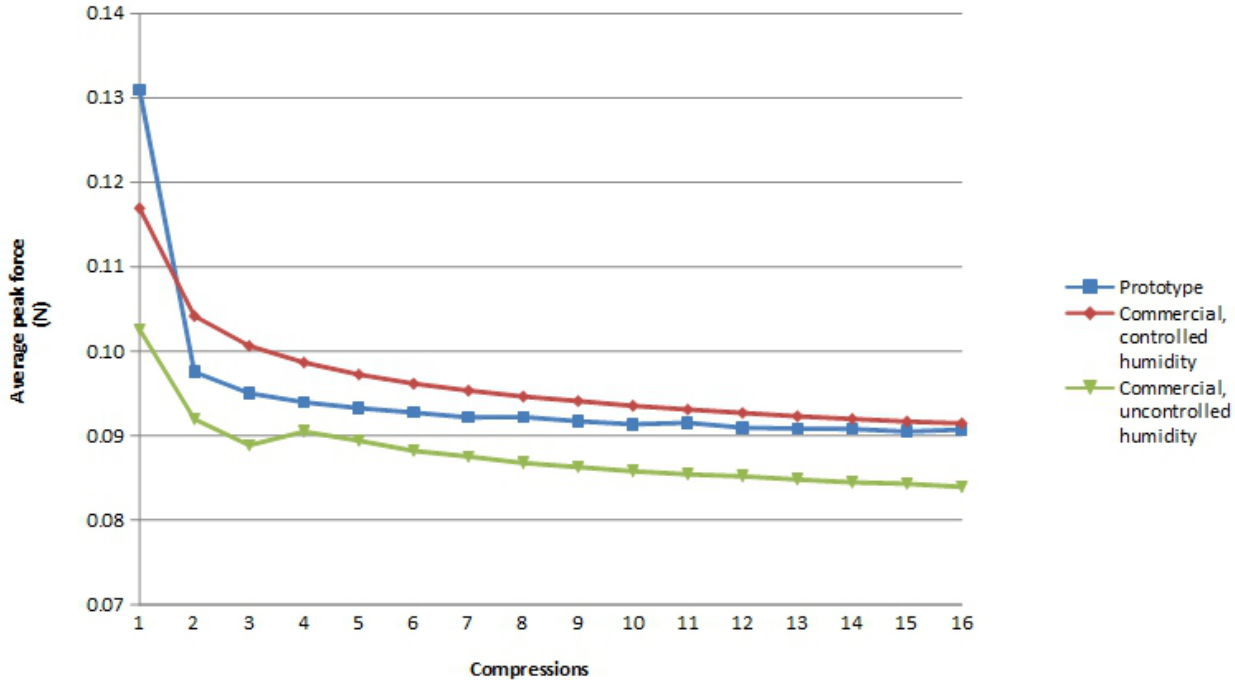


Fig 5. Average peak forces of commercial monofilaments in both indoor humidity condition and controlled condition with fixed humidity at 45% and prototype monofilaments in controlled condition with fixed humidity at 45% after 16 consecutive compressions.

TABLE 1. The average forces and their standard deviations of 16 consecutive compressions of commercial monofilaments put in indoor humidity condition and controlled condition with fixed humidity at 45%, and to compare the effect of humidity condition, the statistic t-test was used to check whether their averages were different or not with the same variance of those groups.

Average peak forces of	Average (N)	Standard deviation (N)	Sample size	Statistic t-test	Statistic F-test
Commercial monofilament in indoor humidity condition	8.29	0.22	40	t-value of 21.262, degree of freedom of 77, p-value = 0, then the averages are different	Same source of population, then assumed no difference of variance
Commercial monofilaments in controlled condition with fixed humidity at 45%	9.23	0.20	39		

TABLE 2. The average forces and their standard deviations of 16 consecutive compressions of commercial and prototype monofilaments commercial monofilaments put in indoor humidity condition and controlled condition with fixed humidity at 45%, and to compare between commercial and prototype monofilaments at fixed 45% humidity condition the statistic F-test was used to check whether two variance were different or not between groups before using the statistic t-test.

In controlled condition with fixed humidity at 45% average peak forces of	Average (N)	Standard deviation (N)	Sample size	Statistic t-test	Statistic F-test
Commercial monofilaments	9.23	0.20	39	Could not be compared because two variances were different	F-value of left tailed test of 0.2840, degree of freedom of 39 and 38, p-value = 0.0001, and then two variances were different
Prototype monofilaments	9.19	0.37	40		

DISCUSSION

After testing on commercial monofilaments, it was statistically confirmed that the null hypotheses was rejected, which means that humidity condition to store monofilaments is the primary concern. Max HH et al.,[8] also found that there were significant changes in buckling force of the Semmes-Weinstein monofilaments in different temperature and humidity conditions. The calibrated monofilament may have deteriorated performance if it is stored in the high humidity condition. Thailand has an average relatively high humidity with high temperature. Therefore, there is a high chance of misdiagnosis if a monofilament is stored in uncontrolled condition as shown in our study. Therefore, using monofilaments and storing them in the uncontrolled humidity condition and then reusing them is not recommended. Our suggestion is that the monofilaments should be disposable, calibrated from the manufacturers and packed in the moisture barrier packaging to ensure their performance. Moreover, the disposable monofilaments tend to be more hygienic than the reusable ones.

For prototype monofilaments, it was shown that monofilament can be possibly made with lower cost with an example of a simple holder like a stamp paper clip as shown in Fig1. The crucial point is the quality control of the manufacturing process to ensure the force of the monofilament and the packaging process to ensure the moisture protection before the use. The buckling force of the monofilament depends on the diameter, length and elastic modulus of the monofilament. These three parameters can be adjusted as appropriate. A holder of the monofilaments can be customized as shown in Fig 6.

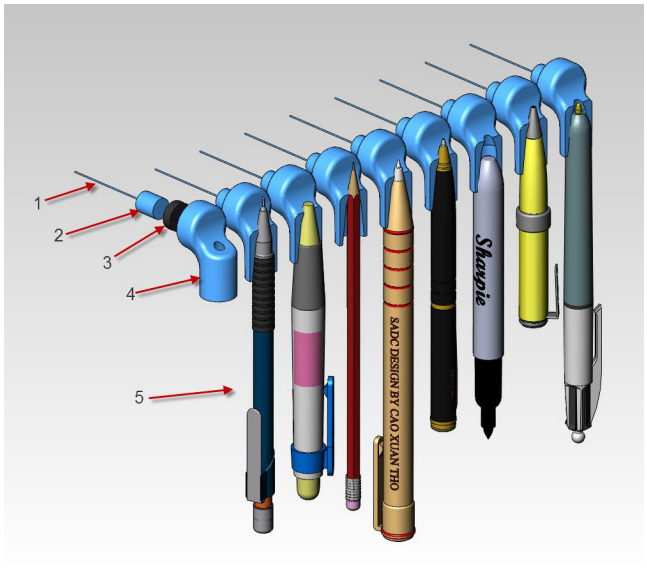


Fig 6. Design examples of local made disposable monofilament.

The effects of the diameter, length of monofilament, elastic modulus of the monofilament and moisture barrier of the package on the performance of the monofilament will be studied in further work.

CONCLUSION

The local made disposable 10-gram Semmes-Weinstein monofilament was developed and tested. Its performance is comparable to the commercial one. However, Thailand's ambient humidity may deteriorate the performance of the calibrated monofilament after storing for a while as shown in our study. Therefore, an affordable and disposable monofilament seems appropriate to Thailand's context. The disposable monofilaments in the sealed packages with good quality control process can ensure their reliable performance to the users.

ACKNOWLEDGMENTS

This study was funded by a grant from National Science and Technology Development Agency (NSTDA), Ministry of Science and Technology. The authors hereby declare no personal or professional conflicts of interest regarding any aspect of this study.

REFERENCES

1. Bundhamcharoen K, Odton P, Phulkerd S, Tangcharoensathien V. Burden of disease in Thailand: changes in health gap between 1999 and 2004. BMC Public Health. 2011;11:53.
2. Reutrakul S, Deerochanawong C. Diabetes in Thailand: Status and Policy. Current Diabetes Reports. 2016;16(3):28.
3. Dros J, Wewerinke A, Bindels PJ, van Weert HC. Accuracy of Monofilament Testing to Diagnose Peripheral Neuropathy: A Systematic Review. Ann Fam Med. 2009;7(6):555-8.
4. Krittiyawong S, Ngarmukos C, Benjasuratwong Y, Rawdaree P, Leelawatana R, Kosachunhanun N, et al. Thailand Diabetes Registry Project: Prevalence and Risk Factors Associated with Lower Extremity Amputation in Thai Diabetics. J Med Assoc Thai. 2006; 89 Suppl 1:S43-8.
5. Tan LS. The clinical use of the 10 g monofilament and its limitations: A review. Diabetes Res Clin Pract. 2010;90(1):1-7.
6. Booth J, Young MJ. Differences in the performance of commercially available 10-g monofilaments. Diabetes Care. 2000;23(7):984-8.
7. Yong R, Karas TJ, Smith KD, Petrov O. The durability of the Semmes-Weinstein 5.07 monofilament. J Foot Ankle Surg. 2000;39(1):34-8
8. Max HH, Inger S, Willem JT. Semmes-Weinstein Monofilaments: Influence of Temperature, Humidity, and Age. J Hand Surg Am. 2011;36(7):1191-6.

PCR-Based Diagnosis of *Neoscytalidium dimidiatum* Infection Using Internal Transcribed Spacer 1 Region of Ribosomal DNA Primers

Charussri Leeyaphan, M.D.*,,***, Koichi Makimura, M.D., Ph.D.*, Chiaki Yamanishi, M.D.* , Sumanas Bunyaratavej, M.D.**, Carren Hau, M.D., Ph.D.***, Yayoi Tada, M.D., Ph.D.***, Wichit Suthammarak, M.D., Ph.D. ****, Supannee Kaewsutthi, M.Sc.****, Sutasinee Phaitoonwattanakij, M.D.**, Shinichi Watanabe, M.D., Ph.D.*****

**Laboratory of Space and Environmental Medicine, Graduate School of Medicine, Teikyo University, Tokyo, Japan, **Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, ***Department of Dermatology, Graduate School of Medicine, Teikyo University, Tokyo, Japan, ****Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: To develop *N. dimidiatum*-specific single PCR-based identification with DNA sequences of nuclear ribosomal internal transcribed spacer (ITS) 1 region primers to facilitate the rapid and accurate detection of *N. dimidiatum*.

Methods: *N. dimidiatum*-specific PCR primers were designed based on the sequence of the internal transcribed spacer 1 region, which is located between 18S and 5.8S nuclear rDNA. Fungal DNA extracted from common causative species for superficial fungal infection including: 2 strains of *N. dimidiatum*, 9 species of dermatophyte (DMP) and 25 species of non-dermatophyte (NDM) colonies grown on culture plates were used for PCR analysis. Also, 30 clinical specimens collected from 30 patients clinically diagnosed with fungal nail and feet infection who attended Dermatology clinic Siriraj Hospital during October 2015 to November 2015 were used for PCR assay.

Results: Using *N. dimidiatum*-specific PCR primers, the PCR product was amplified from two standard strains of *N. dimidiatum*, and there was no amplification from other DMP or NDM species. Regarding sensitivity as lower limit of detection, this PCR method was able to detect 10 pg of *N. dimidiatum* DNA with ethidium bromide staining and could detect *N. dimidiatum* in clinical samples.

Conclusion: This newly developed *N. dimidiatum*-specific PCR identification system is rapid, sensitive, and specific. This diagnostic method will facilitate early and accurate diagnosis and accelerate appropriate treatment in patients with *N. dimidiatum* infection.

Keywords: *Neoscytalidium dimidiatum; N. dimidiatum* infection; *N.dimidiatum*-specific primers; PCR-based diagnosis; nondermatophytes (Siriraj Med J 2018;70: 28-35)

INTRODUCTION

Neoscytalidium dimidiatum is an important causative organism in nondermatophyte (NDM) skin and nail infection worldwide, but especially in the West Indies, South America, West Africa, and Asia.[1-4] NDM onychomycosis has been recently reported to be increasing.[5] The clinical characteristics of superficial fungal skin infection and NDM onychomycosis were reported to be clinically indistinguishable from dermatophyte (DMP) onychomycosis, except that fungal skin infection above the feet patients was not commonly detected in patients with NDM onychomycosis.[1,2,6,7] Mycological culture remains an essential method for isolating causative fungi. Moreover, drug resistance were commonly detected in

Correspondence to: Charussri Leeyaphan
E-mail: charussrilee@gmail.com
Received 4 May 2017 Revised 12 September 2017 Accepted 20 September 2017
doi:10.14456/smj.2018.6

NDM infection, especially in *N. dimidiatum* infection. Standard systemic antifungal treatment used for DMP infections had variable effect on NDM infections.[8] As such, the development of an accurate mycological laboratory technique that enables identification, diagnosis, and proper management of NDM infections is essential.

Diagnosis of *N. dimidiatum* infection is generally made by fungal slide culture, which is always timewasting and requires specially trained personnel. Molecular biological techniques have recently been developed that facilitate the precise and rapid identification of DMPs and NDMs.[4,9,10] Restriction fragment length polymorphism targeting (PCR-RFLP) of the hypervariable V4 domain of the small ribosomal subunit 18S gene was reportedly used to identify *N. dimidiatum*.[4,10] However, species-specific single PCR-based identification for *N. dimidiatum* has not been reported. This PCR method was established to identify different types of fungi by single PCR reaction using a unique amplicon. Accordingly, the aim of this study was to develop *N. dimidiatum*-specific single PCR-based identification with DNA sequences of nuclear ribosomal internal transcribed spacer (ITS) 1 region primers to facilitate the rapid and accurate detection of *N. dimidiatum* infection.

MATERIALS AND METHODS

Primer design

The primer sequence of *N. dimidiatum* was designed using MEGA4 alignment software (http://www.megasoftware.net/download_form) and Primer Express software (Applied Biosystems, Foster City, CA, USA) based on upstream and downstream sequences of the ITS1 region of the ribosomal DNA gene (rDNA) of *N. dimidiatum* in the DDBJ/EMBL/GenBank (accession no. AB490817.1). Oligonucleotides used in this study were designed based on comparisons with sequences of ITS1 rDNA data of DMPs and NDMs [accession numbers: AB042608.1 (*Microsporum duboisii*); AB042607.1 (*M. distortum*); AB042606.1 and AB042605.1 (*M. ferrugineum*); AB049929.1 (*M. gallinae*); AB017178.2 (*M. audouinii*); AB017180.1 (*M. cookie*); AB017177 (*M. gypseum*); AB017179 (*M. canis*); AB017181.1 (*Epidermophyton floccosum*); AB017176.1 (*Trichophyton verrucosum*); AB017174.1 (*T. violaceum*); AB017173.1 (*T. schoenleinii*); AB017172.1 (*T. tonsurans*); AB011453.1 (*T. rubrum*); AB011463 (*T. mentagrophytes*); AJ223852.1 (*Aspergillus niger*); Y14003.1 (*Candida albicans*); LT594689.1 (*Exophiala jeanselmei*); AM412643.1 (*Fusarium solani*); AJ853776.1 (*Scopulariopsis brevicaulis*); and, AB011466 (*Arthroderma vanbreuseghemii*)].

Base sequences specific to *N. dimidiatum* were analyzed with primer 3 output and BLAST® blastn suite software (https://blast.ncbi.nlm.nih.gov) to develop primers. *N. dimidiatum*-specific primers NeoF1 (5'-TGTACCCACCTCTGTTGCTTTG-3') and NeoR1 (3'-TCGATGCCAGAACCAAGAGATC-'5) were made by BEX Co., Ltd. (Tokyo, Japan). The size of the amplified product was estimated to be 173bp.

Preparation of fungal DNA from strains

DMP and NDM fungal strains were used in this study (Table 1). All of these standard strains were maintained in the TIMM culture collection of Teikyo University Institute of Medical Mycology, and most of them were isolated from clinical cases or environment in Japan and part of them were obtained from international culture collections. All fungal strains were grown on SDA at 28°C for 5-14 days. Small amounts of fungal colony on SDA were placed in lysis buffer (200 mMTris-HCl, pH 8.0, with 25 mM EDTA, 0.5%, w/v, sodium dodecyl sulfate, and 250 mMNaCl) and ground with a conical grinder. Then samples were incubated at 100°C for 15 min and mixed with 100 µl of 3.0 M sodium acetate. Samples were maintained at -20°C for 10 min and then centrifuged at 10,000 x g at 4°C for 10 min. Supernatants were extracted once with phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v) and then extracted once with chloroform. DNA was precipitated with an equal volume of isopropanol, washed with 150 µl of 70% ethanol, dried, and suspended in 50 µl of TE buffer (pH 8.0). Aliquots of 1µl of the resulting solutions were then used as templates for the PCR.

Preparation of fungal DNA from clinical specimens

Twenty nail specimens and 10 skin specimens from 30 patients clinically diagnosed with fungal nail and feet infection (respectively) were used for PCR assay. Clinical samples were collected at Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand during the study period October 2015 to November 2015. The protocol for this study was approved by the Siriraj Institutional Review Board (Si 090/2016). Written informed consent was waived, given the nature of the study and the associated anonymity of patient and specimen data.

A portion of each sample was placed on a slide and microscopically studied after clearing in 20% potassium hydroxide. For culture, part of each sample was inoculated in SDA with and without cyclohexamine. Cultures were then incubated at 27°C and examined every 4 days for 6 weeks. Identification of microorganisms was based

TABLE 1. Results of PCR analysis with ITS1 universal and *N. dimidiatum*-specific primer pairs for standard strains

No.	Fungal species	Strain	PCR with ITS1 primers	PCR with *N. dimidiatum* primers
1	*Neoscytalidium dimidiatum*	NBRC110913	+	+
		NBRC110914	+	+
2	*Acremonium curvulum*	NBRC32242	+	-
3	*Alternaria alternata*	NBRC4026	+	-
		NBRC6587	+	-
4	*Arthroderma benhamiae*	SM103(+)	+	-
		SM104(-)	+	-
5	*Arthroderma vanbreuseghemii*	TIMM2789	+	-
6	*Aspergillus flavus*	NBRC4186	+	-
		JCM2061	+	-
7	*Aspergillus fumigatus*	NBRC4057	+	-
		TIMM0108	+	-
8	*Aspergillus niger*	JCM10254	+	-
		NBRC4066	+	-
9	*Candida albicans*	ATCC10231	+	-
		TIMM1768	+	-
10	*Candida glabrata*	ATCC90030	+	-
		NBRC0005	+	-
11	*Candida parapsilosis*	ATCC22019	+	-
		NBRC1396	+	-
12	*Candida tropicalis*	ATCC750	+	-
		NBRC0006	+	-
13	*Chaetomium globosum*	TSY-0369	+	-
14	*Cryptococcus neoformans*	TIMM3147	+	-
		TIMM1835	+	-
15	*Epidermophyton flocosum*	NBRC9045	+	-
		KMU8915	+	-
16	*Exophiala jeanselmei*	NBRC6857	+	-
		TSY-0396	+	-
17	*Fonseceae pedrosoi*	NBRC6071	+	-
		TSY-0394	+	-
18	*Fusarium oxysporum*	NBRC7152	+	-
		TSY-0351	+	-
19	*Fusarium solani*	NBRC5232	+	-
		TSY-0403	+	-
20	*Fusarium verticillioides*	TSY-0219	+	-
21	*Histoplasma capsulatum*	TIMM1846	+	-
22	*Hoetae werneckii*	TIMM4094	+	-
23	*Microsporum canis*	NBRC7863	+	-
		TIMM4092	+	-

TABLE 1. Results of PCR analysis with ITS1 universal and *N. dimidiatum*-specific primer pairs for standard strains (Continued)

No.	Fungal species	Strain	PCR with ITS1 primers	PCR with *N. dimidiatum* primers
24	*Microsporum gypseum*	NBRC5948	+	-
		KMU8642	+	-
25	*Paecilomyces variotii*	NBRC4855	+	-
		TIMM4066	+	-
26	*Penicillium marneffei*	TIMM6206	+	-
27	*Pseudallescherichia boydii*	TIMM0886	+	-
		TIMM0887	+	-
28	*Rhizopus oryzae*	IFM40515	+	-
		IFM62923	+	-
29	*Rhodotorula minuta*	JCM3777	+	-
		TIMM6222	+	-
30	*Scopulariopsis brevicaulis*	NBRC4843	+	-
		TSY-0668	+	-
31	*Scytalidium lignicola*	NBRC104988	+	-
32	*Trichophyton mentagrophytes*	NBRC5466	+	-
		TIMM1189	+	-
33	*Trichophyton rubrum*	NBRC5467	+	-
		TIMM2659	+	-
34	*Trichophyton tonsurans*	NBRC5928	+	-
		KMU8913	+	-
35	*Trichosporon asahii*	NBRC1200	+	-
		TIMM1318	+	-

(+), detected; (-), not detected

on the observation and description of macroscopic and microscopic characteristics of colonies.

QIAamp® DNA Micro Kit (Qiagen GmbH, Hilden, Germany) was used for DNA extraction from nail and skin samples. One µl of DNA solution was used as a template for PCR and sequencing analysis. DNA extraction from *N. dimidiatum* colonies on culture plate and water were used as positive and negative controls, respectively.

Polymerase chain reaction (PCR)

TaKaRa Taq™ (Takara Bio Inc., Tokyo, Japan) was used for PCR. PCR master mix was prepared using 5 µL of 10X PCR buffer, 4 µL of 2.5mMdNTP mix, 0.25 µL of Taq DNA Polymerase, and 38.8 µL of distilled water. One µL of DNA solution and 0.5 µL of each 30 pM specific primer were added to the mixture, which was then placed in a TaKaRa PCR Thermal Cycler (Takara Bio Inc., Tokyo, Japan). Each mixture was heated to 94°C for 4 min and PCR was performed under the following conditions: 94°C, 30 sec; 60°C, 1 min; 72°C, 1 min; 30 cycles. Thermal cycles were terminated by polymerization at 72°C for 10 min. Amplification products were visualized on 1.2% agarose gel containing ethidium bromide. Universal ITS1-specific primers (18SF1: 5'-AGGTTTCCGTAGGTGAACCT-3', 58SR1: 5'-TTCGCTGCGTTCTTCATCGA-3') previously reported by Makimura, et al. were used to confirm DNA detection.[11]

DNA sequencing and analysis

Both strands of each PCR product were directly sequenced using a DNA Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with primers NeoF1 and NeoR1 and an automatic sequencer (Genetic Analyzer 310; Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Sequences were compared with the ITS1 sequence of *N. dimidiatum* using GENETYX-MAC 10 software (https://www.genetyx.co.jp/) to verify a 100% match.

RESULTS

Primer Specificity and Sensitivity

The primer specificity and sensitivity (lower limit of detection) of *N. dimidiatum*-specific PCR for standard DMP and NDM strains were evaluated using specific primers (Table 1). Two strains of *N. dimidiatum* demonstrated band at 173 bp, and DNA sequencing revealed a 100% match with ITS1 region of *N. dimidiatum*. No expression was identified for any other DMPs or NDMs. Fig 1 shows PCR products amplified with universal ITS1 primers 18SF1 and 58SR1 (Fig 1A) and *N. dimidiatum*-specific primers NeoF1 and NeoR1 (Fig1B). DNA of all fungi was amplified successfully with universal ITS1 primers and only *N. dimidiatum* DNA was detected using *N. dimidiatum*-specific primers.

Dilution technique was used to evaluate the lower limit of detection of primers to detect DNA with ethidium bromide staining. *N. dimidiatum*-specific PCR was able to detect 10 pg of *N. dimidiatum* genomic DNA with ethidium bromide staining (Fig 2).

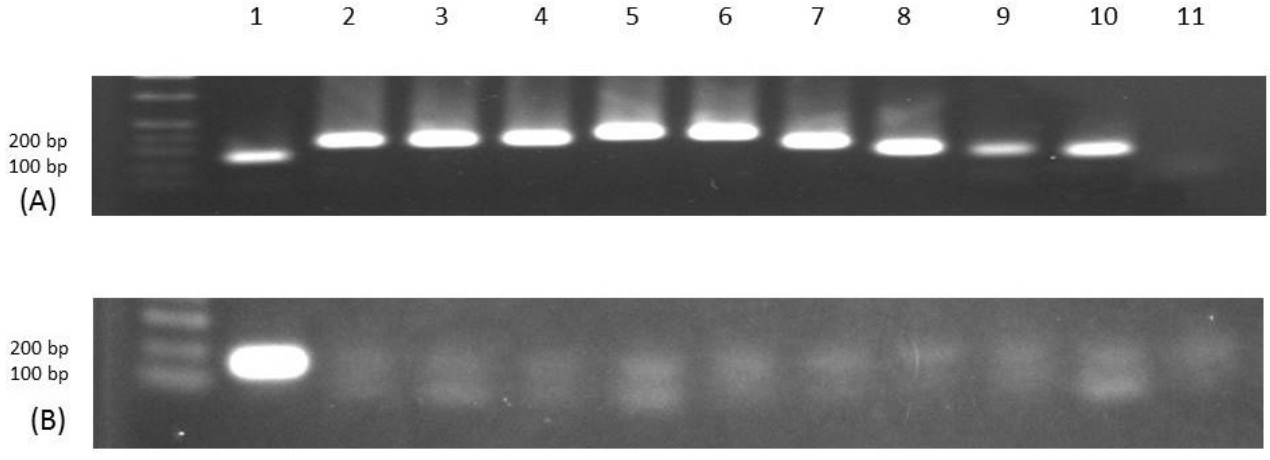


Fig 1. Primer Specificity of *N. dimidiatum*-specific primers: gel electrophoresis images of PCR using ITS1 primers (A); and, *N. dimidiatum*-specific primers (B). M, DNA molecular weight marker (BIONEXUS, USA): (1) *N. dimidiatum*; (2) *T. rubrum*; (3) *T. mentagrophytes*; (4) *T. tonsurans*; (5) *E. floccosum*; (6) *M. canis*; (7) *M. gypseum*; (8) *Aspergillusflavus*; (9) *Fusariumsolani*; (10) *C. albicans*; (11) water (negative control)

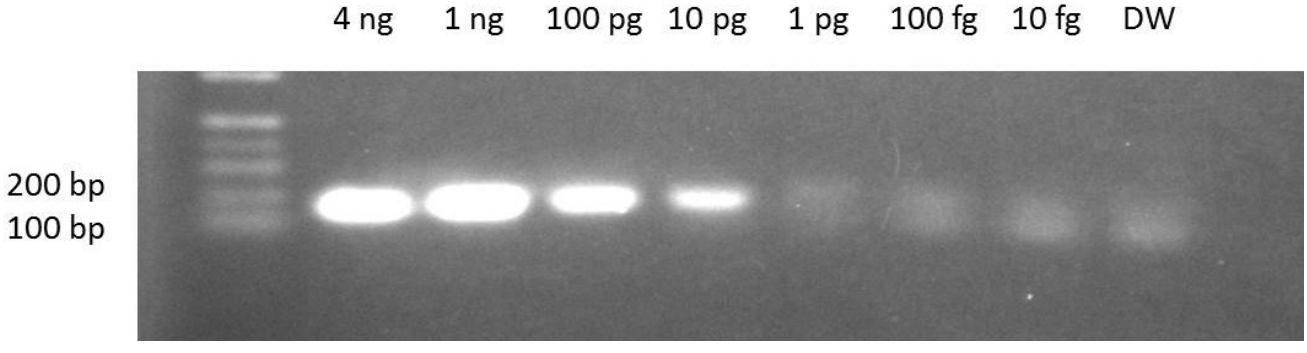


Fig 2. Lower limit of detection of *N. dimidiatum*-specific primers with dilution of *N. dimidiatum* DNA solutions

PCR detection of N. dimidiatum DNA from clinical specimens

DNA extraction and PCR reaction were performed in all clinical samples. *N. dimidiatum*-specific primers and ITS primers were used in PCR reaction (Table 2). *N. dimidiatum* was found from culture in 8 nail and 5 skin samples that demonstrated positive bands with both ITS1 and *N. dimidiatum*-specific primers. DMPs and NDMs were detected from culture in 10 nail and 4 skin samples that all showed ITS1 single bands. There were 4 DMP samples (2 nail and 2 skin) and 3 NDM nail samples that demonstrated single band with *N. dimidiatum*-specific primers. Three samples did not detect fungus from culture, with one sample expressing single bands with ITS1 and *N. dimidiatum*-specific primers. Compared with fungal culture as gold standard, sensitivity and specificity of *N. dimidiatum*-specific primers were 100% and 53%, respectively. Positive predictive value was 62% and negative predictive value was 13%.

TABLE 2. Detection of fungi from clinical samples.

Patient No.	Specimen	Microscopy	Culture	PCR with ITS1 primers	PCR with *N. dimidiatum* primers
1	Nail	+	*N. dimidiatum*	+	+
2	Nail	+	*N. dimidiatum*	+	+
3	Nail	+	*N. dimidiatum*	+	+
4	Nail	+	*N. dimidiatum*	+	+
5	Nail	+	*N. dimidiatum*	+	+
6	Nail	+	*N. dimidiatum*	+	+
7	Nail	+	*N. dimidiatum*	+	+
8	Nail	+	*N. dimidiatum*	+	+
9	Nail	+	*T. rubrum*	+	+
10	Nail	+	*T. rubrum*	+	-
11	Nail	+	*T. mentagrophytes*	+	+
12	Nail	+	*T. mentagrophytes*	+	-
13	Nail	+	*Fusarium* spp.	+	-
14	Nail	+	*Fusarium* spp.	+	-
15	Nail	+	*S. brevicaulis*	+	+
16	Nail	+	*S. brevicaulis*	+	-
17	Nail	+	*A. niger*	+	+
18	Nail	+	*Penicillium* spp.	+	+
19	Nail	+	No growth	+	+
20	Nail	+	No growth	-	-
21	Skin	+	*N. dimidiatum*	+	+
22	Skin	+	*N. dimidiatum*	+	+
23	Skin	+	*N. dimidiatum*	+	+
24	Skin	+	*N. dimidiatum*	+	+
25	Skin	+	*N. dimidiatum*	+	+
26	Skin	+	*T. rubrum*	+	+
27	Skin	+	*T. mentagrophytes*	+	+
28	Skin	+	*T. mentagrophytes*	+	-
29	Skin	+	*Fusarium* spp.	+	-
30	Skin	+	No growth	-	-

(+), detected; (-), not detected

DISCUSSION

N. dimidiatum infection is an important health problem in Thailand and many other tropical countries. Previous studies from Thailand reported that 24.1%-51.6% of onychomycosis cases were NDM onychomycosis, and *N. dimidiatum* was the most common pathogen.[1,12] NDM infection is more difficult to treat and more resistant to several drugs than DMP infection.[8] *N. dimidiatum* is not responsive to most systematic antifungal therapies.[4] Therefore, species identification is essential for both accurate diagnosis and appropriate management. Microscopic examination cannot normally differentiate hyphae between DMPs and

N. dimidiatum. Culture remains the standard method for species identification. However, culture requires at least a week to obtain typical macroscopic and microscopic characteristics. In some cases, subculture is required for exact identification. These culture-related processes delay diagnosis and proper management for a week.[4] In this study, PCR identification with *N. dimidiatum*-specific primers based on ITS1 region required approximately 3 hours to provide accurate identification in patients with superficial skin and nail infection. Moreover, this method is objective and does not require specially-trained personnel to morphologically identify the fungus. This method will facilitate early and accurate diagnosis and proper management in clinical practice.

A previous study reported PCR-RFLP method for discriminating between DMPs and *N. dimidiatum* targeted on subunit 18S rDNA. Also PCR-RFLP demonstrated accurate and rapid result, although this technique used two primer sets and two enzymes for *N. dimidiatum* identification.[4] Although fast and accurate, this 2 enzyme-2 primer technique is limited by the fact that it cannot be performed in most standard laboratories. The present study demonstrated molecular diagnosis using a single PCR method with one primer set that may be prepared in a wide range of clinics and hospitals.

In this study, culture of 7 clinical samples revealed DMPs and other NDMs apart from *N. dimidiatum* within positive band that were acquired using *N. dimidiatum*-specific primers. This may be the result of mixed infection or contamination. Mixed infection of DMP and NDM onychomycosis is increasingly reported using molecular analysis, as antibiotics and cyclohexamine in traditional culture may inhibit NDM growth.[13] Identification of mixed infection would be useful for the prediction of clinical course and prognosis, and would lead to proper management.[14] However, diagnosis criteria for mixed infection are still debated and need to exclude contamination. Further study is required to elucidate this mixed infection.

NDMs have been identified in both soil and plants, so infection or colonization may be acquired by direct contact.[1] Previous study reported that NDMs, such as *Acremonium* spp., *Aspergillus* spp., *Fusarium* spp., and *Scopulariopsis* spp., may colonize in an abnormal nail unit and may be clinically indistinguishable from onychomycosis.[15] However, no prior reports described *N. dimidiatum* colonization in a nail unit. Currently, NDM onychomycosis diagnosis is based on diagnostic criteria that require at least 3 of the following: positive microscopic examination, positive mycological culture, at least two consistent isolations from repeated sampling, dermatophyte exclusion, and histology. To ensure that NDMs are the true pathogens and not contaminants, serial sampling at multiple time points is recommended to confirm the repeated presence of NDMs with or without DMP infection.[6] Molecular techniques will be a powerful and valuable tool for diagnosing NDM onichomycosis.

Fungal hyphae were microscopically detected in all clinical samples in this study, but 3 samples did not detect fungus from culture, with one sample expressing single bands with ITS1 and *N. dimidiatum*-specific primers. Positive microscopic examination would support that this finding was not due to contamination. It may be explained by the low sensitivity of traditional culture or an uneven distribution of fungus in clinical samples.

