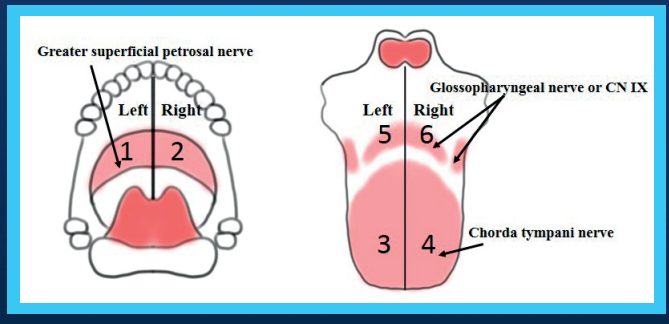
ISSN 2629-995X
Volume 71, Number 2, March-April 2019

SMJ
Siriraj Medical Journal



By Bannapuch Pinkaew, et al.

ORIGINAL ARTICLE

95 **A Comparison of the Bacterial Culture Results of Maxillary Sinus Mucosa and Pus Collections for Chronic Maxillary Rhinosinusitis**
Anan Bedavanija, et al.

102 **Impact Assessment of Smell and Taste Disorders on Quality of Life in Thais Using the SF-36 Health Survey (Thai version)**
Bannapuch Pinkaew, et al.

110 **Risk Scoring System for the Prediction of Postpartum Blood Loss over 300 mL at Chiang Rai Regional Hospital**
Natakorn I-Tuporn, et al.

117 **The Evaluation of Posterior Cingulate Gyrus by Diffusion Tensor Imaging in Alzheimer's Disease Patients Compared with Normal Control Subjects**
Chanon Ngamsombat, et al.

123 **Comparison of Academic Outcomes of Epidemiology and Biostatistics Course between Traditional Lecture and Flipped Classroom Blended Approach in 2nd Year Medical Students: A Retrospective Cohort Study**
On-anong Binsomprasong, et al.

127 **Re – evaluation of Rinne Test with Aluminum Alloy Tuning Fork 256 Hz and 512 Hz**
Patcharaporn Phonpornpaiboon, et al.

131 **Hearing and Balance Survey in Thai Elders**
Samut Chongvisal, et al.

143 **Scintigraphic Detection of Splenosis and Accessory Spleen by Using Tc-99m Labelled Denatured Red Blood Cells**
Apichaya Claimon, et al.

150 **Does Acute Kidney Injury Condition Affect Revised BAUX Score in Predicting Mortality in Major Burn Patients?**
Kusuma Chinaroonchai, et al.

158 **An Association of *Cryptococcus Neoformans/C. gattii* Genotype and HIV Status in Asia: A Systematic Review**
Popchai Ngamskulrungroj, et al.

165 **Clinical Application of Toothbrush Technique for Specimen Collection of Tinea Capitisl**
Lalita Matthapan, et al.

REVIEW ARTICLE

168 **Gummy Smile: A Review of Etiology, Anifestations, and Treatment**
Basel Mahardawi, et al.

Indexed by

Scopus®

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)
Folker 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)
Nam H. CHO (University School of Medicine and Hospital, Korea)

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)

Statistician: 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].

Comparison of Academic Outcomes of Epidemiology and Biostatistics Course between Traditional Lecture and Flipped Classroom Blended approach in 2nd Year Medical Students: A Retrospective Cohort Study

On-anong Binsomprasong, M.Ed.*, Lokachet Tanasugarn, M.D., Pongsakorn Buraphat, M.D.***, Mayuree Homsanit, M.D., Ph.D.****

**Education Department, **Department of Preventive and Social Medicine, ***Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: To compare academic outcomes between integrated flipped classroom (FC) and solely traditional lecture (TL) teaching approach among 2nd year medical students in *Epidemiology and Biostatistics* course.

Methods: General information and academic achievements including grade point average, scores of the basic statistics course and scores of *Epidemiology and Biostatistics* course were extracted from the database of the Education Department. The records were excluded if they belonged to repeated or transferred students. The students in 2014 and 2015 academic year represented the TL group and the FC group respectively. The main outcome was the examination score from the two groups which was converted to a comparable scale out of a hundred.

Results: Six-hundred twenty-eight records were included in the analysis. Based on independent t-test, the TL group showed higher mean score as compared to the FC group (p-value < 0.01). Further comparison using ANCOVA adjusted for age, gender, hometown location, high school location, grade point average and score of a basic statistics course showed a higher score in the TL group (p-value < 0.01).

Conclusion: TL yielded higher examination score than FC from this retrospective study.

Keywords: Flipped classroom; traditional lecture; epidemiology and biostatistics (Siriraj Med J 2019;71: 123-126)

INTRODUCTION

According to a competency-based framework, paradigm shift in medical education transforms traditional lecture (TL) toward other methods to create lifelong learners.[1,2] Depending on co-operation of both students and teachers, active learning curriculum demonstrated long-lasting knowledge by developing intrapersonal skills to solve future problems.[3,4]

The flipped classroom (FC) is an educational method that reverses a student role from a passive learner to an active learner. Certain self-studied resources such as recommended textbooks, articles or even video records

Corresponding author: Mayuree Homsanit
E-mail: mayuree.hom@mahidol.ac.th
Received 1 September 2017 Revised 16 February 2018 Accepted 23 May 2018
ORCID ID: http://orcid.org/0000-0002-9838-5636
http://dx.doi.org/10.33192/Smj.2019.19

are arranged for the students prior to each class. During the class, activities were designed for application or integration of the given assignments. Teachers are not lecturers but they are coaches or supporters.[5] Thus, engagement of both students and teachers are highly required to achieve the excellent outcome.[6,7]

The Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand ("the faculty") provides the *Epidemiology and Biostatistics* course for the second-year medical students. Before 2014 academic year, this course mainly consisted of one-way TL in which instructors were required to deliver the lecture to a large group of audiences (about 300 students). However, "Outcome-Based Curriculum" was implemented in 2014. The new curriculum emphasized the importance of active learning methods and encouraged to eliminate all previous passive teachings. Thus, reduction in TL and integration of FC were implemented to all courses.

Several studies demonstrated that FC could improve knowledge as compared before and after the class[8], or compared with TL.[9] Other evidences[10-12] demonstrated efficacy and advantage of this educational method, although one systematic review focused on medical education suggested that there is no strong evidence for the effectiveness of FC.[13] Inconsistency in learning outcome of FC may come from difference in participants' background, learning culture or heterogeneity of education tools.

As far as we know, there is no evidence in Thailand comparing learning outcome between TC and FC in *Epidemiology and Biostatistics*. The objective of this study was to evaluate changes in *Epidemiology and Biostatistics* learning outcomes after implementing FC in our institute.

MATERIALS AND METHODS

This study was conducted as a retrospective cohort design. The study was approved by Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University (Si 536/2558).

Data retrieval and management

General information and academic outcome of the medical students in 2014 and 2015 academic year were acquired from the Education Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. The background information of each student included gender, age, hometown location, high school location, grade point average (GPA), scores of a basic biostatistics course in pre-medical year and score of *Epidemiology and Biostatistics* course. Any student with repeated grade or transferred credit was excluded from the analysis. We used data of all eligible students (students enrolled in 2014-2015 academic year) for analysis.

Traditional lecture course (TL) vs. Flipped Classroom (FC) course

The 2014 cohort (total students = 319) received TL method. The TL course consisted of thirteen hours of one-way lecture and six hours of workshops. The evaluation consisted solely of examination score. The FC method was implemented in the 2015 group (total students = 313). The course provided eleven hours of interactive lecture and four hours of workshop. The FC course also included one additional online module on revisiting basic statistics which was about three hours. Additionally, two formative evaluations were incorporated in the FC course which were not scored, but were required to be eligible to take the final examination. Both courses were designed based on the same set of learning outcome from the Medical Competency Assessment Criteria for National License 2012 of Thailand.[14] Also, the same set of instructors taught in both courses on each topic. Since the FC group score was categorized as a multiple-choice question (MCQ), attitude, essay, and coursework, we only compared MCQ and essay score with the total score of the TL group (100% of MCQ). Thus, the score of the FC group (50% of MCQ and 50% of short answer question) was converted to a scale of a hundred to be comparable with the TL score. Test analysis of both courses revealed that their MCQ test property fell into the same category with very good average difficulty index and good average discrimination index.

Statistical analysis

All analyses were performed with PASW Statistics for Windows version 18 (SPSS Inc., Chicago, Ill, USA). Descriptive statistics were presented as mean ± SD and n (%). Independent t-test was used to compare the difference between mean of scores. ANCOVA was used to further compare the mean difference with adjustment for age, gender, hometown, high school, grade point average and a score of basic statistics course as covariates.

Power analysis

Because we recruited data of all medical students who met eligibility criteria for this study, no sample size was calculated. We performed power analysis using two-sided test for mean difference. Based on total students enrolled in the TL and FC group (319 vs. 313 students), we estimated that we could retrieve data without missing at 95% of total enrolled students. Using pooled average and standard deviation (SD) of examination score from

a previous study[15], power of the study was estimated to be > 90% at a $\alpha = 0.05$ (difference of pooled means = 4.5, pooled SD of the control group = 5.94, pooled SD of the experimental group = 6.13).