This study has some mentionable limitations. First, this study was a pilot study that used specific primers to detect fungus in clinical samples, so the sample size was relatively small. Further studies with a larger sample size should be performed to validate our results. Second, NDM detection could not be distinguished from true pathogen or colonization in the nail unit. Clinical characteristics, repeated sample examinations, and evaluation of nail histology may be useful to confirm diagnosis and to guide appropriate treatment.

In conclusion, this newly developed *N. dimidiatum*-specific PCR identification system is rapid, sensitive, and specific. This diagnostic method will facilitate early and accurate diagnosis and accelerate appropriate treatment in patients with *N. dimidiatum* infection.

ACKNOWLEDGMENTS

This study was supported by a grant from the Dermatology Society of Thailand.

Conflict of interest declaration

The authors hereby declare no personal or professional conflicts of interest regarding any aspect of this study.

REFERENCES

1. Bunyaratavej S, Prasertworonun N, Leeyaphan C, Chaiwanon O, Muanprasat C, Matthapan L. Distinct characteristics of *Scytalidium dimidiatum* and non-dermatophyte onychomycosis as compared with dermatophyte onychomycosis. J Dermatol. 2015;42(3):258-62.
2. Lacroix C, Kac G, Dubertret L, Morel P, Derouin F, de Chauvin MF. Scytalidiosis in Paris, France. J Am Acad Dermatol. 2003;48(6):852-6.
3. Machouart M, Lacroix C, Bui H, Feuilhade de Chauvin M, Derouin F, Lorenzo F. Polymorphisms and intronic structures in the 18S subunit ribosomal RNA gene of the fungi *Scytalidium dimidiatum* and *Scytalidium hyalinum*. Evidence of an IC1 intron with an His-Cys endonuclease gene. FEMS Microbiol Lett. 2004;238(2):455-67.

4. Machouart-Dubach M, Lacroix C, de Chauvin MF, Le Gall I, Giudicelli C, Lorenzo F, et al. Rapid discrimination among dermatophytes, *Scytalidium* spp., and other fungi with a PCR-restriction fragment length polymorphism ribotyping method. J Clin Microbiol. 2001;39(2):685-90.
5. Welsh O, Vera-Cabrera L, Welsh E. Onychomycosis. Clin Dermatol. 2010;28(2):151-9.
6. Gupta AK, Drummond-Main C, Cooper EA, Brintnell W, Piraccini BM, Tosti A. Systematic review of nondermatophyte mold onychomycosis: diagnosis, clinical types, epidemiology, and treatment. J Am Acad Dermatol. 2012;66(3):494-502.
7. Xavier AP, Oliveira JC, Ribeiro VL, Souza MA. Epidemiological aspects of patients with ungual and cutaneous lesions caused by Scytalidium spp. An Bras Dermatol. 2010;85(6):805-10.
8. Bunyaratavej S, Leeyaphan C, Rujitharanawong C, Surawan TM, Muanprasat C, Matthapan L. Efficacy of 5% amorolfine nail lacquer in *Neoscytalidium dimidiatum* onychomycosis. J Dermatolog Treat. 2016:27(4):359-63.
9. Yoshida E, Makimura K, Mirhendi H, Kaneko T, Hiruma M, Kasai T, et al. Rapid identification of *Trichophyton tonsurans* by specific PCR based on DNA sequences of nuclear ribosomal internal transcribed spacer (ITS) 1 region. J Dermatol Sci. 2006;42(3):225-30.
10. Menotti J, Machouart M, Benderdouche M, Cetre-Sossah C, Morel P, Dubertret L, et al. Polymerase chain reaction for diagnosis of dermatophyte and *Scytalidium* spp. onychomycosis. Br J Dermatol. 2004;151(2):518-9.
11. Makimura K, Murayama SY, Yamaguchi H. Detection of a wide range of medically important fungi by the polymerase chain reaction. J Med Microbiol. 1994;40(5):358-64.
12. Ungpakorn R, Lohaprathan S, Reangchainam S. Prevalence of foot diseases in outpatients attending the Institute of Dermatology, Bangkok, Thailand. Clin Exp Dermatol. 2004;29(1):87-90.
13. Gupta AK, Nakrieko KA. Molecular determination of mixed infections of dermatophytes and nondermatophyte molds in individuals with onychomycosis. J Am Podiatr Med Assoc. 2014;104(4):330-6.
14. De Doncker PR, Scher RK, Baran RL, Decroix J, Degreef HJ, Roseeuw DI, et al. Itraconazole therapy is effective for pedal onychomycosis caused by some nondermatophyte molds and in mixed infection with dermatophytes and molds: a multicenter study with 36 patients. J Am Acad Dermatol. 1997;36(2 Pt 1):173-7.
15. Zaias N, Escovar SX, Rebell G. Opportunistic toenail onychomycosis. The fungal colonization of an available nail unit space by non-dermatophytes is produced by the trauma of the closed shoe by an asymmetric gait or other trauma. A plausible theory. J Eur Acad Dermatol Venereol. 2014;28(8):1002-6.

Mortality Rate and Predictive Factors for Invasive Fungal Rhinosinusitis: Experience in Siriraj Hospital

Premyot Ngaotepprutaram, M.D.*, Pongsakorn Tantilipikorn, M.D.*, Piyamaporn Wongtawornruang, M.D.*, Kanchana Amornphichetkul, M.D., Siri-on Tritrakarn, M.D.***, Popchai Ngamskulrungroj, M.D.****, Jate Lumyongsatien, M.D.***

**Department of Otorhinolaryngology, **Department of Pathology, ***Department of Radiology, ****Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: To elucidate the mortality rate and prognostic factors in patients with invasive fungal rhinosinusitis in Siriraj Hospital.

Methods: Thirty-nine patients with a definitive diagnosis of invasive fungal rhinosinusitis were recruited from October 2003 to September 2014. The mortality rate was retrieved, and the impacts of underlying diseases, clinical presentation, disease extension, fungal types, antifungal drugs, and time to treatment were statistically analyzed.

Results: The overall mortality rate was 23.1%. All patients except one were immunocompromised. Cranial nerve involvement was the most common symptom. The ethmoid sinus was the most commonly affected intranasal site (46.2%), and the majority of extranasal lesions were located in the orbit (17.9%). Most patients were affected by *Aspergillus* spp. (64.1%). Alteration of consciousness and periorbital pain were significant negative prognostic factors [adjusted odds ratio (95% confidence interval), 10.37 (1.31–82.07) and 8.67 (1.30–57.88), respectively]. Other factors such as time to treatment, age, and central nervous system involvement had no effect on mortality.

Conclusion: The mortality rate of invasive fungal rhinosinusitis in this study was 23.1%. Negative prognostic factors were alteration of consciousness and periorbital pain. Clinicians must have a high index of suspicion for invasive fungal rhinosinusitis, and aggressive treatment should be considered.

Keywords: Invasive fungal rhinosinusitis; fungal infection; mucormycosis; aspergillosis; prognosis; mortality (Siriraj Med J 2018;70: 36-43)

INTRODUCTION

Fungal infections of the paranasal sinuses are divided into 2 forms: noninvasive and invasive disease. The noninvasive form is more common and causes chronic rhinosinusitis. In contrast, the invasive form is rapidly progressive and occurs in immunocompromised patients[1,2] such as those with diabetes, prolonged steroid use, and neutropenia. Patients with invasive fungal disease frequently present with more severe symptoms of rhinosinusitis, including fever, facial edema, nasal stuffiness, and discolored nasal discharge. Invasive fungal rhinosinusitis (IFRS) was recently found to be more common than in the past,[3] with high mortality rates ranging from 50% to 80%.[1,2,4]

Early diagnosis of invasive disease is crucial, especially in immunocompromised patients. Pathological examination of suspicious tissue with pathogen identification is mandatory for a definite diagnosis of IFRS. Common causative pathogens are *Aspergillus* spp. and Zygomycetes genera (*Mucor* spp., *Rhizopus* spp., *Rhizomucor* spp.).[1,2] However, culture is the gold standard diagnostic test for identification of fungal species. Surgical intervention is the most important treatment in patients with IFRS. Adequate debridement of infected tissue with systemic

Correspondence to: Jate Lumyongsatien
E-mail: jate.lum@mahidol.ac.th
Received 31 January 2017 Revised 4 April 2017 Accepted 3 May 2017
doi:10.14456/smj.2018.7

antifungal agents and treatment of comorbid diseases provides better results.[4-6]

According to previous studies, negative prognostic factors in patients with IFRS are higher age, central nervous system involvement, changes in consciousness, a delay in starting antifungal drug treatment, and inadequate tissue debridement.[1,2] In a recent systematic review of 52 articles, Turner et al.,[1] studied the mortality rate and risk factors for IFRS in 807 patients and found that the mortality rate was 46.1% and that older age and central nervous system involvement were significant risk factors. Patients with diabetes mellitus had the lowest mortality rate among all comorbidities. Chen et al.,[7] reviewed IFRS comorbidities in 46 patients with hematologic malignancies and found a mortality rate of 41.3%. IFRS developed more commonly in patients with acute myeloid leukemia with prolonged neutropenia.

The mortality rate of IFRS in Thailand varies across different published case series and reports, ranging from 20.0% to 44.7%.[2,8] Soontrapa et al.,[2] reported that the most common coincidental disease was hematologic malignancy. Piromchai and Thanaviratananich[9] studied the impact of the treatment start time in patients with acute IFRS. The study showed an association between the treatment start time and the mortality rate. Starting treatment within 14 days of onset significantly decreased the mortality rate. Patients with IFRS historically received amphotericin B after pathological examination demonstrated tissue invasion by a fungus. The new drug voriconazole was reserved only for patients with a positive *Aspergillus* culture. Infectious disease specialists now tend to prescribe antifungal drugs earlier in the disease course when IFRS is suspected while awaiting a definitive tissue diagnosis. Moreover, voriconazole is now frequently used when the pathologic findings are compatible with *Aspergillus* spp., despite a negative culture. Based on this changing paradigm, we hypothesize that outcomes will improve and mortality will decrease. In the present study, we analyzed the mortality of and predictive factors for IFRS. We also present data that are lacking in other studies,[2,8,9] such as how the site of disease involvement as determined by nasal endoscopy compares with the radiographic and pathologic findings, advanced identification of fungal species by polymerase chain reaction (PCR), and the effects of new antifungal drugs (voriconazole and posaconazole) on the mortality rate.

MATERIALS AND METHODS

This study included 39 consecutive patients with a pathologically confirmed diagnosis of IFRS from October 2003 to September 2014. The Institutional Review Board approved the data collection (Si 808/2556). When tissue culture data were lacking, paraffin blocks of tissue were sent for PCR to identify the type of fungus. The overall mortality rate was presented as a percentage. Prognostic factors were analyzed by the Chi-square test or Fisher's exact test, and any variables with a p-value of <0.05 were selected for multivariate logistic regression. For both univariate and multivariate analysis, a p-value of <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY).

RESULTS

The mortality rate was 23.1%. The 39 patients comprised 19 male and 20 female patients with a mean age of 56.0 ± 15.1 years (range, 5-84 years). The most common underlying disease was diabetes mellitus (69.2%). The other comorbidities were hematologic malignancy (12.8%), human immunodeficiency virus infection (7.7%), systemic lupus erythematosus (5.1%), and CD4 lymphocytopenia (2.6%). Only 1 patient (2.6%) was healthy.

Decreased vision was found in 46.2% of the patients. Headache and periorbital pain were found in 38.5% and 25.6%, respectively. Seven patients (17.9%) had an altered consciousness. Cranial nerve involvement was the most common symptom among all patients (Table 1).

The average admission period was 33 days. The mean time from a provisional diagnosis of IFRS to initiation of systemic antifungal agents and/or surgery was 1.7 and 0.9 days, respectively. Twenty-six patients (68.4%) underwent sinus surgery within 24 hours after the provisional diagnosis, 6 (15.8%) underwent surgery from 24 to 48 hours, and 6 underwent surgery beyond 48 hours. One patient refused to undergo surgery.

With respect to the presentation of signs and symptoms, the patients were divided into a presenting time of ≤28 days (n = 14, 35.9%), 29 to 90 days (n = 10, 25.6%), and >90 days before admission (n = 15, 36.5%).

Physical examination and nasal endoscopy revealed no abnormalities in 4 patients (10.3%). Lesions were confined to the nasal cavity and/or paranasal sinuses in 12 patients (30.7%). The lesions extended to surrounding organs in 23 patients (59.0%) (Table 2). The most common site of involvement was the orbit (46.2%). The lesion distribution rate was 33.3% each in the ethmoid, maxillary, and sphenoid sinuses. Mucosal edema and necrotic tissue were found in 46.2% and 41.2% of patients, respectively. Other findings, such as ulceration, mucosal erythema, bone exposure, and masses, were found in 2.6% to 5.1% of patients.

TABLE 1. Clinical characteristics of patients with invasive fungal rhinosinusitis (n = 39).

Presentation	Patients*
Cranial nerve involvement	19 (48.7)
Decreased vision	18 (46.2)
Headache	15 (38.5)
Periorbital pain	10 (25.6)
Facial pain	7 (17.9)
Alteration of consciousness	7 (17.9)
Facial swelling	6 (15.4)
Nasal congestion	6 (15.4)
Nasal discharge	6 (15.4)
Double vision	5 (12.8)
Fever	4 (10.3)
Epistaxis	3 (7.7)
Cheek numbness	1 (2.6)
Orbital swelling	1 (2.6)
Periorbital swelling	1 (2.6)
Proptosis	1 (2.6)
Seizure	1 (2.6)

Data are presented as n (%). *Some patients probably had more than one sign/symptom.

TABLE 2. Site of involvement in patients with invasive fungal rhinosinusitis.

	Nasal endoscopy/physical examination*	Imaging (CT/MRI)*
Intranasal/PNS lesion		
Ethmoid sinus	13 (33.3)	31 (79.5)
Maxillary sinus	13 (33.3)	25 (64.1)
Sphenoid sinus	13 (33.3)	24 (61.5)
Nasal cavity/septum	11 (28.2)	10 (25.6)
Middle turbinate	10 (25.6)	0 (0.0)
Ostiomeatal unit	2 (5.1)	3 (7.7)
Uncinate process	2 (5.1)	0 (0.0)
Inferior turbinate	1 (2.6)	0 (0.0)
Superior turbinate	1 (2.6)	0 (0.0)
Frontal sinus	0 (0.0)	10 (25.6)
Extranasal/PNS lesion		
Orbit	18 (46.2)	21 (53.8)
Intracranial	6 (15.4)	7 (17.9)
Cavernous sinus	5 (12.8)	4 (10.3)
Palate	4 (10.3)	11 (28.2)
Cheek	2 (5.1)	2 (5.1)

Data are presented as n (%). *Some patients probably had more than one lesion.
Abbreviations: computed tomography = CT, magnetic resonance imaging = MRI, paranasal sinus = PNS

All patients with IFRS in this study had abnormal radiographic findings. Computed tomography or magnetic resonance imaging showed that the lesions were confined to the paranasal sinuses and nasal cavity in only 14 patients (35.9%). The lesions extended to surrounding organs in 25 patients (64.1%). The ethmoid sinus was the most frequently involved paranasal sinus (79.5%) (Table 2). The orbit was the most involved organ outside the nasal cavity and paranasal sinuses (53.8%).

Pathologic examination showed that the ethmoid and maxillary sinuses were the most commonly involved sites (46.2% and 41.0%, respectively) (Table 3). Tissue infarction, vascular invasion, and granulomas occurred in 89.7%, 59.0%, and 7.7% of patients, respectively.

The causative pathogens were identified by tissue culture in 36 patients, and those in the remaining 3 patients were identified by PCR. Positive results were found in 27 patients (culture in 26 and PCR in 1). The fungi in this study were identified as *Aspergillus* spp., Zygomycetes, *Candida* spp., unspecified molds, and *Ramularia* spp. (Table 4).

Treatment options were combinations of surgical interventions and antifungal drugs (Table 5). All patients who underwent nasal and sinus surgery were divided into 3 groups: endoscopic sinus surgery (n = 30), an external approach (n = 3), and a combined endoscopic with external approach (n = 5). The following other extranasal surgical procedures were performed in 11 patients: orbital exenteration (n = 5), craniectomy/craniotomy (n = 2), debridement (n = 2), removal of the orbital wall (n = 1), and internal carotid artery balloon occlusion (n = 1). Thirty-six patients received antifungal drugs. The two most common medications were amphotericin B and voriconazole. Many patients who received voriconazole were administered amphotericin B at the start of treatment before the histological or culture results could be obtained. Some patients were switched from amphotericin B to a lipid formulation or voriconazole because of amphotericin B toxicity.

The mortality rate in this study was 23.1%. Univariate analysis of factors associated with mortality are shown in Table 6. The times to surgery and initiation of antifungal drugs (cut-off at 48 hours) did not affect the results (p = 0.65 and 1.00, respectively). Alteration of consciousness (p = 0.04) and periorbital pain (p = 0.03) had a significant impact on the mortality rate. Both factors were still statistically significant in the multivariate regression model [alteration of consciousness: p = 0.03, adjusted odds ratio (95% confidence interval) = 10.37 (1.31-82.07); periorbital pain: p = 0.03, adjusted odds ratio (95% confidence interval) = 8.67 (1.30-57.88).

TABLE 3. Site of involvement in patients with invasive fungal rhinosinusitis by pathological finding.

Location	Patients*
Ethmoid sinus	18 (46.2)
Maxillary sinus	16 (41.0)
Nasal septum	8 (20.5)
Middle turbinate	7 (17.9)
Sphenoid sinus	7 (17.9)
Orbit	7 (17.9)
Inferior turbinate	6 (15.4)
Uncinate process	4 (10.3)
Floor/lateral wall of nasal cavity	2 (5.1)
Superior turbinate	1 (2.6)
Frontal sinus	1 (2.6)
Ostiomeatal unit	1 (2.6)
Eustachian tube	1 (2.6)
Nasopharynx	1 (2.6)
Posterior choana	1 (2.6)
Cheek	1 (2.6)
Gingivobuccal sulcus	1 (2.6)

Data are presented as n (%). *Some patients probably had more than one lesion.

TABLE 4. Fungal identification from tissue culture and polymerase chain reaction.

	Patients*
Culture	
Negative	10 (25.6)
Positive	26 (66.7)
Aspergillus spp.	15 (38.5)
Aspergillus fumigatus	5 (12.8)
Aspergillus flavus	2 (5.1)
Aspergillus nigar	3 (7.7)
Zygomycetes	9 (23.1)
Not specified	5 (12.8)
Candida spp.	3 (7.7)
Mold	3 (7.7)
PCR	
Negative	2 (5.1)
Positive (*Ramularia* spp.)	1 (2.6)

Data are presented as n (%). *One patient was infected by two types of fungi simultaneously.

TABLE 5. Treatment options and outcomes.

	Patients
Intranasal/paranasal sinus surgery	
Endoscopic sinus surgery only	30 (76.9)
External approach only	3 (7.7)
Combined approach	5 (12.8)
No surgery	1 (2.6)
Extranasal/paranasal sinus surgery	
Orbital exenteration	5 (12.8)
Craniotomy or craniectomy	2 (5.1)
Debridement	2 (5.1)
Partial removal of orbital wall	1 (2.6)
Internal carotid artery balloon occlusion	1 (2.6)
Antifungal drug*	
Amphotericin B	32 (82.1)
Voriconazole	14 (35.9)
Itraconazole	8 (20.5)
Amphotericin B (lipid formulation)	5 (12.8)
Posaconazole	3 (7.7)
Supersaturated potassium iodide	2 (5.1)
No antifungal drug	3 (7.7)
Outcome	
Survival	30 (76.9)
Death	9 (23.1)

Data are presented as n (%). *Some patients received more than one antifungal drug.

TABLE 6. Univariate analysis of factors associated with mortality.

	Mortality rate (%)	OR (95% CI)	p-value
Underlying disease			
Hematologic malignancy	60.0	7.00 (1.00–51.45)	0.07
HIV infection	33.3	1.75 (0.14–21.88)	0.56
Diabetes mellitus	18.5	0.46 (0.10–2.13)	0.42
Autoimmune disease	0.0	1.00	-
CD4 lymphocytopenia	0.0	1.00	-
Presentation			
Neutropenia	66.7	8.29 (0.66–104.89)	0.13
Alteration of consciousness	57.1	7.20 (1.22–42.49)	0.04
Periorbital pain	50.0	6.25 (1.23–31.84)	0.03
Facial swelling	50.0	4.50 (0.72–28.01)	0.12
Cranial nerve palsy	31.6	2.61 (0.55–12.48)	0.27
Headache	20.0	0.75 (0.16–3.59)	1.00
Extension of disease			
Orbit	33.3	4.00 (0.71–22.51)	0.14
Intracranial	28.6	1.43 (0.23–9.01)	0.65
Cavernous sinus	27.3	1.38 (0.28–6.84)	0.69
Palate	25.0	1.13 (0.10–12.36)	1.00
Cheek	0.0	1.00	-
Organism			
Zygomycetes	50.0	4.00 (0.64–25.02)	0.15
Aspergillus spp.	13.3	0.31 (0.05–1.76)	0.25
Pathology			
Vascular invasion	34.8	6.94 (0.76–63.05)	0.11
Therapy			
Posaconazole	66.7	8.29 (0.66–104.89)	0.13
Liposomal amphotericin B	40.0	2.57 (0.36–18.49)	0.57
Extrasinus surgery	36.4	2.63 (0.55–12.55)	0.24
Amphotericin B	28.1	-	0.17
Voriconazole	14.3	0.43 (0.08–2.43)	0.45
Time from admission to surgery	28.6	1.43 (0.23–9.01)	0.65
Time from admission to drugs	23.1	0.85 (0.17–4.17)	1.00

Abbreviations: odds ratio = OR, confidence interval = CI, human immunodeficiency virus = HIV

DISCUSSION

The patients in our study had a lower mortality rate (23.1%) than patients in other studies (43%-80%).[1,2,4,10-12] Our mortality rate was similar to that in studies by Kasapoglu et al.,[5] and Li et al.,[13] who reported mortality rates ranging from 20% to 30 %. DelGaudio and Clemson[6] and Piromchai and Thanaviratananich[9] reported that early treatment of IFRS significantly decreased the mortality rate. Therefore, our favorable outcome probably resulted from prompt management within 24 hours after the provisional diagnosis, although the difference in the time from admission to surgery and the time from admission

to antifungal treatment was not statistically significant.

In this study, the most common underlying diseases associated with IFRS were diabetes mellitus (69.2%) and hematologic malignancy (12.8%). Turner et al.,[1] reported the lowest mortality rate among patients with diabetes mellitus compared with other underlying diseases. Hematologic malignancy was more common in previous studies.[2-4,11,14]

Blurred vision was a common symptom.[11,15] It was often accompanied by headache, periorbital pain, facial pain, facial edema, nasal stuffiness, and rhinorrhea. We found a large number of patients with nervous system involvement characterized by cranial nerve dysfunction (48.7%) and alteration of consciousness (17.9%). Orbital extension in patients with IFRS was associated with a high mortality rate (33.3%), similar to previous studies by Turner et al.,[1] and Ilica et al.[16] The onset of clinical presentations was prolonged more than 28 days before admission in 25 patients. Our mortality rate was lower than that in previous studies because of the slow disease progression in most of our patients.[1,2,4,7,9-11,14]

In this study, most abnormal findings were identified from nasal endoscopy and radiographic imaging in similar proportions. The exception is that the frontal sinus could not be accessed by nasal endoscopy, and the middle turbinate was very difficult to evaluate by imaging alone. Gillespie et al.,[17] reported that the most common site of involvement was the middle turbinate with a diagnostic sensitivity and specificity of 75% and 100%, respectively.[17] The ethmoid sinus was the most common site of infection based on our pathological reports, and this finding corresponded with the endoscopic and radiographic study findings. Thus, in addition to the middle turbinate, the ethmoid sinus may need to be evaluated when clinicians suspect IFRS.

Tissue culture was beneficial for fungal identification and led to specific antifungal drug administration in this study. Among all 39 patients, the causative fungus was identified by tissue culture in 36 patients and by PCR in 3 patients. *Aspergillus* spp. was the most frequent species in this study. One patient was infected by 2 organisms (*Aspergillus* and *Rhizopus* spp.). PCR provided a positive result in only 1 patient. The negative PCR results might have resulted from disintegration of DNA due to aging of the paraffin block, scant fungus within the tissue, prior antifungal drug administration, improper PCR technique, or misinterpretation of the pathological study findings.

Treatment modalities included surgery, systemic antifungal drug administration, and reversal of the preexisting immunocompromised status. DelGaudio and Clemson[6] concluded that early diagnosis and treatment of IFRS could decrease the mortality rate. Adequate surgical debridement of necrotic tissue is a crucial treatment factor.[7,12] Recent reports have shown equal efficacy between endoscopic and external approaches.[5,12] The endoscopic technique results in less tissue trauma and bleeding and is thus appropriate for early treatment of disease that is limited to the paranasal sinus and nasal cavity.

Amphotericin B is still the first-line drug in the treatment of fungal infection because of its broad-spectrum antifungal activity. It covers *Aspergillus* spp. and Zygomycetes, which are common pathogens in IFRS. Patients with nephropathy have been recommended to change to a lipid formulation of amphotericin B. However, if there is evidence of *Aspergillus* infection, voriconazle is the first-choice treatment instead of amphotericin B.[18,19]

In their systematic review, Turner et al.,[1] studied the prognostic factors for mortality in patients with IFRS and found that advanced age and intracranial involvement were negative prognostic factors. Positive factors were diabetes and surgical treatment. In the present study, negative prognostic factors were alteration of consciousness and periorbital pain. According to the low number of deaths and the rarity of the disease, the 95% confidence intervals were broad.

The main limitation of this study is that it was retrospective; therefore, some information was unavailable, such as the degree of pain. Further prospective studies using a visual analog scale are needed to collect data on pain severity. This would allow for determination of which level of pain is associated with mortality. Moreover, the patients in our tertiary hospital may not represent the general population of patients with this disease because they were treated with antifungals or surgery before referral, affecting the pathological study or tissue culture results, and patients with more severe disease tended to be transferred to our tertiary hospital. Thus, selection bias seems inevitable.

In summary, the mortality rate of IFRS in this study was 23.1%. Negative prognostic factors were alteration of consciousness and periorbital pain. Clinicians must have a high index of suspicion for IFRS, and aggressive treatment should be considered. The low overall mortality rate in this study may be explained by early surgical intervention and early antifungal drug administration.

ACKNOWLEDGMENTS

This research project was supported by the Faculty of Medicine Siriraj Hospital, Mahidol University [Grant Number (IO) R015731089]. The authors thank

Mrs. Jeerapa Kerdnoppakhun (research assistant) for her help in coordinating with the institutional review board and managing the document.

Disclosure of financial support: This study was funded by the Faculty of Medicine Siriraj Hospital, Mahidol University.

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

1. Turner JH, Soudry E, Nayak JV, Hwang PH. Survival outcomes in acute invasive fungal sinusitis: a systematic review and quantitative synthesis of published evidence. Laryngoscope. 2013;123:1112-8.
2. Soontrapa P, Larbcharoensub N, Luxameechanporn T, Cheewaruangroj W, Prakunhungsit S, Sathapatayavong B, et al. Fungal rhinosinusitis: a retrospective analysis of clinicopathologic features and treatment outcomes at Ramathibodi Hospital. Southeast Asian J Trop Med Public Health. 2010;41:442-49.
3. Gillespie MB, O'Malley BW Jr., Francis HW. An approach to fulminant invasive fungal rhinosinusitis in the immunocompromised host. Arch Otolaryngol Head Neck Surg. 1998;124:520-26.
4. Valera FC, do Lago T, Tamashiro E, Yassuda CC, Silveira F, Anselmo-Lima WT. Prognosis of acute invasive fungal rhinosinusitis related to underlying disease. Int J Infect Dis. 2011;15:e841-e844.
5. Kasapoglu F, Coskun H, Ozmen OA, Akalin H, Ener B. Acute invasive fungal rhinosinusitis: evaluation of 26 patients treated with endonasal or open surgical procedures. Otolaryngol Head Neck Surg. 2010;143:614-20.
6. DelGaudio JM, Clemson LA. An early detection protocol for invasive fungal sinusitis in neutropenic patients successfully reduces extent of disease at presentation and long term morbidity. Laryngoscope. 2009;119:180-83.
7. Chen CY, Sheng WH, Cheng A, Chen YC, Tsay W, Tang JL, Huang SY, et al. Invasive fungal sinusitis in patients with hematological malignancy: 15 years experience in a single university hospital in Taiwan. BMC Infect Dis. 2011;11:250.
8. Roongrotwattanasiri K, Fooanant S, Naksane L. Invasive fungal rhinosinusitis in Maharaj Nakorn Chiang Mai Hospital. J Med Assoc Thai. 2007;90:2524-8.
9. Piromchai P, Thanaviratananich S. Impact of treatment time on the survival of patients suffering from invasive fungal rhinosinusitis. Clin Med Insights Ear Nose Throat. 2014;7:31-34.
10. Zuniga MG, Turner JH. Treatment outcomes in acute invasive fungal rhinosinusitis. Curr Opin Otolaryngol Head Neck Surg. 2014;22:242-48.
11. Monroe MM, McLean M, Sautter N, Wax MK, Andersen PE, Smith TL, et al. Invasive fungal rhinosinusitis: a 15-year experience with 29 patients. Laryngoscope. 2013;123:1583-7.
12. Tarkan O, Karagün B, Ozdemir S, Tuncer U, Sürmelioğlu O, Cekiç E, et al. Endonasal treatment of acute invasive fungal rhinosinusitis in immunocompromised pediatric hematology-oncology patients. Int J Pediatr Otorhinolaryngol. 2012;76:1458-64.
13. Li Y, Li P, Zhang G. Diagnosis and endoscopic surgery of chronic invasive fungal rhinosinusitis. Am J Rhinol Allergy. 2009;23:622-25.
14. Suslu AE, Ogretmenoglu O, Suslu N, Yucel OT, Onerci TM. Acute invasive fungal rhinosinusitis: our experience with 19 patients. Eur Arch Otorhinolaryngol. 2009;266:77-82.
15. Lee DH, Yoon TM, Lee JK, Joo YE, Park KH, Lim SC. Invasive fungal sinusitis of the sphenoid sinus. Clin Exp Otorhinolaryngol. 2014;7:181-87.
16. Ilica AT, Mossa-Basha M, Maluf F, Izbudak I, Aygun N. Clinical and radiologic features of fungal diseases of the paranasal sinuses. J Comput Assist Tomogr. 2012;36:570-76.
17. Gillespie MB, Huchton DM, O'Malley BW. Role of middle turbinate biopsy in the diagnosis of fulminant invasive fungal rhinosinusitis. Laryngoscope. 2000;110:1832-6.
18. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med. 2002;347:408-15.
19. Pemán J, Salavert M, Cantón E, Jarque I, Romá E, Zaragoza R, et al. Voriconazole in the management of nosocomial invasive fungal infections. Ther Clin Risk Manag. 2006;2:129-58.

The Teratogenic Effects of Dichlorvos on the Development of Chick Embryos

Jantima Roongruangchai, D.D.S., Ph.D.*, Yadaridee Viravud, D.D.S., M.Sc.*, Vasana Plakornkul, D.V.M., M.Sc.*, Kesorn Sripaoraya, M.Sc.*, Wiwat Jorakit, M.Sc.*, Kosol Roongruangchai, M.D., M.Sc.**

**Department of Anatomy, **Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: The purpose of this study was to elucidate the teratogenic effects of dichlorvos on developing chick embryos.

Methods: The fertilized Leghorn hen eggs were divided into two groups: the experimental group which was injected with 0.1 ml of 0.5% and 1% dichlorvos in normal saline and the control group which was injected with 0.1 ml of normal saline after 21 h of incubation. On day 3, 6, and 11, the embryos were collected for studying embryonic dead and abnormalities.

Results: The results showed that the mortality rate increased with the increasing concentration of dichlorvos and time of incubation. The total mount of day 3 had only three primary brain vesicles, small and retarded primordial eye, dilated U-shaped heart looping, bifurcation of spinal cord and trunk when compared with the control. The results in the serial section of day 3 and 6 showed several abnormalities especially the retardation of eye and heart. Day 11 embryo revealed morphological anomalies including hematoma and bone deformation.

Conclusion: Dichlorvos caused congenital abnormalities in chick embryos in 3 categories, the growth retardation, the malformations and the embryonic death which were predicted to cause the same results in contaminated humans. Dichlorvos exposure increases the risk of malformations and embryonic death. The present study revealed that dichlorvos was a powerful teratogenic compound and therefore its use should be limited and pregnant women should avoid contamination with dichlorvos especially in the first trimester.

Keywords: Dichlorvos; organophosphate; teratogen; chick embryo (Siriraj Med J 2018;70: 44-52)

INTRODUCTION

Currently, pesticides are more benefit in everyday life for preventing, destroying, repelling or eliminating pests. They include herbicides, fungicides, insecticides and various other substances used to control pests (U.S.EPA2012).[1] Insecticide dichlorvos, an organophosphate compound produces toxicity primarily by irreversible inhibition of the acetylcholinesterase (AChE) enzyme that stops the function of acetylcholine (ACh), a neurotransmitter, at the synapses. Moreover, the anti-AChE insecticides are known to affect the reproductive system in both female and male. The persons who are exposed to these insecticides can be affected on pregnancy, mentality and health (Gupta RC, 2011).[2] At present, because of the claimed property of rapidly degradation and less remaining in the nature,organophosphate insecticide is the most commonly used around the world although it is highly toxic (Pesticides News 1995).[3]

In 2002, Asmatullah, studied embryotixicity of methylparathion, anorganophosphate insecticide, in developing chick. There were severe gross malformations such as microcephaly, anophthalmia, micromelia, twisted spinal cord and ectopia cordis (Asmatullah, Salah E 2002).[4] In 2004, Szabo *et al* studied the effects of dichlorvos in the chick embryos which were injected directly into the air chamber on day 0 and evaluated on day 19 of incubation.

Correspondence to Kosol Roongruangchai
E-mail: parasite52@hotmail.com
Received 24 February 2017 Revised 21 April 2017 Accepted 26 April 2017
doi:10.14456/smj.2018.8

The rate of mortality was significantly increased with doses.[3] Dichlorvos has many adverse effects to various species, including human, especially a pregnant women, as dichlorvos can cause the adverse effects to baby such as malformations, or spontaneous abortion (Adhikari J, 2011).[5] However, there is no any study to confirm the teratogenicity, toxicity and also the pattern of disability produced by dichlorvos. This study was conducted to demonstrate the teratogenic effects of dichlorvos to the embryo by using chick embryo as an animal model and compared the results with the age related stages of human embryo. Chick embryos are the most suitable animal models for teratogenic screening study as its development stages were proved to be comparable to humans (Hamburger V, Hamilton HL, 1992)[5] and there were plenty of databases of comparison. Moreover, chicken eggs are easily available, chick embryos have short gestation and are also inexpensive and easily manipulated. Animal studies are important as they can lead to the mechanisms of teratogenicity and because when such an agent causes similar patterns of anomalies in several species, human teratogens should also be suspected.

MATERIALS AND METHODS

Ethics statement

According to German animal care guidelines, no IACUC approval was necessary to perform this experiment. According to the local guidelines, only experiment with chick embryos of E18 and more need IACUC approval. However, this study used earlier stages of chick embryos, day 3-day 11.

ACUCGuideline

The Use and Euthanasia Procedures of Chicken/ Avian Embryos

Avian embryos are not considered live animals under PHS policy. However, there is a consensus in the scientific community that at a certain point in development, avian embryos can experience pain. Because that exact point is not known for chicken embryos, chicken use and euthanasia guidelines differ across institutions. Cal Poly Pomona has chosen to adopt a guideline with the belief that pain occurs on or after gestation day 13, in anticipation of reviewing protocols including them. However, this study used earlier stages of chick embryos, day 3-day 11.

Experiment protocol

150 fertilized white leghorn hen (*Gallus domesticus*) eggs were purchased from the Institute of Suwanvajokkasikij Animal Research and Development, Department of Animal Science, Faculty of Agriculture, Kasetsart University. The eggs were randomly divided into 5 groups, 30 each and treated with 0.1 ml of 0.5%. 1% and normal saline at 21 h of incubation into the yolk sac and further incubated until day 3, 6 and 11 of development. All embryos from each stage were recorded the number of deaths and survivals as well as the normal and the abnormal eggs that could be visible externally. The viability was indicated by the heart beating and the blood circulation. The day 3 embryos were processed for total mount and serial section, the day 6 embryos were processed for the serial section and the day 11 embryos were processed for the cartilage and bone staining.

The total mount of day 3 embryos were stained with Mayer's Camine for 30 min and dehydrated with graded series of alcohol for 5 mins each change. Then the specimens were cleared and mounted. The serial sections of day 3 and day 6 were stained with hematoxylin and eosin stains. The cartilage and bone staining of the day 11 were performed by removing the skin and visceral organs and fixed in 4% paraformaldehyde before washing and dehydration with absolute alcohol then transferred to acetone for 24 hr before staining with alcian blue and alizarin red for 3-4 days each at 37°C, then rinsed and transferred to 1% KOH and glycerol. The crown-rump length (CRL), beak, mandible, and other long bones were measured by the red color of the alizarin red, then these were compared with the control and tested statistically using Statistical Package for the Social Science (SPSS) by one way analysis of Variance (ANOVA), the data represented by mean ± SD with significant difference at $p<0.05$.

RESULTS

The effects of dichlorvos on days 3, 6 and 11 chick embryos

The mortality rate

The mortality and survival rates of day 3, 6 and 11 chick embryos were observed by the heart beating and blood circulation. These stages can be compared to the 4th, 5th and 8th weeks of human embryo after fertilization, respectively. All control groups showed 100% survival while the experiment groups showed increases in mortality rates as the concentrations of dichlorvos increased and also corresponding to the advancing age. The highest mortality rate was 74.43% of day 11 in the 1% concentration group.

TABLE 1. Mortality and survival rates of the day 3 chick embryo after treated with different concentrations of dichlorvos and compared with the control which was treated with NSS.

Concentration (%) Control (NSS) (n=7)	Mortality rate (%) 0 (n=0)	Survival rate (%) 100 (n=7)
0.5 (n=14)	28.57 (n=4)	71.43 (n=10)
1 (n=13)	38.46 (n=5)	61.54 (n=8)

*NSS is normal saline solution

TABLE 2. Mortality and survival rates of the day 6 chick embryo after treated with different concentrations of dichlorvos and compared with the control.

Concentration (%) Control (NSS) (n=5)	Mortality rate (%) 0 (n=0)	Survival rate (%) 100 (n=5)
0.5 (n=12)	41.67 (n=5)	58.33 (n=7)
1 (n=13)	46.15 (n=6)	53.85 (n=7)

TABLE 3. Mortality and survival rates of the day 11 chick embryo after treated with different concentrations of dichlorvos and compared with control.

Concentration (%) Control (NSS) (n=4)	Mortality rate (%) 0 (n=0)	Survival rate (%) 100 (n=4)
0.5 (n=7)	57.14 (n=4)	42.86 (n=3)
1 (n=7)	71.43 (n=5)	28.57 (n=2)

Statistic analysis of the body measurement data of the day 6 chick embryo

Measurements of the day 6 chick embryo included body weight, crown rump length (CRL), width of head, width of eye, upper limb and lower limb. All parameters were processed for statistical analysis shown in Table 4.

TABLE 4. Statistic analysis of the body measurements of the day 6 chick embryos after treated with different concentrations of dichlorvos and compared with control.

Group	Control	0.5%	1%
Body weight (g±SE)	0.29±0.02	0.23±0.02	0.22±0.03*
CRL (mm±SE)	12.85±0.29	11.59±0.44	7.69±2.03*
Width of head (mm±SE)	6.60±0.28	5.73±0.29	3.64±0.98*
Width of eye (mm±SE)	3.52±0.07	3.31±0.17	1.55±0.48*
Upper limb (mm±SE)	3.42±0.08	3.25±0.11	1.69±0.50*
Lower limb (mm±SE)	3.83±0.05	3.42±0.13	1.81±0.55*

Data are the mean±standard error. *Statistic mean difference showed significant ($p<0.05$) by one way ANOVAs.

Statistical analysis data of the day 6 chick embryo showed the body weight, the CRL, the width of head, the width of eye, the upper limb and the lower limb of the 1% dichlorvos-treated groups were significantly lower than the control group while 0.5% dichlorvos-treated groups showed no significant difference when compared with the control.

Total mount of the day 3 chick embryo

The effects of dichlorvos on day 3 chick embryo were examined and compared with the control. In the control, the total mount of the day 3 chick embryo can be referred to as Hamburger-Hammilton stage 18 which had approximately 36 somites. The brain showed 5 secondary brain vesicles which comprised telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon. The eye comprised optic cup and lens vesicle at the diencephalic region and the otocyst at the myelencephalon. Anterior and posterior limb buds were presented (Fig 1A). Figs 1B and 1C were 0.5% and 1% dichlorvos treated groups which showed several abnormalities and growth retardation. The brain showed three primary brain vesicles with only cephalic flexure with very small primordial eye which is referred to as microphthalmia. The heart loops were looser with distended heart chambers. Branchial arches showed only one arch. Neural tube appeared as two dense lines which were not parallel. The somites appeared only on the upper part of the body. There was absence of the limb bud and tail fold with the opening of the posterior neuropores. The 1% dichlorvos group showed caudal degeneration. One of the 0.5% and 1% of dichlorvos treated groups showed similar abnormalities, conjoined twins, appeared as the lower part of the body bifurcated (Fig 1D and 1E) and also showed brain retardation and abnormality of eye (microphthalmia and anophthalmia) and heart development. All primordial eyes were extremely small and the heart loop showed abnormal looping, and the brain was under developed.

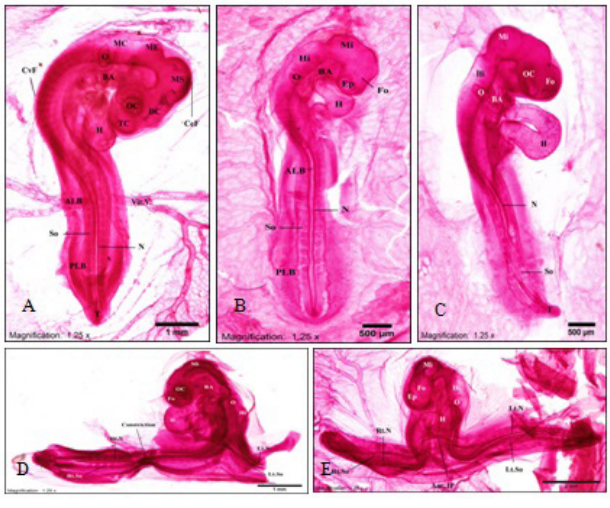


Fig 1. Total mount of the control (A), 0.5% (B) and 1% (C) dichlorvos treated groups, showed normal development in the control and growth retardation in all the experiment groups with severe underdevelopment of brain and the flexures. The eyes were smaller than the control which referred to as microphthalmia. The heart loops were looser U-shaped and distended. The somites were not extend to the caudal end with the absent of limb bud and tail fold. The 1% dichlorvos treated group showed caudal degeneration from the middle of the body. ID and 1E showed similar interesting congenital abnormality, bifurcation of the body or referred to as the conjoined twins, and also showed the retardation of brain formation, microphthalmia, anophthalmia, looser of heart looping (TC) telencephalon, (DC) diencephalon, (MS) mesencephalon, (ME) metencephalon, (MC) myelencephalon, (OC) optic cup, (O) otocyst, (BA) branchial arch, (H) heart, (CeF) cephalic flexure, (CvF) cervical flexure, (So) somite, (N) neural tube, (ALB) anterior limb bud, (PLB) posterior limb bud, (T) tail fold, and (VitV) vitelline vein.

The serial section of the day 3 and day 6 chick embryo

The abnormalities of the eye

The serial section of the day 3 chick embryo in 0.5% (2B) and 1% (2C) dichlorvos treated group (Fig 2) showed severe retardation of the eye when compared with control group (2A). In the control (2A) the optic cup comprised inner nervous layer and outer pigment layer which were closely contacted to each other. The lens placode was completely fused and detached from the surface ectoderm. The lens comprised the anterior lens epithelium and posterior lens fiber which was elongated to obliterate the lens cavity. In the experimental groups, the lens vesicle presented clearly the lens pit (2B and 2C) which indicated that the lens placode did not undergo a process of double fusion to become the lens vesicle. Posterior lens fiber showed no elongation and the lens cavity was widely opened. The optic cup was smaller than the control and showed severe retardation, the intraretinal space was widely separated when compared with the control.

The effects to the eye of the day 6 chick embryo (Fig 2D, 2E and 2F), the optic cup and lens of the control group illustrated in Fig 2D showed oval-shaped double-walled optic cup comprised of 2 layers; outer pigment layer and inner nervous layer. Both layers were outer thin layer and inner thick layer separated by obliterated intraretinal space. The lens vesicle was an oval-shaped, and comprised anterior lens epithelium which was a cuboidal epithelium and posterior lens fiber which was extremely elongated as columnar epithelium. The lens cavity was obliterated in the day 6 chick embryos. The

cornea developed from the mesenchyme and surface ectoderm superficial to the lens. The anterior chamber located between cornea and lens while the vitreous chamber located posterior to the lens. Most of the day 6 chick embryos showed more or less retardation of eye development such as smaller, irregular-shaped optic cup and lens vesicle when compared with the controls. There were wide separation of the intraretinal space and underdeveloped lens (Fig 2E).

One of the 1% dichlorvos group showed severe abnormal eye development, which represented only primitive eye primordial, optic vesicle, and there was no invagination to form double walled optic cup. However, distal part of optic vesicle induced the surface ectoderm to form small lens detached from the surface ectoderm (Fig 2F). This optic primordium originated from the diencephalon and the distal end expanded to form optic vesicle. The right optic vesicle induced the surface ectoderm to form successively a small lens before the development ceased (Fig 2F). The left optic vesicle failed to induce the surface ectoderm to form lens (Fig 4A).

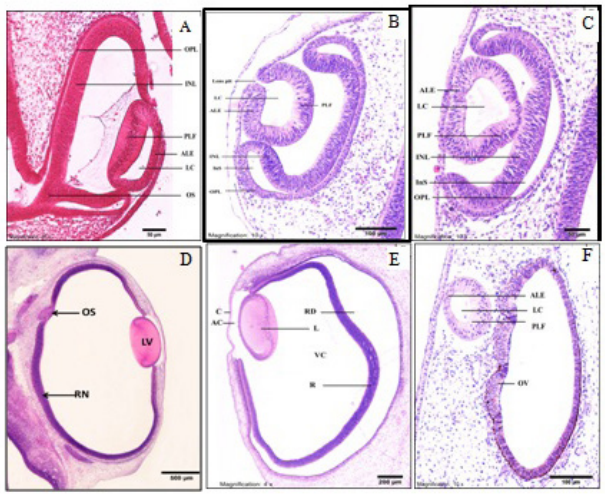


Fig 2. The serial section of the day 3 chick embryo in the control (2A) and the 0.5% (2B) and the 1% (2C) of dichlorvos treated groups: In the control (2A) the optic cup comprised outer pigment layer and inner nervous layer which contacted each other so the intraretinal space was obliterated while in 2B and 2C there were wide separation of intraretinal space. The lens of the control was completely fused and the posterior lens fiber was elongated while the lens placodes of 2B and 2C were incompletely fused left the opening of lens pit. (OPL) outer pigment layer, (INL) inner nervous layer, (PLF) posterior lens fiber, (ALE) anterior lens epithelium, (LC) lens cavity, and (OS) optic stalk, (OC) optic cup, (LV) lens vesicle, (DC) diencephalon, (OPL) outer pigment layer, (InS) Intraretinal space, (INL) inner nervous layer, (PLF) posterior lens fiber, (ALE) anterior lens epithelium, and (LC) lens cavity. The optic cup and lens of the day 6 chick embryos of the control (2D), 0.5% (2E) and 1% (2F) of dichlorvos treated groups, 2E showed retardation of eye development i.e. smaller optic cup, the wide separation of intraretinal space. 2F showed malformation of eye development, no optic cup formed and there was only rudimentary structure of eye primordial. C, cornea, AC, anterior chamber, L lens, RD, retinal detachment, VC, vitreous chamber, R, retina, ALE, anterior lens epithelium, PLF, posterior Lens fiber, LC, lens cavity, OV, optic vesicle.

The multiple abnormalities

Several embryos of both treated groups showed multiple abnormalities. The one with brain anomaly might show anomaly of the internal ear and eye together with the heart and viscerae. The 2 figures below (Figs 3 and Fig 4) showed two types of multiple abnormalities of brain, ear, eye, heart, liver, mesonephros and body wall with trunk degeneration (Figs 3 and 4).

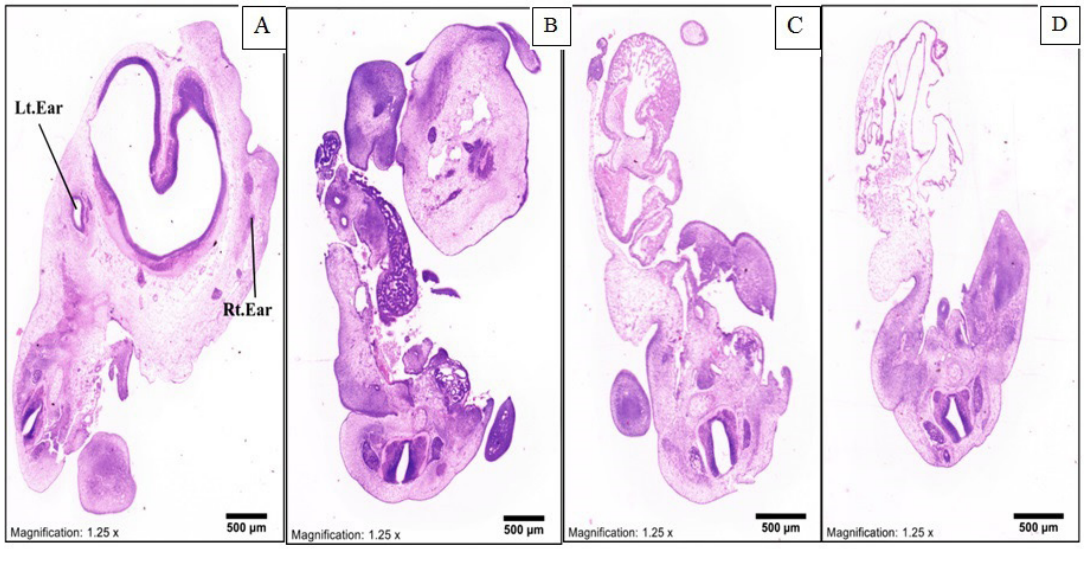


Fig 3. The series of serial section of one of the 6 chick embryo of 1% dichlorvos-treated group, showed very severe retardation and abnormality of the development. The structures in the embryo were dislocated and underdeveloped such as heart, liver, and urogenital system. The ear was developed only at the left side. At the right side, the ear was obliterated and ceased. The eye did not develop in this embryo bilaterally as a result in the bilateral anophthalmia (A). B showed small and underdeveloped liver (L) situated outside the body (ectopia viscerae), C showed heart (H) with ectopia cordis, D showed degenerated trunk. Me, mesonephros.

The 1% of dichlorvos treated group showed multiple abnormalities in the same embryo. Figs 3 and 4 were 2 sets of serial section of 2 embryos of the 1% dichlorvos treated group. Fig 3A cut through the level of myelencephalon where the otocyst formed. This embryo showed twisted and irregular brain and there was an absence of left side optic primordium, while the right otocyst developed adjacent to the myelencephalon. This embryo also showed absence of both primordial eyes as well as unidentified diencephalon and this was the so call bilateral anophthalmia. When tracing caudally to the trunk, this embryo showed a large opening of thoracoabdominal wall which caused the liver and all gut structures to be situated outside the body which was called ectopia viscerae. The mesonephros was rudimentarily situated at the posterior body wall. Fig 3C showed the open body wall and the heart situated outside the body cavity which was called ectopia cordis. The spine showed irregular-shape with the widening of the roof plate. Fig 3D showed degeneration of the lower trunk. This embryo of 1% dichlorvos treated showed absence of eyes and ears with irregularities of brain and spinal cord, ectopia viscerae with small liver and short digestive tract, ectopia cordis and small mesonephros.

Fig 4A showed normal development of otocyst at the level of myelencephalon, while ventrally showed abnormality of both eyes. Abnormality of both optic vesicles failed to form double walled optic cups, and the left side induced the surface ectoderm to form a small lens while the right side failed to form lens (bilateral anophthalmia). Fig 4B showed the heart situated outside the thoracic cavity (ectopia cordis). Figs 4C and 4D showed small and underdeveloped liver and mesonephros.

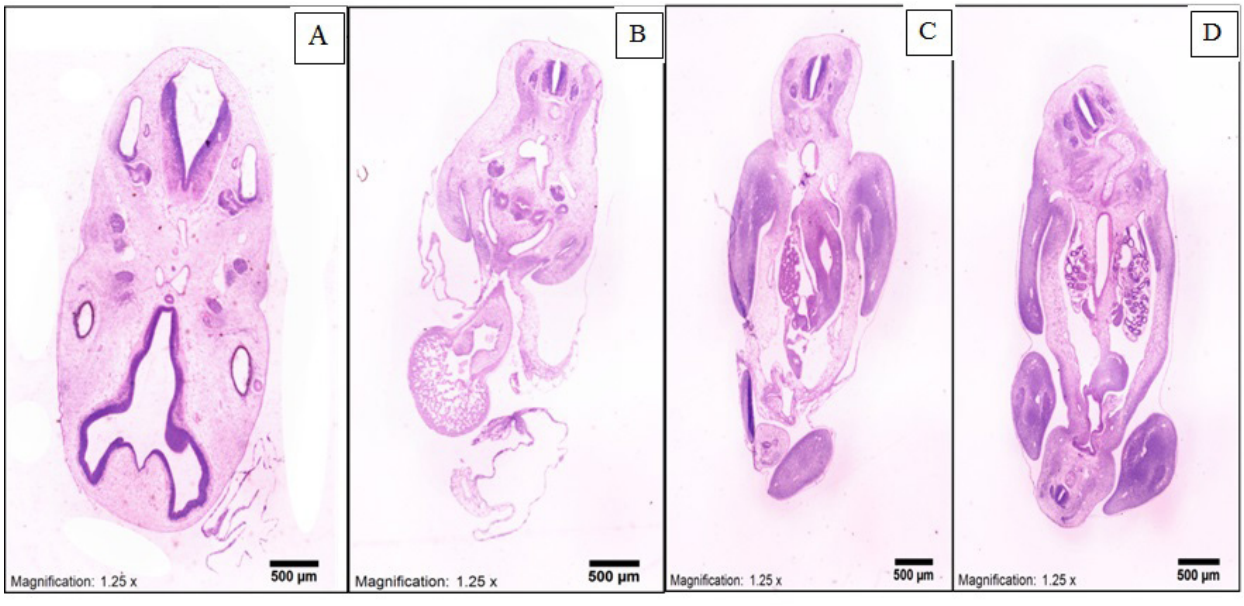


Fig 4. Another series of serial section of the day 6 chick embryo of 1% dichlorvos-treated group showed very severe retardation and abnormality of the development. The structures in this embryo were dislocated and underdeveloped such as heart, liver, and urogenital system. The eye did not develop to the final stage but ceased at the stage of optic vesicle (A), result in the bilateral microphthalmia. Body wall was absent with ectopia cordis (B) while other viscerae developed inside the body but showed small and underdeveloped liver (C) and mesonephros (D). D, diencephalon, OC, optic cup, H, heart, L, liver, Me, mesonephros.

The effects of dichlorvos on the day 11 chick embryo

General features of the 11th day chick embryo

The 11th day chick embryo of the control group shown in Fig 5A was about the Hamburger Hamilton stage 35. Length of beak from anterior edge of nostril to tip of bill could be measured.The length of the third toe was measured to represent the length of digit of the foot. Scales were overlapping on inferior as well as superior surfaces of leg. The feather was well developed all over the body. Fig 5B was the 0.5% of dichlorvos treated group showed smaller and under developed external appearance. It showed shorter beak and smaller eye, shorter upper and lower limbs, and no feather formation. Fig 5C was the 1% of dichlorvos treated group which showed smallest of the three with lower limb and trunk degenerated. Anterior body opened and the viscerae laid outside the body cavity.

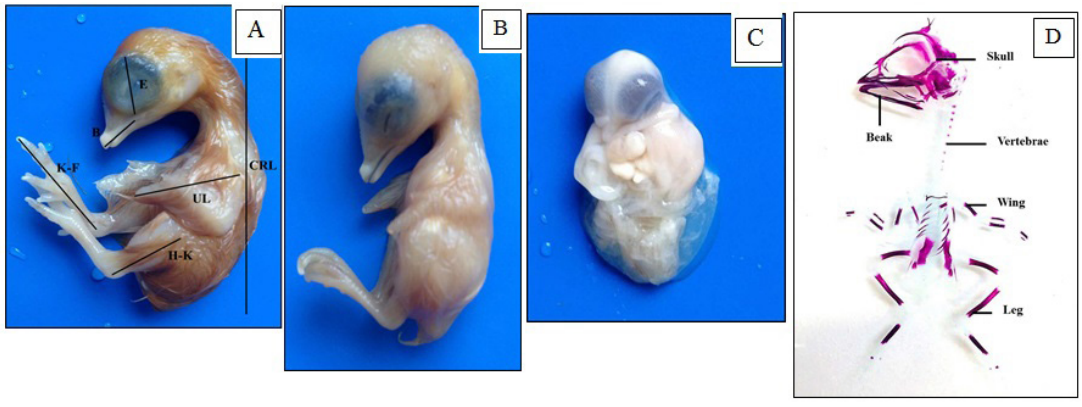


Fig 5. The external appearance of the day 11 chick embryos of the control (A), 0.5% (B) and 1% (C) of dichlovos treated groups, A showed normal development. B showed smaller and no feather germ. C were severely small with under developped limbs and tail. C also showed no anterior body wall with ectopia viscerae. E, eye, B, beak, CRL, crown rump length, UL, upper limb, HK, hip knee, KF. Knee foot, EV, ectopia viscerae. D was the alcian blue and alizali red staining of the cartilages and bones. The red area of bone were measured and test statistically in Table 5.

The alcian blue and alizarin red staining

This procedure was performed to observe the skeletal malformation. The experimental group of the day 11 showed a striking mortality rate in which very few embryos could survive up to 11 days. The survival embryos showed normal appearances when compared with the control and when they were processed for alcian blue and alizarin red staining, the bone and cartilage showed no significant difference as shown in Fig 5D. The ossification centers were stained red while cartilage stained blue. The lengths of the ossification center of each bone were measured by the stereomicroscope, the data were then evaluated for statistical difference between the control, 0.5% and 1% dichlorvos treated groups by mean-+standard error at $p<0.05$ by one way ANOVAs as shown in Table 5.

TABLE 5. Statistic analysis of the bone measurement data of the 11th day chick embryo after treated with different concentrations of dichlorvos and compared with the control.

Group	Control	0.5%	1%
CRL (mm±SE)	45.11±3.20	39.19±3.46	39.51±2.19
Beak (mm±SE)	7.87±0.10	4.73±0.29*	5.54±0.20*
Mandible (mm±SE)	14.58±0.04	12.90±0.17	13.41±0.25
A-P (mm±SE)	21.88±0.13	19.11±0.40*	18.58±0.18*
Lt. humerus (mm±SE)	4.83±0.16	4.26±0.41	4.00±0.23
Rt. humerus (mm±SE)	5.09±0.03	3.94±0.32*	4.04±0.00*
Lt. radius (mm±SE)	4.87±0.09	4.00±0.24	3.64±0.25*
Rt. radius (mm±SE)	5.41±0.03	3.89±0.12*	3.72±0.13*
Lt. ulna (mm±SE)	4.80±0.06	4.13±0.21	3.81±0.16*
Rt. ulna (mm±SE)	4.82±0.05	3.96±0.20*	3.85±0.00*
Lt. metacarpus (mm±SE)	3.33±0.02	2.59±0.16*	2.48±0.02*
Rt. metacarpus (mm±SE)	3.29±0.14	2.66±0.10*	2.69±0.14*
Lt. femur (mm±SE)	7.81±0.03	6.29±0.36	6.06±0.08
Rt. femur (mm±SE)	7.77±0.03	6.07±0.31*	5.84±0.25*
Lt. tibia (mm±SE)	10.44±0.01	8.18±0.40*	7.90±0.02*
Rt. tibia (mm±SE)	10.29±0.04	8.25±0.51*	7.92±0.19*
Lt. metatarsus (mm±SE)	7.26±0.06	5.67±0.33*	5.01±0.44*
Rt. metatarsus (mm±SE)	7.12±0.10	5.41±0.45*	5.02±0.45*
Lt. digit of foot (mm±SE)	10.07±0.05	8.39±0.12	6.92±1.41*
Rt. digit of foot (mm±SE)	10.08±0.04	7.62±0.65	7.80±0.29

Data were the mean±standard error. *Statistic mean difference shows significant ($p<0.05$) by one way ANOVAs.

Statistical analysis of the length of ossification centers of bones which were measured by stereomicroscopy indicated that ossification centers of the experimental group showed significantly lesser bone formation except for the length of mandible, humerus, femur and right digit of foot which showed slightly less, but non- significant when compared with the control. Moreover the 0.5% dichlorvos treated group showed non-significant difference of the length of the left ulna, left radius and left digit when compared with the control group (Table 5).

DISCUSSION

The majority of pesticides do not specifically target the pest only and during their application they also affect non-targetted plants and animals, such as wild bees, butterflies, birds, frogs, fishes, etcetera. Repeated application leads to loss of biodiversity. Many pesticides are not easily degradable, they persist in soil, leach to groundwater and surface water and contaminate the wider environment. Depending on their chemical properties they can enter the organism, bioaccumulate in food chains and consequently influence also human health. For this reason, no one can ensure that such chemical residues were the major causes of the congenital abnormalities which occurred in the babies born in the pesticide treated area.

Dichlorvos (DDVP), the organophosphate (OP) insecticide, is widely used to control pests. Several studies reported that dichlorvos irreversibly inhibited the function of the acetylcholinesterase (AChE), and acetylcholine (ACh) was then uncontrolled and accumulated at the synapse, resulting in increase of the cholinergic receptor activity.[6] The cholinergic receptors comprised muscarinic and nicotinic receptors. The ACh affected the muscarinic receptors of the eye, heart, GI tract, glands, and respiratory system and the nicotinic receptor of the muscle cell. Moreover, the acetylcholine affected to the non-neuronal cell activity[7,8] which presents on the epithelial cell[9] endothelial cell,[10] and tendons[11] including those involved with the cell proliferation and differentiation.[12] In addition, acetylcholinesterase activity is very important to the embryonic development especially the gastrulation and neurulation steps.[13,14,15] Furthermore, the effects of abnormal AChE activity were similar to the effects of the abnormal ACh activity especially cell proliferation, cell migration and differentiation.[16] The abnormality of the embryo could be observed when the function of the acetylcholinesterase was irregular. Although, DDVP is known to be used for several functions, it is very dangerous for the embryo such as anomaly of morphology, visceral abnormality, and the severe congenital malformation.

This result indicated that the mortality rates increased when the concentration of the dichlorvos increased, and also the duration of exposure was longer. This result supported the previous study which reported the adverse effect of dichlorvos to the embryo which depended on the size and species of animal, the concentration of dichlorvos and duration of exposure.[16,17]

Dichlorvos caused congenital abnormalities in chick embryos in 3 categories, the growth retardation, the malformations and the embryonic death which were predicted to cause the same results in contaminated humans. The mortality resembled spontaneous abortion, and growth retardation indicated the intrauterine growth retardation in the pregnant woman and the malformation indicated the human congenital abnormalities. This study illustrated the abnormal development of brain, internal ear, anophthalmia, microphthalmia, irregular heart looping, ectopia cordis, ectopia viscerae, small and underdeveloped liver and mesonephros and caudal degeneration, some of which agree with the previous reports.[3-5]

There were bifurcations of the caudal part of the bodies in two dichlorvos-treated groups. From the previous study[18], they explained about the abnormality of the twinned embryo which produced duplicated posterior. The embryo was formed by bisecting of the primitive streak. Then, both primitive streaks developed and the anterior end was coiled as the head. The concept of the result in this study was adjusted from their idea and the idea from the previous study about the mechanism of dichlorvos toxicity. Dichlorvos is an organophosphate insecticide which inhibits the function of acetylcholinesterase permanently. The AChE activity began from the gastrula stage (H.H stage 5). At the gastrula stage, the AChE was located in the area opaca. During the first neurulation, most AChE reaction was found in the notochord and in the portion of the neural fold.[5-7] Thus, AChE was collected in the notochord. Dichlorvos affected on the notochord at the point caudal to anterior intestinal portal in the late gastrulation period at 21 hours of incubation. The notochord broke and the caudal part of the notochord bifurcated. Each notochord developed and induced neuroectoderm to be a neural tube. In the early stage, cephalic part of neural tube developed into primary brain vesicle which had only one in this embryo. Two caudal parts of neural tube which developed from the bifurcation of caudal part of the notochord developed into two spinal cords. Thus, this embryo had only one head and two bodies. This idea was explained by diagram as shown (Fig 6).

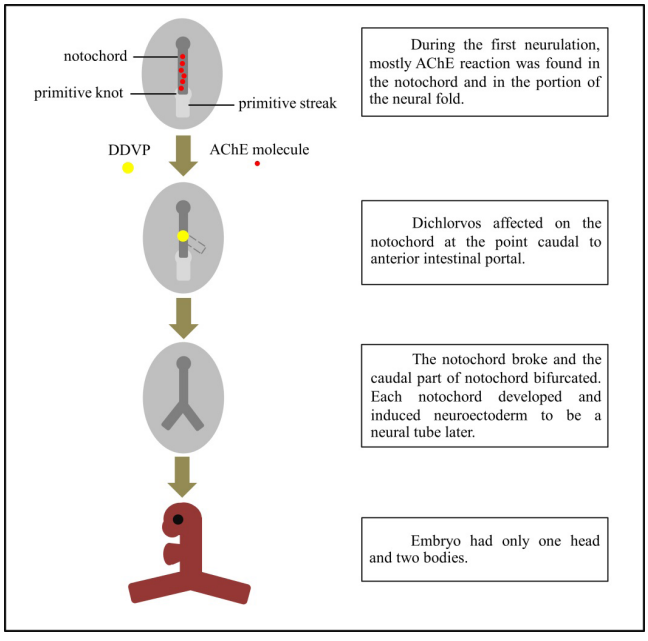


Fig 6. The idea of bifurcation at caudal part of notochord led to develop two bodies in the abnormal day 3 embryos of both experiment groups of this study.

CONCLUSION

Dichlorvos (DDVP) was reported in this experiment to cause several malformations in chick embryos which might cause the same effects to human embryos of the age related period. DDVP caused abnormalities to brain, ear, eye, heart, liver, mesonephros, gonad and anterior abdominal wall which might relate to multiple gene deformities. In the area using the pesticides, DDVP might produce harmful effects to humans in the same way as occurred in animal models. Therefore, there should be strong measure to prevent the toxicity such as the limit of using the chemical, the users themselves must have protective equipment and clothing and enough knowledge. In particular, pregnant women should avoid contamination with the chemicals especially in the first trimester of pregnancy.