RESULTS

Six-hundred and thirty-two records were eligible for the study which comprised of 319 records in the traditional lecture group and 313 records in the flipped classroom group. In the traditional lecture group, three records were excluded: two records belonged to repeated grade students and one record was a transferred student. For the flipped classroom group, one record belonged to a transfer student and was excluded. Therefore, a total of 628 records were included in the analyses. The demographic data of both groups is presented in Table 1.

The independent t-test revealed that the TL group had a higher score than the FC group (p-value < 0.01). Subsequently, ANCOVA with adjustment for covariates showed the same outcome (p-value < 0.01). The details of the comparison are shown in Table 2.

DISCUSSION

This study illustrates the difference in academic outcome between TL and FC method in *Epidemiology and Biostatistics* courses among Thai 2nd year undergraduate medical students. The main finding from this study suggests that TL yields better academic achievement compared to FC in this subject.

It is clear that the FC group had statistically lower examination score even though all the teaching topics were the same and the test analysis illustrated similar test property. Additionally, the ANCOVA with adjusted covariates presented a consistent result and the power analysis guaranteed a sufficient power. Other studies aiming at exploring the effect of implementing FC on the same or related subjects have illustrated the similar academic outcome between the two groups.[16,17] Hence,

TABLE 1. Characteristic of TL and FC groups.

Characteristic	Traditional Lecture group (N = 315)	Flipped Classroom group (N = 313)
Gender: female	153 (48.6)	145 (46.3)
Age (year)	19.63 ± 0.91	19.50 ± 0.58
Hometown located in Bangkok	152 (48.3)	146 (46.6)
High school located in Bangkok	182 (57.8)	188 (60.1)
Grade point average during 1st year	3.30 ± 0.39	3.53 ± 0.31
Score of basic statistics course	60.24 ± 12.72	75.46 ± 10.58

*Data is presented in n (%) for categorical data and mean ± SD for continuous data

TABLE 2. Comparison of academic outcome between TL and FC group.

Score comparison	TL group[1]	FC group[1]	P-value	95% Confidence Interval (CI)
Examination score[2]	77.97 ± 9.44	69.82 ± 10.83	< 0.001	6.56, 9.74
Examination score with adjustment for covariates[3]	77.97 ± 9.44	69.82 ± 10.83	< 0.001	11.16, 14.15

[1]Data is presented in mean ± SD
[2]p-value and 95% CI from independent t-test
[3]p-value and 95% CI from ANCOVA with gender, age, hometown location, high school location, GPA and score of basic statistic course as covariates

our results support the evidence that the integration of FC into the curriculum did not show better academic outcome, but worse.

Nevertheless, a number of studies have showed a significant increase in students' satisfaction in the FC group.[10,13,18] Furthermore, implementation of FC results in the better development of several practical soft skills.[19] Thus, it is clear that integration of FC should not be aimed only at better academic outcome of the students, but also focusing on maturation process of the learning ability of the students to eventually acquire adult learning which is the ultimate outcome for learners in this era. Thus, it is highly suggested that evaluation of these skills should be included in the FC curriculum as well.

This study did have some limitations. Firstly, the active learning policy was implemented in every course for the 2015 cohort. Thus, the result of this study could be affected by the overt workload from other courses as well. Secondly, other factors that could affect the examination score such as examination preparation technique or additional peer tutoring within each cohort were not explored due to the nature of a retrospective study. Nevertheless, this study did retrieve all related objective data, controlled numerous potential confounders and presented consistent findings across statistical analyses which were comparable with other published studies.

CONCLUSION

TL method shows better academic outcome as compared to FC method in the subject of *Epidemiology and Biostatsitics* from the historical records of Thai 2nd year medical students.

ACKNOWLEDGMENTS

We would like to thank the Education Department for the provided data, all the instructors and coordinators of the course and Ms. Saengnapha Tarpick for their support.

Conflict-of-Interest Notification Page: This project was supported by Medical Education Fund. The authors declare that they have no other conflicting interest. MH was the main instructor and OB was the coordinator of both courses, although all data was obtained through Department of Education of the Institute.

REFERENCES

1. Schumacher DJ, Englander R, Carraccio C. Developing the master learner: applying learning theory to the learner, the teacher, and the learning environment. Acad Med 2013;88:1635-45.
2. Collins J. Education techniques for lifelong learning: principles of adult learning. Radiographics: a review publication of the Radiological Society of North America, Inc 2004;24:1483-9.
3. Hurtubise L, Roman B. Competency-based curricular design to encourage significant learning. Curr Probl Pediatr Adolesc Health Care 2014;44:164-9.
4. Inra JA, Pelletier S, Kumar NL, Barnes EL, Shields HM. An active learning curriculum improves fellows' knowledge and faculty teaching skills. Adv Med Educ Pract 2017;8:359-64.
5. Moffett J. Twelve tips for "flipping" the classroom. Med Teach 2015;37:331-6.
6. Sharma N, Lau CS, Doherty I, Harbutt D. How we flipped the medical classroom. Med Teach 2015;37:327-30.
7. Ramnanan CJ, Pound LD. Advances in medical education and practice: student perceptions of the flipped classroom. Adv Med Educ Pract 2017;8:63-73.
8. Tainter CR, Wong NL, Cudemus-Deseda GA, Bittner EA. The "Flipped Classroom" Model for Teaching in the Intensive Care Unit. J Intensive Care Med 2017;32:187-96.
9. Rui Z, Lian-rui X, Rong-zheng Y, Jing Z, Xue-hong W, Chuan Z. Friend or Foe? Flipped Classroom for Undergraduate Electrocardiogram Learning: a Randomized Controlled Study. BMC Med Educ 2017;17.
10. Betihavas V, Bridgman H, Kornhaber R, Cross M. The evidence for 'flipping out': A systematic review of the flipped classroom in nursing education. Nurse Educ Today 2016;38:15-21.
11. Moraros J, Islam A, Yu S, Banow R, Schindelka B. Flipping for success: evaluating the effectiveness of a novel teaching approach in a graduate level setting. BMC Med Educ 2015;15:27.
12. Mortensen CJ, Nicholson AM. The flipped classroom stimulates greater learning and is a modern 21st century approach to teaching today's undergraduates. J Anim Sci 2015;93:3722-31.
13. Chen F, Lui AM, Martinelli SM. A systematic review of the effectiveness of flipped classrooms in medical education. Med Educ 2017;51:585-97.
14. Shin YI, Yang CY, Joo MC, Lee SG, Kim JH, Lee MS. Patterns of using complementary and alternative medicine by stroke patients at two university hospitals in Korea. Evid Based Complement Alternat Med 2008;5:231-5.
15. Hu R, Gao H, Ye Y, Ni Z, Jiang N, Jiang X. Effectiveness of flipped classrooms in Chinese baccalaureate nursing education: A meta-analysis of randomized controlled trials. Int J Nurs Stud 2017;79:94-103.
16. Evans KH, Thompson AC, O'Brien C, Bryant M, Basaviah P, Prober C, et al. An innovative blended preclinical curriculum in clinical epidemiology and biostatistics: Impact on student satisfaction and performance. Acad Med 2016;91:696-700.
17. Galway LP, Corbett KK, Takaro TK, Tairyan K, Frank E. A novel integration of online and flipped classroom instructional models in public health higher education. BMC Med Educ 2014;14.
18. Graham M. Blended learning: A way to engage undergraduate students in learning epidemiology and biostatistics? Australian Epidemiologist 2016;23:16-9.
19. Lin Y, Zhu Y, Chen C, Wang W, Chen T, Li T, et al. Facing the challenges in ophthalmology clerkship teaching: Is flipped classroom the answer? PLoS One 2017;12:e0174829.

Re – evaluation of Rinne Test with Aluminum Alloy Tuning Fork 256 Hz and 512 Hz

Patcharaporn Phonpornpaiboon, M.D., Kanokrat Suvarnsit, M.D., Sarun Prakairungthong, M.D.

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

ABSTRACT

Objective: To study the sensitivity, specificity and accuracy of the 256 Hz and 512 Hz aluminum tuning fork in the detection of conductive hearing loss by quick Rinne test.

Methods: The patients with hearing problems recruited from out-patient unit were tested with the 256 Hz and 512 Hz aluminum quick Rinne test. The audiometry was performed on the same day and the results were compared.

Results: During the study period, 246 ears with conductive hearing loss and 246 ears with non-conductive hearing loss were recruited. The 256 Hz Rinne test had higher sensitivity than the 512 Hz Rinne test (93.83% and 71.95% respectively). The specificity and accuracy of the 512 Hz Rinne test was markedly greater than the 256 Hz Rinne test (91.18 % vs. 26.7% specificity and 83.57% vs. 61.85% accuracy). The 512 Hz Rinne test had sensitivity over 80% when the air-bone gap was equal to or greater than 20 dB. The sensitivity was even higher (more than 90%) if the air-bone gap was equal to or greater than 30 dB.

Conclusion: This study demonstrated that the 512 Hz Rinne test had better accuracy than the 256 Hz Rinne test for the diagnosis of conductive hearing loss. The 512 Hz Rinne test can detect the air-bone gap of 30 dB or greater which indicates the surgical role with the sensitivity more than 90%. We recommend that 512 Hz Rinne test should be used as a screening tool for the detection of conductive hearing loss.