REFERENCES

1. United States Environmental Protection Agency (U.S. EPA). Pesticides. 2012 Sep.[cited 2015 Jan. 7] Available at: http://www.epa.gov/pesticides/
2. Gupta RC, Malik JK, Milatovic D. Chapter 37- Organophosphate and carbamate pesticides, Reproductive and Developmental Toxicology (pp. 471-86). Kentucky, USA: Elsevier, 2011.
3. Asmatullah, Salah E, Andleeb S, Iqbal A. Embryotoxicity of methlyparathion in developing chick. Punjab University J Zoology 2002; 17: 28-34.
4. Szabo R, Keseru M, Fejes S, Budai P, Juhasz E, Pongracz A. Toxicity of a dichlorvos containing insecticide formulation and an atrazine containing herbicide formulation in chicken embryos after individual administration. Commun Agric Appl Biol Sci. 2004;69(4):811-4.
5. Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. 1951. Dev Dyn. 1992;195(4): 231-72.
6. Wessler I, Kirkpatrick CJ. Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans. Br J Pharmacol. 2008; 154(8):1558-71.
7. Wessler I, Kilbinger H, Bittinger F, Unger R, Kirkpatrick CJ. The non-neuronal cholinergic system in humans: expression, function and pathophysiology. Life Sci. 2003;72(18-19):2055-61.
8. Klapproth H, Reinheimer T, Metzen J, Munch M, Bittinger F, Kirkpatrick CJ, et al. Non-neuronal acetylcholine, a signaling molecule synthesized by surface cells of rat and man. Naunun-Schmiedeberg's Arch Pharmacol 1997; 355: 515-23.
9. Kirkpatrick CJ, Bittinger F, Nozadze K, Wessler I. Expression and function of the non-neuronal cholinergic system in endothelial cells. Life Sci. 2003; 72: 2111-16.
10. Danielson P, Andersson G, Alfredson H, Forsgren S. Extensive expression of markers for acetylcholine systhesis and of M2 receptors in tenocytes in therapy-resistant chronic painful patellar tendon tendinosis - a pilot study. Life Sci. 2007; 80: 2235-8.
11. Resende RR, Alves AS, Britto LRG, Ulrich H. Role of acetylcholine receptors in proliferation and differentiation of P19 embryonal carcinoma cells. Exp Cell Res. 2008;314(7):1429-43.
12. Caterina M, Agati PD, Cangialosi MV, Mansueto V, Puccia E. Further data on expression of acetylcholinesterase during oogenesis and embryonic development of Cionaintestinalis and Ascidia malaca (Ascidiacea, Urochordata): a biochemical and histochemical study. Caryologia 2006; 59(4): 388-94.
13. Sandra FM, Gunther SS. Appearance and localization of acetylcholinesterase in embryos of the leech Helobdella Triserialis. J Neurosci. 1981;1(8):901-7.
14. Laasberg T, Neuman T. Changes in the acetylcholinesterase and choline acetyltransferase activities in the early development of the chick embryo. Roux's Arch Developmental Biology 1985; 194: 306-10.
15. Vidal CJ. Expression of cholinesterases in brain and non-brain tumours. Chem Biol Interact. 2005;157-158:227-32.
16. Ural MS, Calta M. Acute toxicity of dichlorvos (DDVP) to Fingerling Mirror Carp, Cyprinuscarpio L. Bull Environ Contam Toxicol. 2005;75(2):368-73.
17. Ural MS, Koprucu SS. Acute toxicity of dichlorvos on fingerling European catfish, Silurus glanis. Bull Environ Contam Toxicol. 2006;76(5):871-6.
18. Bellairs R, Osmond M. The atlas of chick development, second edition. London, UK: Elsevier, 2005.

Is Non-Contrast Enhanced CT of Paranasal Sinus Adequate for the Diagnosis of Complicated Sinusitis?

Siri-on Tritrakarn, M.D.*, Nidcharat Arlaipol, M.D.*, Jate Lumyongsatien, M.D., Suwimon Wonglaksanapimon, M.D.***

**Division of Diagnostic Radiology, Department of Radiology, **Division of Rhinology and Allergy, Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: To evaluate the accuracy of NECT compared with CECT of PNS for the detection of complicated sinusitis.
Methods: A retrospective review of 96 patients (mean age, 51.78; range 20-84 years), including 44 men and 52 women, with clinically suspicious complicated sinusitis who underwent both NECT and CECT of PNS. Analysis and comparison between CT PNS on NECT and CECT for detection of complicated sinusitis were performed.
Results: Complicated sinusitis was detected in 7 out of 96 patients. Orbital complications alone in 5 patients and 2 patients with both orbital and intracranial complications were found. Detection of orbital complication on NECT was 5 out of 7 patients, giving a sensitivity of 71.4% and specificity of 100%. Detection of orbital complication on CECT was 6 out of 7 patients, giving a sensitivity of 85.7% and specificity of 100%. In this presented study NECT could not demonstrate intracranial complication such as cavernous sinus invasion and brain parenchymal lesion.
Conclusion: NECT of PNS shows high accuracy for the diagnosis of orbital complication, but low sensitivity for the detection of intracranial complication.

Abbreviations: Contrast enhanced computed tomography (CECT) and non-contrast enhanced computed tomography (NECT), paranasal sinus (PNS), Hounsfield unit (HU)

Keywords: CT paranasal sinus; complicated sinusitis (Siriraj Med J 2018;70: 53-59)

INTRODUCTION

Complicated sinusitis showed increased prevalence in Thailand and this condition generally occurred after developing acute sinusitis from 3.7 to 20%.[1] This condition tends to be found in immunosuppressive patients such as during periods of chemotherapy, bone marrow transplantation, kidney transplantation or immunodeficient patients, because these patients develop severe leukopenia and are prone to be infected by fungal or severe bacterial infections.[2] Complications of sinusitis are classified into three types: osseous, orbital, and intracranial complications in which invasive fungal sinusitis frequently causes severe orbital and intracranial complications.[3] Computer tomography of paranasal sinus should be performed in patients who are suspected to have complicated sinusitis for pretreatment diagnosis and preparation before endoscopic sinus surgery.[4] However patients who develop complicated sinusitis such as patients with underlying chronic kidney disease or diabetes mellitus or both usually have conditions that are risk for contrast-induced nephropathy (CIN).[5] CT findings of invasive fungal sinusitis in immunocompromised patients from prior study of DelGaudio JM et.al,[6] found severe soft tissue edema of nasal mucosa, bone erosion, orbital invasion,

Correspondence to: Siri-on Tritrakarn
E-mail: jeabtrit@yahoo.com
Received 4 March 2017 Revised 31 May 2017 Accepted 6 July 2017
doi:10.14456/smj.2018.9

facial soft tissue swelling and thickening of periantral fat in which infiltration of periantral fat may be the earliest sign of invasive fungal disease.[7] All of these findings of fungal sinusitis from prior studies can be detected on NECT due to no need for tissue enhancement feature for detecting lesion. This will decrease incidence of contrast-induced nephropathy and decrease radiation exposure from CT examination due to only performing NECT study.

However, there is no prior study that has reported about accuracy of NECT for detection of complicated sinusitis and cut off density value of periantral fat area which suggests this condition. The objective of the present study was to evaluate accuracy of NECT for diagnosis complicated sinusitis by comparison with CECT and definite diagnosis by using endoscopic findings or histopathologic examination.

MATERIALS AND METHODS

This study was approved by Institutional Ethics Committee of Faculty of Medicine Siriraj Hospital, Mahidol University and received projected code (Si 476/2556). The informed consent was waived due to retrospective study design.

In total 96 patients (44 males and 52 females) were enrolled with age over 18 years old and clinically suspected complicated sinusitis who underwent NECT and CECT of Paranasal sinuses (PNS) and images were collected. All imaging studies were available on PAC system of Siriraj Hospital during September 2011 to March 2013. All imaging data was reviewed without clinical information and images interpreted from NECT of PNS and CECT of PNS by separation of groups of images for reducing bias.

All patients were performed CT PNS by using GE LightSpeed VCT 64-slices scanner. CT images were done in thickness range from 1.0 to 1.25 mm. Reformation of images on axial and coronal planes were done and these images were used for interpretation. Post contrast images were also performed after injection of iodinated contrast media with dose about 1.5 cc/ kg and scanned by the same scanner. All CT PNS on NECT and CECT images were available on PACs system of Siriraj hospital.

Demographic data of all patients which included age, sex, patient conditions (immunocompromised or immunocompetent) were collected. The organisms that cause complicated sinusitis, endoscopic findings with detail of complications and final diagnosis of patients were also recorded. The presented study was reviewed by a six-year experienced neuroradiologist with double blind of clinical information and images of patients on CECT during CT evaluation. Images of CT PNS on NECT and CECT were reviewed on axial and coronal reformatted images in soft tissue and bone window. Each NECT scan was evaluated for location of sinus involvement and evidence of extrasinus involvement including infiltration of periantral fat, intraorbital extension, facial soft tissue swelling, and intracranial extension if there was demonstration of vasogenic brain edema. Additional quantitative measurement of HU of periantral fat with calculated difference of HU of periantral fat at the same level for evaluating the cut off value of abnormal fat infiltration. Each CECT scan was also evaluated for extrasinus involvement including orbital area (apex, intraconal, extraconal parts) and intracranial area (cavernous sinus and brain parenchyma).

Definite clinical diagnosis was proved by endoscopic finding, histopathologic and microbiologic reports.

Statistical analysis

All continuous (quantitative) data, including patient's age and different Hounsfield Unit (HU) of bilateral periantral fat were reported as mean ± SD. Sex and causative organisms were collected and reported in number and percentage. Validity of imaging data on NECT and CECT of PNS were calculated by comparison with definite clinical diagnosis (gold standard) and shown in sensitivity, specificity, PPV, NPV and accuracy. Statistical analysis was performed with a statistical software package (PASW statistics 18).

RESULTS

In total 96 patients were enrolled who were met inclusion criteria. The mean age was 51.78 years (range, 20-84 years), SD=13.63. There were 44 males and 52 females. Eighty four patients were immunocompetent hosts. Twelve (12.5%) patients were immunocompromised hosts such as diabetes mellitus, HIV infection, lymphoma, SLE, anaplastic anemia and patients who received chemotherapy due to hematologic malignancy.

Location of sinus involvement showed 31 patients with bilateral pansinusitis, 6 patients with right sided pansinusitis and 6 patients had left sided pansinusitis. Most commonly affected sinuses were maxillary sinus, ethmoid sinus, sphenoid sinus, and frontal sinus, respectively.

Causative organisms of sinusitis were reported in Table 1. There were no differences in frequency of causative organisms between immunocompetent and immunocompromised hosts which included bacteria, fungus and mixed organisms (Table 2).

Clinical records showed sinusitis with complication in 7 out of 96 patients which 5 out of 7 patients had

TABLE 1. Causative organism of sinusitis in immunocompetent and immunocompromised hosts

Organisms	Hosts		Total
	Immunocompetent	Immunocompromised	
Not found	63 (75 %)	4 (33.3%)	67 (69.8%)
Bacteria	10 (11.9%)	2 (16.7%)	12 (12.5%)
Fungus	8 (9.5%)	3 (25%)	11 (11.5%)
Bacteria & fungus	3 (3.6%)	3 (25%)	6 (6.3%)
Total	84	12	96

TABLE 2. Positive causative organism of sinusitis in immunocompetent and immunocompromised hosts

Organisms	Hosts	
	Immunocompetent	Immunocompromised
Bacteria	10 (47.6%)	2 (25%)
Fungus	8 (38.1%)	3 (37.5%)
Bacteria & fungus	3 (14.3%)	3 (37.5%)
Total	21	8

orbital complication alone, and 2 out of 7 patients had orbital and intracranial complications. Causative agents in complicated sinusitis were fungus (n=3), bacteria (n=2) and not found organism (n=2). Patients with orbital complication were diagnosed by signs and symptoms of blindness (n=2), orbital abscess (n=1), orbital cellulitis (n=1) and cranial nerve palsy (n=3).

NECT of PNS showed evidence of sinusitis in all patients in which 4 patients had bilateral pansinusitis, 2 patients had bilateral maxillary sinusitis and 1 patient had bilateral maxillary with left ethmoidal sinusitis.

NECT of PNS could detect orbital complication in 5 out of 7 patients (sensitivity 71.4%, specificity 100%, PPV 100%, NPV 97.8% and accuracy 97.9%). (Table 3) CT findings showed infiltration of periantral fat and orbital invasion in all five patients (Fig 1).

CECT of PNS could detect orbital complication in 6 out of 7 patients (sensitivity 85.7%, specificity 100%, PPV 100%, NPV 98.9% and accuracy 98.9%) (Table 4) by using abnormal soft-tissue enhancement at orbital areas such as orbital apex, extraconal or intraconal parts. The clinical record revealed two patients had intracranial complication, one patient had cavernous sinus thrombosis (Fig 2) and another one patient had cavernous sinus thrombophlebitis with pituitary abscess (Fig 3). Both patients had bilateral pansinusitis. NECT

TABLE 3. The accuracy of non-enhanced computed tomography (NECT) of PNS for detecting orbital complication

Modality	CT Finding	Orbital complication (+)	Orbital complication (-)	Total
NECT	Positive	5	0	5
	Negative	2	89	91
Total		7	89	96

TABLE 4. The accuracy of contrast-enhanced computed tomography (CECT) of PNS for detecting orbital complication

Modality	CT Finding	Orbital complication (+)	Orbital complication (-)	Total
CECT	Positive	6	0	6
	Negative	1	89	90
Total		7	89	96

TABLE 5. Different Hounsfield unit of bilateral periantral fat

Periantral fat	Mean	Std. deviation	Median	Total
Negative complication group	7.67	4.77	7.00	73
Positive complication group	20.26	9.58	19.00	23

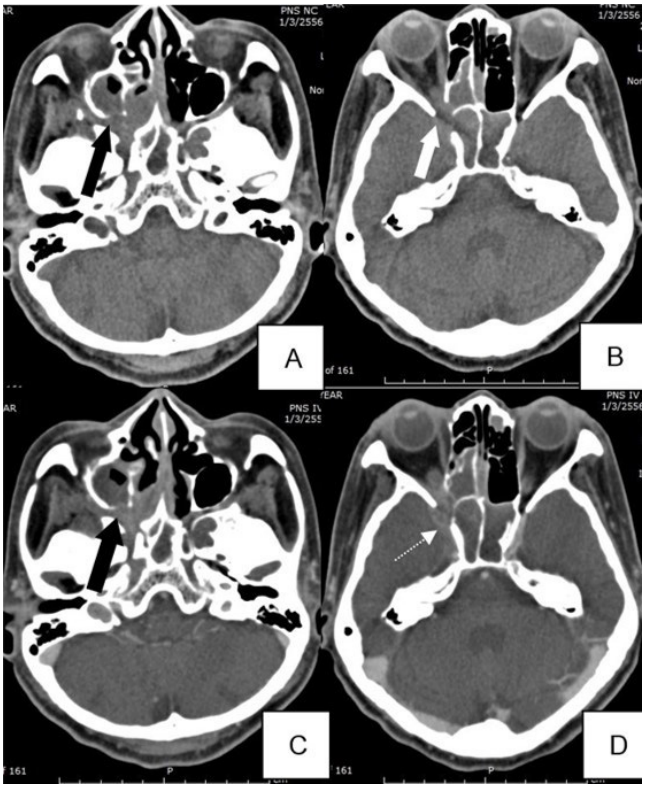


Fig 1. A 63-year-old female underlying poor controlled DM type 2 presented with right sided pansinusitis and right orbital apex involvement from invasive aspergillus infection.
NECT of PNS on image (A)&(B) show fluid filled in right posterior ethmoid sinus and bilateral sphenoid sinuses with demonstrates (A) abnormal soft tissue density lesion inside right pterygomaxillary fissure /pterygopalatine fossa and cause widening of this fissure **(black arrow)**
(B) abnormal soft tissue density lesion also found at right orbital apex and cause widening of orbital apex or superior orbital fissure **(white arrow)**
CECT of PNS on image (C)&(D) show abnormal enhancing soft tissue at the same areas as found on NECT and abnormal bulging of anterior part of right cavernous sinus, which suggestive right cavernous sinus thrombophlebitis **(dash white arrow)**.

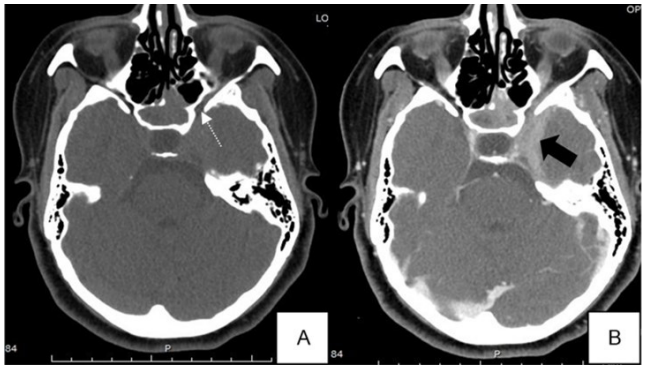


Fig 2. A 31-year-old female presented with left posterior ethmoid and bilateral sphenoid sinusitis and found causative organism from invasive fungal infection. This patient was diagnosed with complicated sinusitis and develop cavernous sinus thrombophlebitis with left orbital apex involvement.
(A) NECT image cannot be demonstrated intracranial complication at left cavernous sinus, but only seen abnormality of abnormal fat infiltration at left orbital apex **(dash white arrow)**.
(B) CECT image shows benefit of contrast study for evaluation intracranial complication due to well demonstrate abnormal enhancement at left cavernous sinus from thrombophlebitis **(black arrow)**.

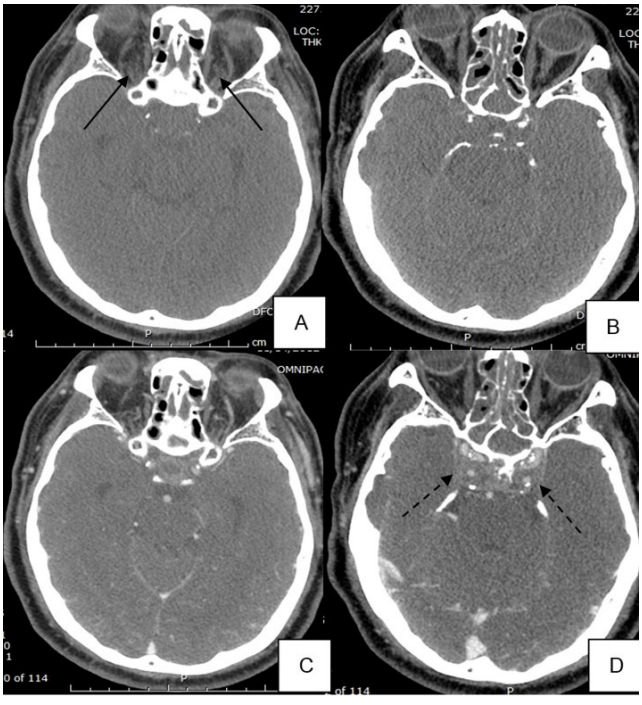


Fig 3. A 63-year-old male presented with bilateral pansinusitis and causative organism from bacterial infection with shown bilateral cavernous thrombosis and orbital involvement from demonstrated retrobulbar fat infiltration.
(A)&(B) NECT image can be detected only orbital complication by demonstrate retrobulbar fat infiltration **(black arrow)**
(C)&(D) CECT image can be demonstrated intracranial complication by detecting abnormal filling defect inside bilateral cavernous sinuses from cavernous sinus thrombosis **(dash black arrow)**

of PNS could not detect two cases with intracranial complication such as cavernous sinus thrombophlebitis, but CECT of PNS could detect in both cases. One case demonstrated abnormal soft tissue enhancement and increased thickening at cavernous sinus which continued from adjacent orbital apex area (Fig 2) and another case showed filling defect inside cavernous sinus with eye congestion from cavernous sinus thrombosis (Fig 3).

The present study also tried to find cut off point value of quantitative measurement of HU of periantral fat infiltration for increasing sensitivity and specificity for detecting early stage of complicated sinusitis from extension of severe infection beyond bony wall of paranasal sinuses which was shown as periantral fat infiltration on CT image, which may be more accurate than visual assessment.

The difference of Hounsfield Unit of bilateral periantral fat in almost all patients with complicated sinusitis should be more than 10 HU (Figs 4 & 5). The significant cut off value of different Hounsfield unit of bilateral periantral fat for which a radiologist can detect abnormality was more than 20.26 HU. (Table 4). Correlation between periantral fat infiltration by using difference of HU of periantral fat area and image findings of sinusitis on NECT may increase confidence for diagnosis of complicated sinusitis.

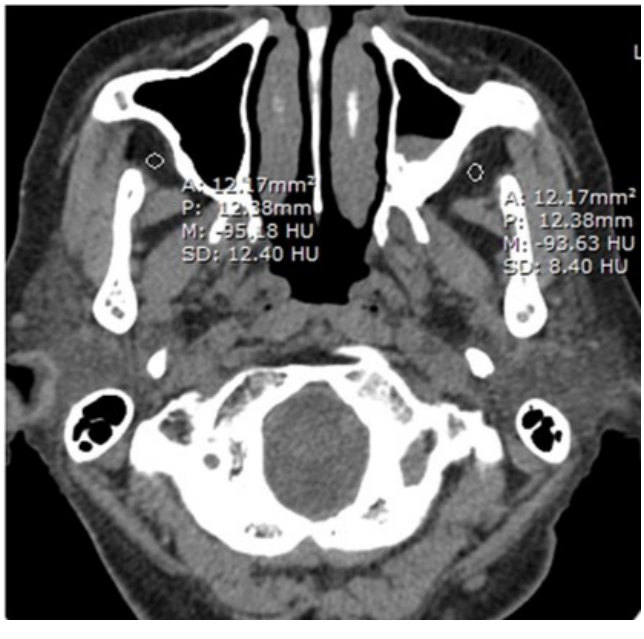


Fig 4. A 53-year-old female patient with chronic bilateral maxillary sinusitis due to detect bilateral maxillary antrums sclerosis and suspected acute sinusitis on top at left side which shows fluid filled inside. NECT of PNS shows no significant different of HU between each sides of periantral fat (different value about 1.55 HU)

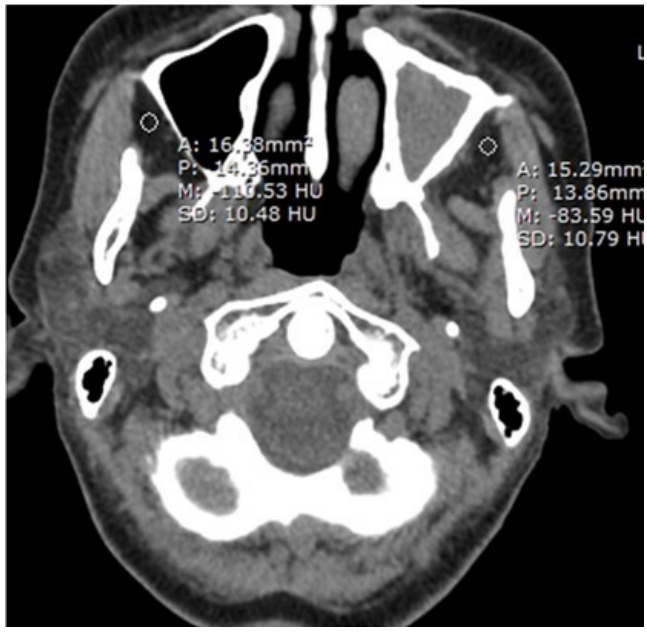


Fig 5. A 59-year-old female with invasive fungal sinusitis at left maxillary sinus. The left maxillary sinus shows evidence of acute on top chronic sinusitis due to detect fluid filled inside with sclerotic change of bony wall. NECT of PNS shows significant different HU between bilateral periantral fat values about 26.94 HU.

DISCUSSION

Severe infection of paranasal sinus will be followed by serious complication especially in patients with immunocompromised or immunocompetent hosts. The causative agents in this condition usually occur from severe bacterial infection such as pseudomonas aeruginosa or Klebsilla or fungal infection such as mucormycosis or aspergillosis.[2] Early diagnosis and appropriated treatment from surgery and medication in case of severe infected sinusitis show more important to safe life and decrease morbidity and mortality of patients. Complicated sinusitis is defined by extension of infection beyond PNS area to adjacent structures such as orbit, adjacent soft tissue or invade into intracranial area at the time of diagnosis.[8] Combination between functional endoscopic sinus surgery (FESS) and CT scan of PNS have benefit and useful for evaluation before treatment due to well demonstrate anatomical and extension of disease such as mass or severe infection.[9] However CECT may be limited in these population due to poor renal function and risk for contrast-induced nephropathy (CIN) that may lead to increased morbidity and mortality rates.[10] Prior study of Pongsakorn et al. showed that limited CT of PNS can be used for surgical roadmap for treatment planning sinus surgery[11], but no previous research study about using NECT of PNS for detecting complicated sinusitis which may be useful for demonstrating lesion before treatment and minimizing the incidence of contrast-induced nephropathy from CECT of PNS.

From the presented study found immunocompromised and immunocompetent patients had bilateral pansinusitis or unilateral pansinusitis as clinical presentation. The first three sinuses that effected from complicated sinusitis were maxillary, ethmoid and sphenoid sinuses, respectively. No significant difference of causative organisms from bacterial and fungus in immunocompromised and immunocompetent patients, but may be from small amount of immunocompromised host when compared with normal host. Main findings of complicated sinusitis in this study were found at periantral area, orbital apex and cavernous sinus without evidence of bony destruction. Del Gaudio et al[6] found that bone erosion and extrasinus extension were highly specific finding of invasive fungal sinusitis. Silverman and Mancuso[7] found that periantral fat infiltration may be earliest sign of invasive fungal sinusitis in the appropriate clinical setting which the presented study also shown this evidence in case of complicated sinusitis by detection abnormal soft tissue infiltration or fat infiltration at periantral area or orbital apex without evidence of bony destruction.

Groppo et al. found sensitivity, specificity, PPV and NPV of CECT for diagnosis of acute invasive fungal sinusitis in immumocompromised patients were 57-69%, 81-83%, 89-93% and 45-67%, respectively.[12] In the presented study showed benefit of NECT of PNS for detection orbital complication due to accuracy about 97.9% which more useful in population who risk to develop contrast-induced nephropathy or severe allergic reaction from contrast.

However, NECT was limited to detect intracranial complication because it depended on enhancing property of soft tissue and vascular structures such as cavernous sinus or brain parenchyma.[13] If clinical suspected intracranial or cavernous sinus involvement, CECT should be performed for better evaluation.

Another additional quantitative datas from this study was the significant different Hounsfield Unit of density value at bilateral periantral fat region for early detecting periantral fat infiltration which Silverman and Mancuso found that this finding may be earliest sign of invasive fungal infection.[7] The cut-off point value of different HU of periantral fat that suggestive complicated sinusitis should be more than 20.26 HU which in non-complicated sinusitis group was measured about 7.67 HU.

Limitations in this study were small number of immunocompromised and immunocompetent patients who had orbital and intracranial complications and positive histopathologic results.

CONCLUSION

NECT of PNS has benefit for diagnosis complicated sinusitis that invade into extrasinus along periantral area and orbital apex in patients who may risk for developing contrast-induced nephropathy such as immunocompromised and immunocompetent host. However CECT of PNS is still necessory for evaluating intracranial complication such as brain parenchyma invasion or cavernous sinus involvement.

REFERENCES

1. Fokkens WJ, Lund VJ, Mullol J. EPOS 2007: European position paper on rhinosinusitis and nasal polyps 2007. A summary for otorhinolaryngologists, 97-101. EPOS. 2006;44(1).
2. Berlinger NT. Sinusitis in immunodeficient and immunosuppressed patients. The Laryngoscope. 1985;95(1):29-33.
3. Epstein VA, Kern RC. Invasive fungal sinusitis and complications of rhinosinusitis. Otolaryngologic Clinics of North America. 2008;41(3):497-524.
4. Bunnag C, Thanaviratananich S, Teeratakulpisan C. Clinical practice guideline for rhinosinusitis in Thais. Siriraj Medical Journal. 2003;55:1-27.

5. Shoukat S, Gowani SA, Jafferani A, Dhakam SH. Contrast-induced nephropathy in patients undergoing percutaneous coronary intervention. Cardiology research and practice. 2010;2010.
6. DelGaudio JM, Swain RE, Muller S, Hudgins PA. Computed tomographic findings in patients with invasive fungal sinusitis. Archives of Otolaryngology–Head & Neck Surgery. 2003;129(2):236-40.
7. Silverman CS, Mancuso AA. Periantral soft-tissue infiltration and its relevance to the early detection of invasive fungal sinusitis: CT and MR findings. American journal of neuroradiology. 1998;19(2):321-5.
8. Groppo ER, El-Sayed IH, Aiken AH, Glastonbury CM. Computed tomography and magnetic resonance imaging characteristics of acute invasive fungal sinusitis. Archives of otolaryngology--head & neck surgery. 2011;137(10):1005-10.
9. Chakravarti A, Naglot S, Dhawan R. Outcome of endoscopic sinus surgery in patients with symptomatic chronic rhinosinusitis with minimal changes on computerised tomography. Indian journal of otolaryngology and head and neck surgery: official publication of the Association of Otolaryngologists of India. 2011;63(4):359-63.
10. Nikolsky E, Aymong ED, Dangas G, Mehran R. Radiocontrast nephropathy: identifying the high-risk patient and the implications of exacerbating renal function. Reviews in cardiovascular medicine. 2002;4:S7-14.
11. Tantilipikorn Pongsakorn, Churojana Anchalee, Chiewwit Pipat, Phuttharak Warinthorn, Lerdlum Sukalaya, Aeumjaturapat Songklot, Tanaviratananich Sanguansak, Assanasen Paraya, Bunnag Chaweewan. The accuracy of limited CT in the evaluation of chronic rhinosinusitis and for endoscopic sinus surgery: a multicenter study. Siriraj Medical Journal, 2009 Jan; 61(1): 3-7.
12. Groppo ER, El-Sayed IH, Aiken AH, Glastonbury CM. Computed tomography and magnetic resonance imaging characteristics of acute invasive fungal sinusitis. Archives of Otolaryngology–Head & Neck Surgery. 2011;137(10):1005-10.
13. Shah NJ, Rathore A. Intracranial extension of fungal sinusitis. Otorhinolaryngol Clin Int J. 2009;1(1):55-61

Correlation between Timed Barium Esophagogram and the Eckardt Stage in Achalasia

Waraporn Boonsomjint, M.D.*, Monthira Maneerattanaporn, M.D., Aphinya Charoensak, M.D.***

**Department of Radiology, **Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: To evaluate the correlation of timed barium esophagogram (TBE) parameters and the Eckardt stage in patients with achalasia.

Methods: This prospective study was done in 29 adult achalasia patients who underwent TBE and were assessed for clinical symptoms according to the Eckardt stage. The association between the Eckardt stage and TBE parameters including the height and the width of barium column, esophageal emptying between 1 and 5 minutes, which was calculated by comparing the area of barium column and esophageal emptying between 0 and 5 minutes, which was calculated from the relative changes in ingested volume to estimated volume of barium column on the 5-min image and this was calculated by using Kruskal-Wallis test. Comparison of mean emptied volume at 1 min. image and mean emptied volume between 1 and 5 minutes was done by using paired samples t-tests.

Results: There was a significant difference in the amount of emptied volume at 1 min image (mean = 118.27, SD = 31.1) and emptied volume between 1 min and 5 min images (mean = 11.41, SD = 9.4); $t(27) = 15.375$, $p < 0.001$. Statistically significant difference in esophageal emptying between 1 and 5 minutes across the Eckardt stages was found ($H = 8.115$, 3 d.f., $p = 0.044$). The difference was statistically significant between the Eckardt stage 2 and 3 ($p = 0.039$). There were no statistically significant differences in other TBE parameters across the Eckardt stages.

Conclusion: No statistically significant difference in TBE parameters across the Eckardt stages was found in the present study. However, TBE is a useful objective method to assess esophageal emptying in patients with achalasia. Interpretation of TBE should be standardized. Calculation of esophageal emptying should include the amount of barium emptied from the esophagus in the first minute.

Keywords: Timed barium esophagogram; Eckardt stage; achalasia (Siriraj Med J 2018;70: 60-65)

INTRODUCTION

Achalasia is an esophageal motility disorder characterized by a failure of the lower esophageal sphincter (LES) to relax during swallowing and the absence of esophageal peristalsis. It is caused by a loss of inhibitory myenteric plexus ganglion cells for which the etiology is unknown.[1] Typical clinical manifestations included dysphagia, regurgitation of undigested food, retrosternal pain, and weight loss. Esophageal manometry is the gold standard for the diagnosis.[2,3] High-resolution manometry (HRM) of the esophagus is a new technique that provides a more precise assessment of esophageal motility than conventional techniques[4,5] which has improved the diagnostic accuracy as well as defined clinically relevant subtypes. Barium swallow is a complimentary diagnostic test and has long been used to diagnose achalasia if the classic radiologic findings are demonstrated. Treatments include medication, botulinum toxin injection, pneumatic dilatation and surgical myotomy. Current treatments aim to reduce the pressure at the LES which improves esophageal emptying and esophageal dilatation. No therapy restores esophageal peristalsis.

Esophageal function can be assessed by esophageal scintigraphy, manometry or barium study. Both esophageal

Correspondence to: Aphinya Charoensak
E-mail: caphinya@gmail.com
Received 20 February 2017 Revised 20 April 2017 Accepted 26 April 2017
doi:10.14456/smj.2018.10

scintigraphy and manometry are more expensive and not widely available, whereas timed barium esophagogram (TBE) is a simple, cheap, noninvasive and widely available method to assess esophageal emptying. TBE has been used as an objective measure of baseline and post-treatment esophageal emptying in achalasia patients. However, techniques and interpretation of TBE are varied and there is no standardized criteria to define treatment response. While some institutions used the height and the width of barium column, others used esophageal emptying calculated by comparing area of barium column on the 1- and 5-min images.[6,7] Prior study by Oezcelik A et al[8] used initial esophageal clearance by comparing area of barium column on the 1-min images obtained before and after myotomy and found that the degree of improved emptying on TBE correlates with clinical outcome. On the contrary, previous studies showed some discordance between symptom improvement and improvement of esophageal emptying on TBE.[9]

There are several disease-specific severity scoring systems in achalasia including HRM-based classification, the Adams's stages, the Eckardt score, and the Vantrappen classification. The Eckardt score tends to be the most useful system in clinical practice.[10] This system grades the four major symptoms of achalasia (dysphagia, regurgitation, retrosternal pain and weight loss). Total symptom scores can be converted to Eckardt clinical stages. Stages 0 and I indicate remission of the disease. On the contrary, stages II and III represent failure of treatment.

The purpose of the present study was to evaluate the correlation of TBE parameters (the height and the width of barium column, esophageal emptying calculated from area of barium column between the 1- and 5-min images and esophageal emptying calculated from the relative changes in ingested volume to estimated volume of barium column on the 5-min image) and the Eckardt clinical stage in patients with achalasia.

MATERIALS AND METHODS

This study was a prospective study which was approved by Institutional Review Board (Si 467/2015). From 1 September 2015 to 31 October 2016, all 33 consecutive adult patients (age >18 years) who were scheduled for TBE were recruited and informed consent was obtained at the time of making an appointment. Three patients were excluded from the study due to their diagnoses of achalasia were not confirmed by HRM. Another patient diagnosed as a nutcracker esophagus was also excluded. Finally, a total of 29 patients with confirmed diagnosis of achalasia by HRM criteria were included.

Demographic and clinical data of patients were recorded which included gender, age, previous treatment, the duration of symptoms and weight loss prior to treatment. The four major clinical symptoms of each patient were assessed by the author on the day of TBE. The frequency of each symptom (dysphagia, regurgitation, retrosternal pain) was graded on a scale ranging from 0 to 3 (0 = none, 1 = occasionally, 2 = daily, 3 = several times a day after each meal). The degree of weight loss was graded on a scale ranging from 0 to 3 (0 = none, 1 = < 5 kg, 2 = 5-10 kg, 3 = > 10 kg). A total symptom scores of 0-1 corresponds to the Eckardt stage 0, of 2-3 corresponds to the Eckardt stage 1, of 4-6 corresponds to the Eckardt stage 2 and score > 6 corresponds to the Eckardt stage 3.

TBE

Patients were advised overnight fasting prior to TBE. TBE was performed by using the fluoroscopic machine. Entire study was performed in the erect posture and left posterior oblique position. A ruler was placed on the left side of fluoroscopic table for the measurement calibration. A fixed volume (150 ml) of low density barium sulfate suspension (45% weight by volume; Solotop 140 mg/300 ml.) was ingested orally within 15-20 seconds. The position of spot film was confirmed under fluoroscopically to include barium filled esophagus, esophagogastric (EG) junction and the ruler. Spot films were taken on 1, 2 and 5 minutes after barium ingestion. If barium was completely emptied from the esophagus on 1-min or 2-min film, no additional spot film was obtained.

Radiological assessments were performed at PACS workstation by the author including measurements of the height and the maximum width of barium column, the mean width of the esophagus and the area of barium column (Fig1). All measurements in each image were calibrated by measuring 100 millimeters scale of a ruler put inside the fluoroscopic view. The height of barium column was measured from top of barium column to EG junction or location of bird-beak. If barium-foam interface was observed in dilated esophagus with retained food content and secretions, the measurement was done

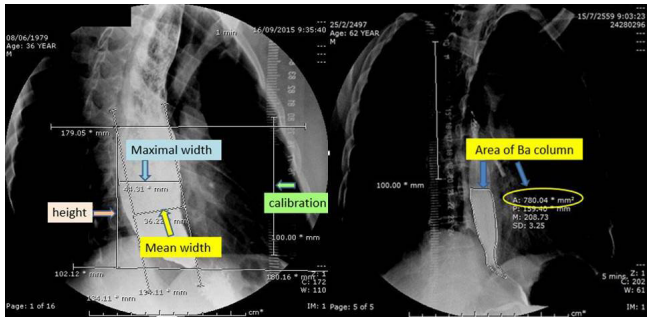


Fig 1. Calibration and measurement in TBE

at superior aspect of barium column which did not include foam. The maximal width was measured at widest part of barium column perpendicular to long axis of the esophagus. The mean width was measured by drawing two lines parallel to the respective outer margins of the barium column from the top to the bottom with the estimated contrast-containing area outside the line equal to the area not containing contrast inside the line. The mean width was recorded as the distance between these two lines. The area of barium column was drawn manually using a freehand tool.

Esophageal emptying between 1 and 5 minutes was calculated by comparing the area of barium column on the 1- and 5-min images. Esophageal emptying between 0 and 5 minutes was calculated from the relative changes in ingested volume (150 ml) to estimated volume of barium column at 5-mins. The estimated volume of barium column was calculated according to the cylindrical formula: (mean radius)^2*3.14 * height. Mean radius is a half value of the mean width.

Statistical analysis

Categorical data were summarized as frequencies and percentages. Continuous variables were summarized parametrically as mean and standard deviation or nonparametrically as box plots with median, 25th, and 75th percentiles (bottom and top of box).

Paired samples t-tests was used to compare the mean emptied volume at 1 min. image and mean emptied volume between 1 min and 5 mins.

Kruskal-Wallis test was used to assess the association between the Eckardt stage and TBE parameters including the height and maximum width of barium column on 5-min image, esophageal emptying between 1 and 5 mins calculated by comparing the area of barium column and esophageal emptying calculated from the relative changes in ingested volume to estimated volume of barium column on the 5-min image.

A p value < 0.05 was considered to be statistically significant.

RESULTS

Demographic and clinical data were shown in Table 1. Of a total 29 patients with achalasia, 55.2 % were female and 44.8 % were male with mean age of 48.6±13.6 years. Most of the patients (72.4%) already had previous treatment before TBE. The Eckardt clinical stages were stage 0 in 8 patients, stage 1 in 9 patients, stage 2 in 9 patients and stage 3 in 3 patients.

The amount of barium emptied at 1 min image

TABLE 1. Demographic and clinical data of 29 patients with achalasia

Age range (mean±SD)	19-70 years (48.6±13.6)
Gender, M/F	13/16
Duration of symptoms, range (mean±SD)	2 – 267 months (40.7±67.2)
Weight loss in 6 months, range (mean±SD)	0-24 kg (6.5±7.2)
Previous treatment, no. of patients (%)	
None	8 (27.6%)
Yes	21 (72.4%)
Open myotomy	1 (3.4%)
Open myotomy and POEM	2 (6.9%)
Pneumatic dilatation and POEM	2 (6.9%)
POEM	16 (55.2%)
Eckardt stage, no. of patients (%)	
Stage 0	8 (27.6)
Stage 1	9 (31.0)
Stage 2	9 (31.0)
Stage 3	3 (10.3)

Abbreviation: POEM = peroral endoscopic myotomy

(150 ml – calculated volume of barium column at 1 min) ranged from 44.9-150 ml. The amount of barium emptied between 1 min and 5 mins ranged from 0.35-37.9 ml. There was a significant difference in the amount of emptied volume at 1 min image (mean = 118.27, SD = 31.1) and emptied volume between 1 min and 5 mins (mean = 11.41, SD = 9.4); $t(27) = 15.375$, $p < 0.001$.

Relation of the Eckardt stage and the height of barium column, and relation of the Eckardt stage and the maximal width of barium column on 5-min image were shown in Figs 2 and 3. The height of barium column and the maximal width of barium column on 5-min image showed no statistically significant differences across the Eckardt stages (H=5.053, 3 d.f., p=0.168; and H=4.546, 3 d.f., p=0.208, respectively).

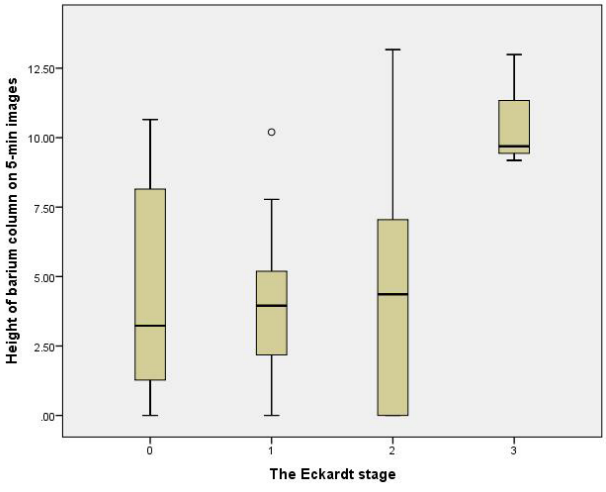


Fig 2. The Eckardt stage and the height of barium column on 5-min image relation. Box-and-whiskers plot represents ranges and the 25th to 75th percentiles, respectively. The horizontal thick lines denote group medians.

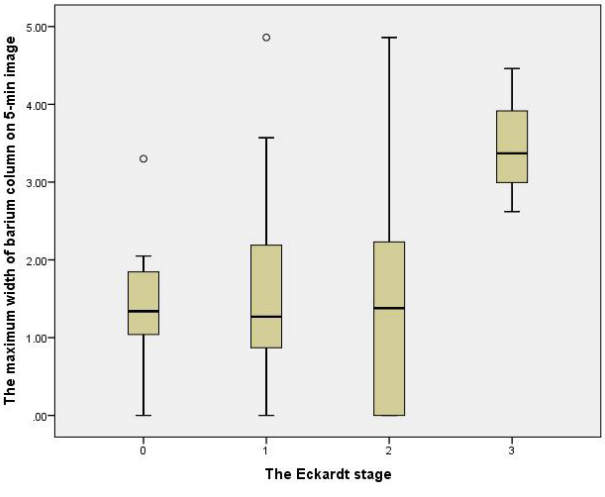


Fig 3. The Eckardt stage and the maximum width of barium column on 5-min image relation. Box-and-whiskers plot represents ranges and the 25th to 75th percentiles, respectively. The horizontal thick lines denote group medians.

Relationhip of esophageal emptying calculated by comparing the area of barium column on the 1- and 5-min images and the Eckardt stages in 28 patients was shown in Fig 4. One patient was not included because barium was completely emptied from the esophagus on 1-min image. There was statistically significant difference in percentage of emptied volume by comparing the area of barium column on the 1- and 5-min images across the Eckardt stages (H=8.115, 3 d.f., p=0.044). The difference was statistically significant between the Eckardt stages 2 and 3 (p=0.039).

Fig 5 showed the relationship of esophageal emptying calculated from the relative changes from ingested volume

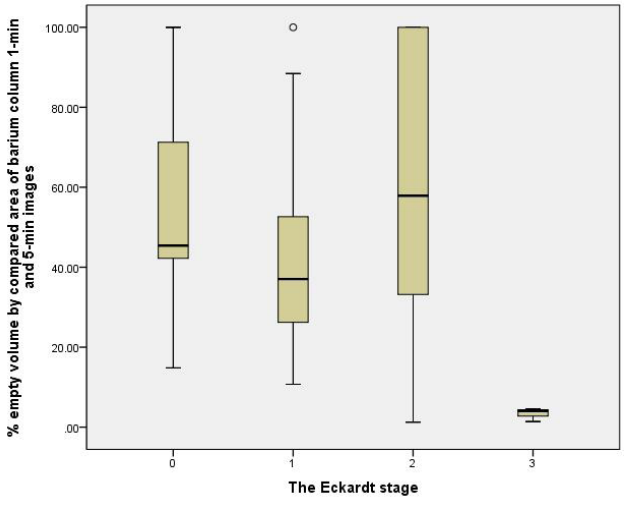


Fig 4. The Eckardt stage and percentage of emptied volume by compared area of barium column on 1-min and 5-min images relation. Box-and-whiskers plot represents ranges and the 25th to 75th percentiles, respectively. The horizontal thick lines denote group medians.

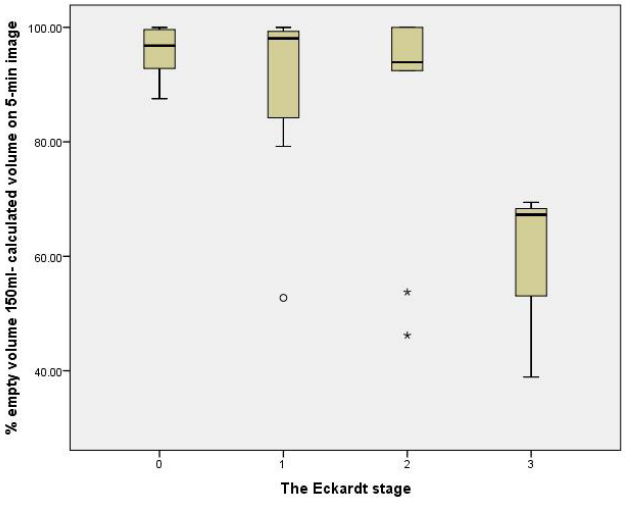


Fig 5. The Eckardt stage and percentage of emptied volume from the relative changes in ingested volume (150 ml) to calculated volume of barium column on the 5-min image relation. Box-and-whiskers plot represents ranges and the 25th to 75th percentiles, respectively. The horizontal thick lines denote group medians.

(150 ml) to estimated volume of barium column on the 5-min image and the Eckardt stages. There was no statistically significant difference in percentage of emptied volume by this method across the Eckardt stages (H=6.102, 3 d.f., p=0.107).

DISCUSSION

The goal of current treatments of achalasia is to reduce LES pressure which improves esophageal emptying, esophageal dilatation and clinical symptoms of patients. Improvement of clinical symptoms, post treatment LES pressure <10 mmHg and >50% improvement over baseline of barium column height at 1 min after swallowing are considered as positive predictors of treatment outcome.[2] Esophageal manometry is costly and lacks availability so that TBE is a widely useful objective tool to evaluate treatment response in patients with achalasia. However, there are some differences in TBE technique and interpretation among institutions and the criteria to define treatment response has not been standardized.

Most institutions used standard radiography machine while our institution used fluoroscopic machine to perform TBE. Standard radiography machine is widely available but fluoroscopic machine has some potential benefits. It can assess and adjust the coverage of barium-filled esophagus and EG junction prior to obtaining the first spot film at 1 min. after swallowing. In addition, if the film is taken while the esophagus is contracted, it can repeat another spot film immediately to get image while the esophagus is relaxed.

Volume of orally ingested barium sulfate suspension used in our institution is a fixed volume at 150 ml. for every patient. Some institutions used adjust volume of barium sulfate suspension range from 100-250 ml. as each patient can tolerate. Using a fixed volume is better to compared pre- and post-treatment TBE. However, the amount of barium sulfate suspension should be enough to adequately fil a dilated esophagus without regurgitation or aspiration. No patient had regurgitation or aspiration in the present study. However, we observed inadequately barium filled marked dilated esophagus in a few cases which caused difficulty in measurement of barium column.

Interpretation of TBE has also varied. Esophageal emptying by TBE can be assessed by measurement of the height, the width, area and volume of barium column. Similar to previous study[11], the present study found that emptying of barium suspension in the first minute is faster than barium emptying from 1min to 5 mins. The authors suggest that the emptied volume (150 ml-volume at 5 min.) represents esophageal emptying better than the emptied volume (volume at 1 min – volume at 5 mins). In addition, we agree with this prior study[11] that calculation of volume by cylindrical formula is better than using area of the barium column.

The present study found no statistically significant differences in TBE parameters across the Eckardt stages except the difference in percentage of emptied volume by comparing the area of barium column on the 1- and 5-min images between the Eckardt stage 2 and 3. However, the present study has some limitations especially the small sample size and the heterogeneity of patient population which included both newly diagnosed achalasia patient and patients with various treatment types. Prior study suggested that improvement of post-treatment clinical symptoms may not correlate to esophageal emptying.[9,12,13] However, Andersson M. et al.,[14] found significant correlations between the height of barium column and symptoms score (dysphagia and chest pain). Oezcelik A. et al[8] also found correlation between the degree of improved emptying on TBE and clinical outcome. Although correlation between clinical symptoms and TBE parameters is still unclear, poor esophageal emptying on TBE is considered as a predictor of treatment failure.[2,15] The further study should compare different patient groups; the newly diagnosed cases and the patients with prior treatment.

CONCLUSION

Although the present study found no statistically significant difference in TBE parameters across the Eckardt stages, TBE is a useful objective method to assess esophageal emptying in patients with achalasia. Interpretation of TBE should be standardized. Calculation of esophageal emptying should include the amount of barium emptied from the esophagus in the first minute.

ACKNOWLEDGMENTS

The authors (MM, AC) were supported by Chalermphrakiat Grant, Faculty of Medicine Siriraj Hospital, Mahidol University. The authors wish to thank Dr. Chulaluk Komoltri for the statistical analysis.

REFERRENCES

1. Vaezi MF, Pandolfino JE, Vela MF. ACG clinical guideline: diagnosis and management of achalasia. Am J Gastroenterol. 2013;108:1238-49.
2. Eckardt AJ, Eckardt VF. Treatment and surveillance strategies in achalasia: an update. Nat Rev Gastroenterol Hepatol. 2011; 8:311-9.
3. Negreanu L, Popescu BO. Evaluation of successful treatment in achalasia with timed barium esophagogram: revisiting an old friend. J Med Life. 2010;3:64-6.

4. Conklin JL. Evaluation of esophageal motor function with high-resolution manometry. J Neurogastroenterol Motil. 2013;19:281-94.
5. Sadowski DC, Broenink L. High-resolution esophageal manometry: a time motion study. Can J Gastroenterol. 2008; 22(4):365-8.
6. Neyaz Z, Gupta M, Ghoshal UC. How to perform and interpret timed barium esophagogram. J Neurogastroenterol Motil. 2013;19:251-6.
7. de Oliveira JM, Birgisson S, Doinoff C, Einstein D, Herts B, Davros W, et al. Timed barium swallow: a simple technique for evaluating esophageal emptying in patients with achalasia. AJR Am J Roentgenol. 1997;169:473-9.
8. Oezcelik A, Hagen JA, Halls JM, Leers JM, Abate E, Ayazi S, et al. An improved method of assessing esophageal emptying using the timed barium study following surgical myotomy for achalasia. J Gastrointest Surg. 2009;13:14-8.
9. Vaezi MF, Baker ME, Achkar E, Richter JE. Timed barium oesophagram: better predictor of long term success after pneumatic dilation in achalasia than symptom assessment. Gut. 2002;50:765–70.
10. Gockel I, Junginger T. The value of scoring achalasia: a comparison of current systems and the impact on treatment-the surgeon's viewpoint. Am Surg. 2007;73:327-31.
11. Andersson M, Kostic S, Ruth M, Lönroth H, Kjellin A, Hellström M, et al. Characteristics of timed barium esophagogram in newly diagnosed idiopathic achalasia: clinical and manometric correlates. Acta Radiol. 2007;48:2-9.
12. Kostic SV, Rice TW, Baker ME, DeCamp MM, Murthy SC, Rybicki LA, et al. Timed barium esophagogram: a simple physiologic assessment for achalasia. J Thorac Cardiovasc Surg. 2000;120:935-46.
13. Eckardt VF, Aignherr C, Bernhard G. Predictors of outcome in patients with achalasia treated by pneumatic dilation. Gastroenterology.1992;103:1732-8.
14. Andersson M, Lundell L, Kostic S, Ruth M, Lönroth H, Kjellin A, et al. Evaluation of the response to treatment in patients with idiopathic achalasia by the timed barium esophagogram: results from a randomized clinical trial. Dis Esophagus. 2009;22:264-73.
15. Rohof WO, Lei A, Boeckxstaens GE. Esophageal stasis on a timed barium esophagogram predicts recurrent symptoms in patients with long-standing achalasia. Am J Gastroenterol. 2013;108:49-55.

Characteristics of Patients Admitted with Tubo-Ovarian Abscess and Surgical Treatment in Siriraj Hospital

Sayan Luangchaichaweng, M.D.*, Chanon Neungton, M.D., Chenchit Chayachinda, M.D., M.Sc. in STIs and HIV**, Dittakarn Boriboonhirunsarn, M.D., M.P.H., Ph.D.**, Vanessa Z. Quinones-Garcia, M.D., M.Sc. in STIs and HIV*****

**Angthong Hospital, Thailand, **Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand, ***Mexican Institute of Sexology, Mexico.*

ABSTRACT

Objective: Tubo-ovarian abscess (TOA) is the most severe form of pelvic inflammatory disease (PID). We aimed to demonstrate the characteristics of patients admitted with TOA in Siriraj Hospital and to assess the prevalence of surgical treatment.

Methods: The descriptive chart reviews were conducted in all patients admitted with TOA during January 2008 - December 2012. One exclusion criterion was that the diagnosis was changed during the admission. Data were presented in n (%), mean ± S.D. Chi-square and Student t-test were used for the comparison between TOA with and without surgical treatment. A $p < 0.05$ was considered statistically significant.

Results: From 122 patients admitted with TOA, 107 cases had the same diagnosis on discharge. Mean age was 33.1+ 11.8 years and mean BMI was 21.9 + 4.9 kg/m^2. Around half of them were nulliparous, 55.1% were married and 67.3% were sexually active. About 14% reported multiple partners. Surgical treatment was performed in 42% of the participants. The characteristics associated with surgical treatment included older age (38.6 ± 11.2 vs. 29.1 ± 10.7 years, $p<0.001$) and abscess size larger than 6 cm (75.6% vs. 22.6%, p<0.001).

Conclusion: Older age and abscess size larger than 6 cm are associated with surgical treatment in patients with TOA.

Keywords: Tubo-ovarian abscess; surgical treatment (Siriraj Med J 2018;70: 66-69)

INTRODUCTION

Tubo-ovarian abscess (TOA) is a subset of pelvic inflammatory disease (PID) which includes abscess formation.[1] TOA has been mainly recognized as severe sequelae of acute PID. It accounts for 41.5% of all patients admitted with PID.[2,3] Previous studies have shown that TOA is associated with longer hospital stay[2-4] and is a negative predictive factor for fertility in reproductive-aged patients.[2]

The initial treatment of TOA is antibiotics administration.[1] The response to antibiotics administration was about 70% of all cases.[5] If there is no clinical response, surgical treatment will be considered.[6] Previous studies have shown that larger abscess size[7-10], high white blood cell (WBC) count[8], increasing patient's age[8] and higher parity[8,9,11] were associated with surgical treatment.

This study aimed to demonstrate the characteristics of patients admitted with TOA in Siriraj Hospital and to assess the prevalence of surgical treatment.

MATERIALS AND METHODS

After ethical approval from Siriraj Institutional Review Board (Si 697/2013), a retrospective cross-sectional study was conducted at the Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital. Patients admitted with TOA in Siriraj Hospital from January 2008 to December 2012 were included into the study. A change of diagnosis at any point was considered an exclusion criterion.

At our institute, as a tertiary teaching hospital,

Correspondence to: Chanon Neungton
E-mail: chanon.neu@mahidol.ac.th
Received 10 October 2016 Revised 13 November 2017 Accepted 20 November 2017
doi:10.14456/smj.2018.11

PID diagnosis was performed using clinical criteria by Centers for Disease Control and Prevention (CDC)[1] and all of those patients with PID were scanned for adnexal mass(es) by bed-side transvaginal ultrasonography. TOA was defined as PID with abscess formation.[1] All patients were admitted and initially treated with intravenous antibiotics according to the regimens of CDC guideline 2015 for treatment of TOA.[1] Clindamycin 900 mg IV every 8 hours plus gentamicin loading dose 240 mg IV drip once daily. The indications for surgical treatment included failure to respond to antibiotic treatment at 72 hours post-treatment and deterioration of clinical signs.[1]

Data were retrieved from medical records, including demographic data, medical history, physical examination, biochemical blood test and ultrasonographic findings. Descriptive statistics, including number (percentage) and mean ± standard deviation (S.D.) were used to describe various characteristics as appropriate. Chi-square test and Student *t*-test were used to compare various characteristics between groups. A *p-value <0.05* was considered statistically significant.

RESULTS

From a total of 122 patients who were admitted with TOA as described on medical charts from January 2008 to December 2012, 107 patients were eligible for the study. During the study period, a total of 45 out of 107 patients (42%) received surgical treatment via exploratory laparotomy.

Study population characteristics are shown on Table 1. Mean age was 33.1±11.8 years and mean BMI was 21.9±4.9 kg/m^2. About 43% were nulliparous, 55.1% were married and 67.3% were sexually active. 14.0% reported multiple partners. Majority of cases used no contraception (42.1%) and 10.3 % had tubal sterilization. Surgical treatment was performed in 45 cases (42.1%). Patients with surgical treatment were significantly older than those with medical therapy (38.6 ± 11.2 vs. 29.1 ± 10.7 years, p<0.001).

Symptoms, signs and laboratory results of study population are described on Table 2. Most common findings were abdominal tenderness (96.3%) and adnexal tenderness (84.1%). Bilateral abscess was found in only

TABLE 1. Demographic data of patients admitted with TOA at Siriraj Hospital a (N=107).

Demographic data	Total	Surgical treatment		*P value*
	(N=107)	Yes (N=45)	No (N=62)	
Age (years)	33.1+11.8	38.6±11.2	29.1±10.7	<0.001
BMI (kg/m^2)	21.9+4.9	22.7±5.2	21.1±4.6	0.129
Nulliparous	46 (43.0)	18 (40.0)	28 (45.2)	0.497
Marital status				0.099
Single	41 (38.3)	12 (26.7)	29 (46.8)	
Married	59 (55.1)	29 (64.4)	30 (48.4)	
Divorced	9 (6.5)	4 (8.9)	3 (4.8)	
Active sexual activity	72 (67.3)	23 (51.1)	47 (75.8)	0.090
Multiple partners	15 (14.0)	6 (13.3)	9 (14.5)	0.918
Contraception				0.444
None	45 (42.1)	16 (35.6)	29 (46.8)	
Hormonal	9 (8.4)	4 (8.9)	5 (8.1)	
Non hormonal intrauterine device	16 (15.0)	5 (11.1)	11 (17.7)	
Tubal sterilization	11 (10.3)	6 (13.3)	5 (8.1)	

Data presented in mean ± S.D. and n (%). *P values* < 0.05 is considered statistically significant.
Abbreviation: BMI = body mass index

TABLE 2. Symptoms, signs and laboratory results of patients admitted with TOA (N=107).

Symptoms, signs and laboratory results	Total cases (107)	Surgical treatment cases		*P value*
		Yes (45)	No (62)	
Abdominal tenderness	103 (96.3)	41 (91.1)	62 (100.0)	0.860
Cervical tenderness	69 (64.5)	28 (62.2)	41 (66.1)	0.960
Uterine tenderness	51 (47.7)	19 (42.2)	32 (51.6)	0.916
Adnexal tenderness	90 (84.1)	33 (73.3)	57 (91.9)	0.514
Abnormal discharge	53 (49.5)	18 (40.0)	35 (56.5)	0.313
WBC >20,000/mm^3	16 (15.0)	10 (22.2)	6 (9.7)	0.074
Neutrophils >80%	60 (56.1)	23 (51.1)	37 (59.7)	0.675
ESR >30 mm/hr	36 (33.6)	9 (20.0)	27 (43.5)	0.449
Diameter of abscess >6 cm	48 (44.9)	34 (75.6)	14 (22.6)	<0.001
Bilaterality of adnexal mass	36 (33.6)	14 (31.1)	22 (35.5)	0.636

Data presented in n (%). *P values* < 0.05 is considered statistically significant.
Abbreviations: ESR = erythrocyte sedimentation rate, WBC = white blood cell

33.6% of the cases. Patients with surgical treatment had higher proportion with WBC >20,000/mm^3 compared with the medical treatment-group with borderline significance (22.2% vs 9.7%, *p=0.074*). Patients with surgical treatment were more likely to have abscess size larger than 6 cm compared to medical treatment group (75.6% vs 22.6%, *p<0.001*). None of the other results were statistically significant.

DISCUSSION

The present study describes the characteristics of patients admitted with TOA at Siriraj Hospital. When there is no clinical response within 72 hours after initiation of antibiotics, surgical treatment might be considered. We found that almost half of patients with TOA required the procedure (42.1%). The rate was higher than that reported in previous studies, ranging from 15.7% to 34.4%.[8-12] This might be partly explained by the fact that this study was conducted in a large, tertiary university hospital where TOA cases were more likely to be complicated than in other settings.

Our study shows that older age is associated with surgical treatment in patients with TOA. The finding is similar to the study conducted by Greenstein et al and the study by Mizushima et al.[7,8] As recurrent PID and incomplete treatment is prevalent among this population[2], TOA formation is likely to be more severe.

In terms of abscess size, those with a size larger than 6 cm are at increased risk for surgical treatment. This is compatible with previous studies which showed more surgical treatment in patients with larger abscess size.[7-10] The efficacy of administered antibiotics is based on their ability to penetrate abscess wall. Larger TOA resulted from chronic, untreated PID. This might lead to some level of resistance to medical treatment. As no definite cut off value of the abscess size has been established, the present study may propose '6 cm' as a predictor for surgical treatment.

The main limitation of this study was the retrospective chart review design which might contain some missing data. The variation among physicians who took care of the patients might affect decision on surgical treatment. Exploratory laparotomy was the only surgical approach in this study while new technologies, such as diagnostic laparoscopy and drainage or drainage of pelvic collection under U/S guide have been reported.[13]

CONCLUSION

Surgical treatment in patients admitted with TOA is prevalent, at 42.1%. The associated factors include older age and abscess size larger than 6 cm.

REFERENCES

1. Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015;64(RR-03):1-137.
2. Chayachinda C, Rekhawasin T. Reproductive outcomes of patients being hospitalized with pelvic inflammatory disease. J Obstet Gynaecol. 2017; 33: 228-32.
3. Topcu HO, Kokanali K, Guzel AI, Tokmak A, Erkilinc S, Umit C, et al. Risk factors for adverse clinical outcomes in patients with tubo-ovarian abscess. J Obstet Gynaecol. 2015; 35(7):699-702.
4. Dewitt J, Reining A, Allsworth JE, Peipert JF. Tuboovarian abscesses: is size associated with duration of hospitalization & complications? Obstet Gynecol Int. 2010;2010:847041.
5. McNeeley SG, Hendrix SL, Mazzoni MM, Kmak DC, Ransom SB. Medically sound, cost-effective treatment for pelvic inflammatory disease and tuboovarian abscess. Am J Obstet Gynecol. 1998;178(6):1272-8.
6. Ross J, Judlin P. 2012 European Guideline for the Management of Pelvic Inflammatory Disease 2012. Available from: http://www.iusti.org/regions/europe/pdf/2012/PID_Treatment_Guidelines-Europe2012v5.pdf. Accessed 30 September 2016
7. Mizushima T, Yoshida H, Ohi Y, Ishikawa M, Hirahara F. Evaluating the risk factors for developing resistance to parenteral therapy for tubo-ovarian abscess: a case-control study. J Obstet Gynaecol Res. 2013;39(5):1019-23.
8. Greenstein Y, Shah AJ, Vragovic O, Cabral H, Soto-Wright V, Borgatta L, et al. Tuboovarian abscess. Factors associated with operative intervention after failed antibiotic therapy. J Reprod Med. 2013;58(3-4):101-6.
9. Kinay T, Unlubilgin E, Cirik DA, Kayikcioglu F, Akgul MA, Dolen I. The value of ultrasonographic tubo-ovarian abscess morphology in predicting whether patients will require surgical treatment. Int J Gynaecol Obstet. 2016;135(1):77-81.
10. Gungorduk K, Guzel E, Asicioglu O, Yildirim G, Ataser G, Ark C, et al. Experience of tubo-ovarian abscess in western Turkey. Int J Gynaecol Obstet. 2014;124(1):45-50.
11. Kuo CF, Tsai SY, Liu TC, Lin CC, Liu CP, Lee CM. Clinical characteristics and treatment outcomes of patients with tubo-ovarian abscess at a tertiary care hospital in Northern Taiwan. J Microbiol Immunol Infect. 2012;45(1):58-64.
12. Halperin R, Levinson O, Yaron M, Bukovsky I, Schneider D. Tubo-ovarian abscess in older women: is the woman's age a risk factor for failed response to conservative treatment? Gynecol Obstet Invest. 2003;55(4):211-5.
13. Revzin MV, Mahan Mathur MS, Dave HB, Macer ML, Spektor M. Pelvic inflammatory disease: Multimodality imaging approach with clinical-pathologic correlation. Radio Graphics. 2016: 36. Available from: https://doi.org/10.1148/rg.2016150202

HBA as a Potential Alternative to the TUNEL Assay for Assessment of DNA Integrity in Asthenozoospermic Spermatozoa

Pitak Laokirkkiat, M.D.*, Paweena Sarakarnkosol, M.D., Isarin Thanaboonyawat, M.D., MMedSci*, Somsin Petyim, M.D., Ph.D.*, Japarath Prechapanich, M.D.*, Suphadtra Phornwilardsiri, M.C.(Genetics)*, Noira Nigmatova, MMedSci.***, Roungsin Choavaratana, M.D.***

**Department of Obstetrics & Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, **Taksin Hospital, Bangkok 10600, Thailand, ***Genome Astana, Astana, Kazakhstan.*

ABSTRACT

Objective: This study was conducted to evaluate the appropriateness of the hyaluronan binding assay (HBA) for assessment of DNA integrity indirectly in asthenozoospermic spermatozoa, by studying its correlation with the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay.

Methods: Thirty asthenozoospermic semen samples of infertile couples were recruited. Semen analysis was performed before being processed by HBA and the TUNEL assay. All the semen parameters were recorded and analyzed.