Keywords: Rinne; tuning fork; accuracy; conductive hearing loss; air-bone gap (Siriraj Med J 2019;71: 127-130)

INTRODUCTION

The tuning fork test for hearing evaluation has long been widely used. Various techniques for tuning fork usability were developed; for instance, Weber test, Bing occlusion test, Schwaback test and Rinne test. However, the Rinne test is primarily used as one of the bedside tools in the evaluation of patients with hearing problem.

Despite the easily accessibility of the audiometry which is considered as a gold standard for hearing diagnosis, tuning fork 256 Hz and 512 Hz were still recommended for general practitioners and otoneurologists for several circumstances; for example, screening for conductive hearing loss, pre-and post- operative evaluation of middle ear surgery[1] and detection of the air-bone gap. Even though, several researches have tried to evaluate the value of tuning fork since 1980s[2], the results had wide variation. One study[3] suggested that a 512 Hz tuning fork should be used for screening patients with conductive hearing loss. However, another study advised to use 256 Hz tuning fork instead.[3] Also, the tuning fork material is another issue to consider for the various results. Many studies didn't mention tuning fork material.[2,3,4] Also, one study used uncommon material such as metal tuning fork which is rarely used in ordinary practice.[5]

Coresponding author: Sarun Prakairungthong
E-mail: tiktono99@gmail.com, tiksarun99@yahoo.com
Received 24 July 2018 Revised 17 January 2019 Accepted 20 January 2019
OCID ID: http://orcid.org/0000-0002-2098-5199
http://dx.doi.org/10.33192/Smj.2019.20

Our study aimed to re-assess the accuracy, specificity, and sensitivity of the 256 Hz and 512 Hz aluminum tuning fork test by performing quick Rinne test as the primary objective. The secondary objectives were the following;

1. To determine how to interpret equivocal Rinne test
2. To determine the results of tuning fork test in comparison with the air-bone gap.

The findings from this study will guide us for the way to evaluate of hearing problem patients in out-patient clinic and primary care unit.

MATERIALS AND METHODS

This cross-sectional study was approved by the Siriraj Institutional Ethical Committee (Si 587/2015). Subjects were recruited from the out-patient otolaryngology clinic during May 2015 to July 2016. Routine history taking and otological examination were performed in individual patients. The patients with the age of 18 years and older suspicious for abnormal hearing were recruited.[6] The exclusion criteria was patients with learning or psychological problems. The procedure was started with the quick Rinne test performed on both ears by a group of well-trained doctors in the tightly closed room (estimate background noise of 53.2 dBA SPL). The examiners were blinded from both patients' diagnosis and otological examination. The Rinne test was performed using 256 Hz and 512 Hz aluminum tuning fork by the loudness comparison method (quick Rinne test).[7]

The vibrating tuning fork was placed firmly behind the pinna (BC position) for few seconds then moved to the front of the ear canal approximately 2 to 2.5 centimeters from the tragus with parallel position to the skull (AC position). The examinee would be asked to compare the loudness of each position. Which site was louder?[2] The results were recorded in 3 categories: negative Rinne test when the examinee heard louder when placing tuning fork on the mastoid bone than when holding in front of the ear (BC>AC), positive Rinne test when the examinee heard louder while holding the fork in front of the ear than placing on the mastoid bone (AC>BC), and equivocal Rinne test when the examinee could not tell the difference between the two positions (AC=BC).

We classified the examinee into 2 groups: conductive hearing loss group and nonconductive hearing loss group. The calculated number for each group was 246 ears which were collected in order to avoid the bias. The profound sensorineural hearing loss ears were excluded from the study.

RESULTS

There were 296 cases recruited in this study. Most of them are women (61.5%). Age ranged between 18 to 87 years with the mean age of 54.4 years (S.D. 15.22). Demographic data are shown in Table 1.

Data recorded when performing the test with 256 Hz tuning fork was mostly negative Rinne test on both conductive hearing loss (92.6%) and nonconductive hearing loss (65.85%) groups. When using 512 Hz tuning fork, the negative Rinne test was recorded in 71.95% of conductive hearing loss subjects. Whereas, the positive Rinne test was reported in 86.5% of nonconductive hearing loss subjects.

The equivocal result was recorded in similar amount of 5% in both conductive and nonconductive hearing loss group either performing with 256 Hz or 512 Hz tuning fork. Interestingly this equivocal result lead to nonconductive situation in 256 Hz tuning fork while this leads to no clue in 512 Hz tuning fork. All results from all tests in this study are shown in Table 2.

The data was calculated with the exclusion of equivocal results for sensitivity, specificity, accuracy, positive predictive value and negative predictive value of 256 Hz and 512 Hz quick Rinne test. Therefore, the number of the tested ears decreased to the final of 464 ears in 256Hz tuning fork group and 465 ears in 512Hz tuning fork group.

As the result, the 256 Hz Rinne tuning fork test has very high sensitivity (93.83%), low specificity (26.7%), and moderate accuracy (61.85%). However, all the data in 512 Hz tuning fork group was higher than 256 Hz tuning fork group except for the sensitivity. (Fig 1)

TABLE 1. Demographic data.

Data	Number (%)
Sex	
Male	114 (38.5)
Female	182 (61.5)
Education	
Uneducated	2 (0.7)
Grade 1-6	75 (25.3)
Grade 7-12	56 (18.9)
Diploma	24 (8.1)
Undergraduate	139 (47%)
Number of used ear	
Unilateral	100 (33.8%)
Bilateral	196 (66.2%)

TABLE 2. Results of 256 Hz and 512 Hz Rinne tuning fork test.

	Conductive hearing loss		Nonconductive hearing loss	
	256 Hz (246 ears) Number (%)	512 Hz (246 ears) Number (%)	256 Hz (246 ears) Number (%)	512 Hz (246 ears) Number (%)
Rinne negative	228 (92.6)	177 (71.95)	162 (65.85)	19 (7.72)
Rinne positive	15 (6.09)	56 (22.76)	59 (23.98)	213 (86.5)
Rinne equivocal	3 (1.21)	13 (5.28)	25 (10.16)	14 (5.69)

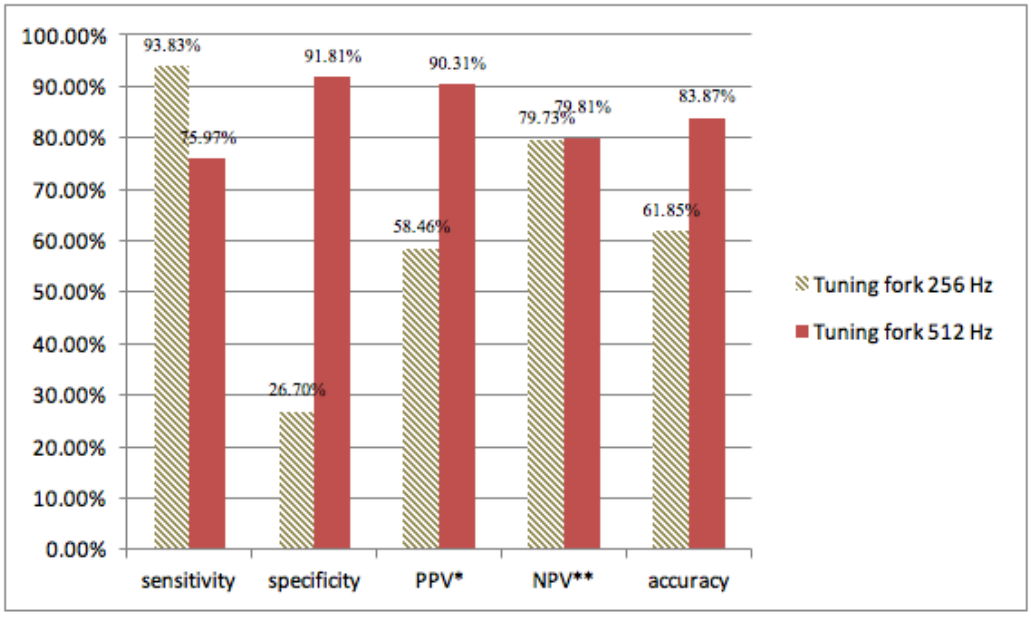


Fig 1. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 256 Hz compare with 512 Hz Rinne tuning test. (*positive predictive value, **negative predictive value)

In this study authors used the average air-bone gap according to Committee on Hearing and Equilibrium guidelines for the evaluation of results of treatment of conductive hearing loss.[9] From the results, different frequencies tuning forks had different power to determine conductive hearing loss. The 256 Hz tuning fork had very high power (92.7%) to detect conductive hearing loss even with only 10 dB air-bone gap. Whilst, 512Hz tuning fork would have a high power (91.9%) to detect conductive problem if the air-bone gap is greater than 30 dB air-bone gap. (Fig 2)

DISCUSSION

Despite the low specificity of 256 Hz tuning fork, the sensitivity of 256 Hz Rinne tuning fork test is greater than 512 Hz tuning fork test (Fig 1). This yield the similar result as previous studies of Burkey et al[2] and Chole et al.[3]

Burkey et al[2] suggested that the sensitivity of equivocal Rinne test in conductive hearing loss can be improved by applying 512 Hz tuning fork.

Chole et al[3] and Browning et al[4] reported that whether they included equivocal results of tuning fork test or not, the sensitivity and specificity were not different. We found that the equivocal Rinne test comprised of 5% of subjects and our data showed that the results did not correlate with either positive nor negative Rinne test.[3,4] For this reason, we did not include the equivocal Rinne test in the calculation for sensitivity and specificity.