Results: The mean age of the patients was 38.1 ± 6.4 years. The average total and progressive motility was 33.5 ± 11.0% and 21.9 ± 6.6%, respectively. The mean TUNEL-positive and hyaluronan-bound sperm were 17.8 ± 6.4% and 57.2 ± 14.5%, respectively. Pearson correlation coefficient showed a high negative correlation between HBA and the TUNEL assay ($r = -0.73$, $p < 0.001$). The intraclass correlation coefficients revealed a high level of agreement between two observers who evaluated the TUNEL-positive and hyaluronan-bound sperm [0.81 (95% CI: 0.65–0.91) and 0.92 (95% CI: 0.84–0.96)], respectively.

Conclusion: The study results showed a high negative correlation between HBA and the TUNEL assay. This suggests that HBA may be used as a viable intact DNA spermatozoa identification method, as a potential alternative to the TUNEL assay, in asthenozoospermia sperm.

Keywords: Spermatozoa; asthenozoospermia; hyaluronan binding assay; TUNEL assay; infertility and DNA fragmentation (Siriraj Med J 2018;70: 70-76)

INTRODUCTION

Asthenozoospermia is one of the causes of male infertility. In a fresh ejaculate, the lower limits of normal semen analysis are defined as 32% progressive motility (5th centile, 95%CI 31-34) and 40% total motility (5th centile, 95%CI 38-42).[1] The prevalence of asthenozoospermia has been reported to be as high as 18.71–24.19%.[2,3] This could be associated with certain factors, such as advanced age,[4] varicocele,[5] smoking, alcohol consumption, drugs, toxic substances and chemotherapy. Sperm velocity has been suggested to be a promising indicator of sperm function and fertility.[6] Diminished sperm motility prevents the sperm from transiting itself through the epididymis, vagina and fallopian tubes to the oocyte, to penetrate the zona pellucida for fertilization.[2] To overcome the low fertilization rate, intracytoplasmic sperm injection

Correspondence to: Isarin Thanaboonyawat
E-mail: isarin.tha@mahidol.ac.th
Received 7 November 2017 Revised 9 January 2018 Accepted 10 January 2018
doi:10.14456/smj.2018.12

(ICSI) has been adopted to select the morphologically normal, motile sperm in asthenozoospermic males. However, a real-time PCR study has shown higher DNA fragmentation in asthenozoospermia than in normal spermia.[7] Several studies have also suggested the association of sperm motility with DNA fragmentation.[8,9] Because ICSI bypasses the natural selection barrier for fertilization, the possibility of transmitting damaged sperm genetic material to conceptus increases, which may affect fetal or postnatal life. Several studies have reported that sperm DNA damage is associated with decreased fertilization rate, embryo quality, implantation rate, increased miscarriage rate, childhood diseases and cancer.[10-16]

DNA integrity analysis is a good diagnostic test for sperm quality, and is useful for predicting pregnancy outcomes.[17,18] High level of DNA fragmented sperm is associated with a significant increase in miscarriage rate[19] and a lower live birth rate.[20] Therefore, finding methods for discriminating DNA-intact spermatozoa from the asthenozoospermic samples, to improve the chance of conception and reproductive health outcomes is a challenging topic.

One of the commonly used standard techniques to reveal sperm DNA integrity is the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay.[12] However, in addition to being time-consuming, requiring special skills and expensive, it is toxic to spermatozoa. Therefore, it is not a suitable test for spermatozoa selection for ICSI.

Accordingly, the appropriate method is to use non-destructive DNA tests or tests that can predict DNA fragmentation indirectly, for example, the hyaluronan binding assay (HBA), which has some advantages. First, it is a test for choosing viable mature sperm in a fresh semen sample. Hyaluronan is the major component of the cumulus oophorus matrix and zona pellucida surrounding the oocyte. Sperm binds to hyaluronan only after it completes its plasma membrane remodeling, cytoplasmic extrusion and nuclear maturation, because hyaluronic acid binding sites fail to appear upon incomplete spermiogenesis.[16] The plasma membrane remodeling ability of mature sperm is necessary for facilitating the zona pellucida or hyaluronic acid binding site.[21] This binding process is important for the selection of the functionally most competent sperm for fertilization in vivo.[16] Biochemical markers indicating sperm development and function, such as sperm creatinine phosphokinase activity and heat shock protein family A (Hsp70) member 2 (HspA2), are properly expressed in hyaluronan-bound sperm. In vitro, HBA is used to evaluate the sperm maturity through assessment of the binding capacity of its head to hyaluronan. Second, HBA is a simple, quick test that provides a cost-effective benefit. Third, HBA has been reported to be associated with the fertilization rate and pregnancy outcomes.[22-25] As the hyaluronan binding ability is related to many semen parameters, such as viability, acrosomal integrity and low aneuploidy frequency, [26,27] this test could be useful for predicting the ability of spermatozoa to fertilize oocytes in vivo, as well as ICSI. Despite there are publications demonstrating poor correlation between HBA test and sperm penetration assay (SPA),[28] which asseses direct fertilising capacity of sperm such as capacitation, acrosome reaction, fusion and penetration of the oolemma and chromatin decondensation; it is controversial whether HBA test would be a sufficient indirect analysis to examine fertilising ability in normal semen samples instead of SPA.[29] Finally, DNA fragmentation is associated with HBA. A recent study has revealed a correlation between HBA and the TUNEL assay[27] in normal semen. Several studies have reported an association of DNA fragmentation with HBA.[22,30] Higher clinical pregnancy rate and birth rate were reported as well.[31] However, there has been insufficient data regarding this association in asthenozoospermia.

The TUNEL assay and HBA work on different definitions of good sperm. The former is used to find intact genetic material, while the latter is used to find mature sperm. This study was conducted to reveal whether the mature sperm is associated with intact DNA contents inside. The purpose of our research was to clarify the correlation between HBA and the TUNEL assay in asthenozoospermia. The results of this study will help in the decision of using HBA to assess sperm DNA integrity, and thus, facilitate the routine sperm selection for ICSI and improve reproductive health outcomes.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Infertility Unit, Department of Obstetrics and Gynecology, Siriraj Hospital from March 2015 to August 2016. The study protocol was approved by the hospital institutional review board (Si 128/2015). The sample size was based on the results of a previous study, which reported a correlation of 0.848 between HBA and the TUNEL assay for DNA integrity.[27] The sample size was calculated to achieve a 95% confidence level and 80% power.

After a written informed consent was granted, the male partners of infertile couples were asked to collect a semen sample by masturbation. Semen was left for liquefaction at room temperature for 30 min. Semen analysis was then performed by computer-aided sperm analysis (CASA; Hamilton Thorne Inc., Beverly, MA, USA). Samples with asthenozoospermia, diagnosed according

to the World Health Organization 2010 criteria,[1] were included in this study. Baseline information and semen analysis results were collected.

Semen processing

Two aliquots were extracted from each liquefied semen sample and subjected to HBA and the TUNEL assay within 90 min of semen collection. Interpretation was made twice on each sample by well-trained personnel without knowledge of the results of each assay, based on 200 spermatozoa.

HBA

HBA testing was performed following the instructions of the manufacturer. Seven to ten microlitres of liquefied semen sample were pipetted and placed near the center of the chamber of an HBA kit slide (HBA® Sperm-Hyaluronan Binding Assay; Biocoat, Inc., Horsham, PA, USA), which was covered with a Cell-Vu gridded cover slip and incubated at 20–30°C for at least 10 and not more than 20 min. The incubation time is needed for all the spermatozoa to contact and bind to the immobilized hyaluronan layer. The Cell-Vu gridded cover slip provides a grid of 100 0.1×0.1 mm^2 squares for counting spermatozoa. Spermatozoa in 10 grid squares were evaluated with light microscopy twice, to determine the percentage of hyaluronan-bound spermatozoa.

The percentage of hyaluronan-bound spermatozoa was calculated by the following formula:

$$\% \text{ bound} = \frac{\text{Bound motile sperm}}{\text{Bound motile sperm} + \text{Unbound motile sperm}} \times 100$$

TUNEL assay

For TUNEL analysis, the semen sample was subjected to TUNEL staining using an In Situ Cell Death Detection Kit (Fluorescein; Roche, Germany). The TUNEL assay was performed following the instructions of the manufacturer: Semen samples were put on slides. The air-dried samples were fixed with a freshly prepared Fixation Solution [4% paraformaldehyde in phosphate buffered saline (PBS), pH 7.4] for an hour at 15–25°C. The slides were then rinsed with PBS and incubated in permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) for 2 min on ice (2–8°C). Then, the slides were washed twice with PBS and dried around the sample. Fifty microliters of TUNEL reaction mixture were placed on the samples. Then 10 µL of 4',6-Diamidine-2'-phenylindole dihydrochloride was added to the samples as well. To ensure a homogeneous spread of the TUNEL reaction mixture across the cell monolayer and to avoid evaporation, samples were covered with parafilm or a coverslip during incubation. The slides were incubated in a humidified atmosphere for 60 min at 37°C in the dark. Next, the slides were rinsed three times with PBS. Samples were directly analyzed by flow fluorescence microscopy.

Two hundred spermatozoa on the TUNEL staining slide were evaluated with fluorescence microscopy (Olympus fluorescence microscope Bx51, Melville, NY, USA) at 40x magnification, to determine the percentage of TUNEL-positive spermatozoa. TUNEL-negative spermatozoa were stained blue and TUNEL-positive spermatozoa were stained bright green (Fig 1), which indicated DNA fragmentation.

The percentage of TUNEL-positive spermatozoa was calculated by the formula:

$$\% \text{ Average positive staining} = \frac{\text{Average of stained apoptotic spermatozoa}}{200} \times 100$$

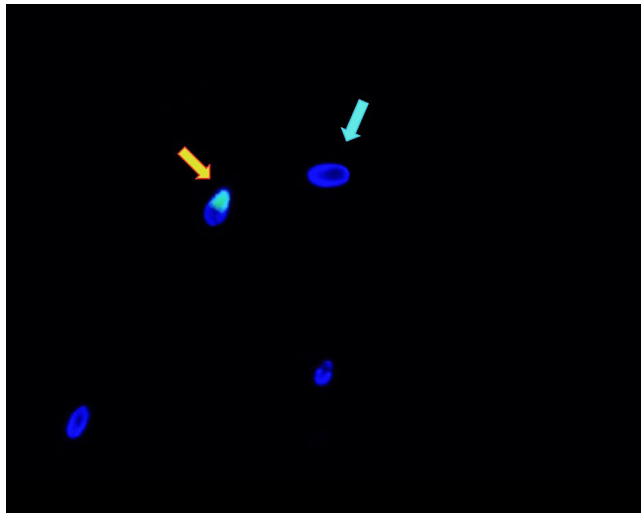


Fig 1. TUNEL-negative spermatozoa was stained by DAPI, the blue fluorescence (light blue arrow), while TUNEL-positive spermatozoa was stained brightly in green fluorescence (yellow arrow). In this picture, the sperm was partially stained by TUNEL indicating partial damage of sperm DNA.

Statistical analysis

The characteristics of the patients and their semen are presented as mean ± SD, or median with interquartile range, as appropriate. Intraclass correlation coefficient (ICC) was used to evaluate the reliability of the TUNEL and HBA scores. Pearson correlation coefficient was used to assess the correlation between the HBA and TUNEL scores. The quantitative variables between the HBA groups were compared using the unpaired t-test or Mann-Whitney U test, as appropriate. Data were analyzed using PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

RESULTS

In total, 463 infertile males were screened for eligibility, of which 30 were enrolled in this study. The mean age of the patients was 38.1 ± 6.4 years. The basic semen parameters are displayed in Table 1. The mean semen volume was 3.0 ± 1.4 mL. The median sperm concentration was 15.5 x 10^6/mL. The mean total and progressive motility were 33.5 ± 11.0% and 21.9 ± 6.6%, respectively. The mean normal sperm morphology was 8.9 ± 5.9%. The mean TUNEL-positive and hyaluronan-bound sperm in the semen samples was 17.8 ± 6.4% and 57.2 ± 14.5%, respectively. In the previous report that studied in the normal semen, there were 5.78 ± 2.28% of TUNEL-positive sperms and 17.08 ± 3.24% of HBA-unbound sperms.[27] The results in the asthenozoospermia seemed to be higher than the normal semen. This suggested the higher DNA fragmentation and lower matured sperms in asthenozoospermia.

TABLE 1. Characteristics of semen parameters (n = 30). Data are presented as mean ± SD or median (min, max), as appropriate.

Factor	Mean ± SD/Median (IQR)
pH	7.8 ± 0.3
Volume (mL)	3.0 ± 1.4
Sperm concentration (10^6/mL)	15.5 (1.5, 67)
Total sperm count (10^6)	37.1 (3.2, 250.5)
Normal morphology (%)	8.9 ± 5.9
Vitality (%)	49.3 ± 12.5
Motility (%)	
Total	33.5 ± 11.0
Progressive	21.9 ± 6.6
Non-progressive	11.6 ± 8.0
Immotile sperm (%)	66.8 ± 11.4

There was no correlation found between the proportion of the TUNEL-positive sperm and the sperm motility (r = 0.2, p = 0.288). However, the percentage of the HBA-bound sperm and the sperm motility (r = -0.317, p = 0.08) tended to be correlated (Fig 2). The percentages of TUNEL-positive and hyaluronan-bound sperm were plotted in a scatter diagram (Fig 3). Pearson correlation coefficient showed a strong negative correlation between the HBA and the TUNEL assay results (r = –0.73, p < 0.001). The intraclass correlation coefficient revealed that there was an adequate level of agreement between two observers who evaluated the percentages of TUNEL-positive and hyaluronan-bound sperm [0.81 (95% CI: 0.65–0.91) and 0.92 (95% CI: 0.84–0.96)], respectively.

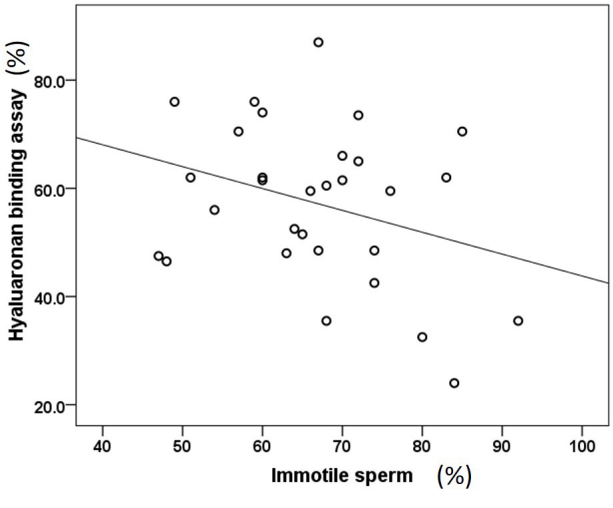


Fig 2. Scatter plots of the percentages of HBA-bound sperm and the sperm motility.

The percentages of HBA-bound sperm tended to be negatively correlated with the immotile sperm number (r = –0.317, p = 0.08), however, is not statistically significant.

DISCUSSION

Since the first IVF baby was born in 1978, the assisted reproduction technology has been developed continuously. Many methods for semen preparation have

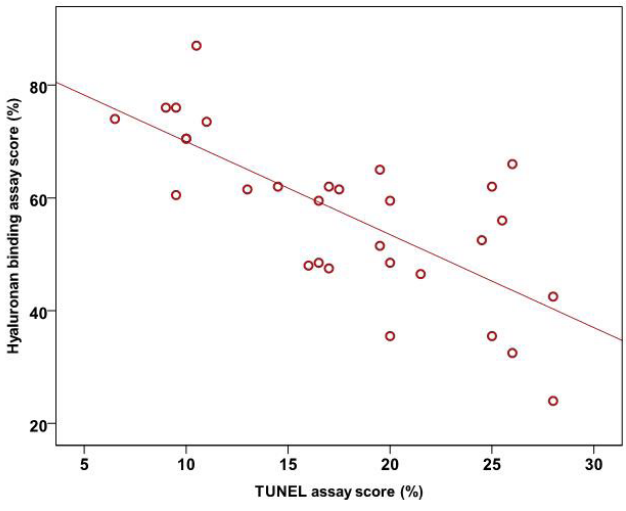


Fig 3. Scatter plot of the percentages of TUNEL-positive and hyaluronan-bound sperm.

The percentages of TUNEL-positive and hyaluronan-bound sperm were plotted in a scatter diagram. Pearson correlation coefficient showed a high negative correlation between the HBA and the TUNEL assay results ($r = -0.73$, $p < 0.001$). The intraclass correlation coefficient revealed that there was a high level of agreement between two observers who evaluated the percentages of TUNEL-positive and hyaluronan-bound sperm [0.81 (95% CI: 0.65–0.91) and 0.92 (95% CI: 0.84–0.96)], respectively.

been developed to select the best viable spermatozoa for fertilization. Injection of damaged sperm was minimized by conventional semen preparation. Both density gradient centrifugation and swim up techniques help filter the sperms with less DNA damage.[32] Combination of a variety of sperm evaluation tests may provide a better selection over standard semen preparation. Selection based on the morphology and motility is used worldwide. However, several studies have reported that spermatozoa with normal morphology may have DNA fragmentation.[33,34] In the case of severe asthenozoospermia, it is difficult to distinguish between live and dead sperm. The mechanical touch technique, which assesses viability by observing tail flexibility and tail recovery after touching it with an ICSI pipette, provides a fertilization rate of 30.3%–73.4%.[35,36] However, there are variations in the criteria for identifying viable spermatozoa using this technique. Thus, a technique for choosing viable sperm with intact DNA in asthenozoospermia is required.

The association between the mature sperm and the integrity of the genetic material was studied. The results showed a high negative correlation between the HBA and the TUNEL assay results [$r(28) = -0.726$, $p < 0.001$]. This is comparable to the results reported by previous studies, which were conducted on normal semen and generally infertile males.[22,27] The difference in the strength of the correlation compared with the study conducted on normal semen is probably because of the difference in the study population, as the present study only enrolled asthenozoospermia patients.

Asthenozoospermia contains a high number of immotile sperm, which includes both the live immotile and the apoptotic or dead sperm. These apoptotic nonviable germ cells were suspected to have DNA fragmentation. However, this study did not find the association between the TUNEL-positive sperm and sperm motility. The result suggested that DNA damage is caused similarly in both motile and immotile groups. On the contrary, the correlation between the HBA and TUNEL test suggested the possibility that DNA damage is more frequent in the immature motile sperm than mature motile sperm in the asthenozoospermia patients. This is in accordance with other studies that have reported low DNA fragmentation in hyaluronan-bound sperm,[24,30,37,38] using green acridine orange fluorescence, Halosperm kit and the TUNEL assay. Several studies reported that both total and progressive sperm motility and normal morphology were highly correlated with HBA scores.[39-41] In the present study, we found that the percentages of HBA-bound sperm had a trend of negative correlation with the immotile sperm number, although it was not statistically significant (Fig 2). This may due to the small sample size for the secondary outcome assessment. Sperm maturation and DNA fragmentation have been suggested as being closely related. Experiments have shown that diminished maturity sperm possess defects in both histone-protamine replacement and DNA fragmentation.[42] The maturation process of spermatogenesis is related to many substances, especially the HSP70 family of proteins (HspA2 in men). HpsA2 is highly expressed during two major stages, first in spermatocytes as a meiotic component within the synaptonemal complex, second in elongating spermatids during terminal spermiogenesis.[21] Low HpsA2 expression during spermiogenesis causes a synaptonemal complex defect, which leads to chromosome aneuploidy. Inadequate delivery of DNA repair enzymes, which may be due to the diminished HpsA2 chaperone activity, leads to DNA fragmentation.[25] Moreover, immature sperm containing excessive cytoplasm, but low HpsA2 level, express insufficient zona binding sites and are unable to bind to hyaluronan.[43] Therefore, live sperm that bind to hyaluronan reflect their maturity and are indirectly suggested to possess intact genetic material, and lack persistent histones and apoptosis.

Thus, HBA, is a simple, time-saving, non-toxic and cost-effective method, which is reasonable to be adopted for evaluating DNA fragmentation indirectly instead

of the TUNEL assay in asthenozoospermia patients. It may help in sperm selection for ICSI, and consequently facilitate our routine fertility treatment and improve reproductive health outcomes.

There were some limitations in this study. The study was conducted to examine the correlation between two tests. As there are several studies which reported lower DNA fragmentation in HBA bound sperm, the DNA fragmentation proportion in the total sperm population and the hyaluronan-bound group in this study were not assessed. Despite the suggestion of intact DNA using HBA selection, its clinical value for fertility and pregnancy outcomes needs to be further evaluated.

In conclusion, the study results showed a strong negative correlation between HBA and the TUNEL assay. The results support the idea that HBA may be used as a viable intact DNA spermatozoa identification method in asthenozoospermic patients.

ACKNOWLEDGMENTS

The authors would like to thank all the laboratory staff for their kind assistance. This research was funded by the Faculty of Medicine Siriraj Hospital, Mahidol University [Grant Number (IO) R015832020].

Declaration of interest statement: The authors confirm that there are no conflicts of interest.

REFERENCES

1. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: World Health Organization; 2010.
2. Curi SM, Ariagno JI, Chenlo PH, Mendeluk GR, Pugliese MN, Sardi Segovia LM, et al. Asthenozoospermia: analysis of a large population. Arch Androl. 2003;49(5):343-9.
3. Luboshitzky R, Kaplan-Zverling M, Shen-Orr Z, Nave R, Herer P. Seminal plasma androgen/oestrogen balance in infertile men. Int J Androl. 2002;25(6):345-51.
4. Rybar R, Kopecka V, Prinosilova P, Markova P, Rubes J. Male obesity and age in relationship to semen parameters and sperm chromatin integrity. Andrologia. 2011;43;(4):286-91.
5. Peluso G, Palmieri A, Cozza PP, Morrone G, Verze P, Longo N, et al. The study of spermatic DNA fragmentation and sperm motility in infertile subjects. Arch Ital Urol Androl. 2013;85;(1): 8-13.
6. Beauchamp PJ, Galle PC, Blasco L. Human sperm velocity and postinsemination cervical mucus test in the evaluation of the infertile couple. Arch Androl. 1984;13;(2-3):107-12.
7. San Gabriel M, Zhang X, Zini A. Estimation of human sperm gene-specific deoxyribonucleic acid damage by real-time polymerase chain reaction analysis. Fertil Steril. 2006;85;(3): 797-9.
8. Bonanno O, Romeo G, Asero P, Pezzino FM, Castiglione R, Burrello N, et al. Sperm of patients with severe asthenozoospermia show biochemical, molecular, and genomic alterations. Reproduction. 2016;152(6):695-704.
9. Dada R, Mahfouz RZ, Kumar R, Venkatesh S, Shamsi MB, Agarwal A, et al. A comprehensive work up for an asthenozoospermic man with repeated intracytoplasmic sperm injection (ICSI) failure. Andrologia. 2011;43;(5):368-72.
10. Borini A, Tarozzi N, Bizzaro D, Bonu MA, Fava L, Flamigni C, et al. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod. 2006;21;(11):2876-81.
11. Evenson D, Wixon R. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. Reprod Biomed Online. 2006;12;(4):466-72.
12. Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod. 1999;14;(4):1039-49.
13. Agarwal A, Allamaneni SS. The effect of sperm DNA damage on assisted reproduction outcomes. A review. Minerva Ginecol. 2004;56;(3):235-45.
14. Shamsi MB, Kumar R, Dada R. Evaluation of nuclear DNA damage in human spermatozoa in men opting for assisted reproduction. Indian J Med Res. 2008;127;(2):115-23.
15. Mokanszki A, Molnar Z, Ujfalusi A, Balogh E, Bazsane ZK, Varga A, et al. Correlation study between sperm concentration, hyaluronic acid-binding capacity and sperm aneuploidy in Hungarian patients. Reprod Biomed Online. 2012;25;(6):620-6.
16. Huszar G. Sperm testing and ICSI selection by hyaluronic acid binding: the hyaluronic acid-coated glass slide and petri dish in the andrology and IVF laboratories. In: Nagy ZP, Varghese AC, Agarwal A, editors. Practical Manual of In Vitro Fertilization: Springer New York; 2012. p. 241-57.
17. Sergerie M, Laforest G, Bujan L, Bissonnette F, Bleau G. Sperm DNA fragmentation: threshold value in male fertility. Hum Reprod. 2005;20;(12):3446-51.
18. Shamsi MB, Imam SN, Dada R. Sperm DNA integrity assays: diagnostic and prognostic challenges and implications in management of infertility. J Assist Reprod Genet. 2011;28;(11): 1073-85.
19. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod. 2012;27;(10):2908-17.
20. Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y. The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. Reprod Biomed Online. 2015;30;(2):120-7.
21. Huszar G, Stone K, Dix D, Vigue L. Putative creatine kinase M-isoform in human sperm is identifiedas the 70-kilodalton heat shock protein HspA2. Biol Reprod. 2000;63;(3):925-32.
22. Pregl Breznik B, Kovacic B, Vlaisavljevic V. Are sperm DNA fragmentation, hyperactivation, and hyaluronan-binding ability predictive for fertilization and embryo development in in vitro fertilization and intracytoplasmic sperm injection? Fertil Steril. 2013;99;(5):1233-41.
23. Huszar G, Ozenci CC, Cayli S, Zavaczki Z, Hansch E, Vigue L. Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. Fertil Steril. 2003;79 Suppl 3:1616-24.

24. Parmegiani L, Cognigni GE, Bernardi S, Troilo E, Ciampaglia W, Filicori M. "Physiologic ICSI": hyaluronic acid (HA) favors selection of spermatozoa without DNA fragmentation and with normal nucleus, resulting in improvement of embryo quality. Fertil Steril. 2010;93;(2):598-604.
25. Huszar G, Jakab A, Sakkas D, Ozenci CC, Cayli S, Delpiano E, et al. Fertility testing and ICSI sperm selection by hyaluronic acid binding: clinical and genetic aspects. Reprod Biomed Online. 2007;14;(5):650-63.
26. Jakab A, Sakkas D, Delpiano E, Cayli S, Kovanci E, Ward D, et al. Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies Fertil Steril. 2005;84;(4):1665-73.
27. Choavaratana R, Mingmitpattanakul P, Sirivatanauksorn V, Makemaharn O, Petyim S. Correlation between Hyaluronan Binding Assay and TUNEL Assay for Sperm DNA Integrity Identification. Siriraj Med J. 2008;60:334-8.
28. Lazarevic J, Wikarczuk M, Somkuti SG, Barmat LI, Schinfeld JS, Smith SE. Hyaluronan binding assay (HBA) versus sperm penetration assay (SPA). Can HBA replace the use of SPA test in male partner screening before in vitro fertilization? Fertil Steril. 2007;88:S376-S7.
29. Lazarevic J, Wikarczuk M, Somkuti SG, Barmat LI, Schinfeld JS, Smith SE. Hyaluronan binding assay (HBA) vs. sperm penetration assay (SPA): Can HBA replace the SPA test in male partner screening before in vitro fertilization? J Exp Clin Assist Reprod. 2010;7:2.
30. Tarozzi N, Nadalini M, Bizzaro D, Serrao L, Fava L, Scaravelli G, et al. Sperm-hyaluronan-binding assay: clinical value in conventional IVF under Italian law. Reprod Biomed Online. 2009;19 (Suppl 3):35-43.
31. Mokanszki A, Tothne EV, Bodnar B, Tandor Z, Molnar Z, Jakab A, et al. Is sperm hyaluronic acid binding ability predictive for clinical success of intracytoplasmic sperm injection: PICSI vs. ICSI? Syst Biol Reprod Med. 2014;60;(6):348-54.
32. Ricci G, Perticarari S, Boscolo R, Montico M, Guaschino S, Presani G. Semen preparation methods and sperm apoptosis: swim-up versus gradient-density centrifugation technique. Fertil Steril. 2009;91;(2):632-8.
33. Avendano C, Franchi A, Taylor S, Morshedi M, Bocca S, Oehninger S. Fragmentation of DNA in morphologically normal human spermatozoa. Fertil Steril. 2009;91;(4):1077-84.
34. Avendano C, Oehninger S. DNA fragmentation in morphologically normal spermatozoa: how much should we be concerned in the ICSI era? J Androl. 2011;32;(4):356-63.
35. Soares JB, Glina S, Antunes N, Jr., Wonchockier R, Galuppo AG, Mizrahi FE. Sperm tail flexibility test: a simple test for selecting viable spermatozoa for intracytoplasmic sperm injection from semen samples without motile spermatozoa. Rev Hosp Clin Fac Med Sao Paulo. 2003;58;(5):250-3.
36. de Oliveira NM, Vaca Sanchez R, Rodriguez Fiesta S, Lopez Salgado T, Rodriguez R, Bethencourt JC, et al. Pregnancy with frozen-thawed and fresh testicular biopsy after motile and immotile sperm microinjection, using the mechanical touch technique to assess viability. Hum Reprod. 2004;19;(2):262-5.
37. Mongkolchaipak S, Vutyavanich T. No difference in high-magnification morphology and hyaluronic acid binding in the selection of euploid spermatozoa with intact DNA. Asian J Androl. 2013;15;(3):421-4.
38. Yagci A, Murk W, Stronk J, Huszar G. Spermatozoa bound to solid state hyaluronic acid show chromatin structure with high DNA chain integrity: an acridine orange fluorescence study. J Androl. 2010;31;(6):566-72.
39. Ye H, Huang GN, Gao Y, Liu DY. Relationship between human sperm-hyaluronan binding assay and fertilization rate in conventional in vitro fertilization. Hum Reprod. 2006;21;(6): 1545-50.
40. Liu DY, Baker HW. [Assessment of human sperm function and clinical management of male infertility]. Zhonghua Nan Ke Xue. 2007;13;(2):99-109.
41. Rashki Ghaleno L, Rezazadeh Valojerdi M, Chehrazi M, Sahraneshin Samani F, Salman Yazdi R. Hyaluronic Acid Binding Assay Is Highly Sensitive to Select Human Spermatozoa with Good Progressive Motility, Morphology, and Nuclear Maturity. Gynecol Obstet Invest. 2016;81;(3):244-50.
42. Sati LG, Ovari L, Demir R, Ward DC, Bray-Ward P, Huszar G. Persistent histones in immature sperm are associated with DNA fragmentation and affect paternal contribution of sperm: A study of aniline blue staining, fluorescence in situ hybridization (FISH) and DNA nick translation. Fertil Steril 2004;82: (Suppl 2):S52.
43. Huszar G, Vigue L. Correlation between the rate of lipid peroxidation and cellular maturity as measured by creatine kinase activity in human spermatozoa. J Androl. 1994;15;(1): 71-7.

Numb Chin Syndrome: An Uncommon Presentation of Metastatic Non-Small Cell Lung Cancer Diagnosed by ^{18}F-FDG PET/CT Scan

Achiraya Teyateeti, M.D.*, Titiporn Jiranantanakorn, M.D., Ajalaya Teyateeti, M.D.*, Pawana Pusuwan, M.D.***

**Department of Radiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, **Diagnostic Radiology and Nuclear Medicine Unit, National Cancer Institute, Bangkok, Thailand.*

ABSTRACT

Objective: To present an uncommon presentation of metastatic non-small cell lung cancer at mandible causing numb chin syndrome (NCS), diagnosed by PET/CT scan.

Case presentation: A 72-year-old man presented with decreased sensation of left lower lip without motor deficit or taste alteration, so called NCS. Work up was initially focused on benign etiology as patient had no known malignancy. However, malignancy was suspected after excluding all possible benign causes. Further investigation showed high level of adenocarcinoma markers and a lytic lesion at sacrum leading to provisional diagnosis of bone metastasis with unknown primary cancer. The subsequent fluorine-18 fluorodeoxyglucose positron emission tomography/computerized tomography (^{18}F-FDG PET/CT scan) showed a pulmonary mass, representing primary lung cancer with multiple metastatic sites, including the destructive lesion at left mandible which was a cause of NCS.

Conclusion: NCS could be an atypical presentation of malignancy. Among several investigations for NCS, PET/CT scan may rarely be used. However, PET/CT scan may provide substantial benefits, particularly for metastatic disease with unknown primary cancer.

Keywords: Numb chin syndrome; mandibular metastasis; F-18 FDG PET/CT scan; non-small cell lung cancer (Siriraj Med J 2018;70: 77-80)

INTRODUCTION

NCS also known as mental nerve neuropathy is a sensory neuropathy characterized by altered sensation and numbness along the distribution of the mandibular branch of trigeminal nerve (CN V_3). Common etiologies are odontogenic problems or procedures and malignancy involving distribution of CN V_3 or mandible.

In this case report, we present a case of NCS due to metastatic non-small cell lung cancer (NSCLC). Our patient presented with numbness of left lower lip, abnormal tumor markers and sacral metastasis without previous history of malignancy. The PET/CT scan was performed to identify primary tumor and the cause of NCS was proved as bone metastasis.

CASE PRESENTATION

A 72-year-old man, presented with numbness of left lower lip for few months. He had history of right Bell's palsy diagnosed 20 years ago, well-controlled psoriasis and smoking for 50 pack-years. Remarkable findings from physical examination were decreased sensation along the distribution of CN V_3 and decreased skin fold below left chin. He denied history of malignancy and odontogenic problems. Magnetic resonance imaging (MRI) of brain performed to evaluate pathology of trigeminal nerve was unremarkable. His pertinent laboratory testing were hemoglobin 10.9 g/dl (normal 12-18), hematocrit 34.6 % (normal 37-52), red blood cell 3.76x10^6/µl (normal 4.2-5.4), erythrocyte sedimentation rate (ESR) 96 mm/hr

Correspondence to: Pawana Pusuwan
E-mail: dbsgtvxq@hotmail.com
Received 28 August 2017 Revised 10 October 2017 Accepted 6 November 2017
doi:10.14456/smj.2018.13

(normal 0-15), C-reactive protein (CRP) 8.76 mg/L (normal <3.02), serum iron 4.8 µmol/L (normal 9-29), total iron binding capacity (TIBC) 36.9 µmol/L (normal 45-70), transferrin saturation 13% (normal 30-50), ferritin 829.4 ng/ml (normal 30-400), prostate-specific antigen (PSA) 3.21 ng/ml (normal 0-4), alpha-fetoprotein (AFP) 1.87 IU/ml (normal 0-5.8), CEA 137.3 ng/ml (normal 0-3.4) and CA 19-9 267.1 U/ml (normal 0-39). Abnormal tumor markers along with history of bowel habit change led to esophagogastroduodenoscopy (EGD), colonoscopy and contrast-enhanced CT scan of whole abdomen. Both EGD and colonoscopy failed to identify malignancy. However, CT scan of whole abdomen revealed bony destruction with soft tissue formation at sacrum (Fig 1).