Rinne's sensitivity of detecting conductive hearing loss in each range of air-bone gap is essential for clinical

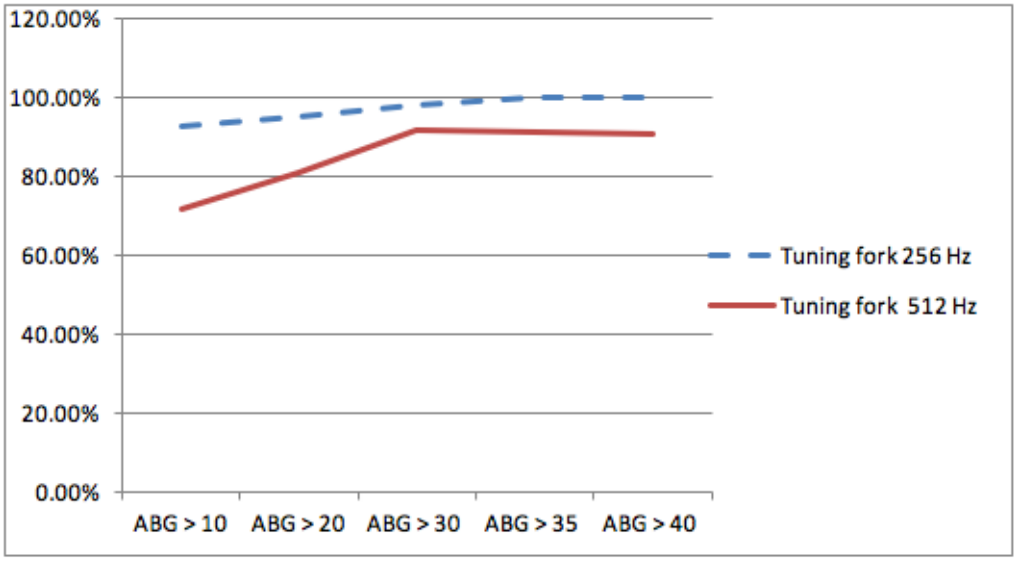


Fig 2. Sensitivity of air-bone gap detection of 256 Hz and 512 Hz Rinne tuning fork.

appliance. Crowley et al[8] studied between the Rinne tuning fork test 256, 512, 1024, and 2048 Hz in 153 ears and air-bone gap detection. He concluded that the air-bone gap equal to 20 dB or over was necessary for Rinne test to distinguish a conductive hearing loss. Burkey et al[1] studied the 512 Hz Rinne test of conductive hearing loss ears, and he concluded that Rinne's sensitivity for the detection of conduction loss equal to 20 dB or greater was 94.9%. Our study showed that 512 Hz tuning fork can detect 20 dB conductive problem or greater with the sensitivity of 81%. Moreover, the sensitivity reached to 92.3% if the conductive problem was 30 dB or greater. Therefore, we can conclude that the 512 Hz tuning fork is appropriate for pre-operative and post-operative evaluation in conductive hearing loss patients or unquestionable otosclerotic patients.

CONCLUSION

This study showed two important results. Firstly, the study showed that the 512 Hz Rinne test has more accuracy than 256 Hz Rinne test and can be a workhorse of screening conductive hearing loss in clinical practice. Secondly, the 512 Hz tuning fork can detect air-bone gap of 30 dB or greater which indicates surgical role with the sensitivity of 92.3%.

REFERENCES

1. Shea PF, Ge X, Shea JJ, Jr. Stapedectomy for far-advanced otosclerosis. Am J Otol 1999;20:425-9.
2. Burkey JM, Lippy WH, Schuring AG, Rizer FM. Clinical utility of the 512-Hz Rinne tuning fork test. Am J Otol 1998;19:59-62.
3. Chole RA, Cook GB. The Rinne test for conductive deafness. A critical reappraisal. Arch Otolaryngol Head Neck Surg 1988; 114:399-403.
4. Browning GG, Swan IR. Sensitivity and specificity of Rinne tuning fork test. BMJ 1988;297:1381-2.
5. Stankiewicz JA, Mowry HJ. Clinical accuracy of tuning fork tests. Laryngoscope 1979;89:1956-63.
6. MacKechnie CA, Greenberg JJ, Gerkin RC, McCall AA, Hirsch BE, Durrant JD, et al. Rinne revisited: steel versus aluminum tuning forks. Otolaryngol Head Neck Surg 2013;149:907-13
7. Golabek W, Stephens SD. Some tuning fork tests revisited. Clin Otolaryngol Allied Sci 1979;4:421-30.
8. Crowley H, Kaufman RS. The Rinne tuning fork test. Arch Otolaryngol 1966;84:406-8
9. Committee on Hearing and Equilibrium guidelines for the evaluation of results of treatment of conductive hearing loss. American Academy of Otolaryngology-Head and Neck Surgery Foundation, Inc. Otolaryngol Head Neck Surg 1995;113:186-7.

Hearing and Balance Survey in Thai Elders

Samut Chongvisal, M.D., FCOT.*, Walop Supavanich, M.D., FCOT., Kanthong Thongyai, M.D., FCOT., MA.*, Sarun Prakairungthong, M.D., FCOT.*, Suvajana Atipas, M.D., FCOT.*, Siriporn Limviriyakul, M.D., FCOT., M.Sc.***

**Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, **Department of Otorhinolaryngology, Banphaeo Hospital, Samutsakorn 74120, Thailand.*

ABSTRACT

Objective: To survey hearing and balance problems of a sampling group of Thai elders in the central region of Thailand.
Methods: A population study was people aged 60 and over who agreed to join the survey at the 14 elderly social associations. The questionnaires were used as a self-report for hearing and balance problems. After ears examination by ENT specialists, the participants underwent air-conduction audiometry for hearing screening.
Results: 828 participants 259 (31.3%) males and 569 (68.7%) females, age ranged from 60 to 97 (median=68, mean ± SD = 69.65 ± 6.89 years), had completed screening program for hearing and balance problems using both questionnaires and examinations. There was a statistically significant correlation between self-report hearing loss and severe or profound hearing loss in both ears (P <0.05). All age groups reported dizziness and vertigo but the elderly over 75 years reported more falls with a statistical significance (P=0.021). The elderly who did not report dizziness or vertigo had a1.1-1.6 times higher fall histories than those who reported (P <0.001). The participants who had hypertension and dyslipidemia reported more falls with statistical significances (P=0.025 and 0.036 respectively).
Conclusion: Our hearing and balance questionnaires may be used as a tool for screening at a primary care for the elderly > 70 years old especially in those with underlying diseases. Hearing and balance screening will provide early detection and primary intervention to minimize the negative impact in the elderly's daily life.

Keywords: Balance; hearing loss; falls; elderly; ear pathology; aging; screening (Siriraj Med J 2019;71: 131-142)

INTRODUCTION

Definition of older or elderly persons varies depending on definition among countries or organizations. Gorman M.[1] defined 'old' as 'the aging process is biological reality which has its own dynamic, largely beyond human control. However, it is also subject to the constructions by which each society makes sense of old age.' In the developed country, age of retirement is about 60 to 65 years old, nevertheless, the United Nations (UN) agreed age over 60 years to be the older population.[2] Age of retirement in Thailand depends on organization in which private employees average about 55 and government officers are about 60 years old.[3] Thai elderly are defined according to Elderly Act, Article 3 and the Department of Older Persons, which was 60 years old and over. In 2015, UN reported 12% of the global population aged 60 and over was expected to rise to be a quarter of the global population by 2050.[4] Asia contains about 56% of the global elderly population.[5] Help Age international global network reported elderly population in Thailand in 2012 was about 13.7% of population and was expected to reach 31.8% in 2050.[6]

Hearing loss in elderly was called 'presbycusis' (or age-related hearing loss), which literally means 'hearing in the older age'.[7] According to WHO classification, hearing impairment was classified into normal (0-25 dB), mild (26-40 dB), moderate (41-60 dB), severe (61-80dB) and profound (>80 dB) hearing loss.[8] Hearing disability in

Coresponding author: Siriporn Limviriyakul
E-mail: siriporn.lim@mahidol.ac.th
Received 21 September 2017 Revised 25 May 2018 Accepted 30 June 2018
ORCID ID: http://orcid.org/0000-0001-5872-8625
http://dx.doi.org/10.33192/Smj.2019.21

Thailand was defined as pure tone average at 0.5,1,2 KHz ≥ 40 dB on the better ear. Overall prevalence of presbycusis in the United states, was defined as hearing loss > 25 dB which was about 23% at age range between 65-75 years and 40% of people over 75 years old.[9] Otological center, Bangkok Unit (1991)[10] reported hearing loss 40.4% of Thai elderly at age more than 60 years. Bunnag C. (2002)[11] reported 52% hearing loss in Thai elders. Hearing loss can affect elderly daily life especially communication and socialization.

Dizziness and vertigo are the common complaints in the elderly. Falls are common causes of injuries among elderly and led to many serious consequences in older people. Elderly over 65 years of age have risk around one-third of older people living in the community of falling at least once per year.[12] One in ten of Thai elderly experienced falls accidentally both inside and outside their home.[5] Falls prevention by early detection and intervention will decrease serious injuries.

The Otological Center, Bangkok Unit (OCBU) was the first International Federation of Otorhino-laryngology Society and International Society of Audiology (IFOS/ISA) recognized center for prevention of hearing impairment and deafness. The mission of OCBU is 'better hearing and balance for all'. As mentioned above, elderly is a major healthcare problems, so screening of hearing and balance problems in terms of ENT specialist are primary community preventive intervention for hearing loss and falls. After retirement, the elderly in Thailand usually join the elderly social association nearby their houses. These gatherings create proper activities for their own sake, group and community as well as the health promotion to both update proper knowledge and activities such as group exercise at the associations, so we surveyed hearing and balance problems to find out their hearing and balance problems that could be treated and rehabilitated to prevent disability that might happen.

Objective: to survey hearing and balance problems of sampling group of elderly in the central region of Thailand.

Outcome measurement

Prevalence of ear pathology, hearing and balance problems in older population in the central region of Thailand.

Setting

Fourteen elderly social associations in Samutsakorn province as a sampling group for central region of Thailand, which is around 100 kilometers far from Siriraj Hospital.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board and Ethical Committee of Faculty of Medicine Siriraj Hospital (Si 463/2014).

Population study

The population study were people age 60 and over who agreed to join the survey at elderly social associations in Samutsakorn province. The entire sampling population was conducted at 14 elderly social associations: Tung Kralok, Kanchana, Rong Khae, Wat Rang Nin Pradit, Yok Krabud, Lak Sam, Am Phang, Don Sakae, Lak Song, Nong Song Hong, Prathumthong Pattana, Khong Tan, Kaset Pattana, and Jet Rew.

Exclusion criteria

Elderly who did not agree to join survey.

Methods

The representatives of the elderly social society announced the 'screening days for hearing and balance". The study was conducted during January to December, 2014. All volunteers had to finish questionnaires as a self-report for hearing and balance problems designed by OCBU for elderly survey in 2002.[11] There were 16 questions, which were occupation, underlying diseases, hearing, ear and balance problems. After ear examination by ENT specialists, they underwent air-conduction audiometry for hearing screening. The specialists filled the ear examination and audiological testing forms (Appendix).

Trained audiometricians performed air conduction hearing thresholds measuring both ears in a silent room which ambient noise less than 50 dBA[13] with a portable audiometer and headphone.

After ear, hearing and balance screening, participants with ear, hearing or balance problems received the referral forms to access the direct channel to the ENT clinics in the hospital affiliations.

Statistical analysis

We used IBM® SPSS Version 20 to analyze data. Descriptive statistic was used for characteristics of patients, pathological ear findings, hearing and balance problems. Self-report hearing, degree of hearing loss, hearing disability and falls were analyzed using Pearson Chi square test. Continuous data more than 2 groups was analyzed by Krusal-Wallis test. $P < 0.05$ was considered as statistical significance.

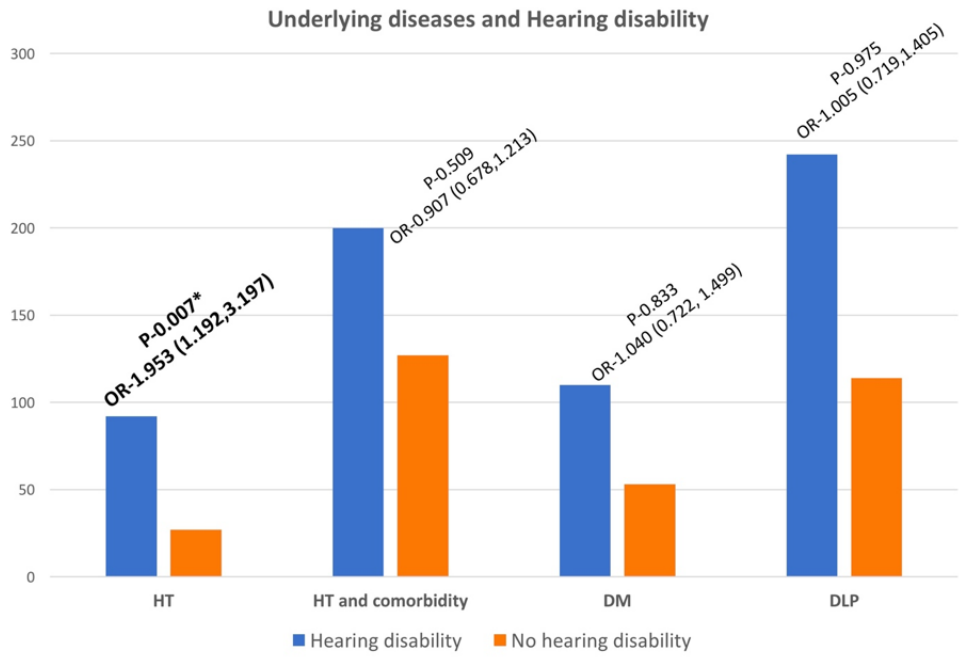


Fig 1. Underlying diseases and hearing disability

RESULTS

There were 1,560 out of 5,888 members (26.5%) of 14 elderly social associations who participated in service survey. The hearing and balance screening questions as well as examination were completed in 828 members. Demographic information showed 259 males (31.3%) and 569 females (68.7%); with age range 60 - 97 (median: 68, mean ± SD:69.65 ± 6.89) years old. Past occupation was divided into not working and working in noisy area at least 20 years. Elderly who did not work in noisy areas were 9 (1.08%) as office workers, 19 (2.29%) as house workers and 800 (96.9%) as others (e.g. freelances, businessmen, farmers) and those who worked in noisy areas were 9 noisy area workers (1%), and 1 soldier (0.1%). Two hundred and eighty (33.8 %) participants had underlying chronic diseases, which were 118 (14.3%) hypertension (HT), 314 (37.9%) HT and other comorbid diseases (diabetes mellitus; DM, dyslipidemia; DLP, chronic renal failure; CRF), 19 (2.3%) DM, 72 (8.7%) DLP, and 5 (0.6%) other diseases.

Questionnaires about ear and hearing problems demonstrated that elderly had three common complaints which were ear itching, hearing loss and tinnitus; 449 (54.2%), (278 (33.6%) and 158 (19.1%), respectively. They had reported balance problems, which were vertigo 299 (36.1%), dizziness 89 (10.7%) and fall 59 (7.1%), Table 1.

Pathological findings were 133 (16.1%) earwax impaction, 19 (2.3%) otitis externa, 18 (2.2%) ear drum perforation, 12 (1.4%) ear drum bulging, 8 (1%) foreign body in the ear, 4 (0.5%) auricular malformations (Table 1).

Elderly had 159 (19.2%) unilateral and 442 (53.4%) bilateral hearing loss. This study classified elderly people into three age ranges for hearing problems, which were 60-70, 71-80 and >80 years. PTA of better hearing were (Mean ± SD) 31.44 ±11.6, 36.52 ± 12.91 and 41.83 ± 11.37 dBHL, respectively, Table 2. There were statistically significant differences in each age group as age increased ($P < 0.001$). Hearing disability found 293 (58.5%), 208 (41.5%), and 652 (15.1%) in 60 -70, 71- 80 and > 80 years, respectively. There were statistical significances in PTA and hearing disability in each age group ($P < 0.001$) (Table 2). Hypertension (N-92, 34.1%) showed a statistical significance (P-0.007, OR-1.953 (1.192, 3.197)) in hearing disability group, but not in DM, DLP and HT with other comorbidities, Fig 1. Self-report hearing problems showed statistical significance to severe and profound hearing loss in both ears ($P < 0.05$) (Table 3). Elderly reported balance problems 131 (15.8%), 128 (15.5%), 91 (10.9%) and 74 (8.9%) in age group of 60-65, 66-70, 71-75 and > 75 years, respectively. There was no statistical significance in each group except falls in age group of > 75 years (P- 0.021) (Table 4). Fifty-nine fallers did not have history of vertigo nor dizziness with statistical significance ($P< 0.001$). Fallers had underlying diseases, which were 5 (8.5%) DM, 6 (27.3%) HT, 26 (61.9%) HT with comorbidity and 34 (57.6%) DLP (Table 5). There were statistical significances in underlying diseases between fallers and non-fallers in DM and DLP (P- 0.025 and 0.036).

TABLE 1. Questionnaires on hearing and balance problems.

Questions	Number (%) (N=828)
Family History of hearing loss	90 (10.9%)
Ever exposure to noise	453 (54.7%)
Duration of exposure (years)	
Often got cold	211 (25.4%)
Ear and hearing problems	
Ear itching	449 (54.2%)
Self-report Hearing loss	278 (33.6%)
Duration of hearing loss (years) (Mean±SD)	0.36 ± 2.86
Tinnitus	158 (19.1%)
Otalgia	92 (11.1%)
Otorrhea	35 (4.2%)
Ear trauma	20 (2.4%)
Ear surgery	1 (0.1%)
Balance problems	
Vertigo	299 (36.1%)
Dizziness	89 (10.7%)
Fall	59 (7.1%)
Ear pathology	
Earwax impaction	133 (16.1%)
Otitis externa	19 (2.3%)
Ear drum perforation	18 (2.2%)
Foreign body	8 (1%)
Ear drum bulging	12 (1.4%)
Auricle malformation	4 (0.5%)

DISCUSSION

This study recruited participants from age of 60 years who met the criteria. There were 828 out of 1560 (53.08%) elderly who participated the hearing and balance screening. From demographic data women attended screening test about 2 times than men (female: male-569:259), which meant that female elderly are more likely to be member of elderly social association and took care of their health more than men. Report of Thai elderly showed women had life expectancy longer than men[5,14] (mean age; female-79 and male-72 years), which were elderly sex ratio of 80 males for every 100 females.