The PET/CT scan was subsequently performed to identify primary malignancy. The study revealed a 3.1 x 3.4 cm. hypermetabolic spiculated mass at anterior segment of left upper lung, a 2.4 x 2.1 cm. hypermetabolic left hilar lymph node (Fig 2), multiple hypermetabolic pulmonary nodules, multiple hypermetabolic foci at several vertebrae, bilateral 7th ribs, right proximal humerus, pelvic bones and both femori and bony destruction with soft tissue forming at left ramus of mandible (Fig 3), left scapula (Fig 4), left manubrium and sacrum. These findings most likely represented primary lung cancer with mediastinal lymph node, intrapulmonary and bone metastases.

He underwent ultrasound-guided biopsy at posterior aspect of left scapula. Histopathologic findings were adenocarcinoma, moderately differentiated with positive mucin, positive CK7, negative CK20 and negative TTF-1. He received palliative external beam radiotherapy at left scapula, left mandible, right femur and sacrum followed by palliative chemotherapy and tyrosine kinase inhibitor. His disease rapidly progressed and he developed several complications during hospital admission including deep vein thrombosis at left brachial and left axillary vein, upper gastrointestinal (GI) bleeding due to stress ulcer and coagulopathy, malignant pleural effusion, obstructive pneumonitis and delirium. He died of sepsis 5 months after his first presentation of NCS.



Fig 1. Whole abdominal CT scan showed osteolytic lesion with soft tissue formation at sacrum.

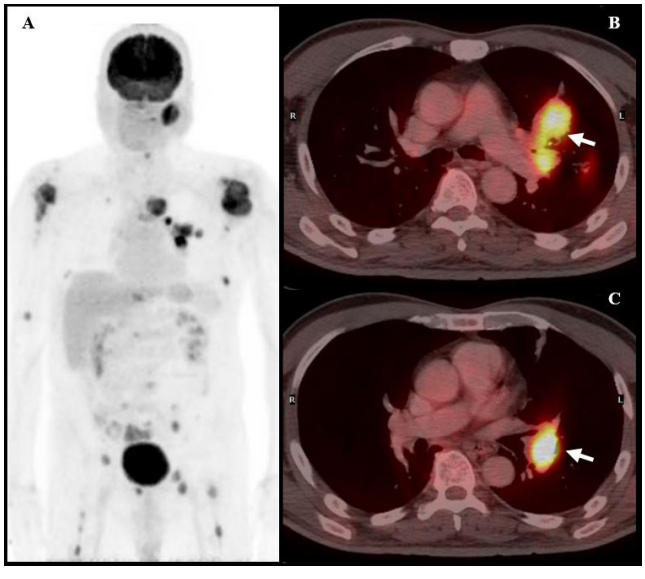


Fig 2. PET/CT scan (A) Maximum intensity projection image showed multiple hypermetabolic lesions throughout the body, (B) axial view of fused image showed a 3.4 cm. hypermetabolic lung mass at left upper lobe and (C) axial view of fused image showed a 2.4 cm. hypermetabolic left hilar lymph node.

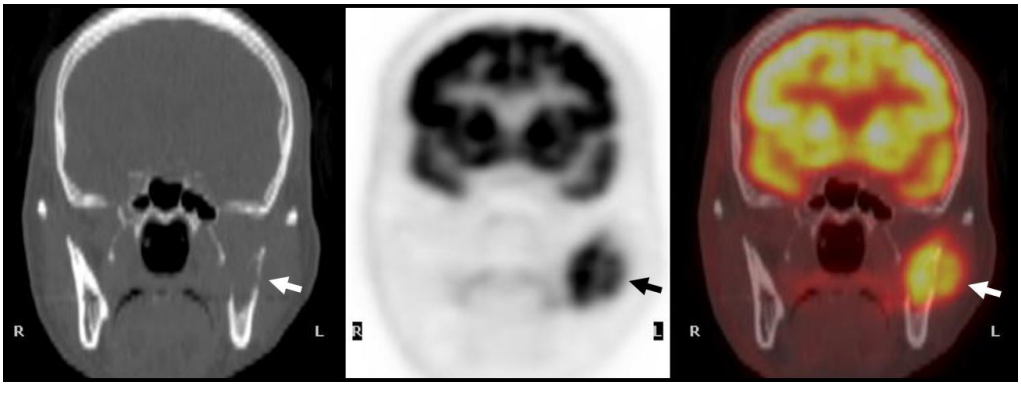


Fig 3. Coronal view of CT image, PET image and fused image showed hypermetabolic bone destruction at left ramus of mandible (arrow) that caused NCS in this patient.

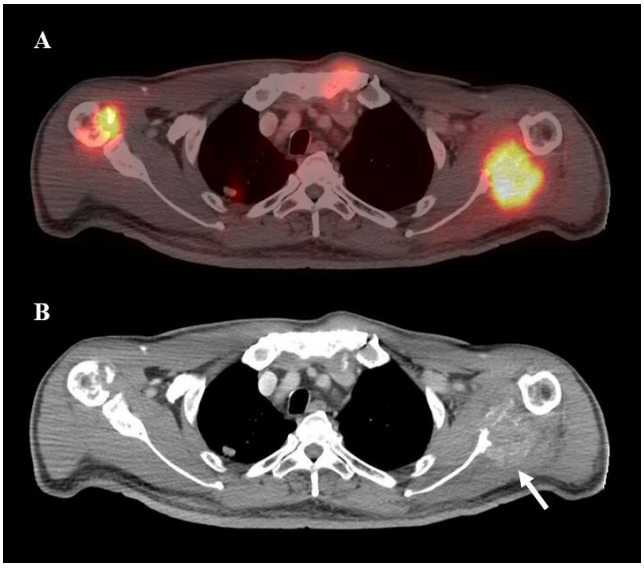


Fig 4. PET/CT scan (A) Axial view of fused image showed hypermetabolic bone lesions at sternum, right humeral head and left scapula and a hypermetabolic pulmonary nodule at right upper lobe. (B) CT image show bone destruction at right humeral head and destructive lesion with soft tissue formation at left scapula where the ultrasound-guided biopsy was performed (arrow).

DISCUSSION

NCS is a pure sensory neuropathy only affecting the sensation along the distribution of mandibular branch of trigeminal nerve (CN V) without motor involvement and alteration of taste. The trigeminal nerve is an intracranial structure, which divides into 3 branches - ophthalmic branch (CN V_1), maxillary branch (CN V_2) and mandibular branch (CN V_3). Mandibular branch exits the skull through foramen ovale and divides into several branches. One of them is an inferior alveolar nerve that continues through the mental foramen of mandible and becomes mental nerve supplying sensation of chin and lower lip area. Regarding the pathway of mandibular branch, causes of NCS could be considered as intracranial and extracranial level. Intracranial cause or selective involvement – e.g. from leptomeningeal metastasis at the level of trigeminal root, is rare.[1-4] In contrast, extracranial causes or literally direct involvement of mandibular region are more common. Odontogenic causes are always the first to be considered. Other concerned causes are primary bone tumor at mandible, head and neck cancer with mandibular involvement, bone metastasis to mandible and systemic diseases – e.g. sarcoidosis, multiple sclerosis and amyloidosis.[5]

Diagnosis of NCS is mainly based on clinical and physical examination, but the cause of NCS may require further investigations. A very useful screening test is a panoramic radiograph of mandible which helps excluding odontogenic conditions and may reveal osteoblastic or osteolytic lesions in approximately 40% of NCS patients with malignancy.[6-7] CT scan of brain including base of skull and mandible may be considered for evaluation of intracranial causes, base of skull destruction and extracranial causes missed by panoramic radiograph of mandible.[6,8] MRI may also assist in identifying involvement of leptomeninges or inferior alveolar nerve.[6] In extremely rare case, when all non-invasive tests have failed to identify cause, surgical exploration may be required.[9]

Regarding the presented case, signs and symptoms were quite typical for NCS but identifying cause was challenging. We initially focused on intracranial cause and MRI of brain was chosen. Nevertheless, MRI of brain showed no explainable cause of NCS. Differential diagnosis then focused on extracranial causes. Patient denied odontogenic problem which is the most common cause of NCS. Thus, further investigations emphasized on malignancy. Local mandibular invasion from primary head and neck cancer was excluded because the only abnormal finding from physical examination was decreased sensation along CN V_3. Primary bone tumor was also less concerned due to its high incidence in young patients. Therefore, the most likely cause of NCS at that time was mandibular metastasis from cancer of unknown primary (CUP).

Evaluation of CUP in this patient began with tumor markers. In the studies of tumor markers - CEA, AFP, PSA, squamous-cell carcinoma antigen, CA 19-9, CA 15-3 and CA 125 in CUP with bone metastasis, these tumor markers match with histological diagnosis in 70% of cases.[10] This patient had high serum CA 19-9 and CEA and bowel habit change. As a result, investigation focused on GI malignancy. However, the only remarkable finding from GI work up was an osteolytic lesion with soft tissue formation at sacrum on CT scan and his bowel symptoms also resolved spontaneously.

With evidence of elevated adenocarcinoma markers, an osteolytic lesion and history of smoking for 50 pack-years, provisional diagnosis was metastatic NSCLC and cause of NCS was suspected to be mandibular metastasis. To prove our assumption, PET/CT scan was an imaging of choice. Firstly, PET/CT scan has a role in detection of primary tumor in CUP. According to a study by Kwee et al., primary tumor detection rate of PET/CT scan ranged from 22-73% with an average of 37% and common sites of primary tumor were lung (33%) followed by oropharynx (16%), pancreas (4.9%), breast (4.3%) and colon (3.7%).[11] In this patient, PET/CT scan successfully revealed a hypermetabolic lung mass supporting a diagnosis of primary NSCLC.

Secondly, PET/CT scan provides a complete staging in a single study. Instead of performing CT scan of chest including upper abdomen for evaluation of intrathoracic lesion, liver and adrenal metastasis and ^{99m}Technetium methyl diphosphonate (^{99m}Tc-MDP) bone scintigraphy for evaluation of bone metastasis, performing PET/CT scan solely may save time and expense for investigation. Regarding PET/CT scan of this patient, evidence of a lung mass, mediastinal lymph nodes involvement and multiple metastatic lesions were sufficient to diagnose stage IV disease without requirement of additional imaging.

Thirdly, PET/CT scan is a whole body imaging. Mandibular metastasis is rare accounting for only 1% of all metastatic sites.[12] Therefore, presence of other bone lesions could support bone metastasis and led to diagnose of mandibular metastasis more confidently. Bone scintigraphy can evaluate bone lesions throughout the body the same as PET/CT scan. However, abnormal lesions on bone scintigraphy may require additional single photon emission computed tomography with computed tomography (SPECT/CT imaging) to confirm their exact locations or to characterize their natures. Therefore, PET/CT scan which simultaneously provides both functional and anatomical imaging in a single study seems to be more appropriate than bone scintigraphy in this specific circumstance. Regarding the presented case, PET/CT scan revealed multiple hypermetabolic bone lesions, including at left mandible raising the suspicion of bone metastasis. CT image of PET/CT scan clearly demonstrated a destructive bone lesion with soft tissue formation at left ramus of mandible, supporting mandibular metastasis as an etiology of NCS.

Lastly, PET/CT scan may aid in biopsy site selection.[13-15] PET/CT scan guided biopsy of hypermetabolic bone metastasis in lung cancer provided a diagnostic success rate of 96.1% at first attempt and 100% for overall.[15] Regarding the presented case, PET/CT revealed several hypermetabolic lesions, including a lung mass, pulmonary nodules, mediastinal lymphadenopathies and bony lesions. Nevertheless, PET/CT guided biopsy was the most appropriate for a biopsy site at left scapula and histopathological findings confirmed the provisional diagnosis of NSCLC.

This case report presents the diagnostic process of NCS and NSCLC by PET/CT scan. However, one should be reminded that advanced imaging is not always required. Osteolytic lesion of mandible and lung mass might be seen even on the simplest test as plain radiograph. Therefore, routine imaging should not be overlooked, as it may be adequate for diagnosis and helps avoiding delay in treatment and expensive investigation.

In conclusion, presentation of NCS may be a red flag of malignancy. For an elderly patient with history of smoking, a rare condition – NSCLC with mandibular metastasis is a possible diagnosis. Investigation of NCS may begin with panoramic radiograph of mandible as a screening test and advanced imaging may be applied accordingly. PET/CT scan is rarely used for diagnosis of NCS. However, in specific circumstances, PET/CT scan can provide benefits in evaluation of unknown primary tumor, staging before treatment and provide guidance for biopsy.

REFERENCES

1. Kuroda Y, Fujiyama F, Ohyama T, Watanabe T, Endo C, Neshige R, et al. Numb chin syndrome secondary to Burkitt's cell acute leukemia. Neurology. 1991;41:453-4.
2. Biasotto M. Numb chin syndrome as the presenting symptom of carcinomatous meningitis. Ann Oncol. 2008;19:599-601.
3. Fenaux P, Lai JL, Miaux O, Zandecki M, Jouet JP, Bauters F. Burkitt cell acute leukaemia (L3 ALL) in adults: a report of 18 cases. Br J Haematol. 1989;71:371-6.
4. Divya KS, Moran NA, Atkin PA. Numb Chin Syndrome: a case series and discussion. Br Dent J. 2010;208:157-60.
5. Narendra H, Ray S. Numb chin syndrome as a manifestation of metastatic squamous cell carcinoma of esophagus. J Cancer Res Ther. 2009;5:49-51.
6. Marinella M A. Numb Chin Syndrome: A Subtle Clue to Possible Serious Illness. Hosp Physician. 2000; 36:54-6.
7. Massey EW, Moore J, Schold SC. Mental neuropathy from systemic cancer. Neurology. 1981;31:1277-81.
8. Lossos A, Siegal T. Numb chin syndrome in cancer patients: etiology, response to treatment, and prognostic significance. Neurology. 1992;42:1181-4.
9. Friedrich E. Mental neuropathy (numb chin syndrome) leading to diagnosis of metastatic mediastinal cancer. Anticancer Res. 2010;30:1819-21.
10. Destombe C, Botton E, Le Gal G, Roudaut A, Jousse-Joulin S, Devauchelle-Pensec V, et al. Investigations for bone metastasis from an unknown primary. Jt Bone Spine. 2007;74:85-9.
11. Kwee TC, Kwee RM. Combined FDG-PET/CT for the detection of unknown primary tumors: systematic review and meta-analysis. Eur Radiol. 2009;19:731-44.
12. Irani S. Metastasis to the Jawbones: A review of 453 cases. J Int Soc Prevent Communit Dent. 2017;7:71-81.
13. Klaeser B, Wiskirchen J, Wartenberg J, Weitzel T, Schmid RA, Mueller MD, et al. PET/CT-guided biopsies of metabolically active bone lesions: applications and clinical impact. Eur J Nucl Med Mol Imaging. 2010;37:2027-36.
14. Cornelis F, Silk M, Schoder H, Takaki H, Durack JC, Erinjeri JP, et al. Performance of intra-procedural 18-fluorodeoxyglucose PET/CT-guided biopsies for lesions suspected of malignancy but poorly visualized with other modalities. Eur J Nucl Med Mol Imaging. 2014;41:2265-72.
15. Guo W, Hao B, Chen HJ, Zhao L, Luo ZM, Wu H, et al. PET/CT-guided percutaneous biopsy of FDG-avid metastatic bone lesions in patients with advanced lung cancer: a safe and effective technique. Eur J Nucl Med Mol Imaging. 2017;44:25-32

Concomitant Primary and Secondary Syphilis: A Case Series from STD Clinic, Siriraj Hospital

Sukhum Jiamton, M.D., M.Sc., Ph.D.*, Charussri Leeyaphan, M.D.*, Natchaya Junsuwan, M.D.*, Pattriya Chanyachailert, M.D.*, Viboon Omcharoen, B.Sc.*

**Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: To emphasize the importance of dermatologists in identifying the presence of concomitant primary and secondary syphilis in the same patient in order to effectively diagnose the disease and provide adequate treatment.

Case presentation: The diversity of the clinical manifestations of syphilis is very common; however, atypical presentations of syphilis have a chance to occur. Such concomitant primary and secondary syphilis, which develops chancre concomitant rash is an uncommon manifestation. Cases of atypical syphilis have been described most frequently in patients with concomitant human immunodeficiency virus (HIV) infection and men who have sex with men (MSM).This report presents the cases of 8 patients with primary and secondary syphilitic lesions. These cases are representative because of their clinical presentation, age range, gender distribution and diagnostic approach.

Conclusion: The concomitant presence of primary and secondary syphilis in the same patient is unusual. The importance of our cases is to highlight some of the differences in clinical manifestations in HIV-infected men and HIV-uninfected men that might be important for early diagnosis for managing and curing such patients.

Keywords: Concomitant primary and secondary syphilis; syphilis (Siriraj Med J 2018;70: 81-86)

INTRODUCTION

Syphilis is a sexually transmitted infection caused by the spirochete *Treponema pallidum*. Humans are the only natural host for this organism.[1] Transmission occurs mainly through microabrasions in mucosal membranes or skin, enabling the organisms to rapidly enter the bloodstream to disseminate to other tissues.[2] Syphilis has three clinical stages. The primary stage is characterized by a single chancre that manifests approximately 90 days after exposure and remits spontaneously within two to eight weeks. The secondary stage occurs between 2 and 12 weeks after exposure, when a rash develops on several parts of the skin.

The rash subsides spontaneously with no treatment when the condition enters its latent stage. Also, called late phase and rarely observed today, the tertiary stage is characterized by gummata and/or neurosyphilis that emerge three years or later after exposure.[3] The pathogenesis of syphilis begins with *T. pallidum* replication at the site of initial inoculation, dividing once every 30-33 hours, inducing a local inflammatory response that results in a painless chancre approximately 3-6 weeks after initial infection. In each chancre, proliferating spirochetes are surrounded by immune cells, including CD4+ and CD8+ T cells, plasma cells, and macrophages, which produce IL-2 and IFN-γ cytokines, indicating a Th1skewed response. Tissue necrosis and ulceration occur due to small vessel vasculitis, and trafficking of immune cells causes a non-tender regional lymphadenopathy. Within 3-8 weeks, the chancre heals, indicating clearance of *T. pallidum* locally. However, by this time, *T. pallidum* has spread systemically to multiple tissues and organs, setting the stage for secondary syphilis.[2] Accordingly, the concomitant presence of primary and secondary

Correspondence to: Sukhum Jiamton
E-mail: sukhum.jiamton@gmail.com
Received 16 February 2017 Revised 20 April 2017 Accepted 26 April 2017
doi:10.14456/smj.2018.14

syphilis in the same patient is unusual.[4] Both primary and secondary syphilis diagnoses are accomplished by serologies or darkfield microscopy.[5,6]

Recently, an increase in the rate of syphilis has been observed in MSM having HIV coinfection.[7,8] In Thailand, syphilis has been considered as a major sexually transmitted infection.[9] Prevalence of syphilis in MSM is significantly associated with young population and people with HIV infection.[10] Moreover, in the early stage of syphilis, men infected with HIV and genital ulcers resulting from syphilis may have secondary syphilis with concurrent primary chancres, and multiple chancres.[11] Here, we have discussed eight cases of concomitant primary and secondary syphilis identified at Sexual Transmitted Disease (STD) clinic, Siriraj Hospital with different risk factors and unusual clinical manifestations.

The study would help in curing similar patients with appropriate treatment.



Fig 1. Multiple discrete erythematous maculopapular rash on trunk

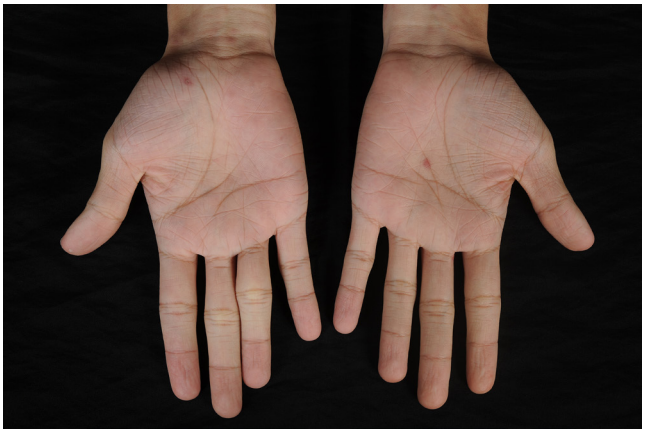


Fig 2. Maculopapular rash on both palms



Fig 3. Multiple discrete erythematous plaque with shallow ulcer on scrotum and shaft of penis

CASE PRESENTATION

Case 1: A 23-year-old male presented to the STD clinic with complaint rash over both palms and soles for three-week duration. He had history of MSM, which included unprotected sexual intercourse with multiple sex partners.

On a physical examination, he was afebrile and had no lymphadenopathy. There were multiple discrete erythematous macules and papules on both palms and soles together with painless solitary whitish papule on erythematous base at his perianal area. There was no oral lesion or other abnormalities.

The Venereal Disease Research Laboratory test (VDRL) was reactive at the titer of 1:64. The Treponema pallidum particle agglutination assay (TPHA) was also reactive at the titer more than 1:80. Perianal ulcer was scraped positive for spirochetes on dark field microscopy. HIV serology of the patient was negative. He was diagnosed with concomitant primary and secondary syphilis. The patient was treated with a single dose of intramuscular benzathine penicillin 2.4 mega units. The follow up of VDRL test three months after completion of the treatment showed remarkably decreased titer to 1:2. The repeated VDRL titer after six months was weakly reactive.

Case 2: A 17-year-old male known with amniotic band syndrome presented to the STD clinic with complaint rash over trunk, extremities, palms, soles and a genital chancre for one-month duration. He had previous history of heterosexual activity and unprotected sexual intercourse with multiple sex partners.

On a physical examination, he was afebrile, had multiple 1-2 cm palpable lymph nodes at both groins and generalized discrete erythematous papules and plaques on the trunk, extremities, palms and soles with multiple erosions and whitish patches on the glans penis.

VDRL test was 1:64, with positive TPHA test as well. Chancre at glans penis was scraped positive on dark field microscopy. Urine PCR for chlamydia trachomatis was positive. Urine PCR for gonococcal urethritis and HIV serology were negative.

The patient was diagnosed as concomitant primary

and secondary syphilis with nongonococcal urethritis. He was treated with single dose intramuscular injection of 2.4 mega units benzathine penicillin, 2 g ceftriaxone and 1 g orally of azithromycin. The follow up of VDRL test three months after completion of the treatment showed remarkably decreased titer to 1:8.

Case 3: A 34-year-old male with HIV infection (126 CD4 cells/mm^3, HIV viral load 2,100,100 copies/ml), involved in bisexual activity and unprotected sexual intercourse with multiple sex partners, presented to the STD clinic with pharyngeal discomfort for 2 weeks. He had history of late latent syphilis and was treated with intramuscular injection of benzathine penicillin 2.4 mega units weekly for three doses in the past 5 years.

On a physical examination, he was afebrile, had no lymphadenopathy and had few discrete scaly erythematous plaques on both palms and soles, multiple whitish patches at posterior pharynx, ill-defined erythematous erosion at glans penis and skin colored verrucous papules at perianal area.

VDRL test was reactive at 1:256. TPHA was also reactive with the titer more than 1:80. Whitish patch at posterior pharynx was scraped positive for spirochetes on dark field microscopy. Tzanck's smear and HSV Ag tests on specimen taken from posterior pharynx were negative. He was diagnosed reinfection of concomitant primary and secondary syphilis with HIV infection. The patient was treated with intramuscular injection of benzathine penicillin 2.4 mega units weekly for three doses because he had recurrent syphilitic infection and HIV infection. The follow up of VDRL test one month after completion of the treatment showed remarkably decreased titer to 1:128. The repeated VDRL titer after three months was not available because he was lost to follow-up.

Case 4: A 25-year-old male with HIV infection (400 CD4 cells/mm^3) was involved in homosexual activity and unprotected sexual intercourse with multiple sex partners. He had history of latent syphilis and was treated with intramuscular benzathine penicillin 2.4 mega units weekly for three doses in the past 1 year. He presented to STD clinic with complaint rash on palms, soles and genitalia for 12 days.

On a physical examination, he was afebrile, had no lymphadenopathy and had multiple discrete scaly erythematous patches and plaques on both palms and soles with discrete erythematous plaques on shaft of penis and scrotum.

VDRL test was reactive at titer of 1:64. TPHA was also reactive with the titer more than 1:80. Dark field microscopy of a specimen scraped from an erythematous plaque at the genitalia was positive. He was diagnosed reinfection concomitant primary and secondary syphilis with HIV infection.

The patient was treated with intramuscular benzathine penicillin 2.4 mega units single dose. The follow up of VDRL test three months after completion of the treatment showed the titer of 1:16.

Case 5: A 21-year-old male student with a history of HIV infection (400 CD4 cells/mm^3) was involved in homosexual activity, protected sexual intercourse with multiple sex partners and intravenous drug usage. He had history of early syphilis and was treated with benzathine penicillin 2.4 mega units intramuscular injection single dose. He presented to the STD clinic with complaint of generalized erythematous rash on palms, trunk and extremities for 3 days.

On a physical examination, he was afebrile, had no lymphadenopathy and had generalized discrete erythematous maculopapular rash on his trunk, both palms, lower extremities and multiple discrete erythematous maculopapular rash with ulcer on the scrotum and shaft of penis. No other abnormalities were observed.

VDRL test was reactive at 1:256, with positive TPHA test as well. Chancre at his scrotum was scraped positive for spirochetes on dark field microscopy. Tzanck's smear was negative for multinucleated giant cell. The patient was treated with intramuscular benzathine penicillin 2.4 mega units single dose. The follow up of VDRL titer was not available because he was lost to follow-up.

Case 6: A 37-year-old male was involved in homosexual activity and protected sexual intercourse with multiple sex partners. He presented to STD clinic with erythematous erosion on the genitalia for 2 weeks.

On physical examination, he was afebrile with ill-defined erythematous erosion on the shaft of penis. He was diagnosed herpes simplex virus infection. The patient was treated with acyclovir 1200 mg/day.

One week later genital rash was improved but he had new rash on both hands and legs. On a physical examination, he had palpable 1-2 cm lymph nodes at both groins, few discrete erythematous macules at both palms and soles with ill-defined erythematous erosion at glans of penis.

VDRL test was reactive at 1:128. TPHA was also reactive at the titer more than 1:80. Chancre at glans penis was scraped positive for spirochetes on dark field microscopy. HIV serology of the patient was not done

because he refused to do the test. The patient was treated with intramuscular benzathine penicillin 2.4 mega units single dose. The follow up of VDRL test three months after completion of the treatment showed remarkably decreased titer to 1:16. The repeated VDRL titer after six months was 1:8.

Case 7: A 22-year-old male presented to the STD clinic with genital rash for six months and rash on both palms and soles for one month. He had history of heterosexual, unprotected sexual intercourse with sexual partner with secondary syphilis. On a physical examination, he was afebrile and had no lymphadenopathy. There were multiple discrete brownish patches and plaques on both palms and soles with multiple discrete erythematous plaques on the scrotum. There was no oral lesion or other abnormalities.

VDRL was reactive at the titer of 1:32, with positive TPHA test as well. Genitalia was scraped positive for spirochetes on dark field microscopy. HIV serology of the patient was negative. He was diagnosed as concomitant primary and secondary syphilis. The patient was treated with intramuscular benzathine penicillin 2.4 mega units for single dose. The follow up of VDRL test three months after completion of the treatment showed remarkably decreased titer to 1:8.

Case 8: 24-year-old male with HIV infection (560 CD4 cells/mm^3) was involved in homosexual activity and unprotected sexual intercourse with multiple sex partners. He presented to the STD clinic with rash on genitalia and both palms and soles for one-week duration. He had history of late latent syphilis and was treated with intramuscular benzathine penicillin 2.4 mega units weekly for three doses in the past 5 years. On a physical examination, he was afebrile, had no lymphadenopathy and had few discrete scaly erythematous plaques on both palms and soles, ill-defined erythematous erosion at glans penis, multiple whitish patches at posterior pharynx and skin colored verrucous papules at perianal area.

VDRL test was reactive at 1:512, with positive TPHA test as well. Whitish patch at posterior pharynx was scraped positive for spirochetes on dark field microscopy. Tzanck's smear and HSV Ag test were negative in specimen scraped from posterior pharynx. He was diagnosed reinfection concomitant primary and secondary syphilis with HIV infection.

The patient was treated with intramuscular benzathine penicillin 2.4 mega units weekly for three doses because he had recurrent syphilitic infection and history of HIV infection. The follow up of VDRL test was not available because he was lost to follow-up.

DISCUSSION

These 8 cases of concomitant primary and secondary syphilis highlight the diversity of clinical presentations and potentially devastating consequences of this syndrome. Although the majority of recently published cases are in HIV-infected men who had sex with men[12], it can also happen in a HIV-negative man. This clinical feature is challenging to diagnose because concomitant primary and secondary syphilis is an uncommon manifestation of syphilis.[13] Secondary syphilis with concurrent primary chancre is more likely to develop in HIV-infected men than HIV-uninfected men. This scenario can happen because of rapid progression of the disease from primary to secondary stage in HIV infected patient or delayed healing of genital ulcer.[12]

Our cases here showed that concomitant primary and secondary syphilis were detected among 6 MSM cases and 4 HIV-infected cases. In case of syphilis and HIV coinfection, all cases had past history of syphilis.

However, in this case series, we also found 3 cases of concomitant primary and secondary syphilis in HIV-negative men with unknown pathogenesis.

Treatment of syphilis in HIV-infected and non-HIV cases is similar. Penicillin continues to be the first-line therapy for all stages of syphilis.[14] A single dose of benzathine penicillin G 2.4 million units intramusculary is currently used to treat primary, secondary, and early latent syphilis. If nontreponemal titers do not decline 4-fold, if there is a 4-fold increase, or if signs or symptoms persist or recur, treatment is considered to fail. In those cases, CSF should be sampled, and treatment should be administered as in HIV-uninfected patients (weekly injections, unless CSF examination indicates neurosyphilis). If additional follow-up cannot be ensured, additional treatment is recommended.[15] Our third case was treated with benzathine penicillin G 2.4 million units intramuscularly once weekly for 3 weeks due to history of recurrent syphilis and HIV infection. In conclusion, the concomitant presence of primary and secondary syphilis in the same patient is unusual. The importance of our cases is to highlight some of the differences in clinical manifestations in HIV-infected men and HIV-uninfected men that might be important for early diagnosis for managing and curing such patients.

ACKNOWLEDGMENTS

The authors are grateful to Professor Kanokvalai Kulthanan, Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University for patients' information.

TABLE 1. Summary of Key Factors in Each Case

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Patient age, sex	23, male MSM	17, male Heterosexual	34, male MSMW	25, male MSM	21, male MSM	37, male MSM	22, male Heterosexual	24, male MSM
HIV status CD4	Negative	Negative	Positive 126 cells/mm^3	Positive 400 cells/mm^3	Positive 400 cells/mm^3	Not available	Negative	Positive 560 cells/mm^3
Presenting symptoms	Rash for 3 months	Rash and chancre for 1 month	Discomfort at pharynx for 2 weeks	Rash for 12 days	Rash for 3 days	Rash for 2 weeks	Rash for 1 month	Rash for 1 week
Physical examination	Multiple discrete erythematous macule and papule on both palms and soles, Painless solitary whitish papule on erythematous base at perianal area	Generalized discrete erythematous papule and plaque at trunk, extremities, palms and soles with multiple erosion and whitish patches at glans penis	Few discrete scaly erythematous plaque at both palms and soles, multiple whitish patch at posterior pharynx, illdefined erythematous erosion at glans of penis, and skin color verrucous papule at perianal area	Multiple discrete scaly erythematous patch and plaque on both palms and soles with discrete erythematous plaque at shaft of penis and scrotum	Generalized discrete erythematous maculopapular rash on trunk, both palms, lower extremities and multiple discrete erythematous maculopapular with ulcer at scrotum and shaft of penis	Few discrete erythematous macule at both palms and soles with ill-defined erythematous erosion at glans of penis	Multiple discrete brownish patch and plaque on both palms and soles with multiple discrete erythematous plaque on scrotum	Discrete scaly erythematous plaque at both palms and soles, ill-defined erythematous erosion at glans of penis, multiple whitish patch at posterior pharynx and skin color verrucous papule at perianal area.
Baseline VDRL Treatment	1:64 PCN IM weekly ×1	1:64 PCN IM weekly ×1	1:256 PCN IM weekly ×3	1:64 PCN IM weekly ×1	1:256 PCN IM weekly ×1	1:128 PCN IM weekly ×1	1:32 PCN IM weekly ×1	1:512 PCN IM weekly ×3
Clinical outcome	Symptom resolution	Symptom resolution	Lost to follow-up	Symptom resolution	Lost to follow-up	Symptom resolution	Symptom resolution	Lost to follow-up

Abbreviations: Pt = indicates patient; MSMW = man reporting sex with men and women; MSM = man reporting sex with men; PCN = penicillin.