The most common ear and hearing problems from questionnaires was ear itching (54.2%), which might be caused by local or systemic diseases. The most common pathological otologic finding was earwax impaction (133 out of 828; 16.1%) which may associate with hearing loss, tinnitus, itching, otalgia, discharge or dermatologic diseases.[15] Our report showed earwax impaction about 16.1%, which was the same as previous reports (19 to 65% of patients over 65 years old).[11,15]

This study classified participants into 3 groups (60-70, 70-80, > 80 years) to clarify hearing problems in each decade of life. It can be seen that PTA increased as

TABLE 2. Pure tone average and hearing disability in each age group.

Age (years)	Right (dBHL)		Left (dBHL)		Hearing disability (Better ear ≥ 40 dB)	OR
	Mean	SD	Mean	SD		
60 - 70	31.44	11.6	32.16	12.33	293 (58.5%)	1
71 - 80	36.52	12.91	37.03	14.15	208 (41.5%)	2.18 (1.57, 3.03) **P < 0.001***
> 80	41.83	11.37	45.04	16.45	52 (15.1%)	6 (2.53, 14.24) **P < 0.001***
P value	**< 0.001***		**< 0.001***			
Overall	33.2	12.33	34.1	13.71		
Min	0		10			
Max	85		100			
Median	32.25		32.5			
Mean (SD)	33.85 (12.45)		34.67 (13.74)			

Median ambient noise (dBA) 48.8 dB, *statistical significance P < 0.05, Kruskal-Wallis test, Chi- square test

TABLE 3. Self-report and degree of hearing loss.

Degree of hearing loss (dB)	Self-report hearing loss		Crude OR (95%CI)	P-value
	No	Yes		
Right ear				
Normal	153 (27.8%)	73 (26.3%)	1	
Mild (26-40)	276 (50.2%)	118 (42.4%)	0.896 (0.63,1.28)	0.542
Moderate (41-60)	111 (20.2%)	71 (25.5%)	1.341 (0.89,2.02)	0.159
Severe (61-80)	0 (0%)	3 (1.1%)	2.73 (1.14,6.5)	0.002*
Profound (>80)	0 (0%)	3 (1%)	0.32 (0.27,0.39)	0.013*
Left ear				
Normal	146 (26.5%)	66 (23.7%)	1	
Mild (26-40)	270 (49.1%)	123 (44.2%)	1.00 (0.70,1.45)	0.967
Moderate (41-50)	113 (20.5%)	65 (23.4%)	1.27 (0.84,1.94)	0.262
Severe (61-80)	20 (3.6%)	20 (7.2%)	2.21 (1.12,4.39)	0.021*
Profound (>80)	1 (0.2%)	4 (1.4%)	8.85 (0.97,80.7)	0.021*

*Statistical significance P<0 .05, Pearson Chi-square test, Fisher's exact for cell <5

TABLE 4. Age and balance problems.

Age (years)	Balance problems			
	Dizziness	Vertigo	Falls	Total (424 out of 828)
60 - 65	29 (22.1%) OR-1	88 (67.2%) OR-1	14 (10.7%) OR-1	131 (15.8%)
66-70	17 (13.3%) OR-1.856 (0.963, 3.578) P - 0.18	91 (71.1%) OR-0.832 (0.496,1.411) P – 0.496	20 (15.6%) OR-1.044 (0.993, 1,097) P – 0.083	128 (15.5%)
71-75	25 (27.5%) OR - 0.751 (0.405,1.393) P - 0.362	60 (65.9%) OR – 1.057 (0.611, 1.863) P – 0.487	6 (6.6%) OR-1.359 (0.512, 3.610) P- 0.573	91 (10.9%)
> 75	18 (24.3%) OR – 1.248 (0.647, 2.408) P – 0.509	37 (50%) OR – 1.262 (0.729, 2.184) P – 0.405	19 (25.7%) R – 0.438 (0.213, 0.898) **P-0.021***	74 (8.9%)

*Statistical significance P <0.05, Pearson Chi-square test

age increased with statistical significances in this study. Elderly in age group of 71-80 and > 80 years had hearing disabilities about 2 (OR - 2.18, P < 0.001) and 6 (OR 6, P <0.001) times more or less than those in age group of 60 -70 years, Table 2. The most common underlying disease in this study was HT, which was related to hearing disability about 1.9 times (OR- 1.192, 3.197) more or less than others. Common severity of hearing loss in study population was mild (~40-50%) and moderate (~25%) by the audiometric evaluation, Table 3. Nevertheless, old people reported their hearing loss when the severity of hearing loss became severe or profound more or less than those who had no hearing loss, Table 3 (Crude OR ~2-8, P<0.05 both ears). Elderly in Thailand seem likely to refuse hearing aids when they had hearing problems. They believed it was the stigmata of the handicapped and dependent elderly, so their guardians would bring them when they had difficulties in communication with them. Although Thai government have offered free hearing aids to whom register for the handicapped certification, some get it, but do not use it.

Balance disorders: dizziness and vertigo; were found to be common complaints in all age groups, but more common in elderly.[16] More than one third of adults over 65 years old had reported falls secondary to dizziness and vertigo each year according to the Center of Disease Control and Prevention.[17] Thailand has become an aging society, so Thai government has planned to extend the retired age from 60 to 65 years old. This study tried to clarify the age-related balance problems which would disturb their daily activities and work life. From the reason above, this study classified 5 years for each age group. Although there were no statistical differences in report of dizziness and vertigo in all age groups, the elderly age group of > 75 years old had reported falls more than other age groups with a statistical significance (P- 0.021). An international consensus statement defined a fall as "an unexpected event in which the participant

TABLE 5. History of vertigo or dizziness correlated with falls.

History	Number (%)		Crude OR	P-value
	Fallers	Non-fallers	(95% CI)	
Vertigo				
Yes	0 (0%)	299 (77.1%)		
No	59 (38.9%)	89 (22.9%)	1.663 (1.458,1.896)	**<0.001***
Dizziness				
Yes	0 (0%)	89 (22.9%)		
No	59 (100%)	299 (77.1%)	1.197 (1.143,1.254)	**<0.001***
Underlying diseases				
Diabetes	5 (8.5%)	158 (20.5%)	0.358 (0.141, 0.910)	**0.025***
Dyslipidemia	34 (57.6%)	335 (43.6%)	1.762 (1.031,0.010)	**0.036***
Hypertension	6 (27.3%)	113 (30%)	0.876 (0.334,2.297)	0.788
Hypertension with co-morbidity (DM, DLP, CRF)	26 (61.9%)	288 (52.2%)	1.49 (0.782,2.838)	0.223

Abbreviations: DM = diabetes mellitus, DLP = dyslipidemia, CRF = chronic renal failure
*Statistical significance P <0.05, Pearson Chi-square test

comes to rest on the ground, floor or lower level".[18] Those who reported dizziness and vertigo in this study were not the fallers comparing to those who did not report with a statistical significance (OR – 1.2-1.6, P < 0.001). This meant participants who did not have symptoms of dizziness nor vertigo, might not concern about falling problems. Prior studies found a marked reduction of hair cell counts within the cristae of semicircular canals as early as 50-60 years of age and a significant reduction of vestibular ganglion cells as early as 60 years of age.[17] Thus, the deterioration of peripheral vestibular functions and central compensation can no longer be compaensated at some critical points.[19] The rate of falls in the well-defined general older population is about 10.4-18.7% in Thailand.[20] Prasansuk S, et al., and Downton J.,[16,21] reported percentage of falling in the elderly each year about 28-35%, 35% and 32-42% in 65+, 70+ and 75+ years. Our study elderly reported falls 10.7%, 15.6%, 6.6% and 25.7% at age of 60-65, 66-70, 71-75 and >75 years, respectively. The percentage of our study was about half of the previous studies, because of the less number of study population. Nevertheless, the peak incidence of falls in our study was over 75 years (25.7%), which was nearly the same as previous reports.[21]

Dizziness, vertigo and falls may be secondary to comorbid conditions other than aging. Comorbid conditions (Intrinsic factors) that we considered in this study were underlying diseases, which showed statistical significances in DM and DLP between fallers and non-fallers. Nicholson M, et al., and Walley M, et al.,[22,23] showed the deficits of vestibulo-ocular reflex and proprioceptive loss in diabetic patients which caused the high percentage of fall in these patients. Dyslipidemia could lead to atherosclerosis. Prior study reported the small vascular diseases related to brain changes impacted on gait and speed in healthy elderly.[16,24] The extrinsic factors was environmental area of their dwellings that was not a part of our study. Many interventions have

been proposed to prevent falls which are exercise which was found to be a benefit of performance of head and neck exercise as Prasansuk S, et al., reported[16], medication (both supplement and treatment), surgery for correction (e.g. cataract extraction), fluid/nutrition supplement, psychological support, environment/assistive technology and knowledge/ educational interventions.[18] However, there are many factors that cause balance problems individually. Old Thai people usually spend their retired life at home with their families, who take care of them. Thus, family may be the one who notice their problems as well as providing environmental area for primary prevention of falls.

CONCLUSION

Hearing and balance problems unusually cause negative impact effects in elderly daily's life. Hearing rehabilitations will resolve their communications, social and psychological problems. Prevention of falls include individual and environmental adjustments, and decreasing the complications of chronic diseases in older people. Our hearing and balance questionnaires may be used as a tool for screening at a primary care for an elderly > 70 year especially in those with underlying diseases. The primary care and elderly social associations should be alerted by giving them education about hearing and balance problems. This study supports the information to the health policy decision for elderly in central region, and all of Thailand which is growing to become an aging society in the near future.

ACKNOWLEDGMENTS

This study was conducted as part of strengthening networking of the Otological Center: Bangkok Unit. We thanks all of our residents who graduated in, 2014.

Conflicts of interest: The authors have no conflicts of interest and did not receive any funding from profited funders.

REFERENCES

1. Gorman M. The ageing and development report: poverty, independence and the world's older people. In: Randel J, editor. Earthscan Publication Ltd.; 1999. p. 3-21.
2. World Health Organization (WHO). Definition of an older or elderly person 2016. Available from: http://www.who.int/healthinfo/survey/ageingdefnolder/en/.
3. Kanchanachitra M, Jarassit S, Kanachanachitra C. To Retire or not to Retire, Whose choice Is It? The 8th International Conference of Population and Social Research Royal River Hotel: Mahidol University; 2012. p. 341-57.
4. United Nations. Department of Economic and Social Affairs, Population Division. World Population Prospects: The 2015 Revision, Volume II: Demographic Profiles (ST/ESA/SER. A/380)2015.
5. Prasartkul P. Situation of the Thai Elderly 2015. Nakhon pathom: Institute for Population and Social Research, Mahidol University; 2015.
6. Knodel, John, Chayavan N. The Changing Well-being of Thai Elderly: An Update from the 2011 Survey of Older Person in Thailand. HelpAge International;2013.
7. Roth TN. Aging of the auditory system. Handb Clin Neurol. 2015;129:357-73.
8. World Health Organization (WHO). Grading of hearing impairment 2017. Available from: http://www.who.int/pbd/deafness/hearing_impairment_grades/en/.
9. Seidman MD, Ahmad N, Bai U. Molecular mechanisms of age-related hearing loss. Ageing Res Rev 2002;1:331-43.
10. Prasansuk S. Report on prevalence of hearing disability and ear diseases in Thailand Bangkok Unit: Otological Center, Department of Otorhinolaryngology, Mahidol University; 1991.
11. Bunnag C, Prasansuk S, Nakorn AN, Jareoncharsri P, Atipas S, Angsuwarangsee T, et al. Ear diseases and hearing in the Thai elderly population. part II. A one year follow-up study. J Med Assoc Thai 2002;85:532-9.
12. Gillespie LD, Robertson MC, Gillespie WJ, Sherrington C, Gates S, Clemson LM, et al. Interventions for preventing falls in older people living in the community. Cochrane Database Syst Rev 2012:Cd007146.
13. Frank T. ANSI update: maximum permissible ambient noise levels for audiometric test rooms. Am J Audiol 2000;9:3-8.
14. WHO. THAILAND http://www.who.int/countries/tha/en/ [25 May 2018].
15. Roland PS, Smith TL, Schwartz SR, Rosenfeld RM, Ballachanda B, Earll JM, et al. Clinical practice guideline: cerumen impaction. Otolaryngol Head Neck Surg 2008;139:S1-S21.
16. Prasansuk S, Siriyananda C, Nakorn AN, Atipas S, Chongvisal S. Balance disorders in the elderly and the benefit of balance exercise. J Med Assoc Thai 2004;87:1225-33.
17. Zalewski CK. Aging of the Human Vestibular System. Semin Hear 2015;36:175-96.
18. Hopewell S, Adedire O, Copsey BJ, Sherrington C, Clemson LM, Close JCT, et al. Multifactorial and multiple component interventions for preventing falls in older people living in the community. Cochrane Database Syst Rev 2016:CD012221.
19. Baloh RW, Jacobson KM, Socotch TM. The effect of aging on visual-vestibuloocular responses. Exp Brain Res 1993;95:509-16.
20. Romli MH, Tan MP, Mackenzie L, Lovarini M, Suttanon P, Clemson L. Falls amongst older people in Southeast Asia: a scoping review. Public Health 2017;145:96-112.
21. Downton J. Falls. In: Luxon L, editor. Textbook od Audiological Medicine; Clincal aspect of hearing and balance. United Kingdom: Martin Dunitz; 2003. p. 831-40.
22. Nicholson M, King J, Smith PF, Darlington CL. Vestibulo-ocular, optokinetic and postural function in diabetes mellitus. Neuroreport 2002;13:153-7.
23. Walley M, Anderson E, Pippen MW, Maitland G. Dizziness and Loss of Balance in Individuals With Diabetes: Relative Contribution of Vestibular Versus Somatosensory Dysfunction. Clin Diabetes 2014;32:76-7.
24. Pinter D, Ritchie SJ, Doubal F, Gattringer T, Morris Z, Bastin ME, et al. Impact of small vessel disease in the brain on gait and balance. Sci Rep 2017;7:41637.

Appendix

Hearing and Balance Questionnaires

Otological Center: Bangkok Unit, Faculty of Medicine Siriraj Hospital, Mahidol University

Record NO…….. **Sex**……

Date……

1. Occupations: work at least 20 years
   - ☐ Office worker…….years
   - ☐ Noisy area worker e.g. Factory ……years
   - ☐ Policeman……..yeas
   - ☐ Solider……..years
   - ☐ House worker……..years
   - ☐ Others……
2. Do you have hearing loss?
   - 2.1 Right ☐ No ☐ Yes……years
   - 2.2 Left ☐ No ☐ Yes……. years
   - 2.3 Hearing loss in family history ☐ No ☐ Yes
3. Have you ever expose to loud noise? ☐ No ☐ Yes
4. Noise in ears (tinnitus) Right ☐ No ☐ Yes Left ☐ No ☐ Yes
5. Otalgia Right ☐ No ☐ Yes Left ☐ No ☐ Yes
6. Ear discharge Right ☐ No ☐ Yes Left ☐ No ☐ Yes
7. Previous ear surgery Right ☐ No ☐ Yes Left ☐ No ☐ Yes
8. Ear itching Right ☐ No ☐ Yes Left ☐ No ☐ Yes
9. Ear trauma e.g. severe head trauma, beating or slapping at ears Right ☐ No ☐ Yes Left ☐ No ☐ Yes
10. Ototoxic drugs ☐ No ☐ Yes …….
11. Often get a cold ☐ No ☐ Yes
12. Have you ever had spinning sensation? (Vertigo) ☐ No ☐ Yes
13. Have you ever had dizziness? ☐ No ☐ Yes
14. Have you ever fallen? (Faller/Nonfaller) ☐ No ☐ Yes
15. How often you feel dizziness or spinning sensation? ☐ No ☐ Sometime ☐ Off and on ☐ Usually
16. Do you have these following diseases?
    1. Diabetes ☐ No ☐ Yes
    2. High blood pressure (Hypertension ☐ No ☐ Yes
    3. High blood lipid profile (Dyslipidemia) ☐ No ☐ Yes
    4. Anemia ☐ No ☐ Yes
    5. Kidney diseases ☐ No ☐ Yes

Ear Examination form

Record no…….. Date……..

Examiner……

Otoscopic examination

	Right ear		**Left ear**	
Auricle Malformation	☐ No	☐ Yes	☐ No	☐ Yes
Otitis externa	☐ No	☐ Yes	☐ No	☐ Yes
Ear Wax	☐ No	☐ Yes	☐ No	☐ Yes
Foreign body	☐ No	☐ Yes	☐ No	☐ Yes
Otorrhea	☐ No	☐ Yes	☐ No	☐ Yes
Fungi	☐ No	☐ Yes	☐ No	☐ Yes
Eardrum perforation	☐ No	☐ Yes	☐ No	☐ Yes
Red and Bulging	☐ No	☐ Yes	☐ No	☐ Yes
Retraction>moderate	☐ No	☐ Yes	☐ No	☐ Yes
Effusion	☐ No	☐ Yes	☐ No	☐ Yes
Other: specify……	☐		☐	
Normal Findings	☐		☐	

Audiological testing

Record no……. Date…….