REFERENCES

1. Lafond RE, Lukehart SA. Biological basis for syphilis. Clin Microbiol Rev. 2006;19:2949.
2. Ho EL, Lukehart SA. Syphilis: using modern approaches to understand an old disease. J Clin Invest. 2011;121(12):4584-92.
3. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015; 64(RR-03):1-137.
4. Schon MP, Bertsch HP. IMAGES IN CLINICAL MEDICINE. Primary and Secondary Syphilis. N Engl J Med. 2016;374(4):372.
5. Goldsmith L, Katz S, Gilchrest B, Paller A, Leffell D, Wolff K. Fitzpatrick's Dermatology in General Medicine, Eighth Edition, 2 Volume set: McGraw-Hill Education; 2012.
6. Eickhoff CA, Decker CF. Syphilis. Dis Mon. 2016;62(8):280-6.
7. Read P, Fairley CK, Chow EP. Increasing trends of syphilis among men who have sex with men in high income countries. Sexual Health. 2015;12(2):155-63.
8. Foggia M, Gentile I, Bonadies G, Buonomo AR, Minei G, Borrelli F, et al. A retrospective study on HIV and syphilis. Infez Med. 2014;22(1):26-30.
9. HIV and syphilis infection among men who have sex with men--Bangkok, Thailand, 2005-2011. MMWR Morb Mortal Wkly Rep. 2013;62(25):518-20.
10. Patton ME, Su JR, Nelson R, Weinstock H. Primary and secondary syphilis--United States, 2005-2013. MMWR Morb Mortal Wkly Rep. 2014;63(18):402-6.
11. CDC. Case definitions for infectious conditions under public health surveillance. MMWR Recomm Rep. 1997;46(RR-10):1-55.
12. Rompalo AM, Joesoef MR, O'Donnell JA, Augenbraun M, Brady W, Radolf JD, et al. Clinical manifestation of early syphilis by HIV status and gender: Results of the syphilis and HIV study. Sex Transm Dis. 2001;28:158-165.
13. Zetola NM, Klausner JD. Syphilis and HIV infection: an update. Clin Infect Dis. 2007;44(9):1222-8.
14. Zetola NM, Engelman J, Jensen TP, Klausner JD. Syphilis in the United States: an update for clinicians with an emphasis on HIV coinfection. Mayo Clin Proc. 2007;82(9):1091-102.
15. Kofoed K, Gerstoft J, Mathiesen LR, Benfield T. Syphilis and human immunodeficiency virus (HIV)-1 coinfection: influence on CD4 T-cell count, HIV-1 viral load, and treatment response. Sex Transm Dis. 2006;33(3):143-8.

Outcomes of Expectant Management in HIV-Infected Pregnancy with Preterm Premature Rupture of Membranes at Less Than 34-Week Gestation: A Case Series

Nalat Sompagdee, M.D.*, Chenchit Chayachinda, M.D.*, Francois W D Venter, M.D., Kanokwaroon Watananirun, M.D.***

**Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, **Wits Reproductive Health and HIV Institute, Department of Medicine, University of the Witwatersrand, Johannesburg, South Africa.*

ABSTRACT

Objective: To present outcomes of expectant management (EM) in HIV-infected pregnancy with preterm premature rupture of membranes (PPROM) at less than 34-week gestation.

Case presentation: During January 2008-December 2015, there were 513 HIV-infected pregnant women giving birth at Siriraj Hospital, Thailand. Ten of them presented with PPROM at GA <34 weeks and six women received EM. The deliveries took place at GA 28 $^{2/7}$ - 33 $^{5/7}$ weeks. The longest interval of ROM was 15 days and the highest on-admission viral load was 633,000 copies/mL. Three of them had antepartum highly active antiretroviral therapy (HAART) for at least four weeks prior to the delivery. Mode of delivery included 3 vaginal deliveries and 3 caesarean sections. All infants' HIV molecular tests were negative at birth. The longest follow-up interval was 12 months and HIV vertical transmission remained negative.

Conclusion: Expectant management in HIV-infected women with PPROM at GA <34 weeks may be sensible because complications of prematurity outweigh the risk of vertical HIV transmission.

Keywords: Expectant management; HIV; preterm premature ruptured of membranes; vertical transmission (Siriraj Med J 2018;70: 87-90)

INTRODUCTION

Preterm premature rupture of membranes (PPROM) is defined as spontaneous rupture of membranes (ROM) before 37 completed weeks of gestational age (GA) and before labour onset.[1] The management has to weigh up the risks and benefits of expectant management (EM),[2] which includes antibiotics to prolong latency period and prophylaxis for Group B Streptococcus infection and a course of corticosteroids to promote fetal lung maturity.[1]

For HIV-infected pregnancy, the other concern is HIV vertical transmission. The usual rate of vertical transmission without any intervention is 35-40%. The transmission rate can be 1% or less in pregnant women complying with their anti-retroviral therapy and other perinatal recommendations.[3] Previous studies demonstrated the higher risk of vertical transmission in ROM >4 hours[4] and showed a 2% increase in transmission risk every hour following ROM in term pregnancy.[5] Accordingly, HIV-infected pregnancy with PPROM tends to be terminated early. However, there is no consensus management for HIV-infected pregnancy with PPROM at GA <34 weeks. Thus, this case series aimed to reveal the outcomes of managing this population expectantly.

Correspondence to: Kanokwaroon Watananirun
E-mail: kanokwaroon.wat@mahidol.ac.th
Received 4 December 2017 Revised 29 December 2017 Accepted 29 December 2017
doi:10.14456/smj.2018.15

CASE PRESENTATION

After ethical approval (Si 568/2559 (EC2)), medical records of all HIV-infected pregnant women who delivered during January 2008 to December 2015 in Siriraj Hospital, Thailand, which is a tertiary hospital with 9,000 deliveries per year, were reviewed. Focusing on those who presented with PPROM at GA <34 weeks who received EM, baseline characteristics, maternal outcomes and neonatal outcomes were retrieved.

During January 2008 to December 2015, there were 513 HIV-infected pregnant women delivered at Siriraj Hospital. Ten of them presented with PPROM at GA <34 weeks and 6 women received EM. Maternal mean age (±standard deviation) was 20.6±6.8 years. Three patients attended antenatal care (ANC) before GA 24 weeks. One patient had a twin gestation. All patients were not aware of their HIV serostatus prior to pregnancy and two had never had any ANC. Highly active antiretroviral therapy (HAART) was initiated by second trimester in 3 women (case nos. 2, 5, 6), including zidovudine (AZT)+lamivudine (3TC)+lopinavir/ritonavir (Kaletra®) for case no. 2, GPO vir Z (nevirapine (NVP)/AZT/3TC) for case no. 5 and tenofovir (TDF)+3TC+efavirenz (EFV) for case no. 6. One patient had anogenital wart. None of the patients had opportunistic infections, hepatitis B/C nor syphilis.

The GA at PPROM was 26 $^{1/7}$ - 33 $^{3/7}$ weeks. The interval to delivery was 2-15 days. Case nos. 2, 5, 6 continued the same HAART regimen while the rest commenced AZT+3TC+Keletra® regimen. Intrapartum AZT was given to all women except for case no. 1. As this case occurred before implementation of national guideline for intrapartum AZT.[6] All women received a full course of steroids. All but case no. 3 received antibiotics to prolong latent phase of labour. Three cases underwent caesarean section due to non-reassuring fetal status.

All infants' birthweights were appropriate for GA. Neonatal complications appeared related with fetal prematurity rather than HIV infection. Infant born to case no. 1 had birth asphyxia, respiratory distress syndrome (RDS), sepsis, jaundice, hypoglycemia, Varicella Zoster infection, Rotaviral enteritis and bronchopulmonary dysplasia. Infant born to case no. 2 had sepsis, jaundice, hypoglycemia, Pseudomonas conjunctivitis. Infant born to case no. 3 was healthy. Infant born to case no. 4 had sepsis, jaundice, intraventricular hemorrhage and RDS. Infants born to case no. 5 and no. 6 had jaundice and hypoglycemia.

All infants received AZT syrup for four weeks after birth and exclusive formula feeding. All infants' HIV molecular tests were negative at birth. The longest follow-up interval was 12 months and HIV vertical transmission remained negative.

DISCUSSION

All of our case series occurred before the implementation of guideline to follow up baby born to HIV-infected mother for the period of 18 months. With limited available data (follow up period of 12 months), our case series shows that EM for HIV-infected pregnancy with PPROM at GA <34 weeks results in no vertical transmission. Maternal and fetal complications appear more related to prolongation of ROM and prematurity rather than HIV infection. The findings were compatible with a previous report in 2006 that HIV-infected pregnant women who received HAART at the onset of PPROM did not transmit HIV to their offsprings.[7]

Other factors which increased the transmission rate included high viral loads, low CD4 cell count, coincident sexually transmitted infections and chorioamnionitis.[8,9] In addition, vaginal delivery has been known to enhance the transmission due to prolonging infant's exposure to the contaminated maternal blood and vaginal fluid.[9] However, there is no such effect in our series.

Currently, HAART regimen is recommended for all pregnant women at all gestational ages[10] to eliminate vertical transmission of HIV. However, pregnancy with no/poor ANC should be an issue if interest as there is much higher prevalence of HIV infection and preterm birth.[11] In addition, the greatest likelihood of the vertical transmission occurs in new HIV infection during pregnancy.[12] The present study supports that, even in real settings like Thailand where no/ poor ANC at up to 2% of deliveries were reported,[11] and the benefit of prolonging pregnancy outweighs the risk of the vertical HIV transmission.

CONCLUSION

Expectant management in HIV-infected women with PPROM at GA <34 weeks may be sensible because complications of prematurity outweigh the risk of vertical HIV transmission.

Declaration of interest: The authors report no conflicts of interest.

TABLE 1. Expectant management and pregnancy outcomes in HIV-infected pregnancy with preterm premature rupture of membranes at less than 34-week gestation.

Case No.	GA at first ANC	GA at initiation of HAART (weeks)	GA at PPROM	Viral load at PPROM (copies/mL)	CD4 count at PPROM (cells/mm^3)	HAART at PPROM	Interval to delivery	Mode of delivery	Chorioamnionitis	Birth weight (g)	Vertical transmission
1	No ANC	None	26 1/7	633,000	38	AZT +3TC+ Kaletra®	15 days	C/S	Yes	920	*Negative at birth, 1, and 2 mo **Negative at 1 yr
2	23 1/7	23 1/7	32 2/7	N/A	N/A	AZT +3TC+ Kaletra®	2 days	C/S	No	1,840	*Negative at birth, 1, and 2 mo **Positive at 1 yr
3	No ANC	None	31	3,409	596	AZT +3TC+ Kaletra®	2 days	SVD	No	2,160	*Negative at birth
4	24	None	30 1/7	3,960	286	AZT +3TC+ Kaletra®	4 days	SVD	No	1,250	*Negative at birth, 1, 2, 4, and 6 mo
5	7	8	33 1/7	430	256	GPO vir Z	2 days	C/S	No	1,810 1,730	A/B: *negative at birth and 1, 2, and 4 mo
6	10	20	33 3/7	N/A	N/A	TDF+3TC +EFV	2 days	SVD	No	1,890	*Negative at birth, 1, and 2 mo

* HIV DNA PCR detection ** HIV antibody detection (ELISA)

Abbreviations: PPROM: preterm premature rupture of membranes; GA: gestational age; ANC: antenatal care; C/S: caesarean section; HAART: highly active antiretroviral therapy; AZT: zidovudine; 3TC: lamivudine; Kaletra: lopinavir/ritonavir; GPO Vir Z: nevirapine/lamivudine/zidovudine; TDF: tenofovir disoproxil fumarate; EFV: efavirenz; N/A: data not available.

REFERENCES

1. American College of O, Gynecologists' Committee on Practice B-O. Practice Bulletin No. 172: Premature Rupture of Membranes. Obstet Gynecol. 2016;128(4):e165-77.
2. Medina TM, Hill DA. Preterm premature rupture of membranes: diagnosis and management. Am Fam Physician. 2006;73(4): 659-64.
3. Rimawi BH, Haddad L, Badell ML, Chakraborty R. Management of HIV Infection during Pregnancy in the United States: Updated Evidence-Based Recommendations and Future Potential Practices. Infect Dis Obstet Gynecol. 2016;2016:7594306.
4. Landesman SH, Kalish LA, Burns DN, Minkoff H, Fox HE, Zorrilla C, et al. Obstetrical factors and the transmission of human immunodeficiency virus type 1 from mother to child. The Women and Infants Transmission Study. N Engl J Med. 1996;334(25): 1617-23.
5. International Perinatal HIVG. Duration of ruptured membranes and vertical transmission of HIV-1: a meta-analysis from 15 prospective cohort studies. AIDS. 2001;15(3):357-68.
6. Phanuphak N LR, Chokephaibulkit K, Voramongkol N, Boonsuk S, Limtrakul A, et al. Thai national guidelines for the prevention of motherto-child transmission of HIV: March 2010. Asian Biomed. 2010;4(4):529-40.
7. Aagaard-Tillery KM, Lin MG, Lupo V, Buchbinder A, Ramsey PS. Preterm premature rupture of membranes in human immunodeficiency virus-infected women: a novel case series. Infect Dis Obstet Gynecol. 2006;2006:53234.
8. Teasdale CA, Marais BJ, Abrams EJ. HIV: prevention of mother-to-child transmission. BMJ Clin Evid. 2011;2011.
9. Tovo PA, de Martino M, Gabiano C, Galli L, Cappello N, Ruga E, et al. Mode of delivery and gestational age influence perinatal HIV-1 transmission. Italian Register for HIV Infection in Children. J Acquir Immune Defic Syndr Hum Retrovirol. 1996;11(1): 88-94.
10. Bureau of AIDS, TB and STIs. Prevention of mother-to-child HIV transmission. Thailand national guidelines on HIV/AIDs treatment and prevention. 2017;243-92.
11. Tiengladdawong P, Chayachinda C, Saysukanun P, Chaemsaithong P. Sexually transmitted infections and pregnancy outcomes in women without antenatal care at Siriraj Hospital. Siriraj Med J. 2017;Article in Press.
12. Goulder PJ, Lewin SR, Leitman EM. Paediatric HIV infection: the potential for cure. Nat Rev Immunol. 2016;16(4):259-71.

Secondary Acute Angle-Closure Attack: Diagnosis and Management

Sakaorat Petchyim, M.D.

Department of Ophthalmology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Patients may come with acute angle-closure attack that mimics primary disease, but actually there was a hidden cause. Mostly, fellow eye with wide open angle might be a clue for secondary cause. However, an area with high prevalence of narrow angle makes it harder to notice while fellow eye has narrow angle too. The most important thing in order to approach acute angle-closure attack is to know a possible underlying cause that should be looked for. This article will simplify various pathologies that could result in secondary acute angle-closure attack. Clinical presentation, special investigation and treatment were all summarized.

Keywords: Secondary acute angle-closure attack; secondary acute attack; diagnosis; management (Siriraj Med J 2018;70: 91-94)

INTRODUCTION

The definition of acute angle-closure attack is at least 2 of the following symptoms: ocular pain, nausea or vomiting, and a history of intermittent blurring of vision with halos; and at least 3 of the following signs: IOP greater than 21 mm Hg, conjunctival injection, corneal epithelial edema, shallower chamber in the presence of occlusion and mid-dilated nonreactive pupil. If patients with acute angle closure have glaucomatous optic neuropathy, it is defined as acute angle closure glaucoma. Acute angle closure can be classified into 2 categories due to presenting of identifiable causes. A term 'Primary' represents acute angle closure with no identifiable cause and 'Secondary' belongs to another category that had definite causes. To the best of my knowledge, there is no review article for secondary angle closure in an acute setting. This article will be the first to review diagnosis and management of secondary acute angle closure.

Primary vs. secondary

When patients come with acute angle closure, is there any clue to tell us that this is secondary? The answer is yes because there are several histories and physical examinations that could lead you to identify possible causes. First, if the following eye has wide open angle that acute angle-closure attack could be secondary. Second, bilateral involvement is an alert of underlying systemic causes. There are many conditions that could result acute angle-closure attack and they have their own characteristics. Therefore, it would be easy to approach patients according to the possible pathologies.

Pathologies

Spontaneous suprachoroidal hemorrhage

Suprachoroidal hemorrhage can happen either as a complication of intraocular surgery or spontaneously. Spontaneous cases can present with acute angle-closure attack in outpatient setting and it should be differentiated from primary acute angle-closure attack. Although, the condition is rare, it could end up with devastating result.

Any pathology that could cause massive suprachoroidal hemorrhage, also results in abrupt lens-iris diaphragm movement forward due to annular hemorrhagic ring.[4] Suspicious predisposing factors of

Correspondence to: Sakaorat Petchyim
E-mail: sakaoratpoy012@gmail.com
Received 24 May 2017 Revised 8 September 2017 Accepted 16 October 2017
doi:10.14456/smj.2018.16

spontaneous suprachoroidal hemorrhage are ocular pathologies (such as age related macular degeneration (AMD), polypoidal choroidal vasculopathy (PCV)[1-3], ateriovenous malformation), anticoagulants usage, blood dyscrasia[22], or systemic hypertension.[3] Patients should be asked for history of those factors. Patients usually present with very poor visual acuity compared to primary acute angle-closure attack. As the result from review of literature on PCV/AMD/other related cases complicated by secondary ACG, there were 9 patients (8 patients had no PL visual acuity, 1 patient with hand motion).[2] Mechanism of angle closure can be complicated as it might not result from one mechanism. Pupillary and non-pupillary mechanism could play their role; thus, iris bombe or uniformly shallowed anterior chamber could be found. Ultrasound might benefit in suspicious secondary acute angle-closure attack cases since it is not invasive and easily accessible. Management of such cases are quite difficult and no consensus due to nature of the conditions which are severe and rare. Aim of treatment may be to reduce ocular pain rather than good visual acuity. Anti-glaucoma medications, laser peripheral iridotomy, laser iridoplasty, sclerotomy drainage, or diode laser cyclodestructive procedure have been used, but the results were poor. Pesin SR et al., documented in the review of 9 patients with acute angle-closure attack which resulted from spontaneous suprachoroidal hemorrhage that, most patients end up with phthisis bulbi[2] and one case was enucleated as an attempt to control pain.[4]

Ciliochoroidal effusion

Any pathology that can cause ciliochoroidal effusion can result in anterior ciliary body rotation and lens-iris diaphragm movement forward. Ciliochoroidal effusion can be caused by medications, ocular inflammation, venous congestion, systemic diseases and unknown etiologies. Uniformly shallow anterior chamber without iris bombe is an important sign. Treatment of an underlying cause is important. Completely different from primary angle closure, It should be highlighted that laser peripheral iridotomy does not work and topical miotics should be avoided due to mechanism of angle closure that is caused from anterior rotation of ciliary body not pupillary block.

Medication-induced ciliochoroidal effusion

Topiramate, Zonisamide, Chlorthalidone, Acetazolamide, Hydrochlorothiazide, Methazolamide, and Indapamide are sulfonamide derivative drugs that are used to treat various conditions such as seizure, migraine, systemic hypertension, microbial infection, reduce intraocular pressure and they have been reported to cause acute angle closure glaucoma.[6-9] The underlying pathogenesis is unknown, with increased lens thickness due to changing in osmotic state of crystalline lens and forward crystalline lens displacement as the earlier theory that tried to explain underlying pathogenesis.[17] After that, there were evidences from several case reports that acute angle closure could be induced by sulfonamide derivatives which could happen in pseudophakic eye 6 and Craig et al.,[18] showed that lens thickness only accounts for 9 to 16% of anterior chamber shallowing. Therefore, they support idiosyncratic inflammation of uveal tissue that causes ciliary body swelling and anterior rotation. The nature of idiosyncratic reactions is unpredictable and does not depend on dosage, so it is hard to study a definite biochemical mechanism. Inflammation of uveal tissue, especially in anterior part, and ciliary processes, result in anterior rotation of ciliary body and move the lens-iris diaphragm forward. Moreover, it causes zonular relaxation which results in crystalline lens thickening, thus a patient may suffer from myopic shift. Clinically, most of the cases are bilateral 20 and the risk of developing angle closure glaucoma is within the first month after drug administration.[19] Acute bilateral blurred vision from acute myopic shift might be the first clinical complaint. The patient may less frequently present with angle closure symptoms. IOP measurement, gonioscopy along with a temporal relationship between initiating or increasing the dose of a sulfonamide derivative and onset of symptoms will help in making diagnosis. B-scan ultrasound can document ciliochoroidal effusion. Subtle ciliochoroidal effusion could be detected by ultrasound biomicroscope. Discontinuing of an offending medication is the mainstay treatment of such cases. Topical anti-glaucoma medications are used for controlling intraocular pressure. Prostaglandin derivatives should be avoided because ciliochoroidal effusion may be mediated by prostaglandin pathway. Topical miotics are contraindicated since they can cause ciliary spasm and worsen symptoms.[20] On the other hand topical cycloplegics can be used to combat myopic shift.[21] Topical steroid is recommended in cases with anterior chamber inflammation. In severe cases, systemic steroid and intravenous mannitol are worth trying. The symptom usually improves within hours to days. Since a mechanism is not a pupillary block, laser iridotomy could not help in relieving angle closure. Laser iridoplasty, choroidal drainage and trabeculectomy should be reserved for medical recalcitrant cases.[8]

Myelofibrosis, leukemia, lymphoma

Albert et al., reported blilateral acute angle closure glaucoma complicating primary myelofibrosis. Moreover, there are documented cases of leukemia and lymphoma presenting with acute angle closure.[23-25] The mechanism is uveal effusion that may result from cellular choroidal infiltration or paraneoplastic syndrome.[23] It can be unilateral or bilateral involvement, but one should be alerted for systemic conditions when dealing with bilateral acute angle closure. Treatment of the underlying causes with immunomodulation or chemotherapy along with controlling intraocular pressure are recommended.

Posterior scleritis

Posterior scleritis can cause annular ciliochoroidal effusion which leads to acute angle closure.[26-29] Treatment of posterior scleritis with systemic steroid should be considered. For controlling intraocular pressure, management should follow that of acute angle closure from ciliochoroidal effusion.

Others systemic diseases

Systemic lupus erythematosus choroidopathy[30], acquired immune deficiency syndrome[32-39], uveal effusion syndrome[40-41], Vogt-Koyanagi-Harada syndrome (Harada disease)[42-44], septic condition of Korean hemorrhagic fever [45], and campylobacter jejuni infection[31] have been reported to be associated with ciliochoroidal effusion.

Lens-induced

Subluxated crystalline lens can occur in patients who have the following risk factors which can cause zonular weakness such as trauma, pseudoexfoliation syndrome, connective tissue disease[49], retinitis pigmentosa, or post vitreoretinal surgery.[47] For intraocular pressure regulation, cataract surgery with intraocular lens implantation is an effective procedure.[48]

Aqueous misdirection syndrome

This form of glaucoma can occur post-surgically or spontaneously. Clinical features are raised intraocular pressure, uniformly shallow anterior chamber and patent peripheral iridotomy. The pathophysiology is unknown. Patients with primary angle-closure glaucoma who underwent surgery especially glaucoma drainage surgery were recommended to follow up closely during the early post-operative period. There are medications, laser and surgical management, so these should be considered according to lens status and severity.[46]

Miscellaneous

There are several rare conditions that have been documented in case reports that can result in acute angle closure. Seongyong Jeong et al., reporteda case with acute angle closure after intravitreal bevacizumab injection[50]; thus, there were several possible causes. First, pupil dilation before procedure may precipitate acute attack. Second, retinal vein occlusion might be associated with narrow angle after intravitreal injection had increase pressure from posterior of the eye, so it could result in angle closure. Le Du B et al., reported acute angle closure attack secondary to BrightOcular© cosmetic iris implant.[51] Snake bite has been documented as a cause of acute angle closure, and although it is rare, it should be kept in mind because it needs timely recognition and management. Proposed mechanism is capillary leakage syndrome, which results in ciliochoroidal effusion.[52]

ACKNOWLEDGEMENTS

Thank you for one of my patients who urged me to find out about this rare condition that could result in acute angle-closure attack.

REFERENCES

1. Pesin SR, Katz LJ, Augsburger JJ. Acute angle-closure glaucoma from spontaneous massive hemorrhagic retinal and choroidal detachment. An updated diagnostic and therapeutic approach. Ophthalmology. 1990;97(1):76-84.
2. Baskaran P, Sindal MD, Dhoble P, Ramakrishnan S, Rengaraj V, Ramulu P. Acute angle closure glaucoma secondary to polypoidal choroidal vasculopathy - a devastating complication. GMS Ophthalmol Cases. 2017;7:Doc01.
3. Chen SN, Ho CL, Ho JD. Acute angle-closure glaucoma resulting from spontaneous hemorrhagic retinal detachment in age-related macular degeneration: case reports and literature review. Jpn J Ophthalmol. 2001;45(3):270-5.
4. Lee YJ, Kang SM, Kang IB. Acute angle-closure glaucoma from spontaneous massive hemorrhagic retinal detachment. Korean J Ophthalmol. 2007;21(1):61–64.
5. Stuart Fourman MD, Angle-closure glaucoma complicating ciliochoroidal detachment, Ophthalmology. 1989;96(5):646-653.
6. Durai I, Mohan Dhavalikar M, Anand CP, Ganesh V, Krishnadas R. Bilateral. Silmutaneous, Acute angle closure glaucoma in pseudophakia induced by Chlorthalidone. Case Rep Ophthalmol Med. 2016;2016:3713818.
7. Mancino R, Varesi C, Cerulli A, Aiello F, Nucci C. Acute bilateral angle-closure glaucoma and choroidal effusion associated with acetazolamide administration after cataract surgery. J Cataract Refract Surg. 2011;37(2):415-7.
8. Weiler DL. Zonisamide-induced angle closure and myopic shift. Optom Vis Sci. 2015;92(2):e46-51.
9. Willett MC, Edward DP. Refractory topiramate-induce angle-closure glaucoma in a man: a case report. J Med Case Rep. 2011;5:33.
10. Lee GC, Tam CP, Danesh-Meyer HV, Myers JS, Katz LJ. Bilateral angle closure glaucoma induced by sulphonamide-derived medications. Clin Experiment Ophthalmol 2007; 35: 55–8.

11. Roh YR, Woo SJ, Park KH. Acute-onset bilateral myopia and ciliochoroidal effusion induced by hydrochlorothiazide. Korean J Ophthalmol 2011; 25: 214–7.
12. Soylev MF, Green RL, Feldon SE. Choroidal effusion as a mechanism for transient myopia induced by hydrochlorothiazide and triamterene. Am J Ophthalmol 1995; 120: 395–7.
13. Kwon SJ, Park DH, Shin JP. Bilateral transient myopia, angle-closure glaucoma, and choroidal detachment induced by methazolamide. Jpn J Ophthalmol 2012; 56: 515–7.
14. Aref AA, Sayyad FE, Ayres B, Lee RK. Acute bilateral angle closure glaucoma induced by methazolamide. Clin Ophthalmol 2013; 7: 279–82.
15. Vegh M, Hari-Kovacs A, Rez K, Tapaszto B, Szabo A, Facsko A. Indapamide-induced transient myopia with supraciliary effusion: case report. BMC Ophthalmol 2013; 13: 58.
16. Blain P, Paques M, Massin P, Erginay A, Santiago P, Gaudric A. Acute transient myopia induced by indapamide. Am J Ophthalmol 2000; 129: 538-40.
17. Sen HA, O'Halloran HS, Lee WB. Case reports and small case series: topiramate-induced acute myopia and retinal striae. Arch Ophthalmol 2001; 119: 775-7.
18. Craig JE, Ong TJ, Louis DL, Wells JM. Mechanism of topiramate-induced acute-onset myopia and angle closure glaucoma. Am J Ophthalmol 2004; 137: 193-5.
19. Ho JD, Keller JJ, Tsai CY, Liou SW, Chang CJ, Lin HC. Topiramate use and the risk of glaucoma development: a population-based follow-up study. Am J Ophthalmol. 2013; 155: 336–41.
20. Abtahi MA, Abtahi SH, Fazel F, Roomizadeh P, Etemadifar M, Jenab K, et al. Topiramate and the vision: a systematic review. Clin Ophthalmol. 2012; 6: 117–31.
21. van Issum C, Mavrakanas N, Schutz JS, Shaarawy T. Topiramate-induced acute bilateral angle closure and myopia: pathophysiology and treatment controversies. Eur J Ophthalmol. 2011; 21: 404-9
22. Shaikh A, Parulekar M, James B. Acute suprachoroidal haemorrhage with acute angle closure glaucoma as a presenting sign of chronic myelomonocytic leukemia. Eye (Lond). 2002;16(5):651-3.
23. Cristol SM, Baumblatt JG, Icasiano E, Grajewski AL, Olivier MM, Parrish RK. Bilateral acute angle-closure associated with systemic lymphoma: a report of 2 cases. J Glaucoma. 2011;20:115–117.
24. Tumuluri K, Woo T, Crowston J, Healey PR, Gottlieb D, Maloof AJ. Bilateral leukemic orbital infiltration presenting as proptosis and narrow-angle glaucoma. Ophthal Plast Reconstr Surg. 2004;20:248–250
25. Baillif S, Cornut PL, Girard C, Ghesquieres H, Perard L. Bilateral angle-closure glaucoma and multifocal choroiditis as a first presentation in Hodgkin lymphoma. Retin Cases Brief Rep. 2011;5:201–205.
26. Quinlan MP, Hitchings RA. Angle-closure glaucoma secondary to posterior scleritis. Br J Ophthalmol. 1978;62:330-335.
27. Benson WE. Posterior scleritis. Surv Ophthalmol 1988;32:297–316.
28. Fourman S. Angle-closure glaucoma complicating ciliochoroidal detachment. Ophthalmology. 1989;96:646–653.
29. Ikeda N, Ikeda T, Nomura C, Mimura O. Ciliochoroidal effusion syndrome associated with posterior scleritis. Jpn J Ophthalmol. 2007;51:49-52.
30. Han YS, min Yang C, Lee SH, Shin JH, Moon SW, Kang JH. Secondary angle closure glaucoma by lupus choroidaopathy as an initial presentation of systemic lupus erythematosus : case report. BMC Ophthalmol. 2015 29;15:148.
31. Mukherji S, Ramanathan S, Tarin S. Uveal effusuin associated with campylobacter jejuni infection presenting as bilateral angle closure glaucoma. J Glaucoma. 2011 Dec;20(9):587-8.
32. Ullman S, Wilson RP, Schwartz L. Bilateral angle-closure glaucoma in association with the acquired immune deficiency syndrome. Am J Ophthalmol. 1986;101:419–424.
33. Williams AS, Williams FC, O'Donnell JJ. AIDS presenting as acute glaucoma. Case report. Arch Ophthalmol. 1988;106:311–312.
34. Meige P, Cohen H, Morin B, Athlani A. Acute bilateral glaucoma in an LAV-positive subject. [Article in French] Bull Soc Ophtalmol Fr. 1989;89(3):449-54.
35. Koster HR, Liebmann JM, Ritch R. Acute angle-closure glaucoma in a patient with acquired immunodeficiency syndrome successfully treated with argon laser peripheral iridoplasty. Ophthalmic Surg. 1990;21:501–502.
36. Nash RW, Lindquist TD. Bilateral angle-closure glaucoma associated with uveal effusion: presenting sign of HIV infection. Surv Ophthalmol. 1992;36:255–258.
37. Joshi N, Constable PH, Margolis TP, Hoyt CS, Leonard TJ. Bilateral angle closure glaucoma and accelerated cataract formation in a patient with AIDS. Br J Ophthalmol. 1994;78:656–657.
38. Zambarakji HJ, Simcock PR. Bilateral angle closure glaucoma in HIV infection. J R Soc Med. 1996;89:581–582.
39. Fritz MH, Kesert M. Glaucoma following the ingestion of sulfathiazone. Am J Ophthalmol. 1947;30(2):197.
40. Fourman S. Angle-closure glaucoma complicating ciliochoroidal detachment. Ophthalmology. 1989;96:646–653.
41. Branda L, Hatt M. Bilateral angle block glaucoma in spontaneous choroid detachment. Klin Monatsbl Augenheilkd. 1990;196:423–424.
42. Shirato S, Hayashi K, Masuda K. Acute angle closure glaucoma as an initial sign of Harada's disease: report of two cases. Jpn J Ophthalmol. 1980;24:260–266.
43. Kimura R, Sakai M, Okabe H. Transient shallow anterior chamber as initial symptom in Harada's syndrome. Arch Ophthalmol. 1981;99:1604-6.
44. Kimura R, Kasai M, Shoji K, Kanno C. Swollen ciliary processes as an initial symptom in Vogt-Koyanagi-Harada syndrome. Am J Ophthalmol. 1983;95:402–403.
45. Cho IH, Chang JH, Choo EJ. Bilateral simultaneous angle-closure glaucoma associated with septic condition of Korean hemorrhagic fever(KHF). J Glaucoma. 2015;24(1):81-3.
46. Shahid H, Salmon JF. Malignant glaucoma: a review of the modern literature. J Ophthalmol. 2012;2012:852659.
47. Hoffman RS, Snyder ME, Devgan U, Allen QB, Yeoh R, Braga-Mele R; ASCRS Cataract Clinical Committee; Challenging/Complicated Cataract Surgery Subcommittee. Management of the subluxated crystalline lens. J Cataract Refract Surg. 2013;39(12):1904-15.
48. Haberle H, Pham DT, Weller A. Lens subluxation and glaucoma : indications and hazards of cataract surgery. Ophthalmologe. 2000;97(1):47-50.
49. Lim SH, Son JH, Cha SC. Acute angle-closure glaucoma in a highly myopic patient secondary to Weil-Marchesani syndrome : histopathologic lens features. Int Ophtahlmol.2016;36(6):921-924.
50. Jeong S, Sagong M, Chang W. Acute angle closure attack after an intravitreal bevacizumab injection for branch retinal vien occlusion : a case report. BMC Opthalmol. 2017; 17: 25.
51. Le du B, Boukhrissa M, Nordmann JP. Acute angle closure attack secondary to Bright ocular cosmetic implant and subsequent subluxation of contralateral iris implant. J Fr Ophthalmol. 2016;39(6).
52. Kulkarni C, George TA, Av A, Ravindran R. Acute angle closure glaucoma with capillary leak syndrome following snake bite. J Clin Diagn Res. 2014;8(10):VC01-VC03.