Audiometric no_____ Ambient_____dB _____

Air conduction threshold

Right ear	AC/(BC)	Left ear
_____	500 Hz	_____
_____	1000 Hz	_____
_____	2000 Hz	_____
_____	400 0Hz	_____

Tester _____

Tympanometry

Right ear		Left ear
☐	Compliance	☐
☐	Negative middle ear pressure	☐
☐	Flat	☐
☐	Stapedial reflex Positive	☐

Remark _____

Treatment:

1. No treatment ☐
2. Action needed ☐
   - 2.1 Medication ☐
   - 2.2 Suction ☐
   - 2.3 Hearing aids ☐
   - 2.4 Removal earwax/foreign body/_____ ☐
   - 2.5 Surgery Referral ☐ Urgent ☐ Non-Urgent
   - 2.6 Other c Specify_____

Follow up:_____

Refer to:_____

Referred form

Otological Center: Bangkok Unit, Faculty of Medicine Siriraj Hospital, Mahidol University

And Ear Disease Foundation (Thailand)

Date _____

Name of patient _____

Result	Right		Left
1. External ear	_____		_____
2. Middle ear	_____		_____
3. Ear drum	_____		_____
4. Hearing		Hz	
	_____	500	_____
	_____	1000	_____
	_____	2000	_____
	_____	4000	_____

Diagnosis_____

Treatment provided

1._____

2._____

Referred for

_____

Doctor_____

Scintigraphic Detection of Splenosis and Accessory Spleen by Using Tc-99m Labelled Denatured Red Blood Cells

Apichaya Claimon, M.D. *, Napaporn Tojinda, M.Sc. *, Ninmanee Taweewatanasopon, B.Sc. *, Pornphit Boonkhon, B.Sc.*, Boontham Amornkitticharoen, M.Sc. *, Sirilak Wiriyaakradecha, M.Sc., Shanigarn Thiravit, M.D.***

**Department of Radiology, Faculty of Medicine Siriraj Hospital, **Department of Clinical Microscopy, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: To evaluate the usefulness of Tc-99m-labelled denatured red blood cell scintigraphy (DRBCS) in the detection of splenosis and accessory spleen.

Methods: This retrospective study reviewed 7 patients who were suspected of splenosis and accessory spleen during 2007-2014. Six patients had undergone total splenectomy before the DRBCS study. Multi-planar, SPECT and SPECT/CT images of DRBCS were reviewed by visual analysis. Image findings were correlated with other imaging modalities and clinical presentations at follow-up.

Results: DRBCSs were positive in 6 patients; 5 splenosis and 1 accessory spleen. A single lesion was detected in each patient. Locations were in splenic bed (5 patients) and in pelvic cavity (1 patient). Mean lesion size was 3.7 + 2.4 cm. SPECT or SPECT/CT imaging could eliminate false-negative results in 2 patients. Lesion uptake intensity in 4 patients was higher than in the liver. Two patients had subsequently undergone resection of the lesions and pathological examinations confirmed the diagnosis of splenosis and accessory spleen. Sensitivity of DRBCS was comparable with other imaging modalities. However, DRBCS was more specific for the splenic tissue.

Conclusion: DRBCS is useful for the detection of splenosis and the accessory spleen. Detection sensitivity of DRBCS is better than ultrasonography(US). DRBCS is more specific to the splenic tissue, compared with US and CT. Furthermore, SPECT/CT imaging increases sensitivity, lesion localization and characterization. Clinical impact of DRBCS is apparent including pre-surgical localization, elimination of additional follow-up imaging and invasive procedure.

Keywords: Accessory spleen; denatured red blood cell; immune thrombocytopenia; splenosis; spleen scintigraphy (Siriraj Med J 2019;71: 143-149)

INTRODUCTION

The spleen is the largest lymphoid organ which plays an important role in regulating immune cells and mediators, removing circulating microorganisms through phagocytosis and filtering damaged cells and foreign material.[1] Splenosis is the acquired condition from autotransplantation of splenic tissue that usually occurs after splenic trauma or splenectomy. In contrast, the accessory spleen is a congenital anomaly of the spleen.[2] These conditions can present a diagnostic challenge and can be particularly difficult to distinguish by using conventional imaging modalities such as ultrasonography (US), computed

Corresponding author: Shanigarn Thiravit
E-mail: thiravit.mahidol@gmail.com
Received 19 October 2017 Revised 6 February 2018 Accepted 9 February 2018
ORCID ID: http://orcid.org/ 0000-0002-4347-8683
http://dx.doi.org/10.33192/Smj.2019.22

tomography (CT) and magnetic resonance imaging (MRI). Improved characterization may be obtained by using superparamagnetic iron oxide as a contrast-enhancing agent in MRI.[3] In contrast, spleen-specific imaging using Tc-99m labeled denatured red blood cells scintigraphy (DRBCS) is a more simple technique that can definitely distinguish splenic tissue from other benign/malignant non-splenic origin according to intrinsic physiologic uptake of DRBC by functioning splenic tissue.

DRBCS was introduced as a spleen-specific tracer since 1967 and has been shown to be of value in the detection of both native spleen and accessory spleen.[4] Clinical indications for DRBCS include congenital/ functional asplenia, accessory spleen, space-occupying lesion in the spleen, refractory immune thrombocytopenia (ITP) after splenectomy, and thoracic/ abdominopelvic mass suspected for the accessory/ectopic spleen.

The objective of this study was to evaluate the usefulness of DRBCS in the detection of splenosis and accessory spleen by 1) reporting the DRBCS findings and compare with the other imaging modalities and 2) investigating the clinical impact of DRBCS in the patients with suspected splenosis and accessory spleen.

MATERIALS AND METHODS

This retrospective study was approved by The Institutional Review Board (Si 085/2015). We searched our internal clinical database for the time period between 2007 and 2014 and identified 8 patients who had undergone DRBCS from our Nuclear Medicine Unit. The inclusion criteria were the patients who had undergone DRBCS for the evaluation of splenosis or accessory spleen. We excluded 1 patient who had undergone DRBCS for the evaluation of the space-occupying lesion in the native spleen. Our final study cohort comprised of 7 patients.

DRBCS protocol

Preparation of Tc-99m DRBC

1. Reconstitute stannous kit with normal saline and intravenously inject to a patient.
2. After 20-30 min, draw blood of 10 ml into a heparinized syringe.
3. Using a lead glass shielding, add 20 mCi of $Na^{99m}TcO_4$ to the heparinized whole blood.
4. After 5 min, incubate the tube in a constant-temperature water bath at 50 ± 1°C for 20 minutes with periodical shaking.
5. Centrifuge, wash and reconstitute with NSS.
6. Check the percentage of DRBC and re-inject to the patient. Denaturation of RBC was technically successful in all patients. In every patient, no complications occurred after the re-injection. The preparation was tested in one of our research team as a healthy volunteer and the images shown a normal distribution (Fig 1).

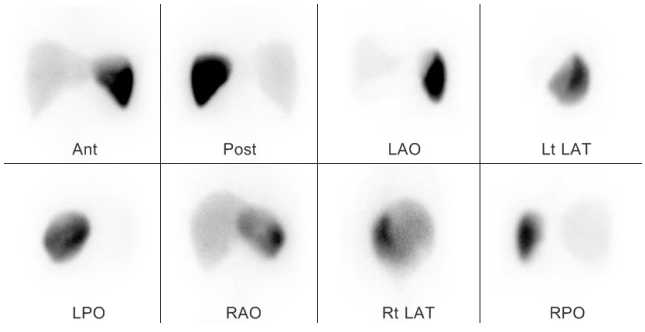


Fig 1. DRBCS of a normal volunteer. The uptake in spleen is homogeneously intense. Liver uptake is faint. No marrow uptake is observed.
Abbreviations: Ant: Anterior; Post: Posterior; LAO: Left anterior oblique; Lt LAT: Left lateral; LPO: Left posterior oblique: RAO: Right anterior oblique: Rt LAT: Right lateral; RPO: Right posterior oblique.

DRBCS imaging protocol

Scintigraphy began 30 minutes after the injection of DRBC, using a gamma camera equipped with a high-resolution, low-energy collimator. Static planar images of the abdomen were obtained in anterior, posterior, both anterior and posterior oblique (LAO, LPO, RAO, RPO), and both lateral views in all patients with the same imaging acquisition protocol. After planar imaging, single photon emission computed tomography (SPECT) and SPECT/CT imaging was obtained over the abdomen in the selected patients. One patient (patient No.6) was obtained planar imaging only. Two and 4 patients were performed SPECT and SPECT/CT imaging sequentially after the planar scans.

Image analysis

DRBCS images were retrospectively analyzed on a printed film or electronic archiving system by a nuclear medicine physician who was unaware of other clinical data. The following findings were determined by visual analysis: (1) the presence or absence of abnormal tracer uptake suggesting splenosis or accessory spleen; (2) the intensity of uptake (compared with the liver uptake); and (3) the location of the abnormal uptake lesion(s). Lesion size was measured on the SPECT/CT images. In patients without SPECT/CT imaging, lesion size was evaluated by other imaging modalities. Other imaging data (the US in 4 patients and contrast-enhanced CT in 1 patient) were reviewed by a radiologist. The DRBCS

findings were compared with other imaging modalities and pathological findings as a gold standard.

Statistical analysis

The analyses were performed by using statistical software (SPSS version 18.0; SPSS Inc., Chicago, IL, USA). All continuous (quantitative) data, including the patient's age and lesion size, were summarized.

RESULTS

Four patients were male and 3 were female. The median age was 31 years (range 5 – 70 years). Three patients were children; age ranged from 5-8 years. Six patients had undergone total splenectomy prior to the DRBCS; 1 of these had undergone 2 sessions of splenectomy according to refractory ITP (patient No. 4). None of the study population had a history of abdominal trauma. The clinical indications for performing DRBCS were to localize residual splenic tissue causing refractory ITP post-splenectomy (5 patients), to differentiate between splenosis and metastatic cancer (1 patient) and to evaluate pre-operatively for the biopsy confirmed accessory spleen (1 patient). Time intervals between splenectomy and DRBCS were within 1 year in 3 patients, 3 years in 1 patient and 33 years in 2 patients.

Of the 5 patients with refractory ITP, 1 patient had secondary ITP due to HCV infection (patient No. 3), 1 patient had acute ITP with a subdural hematoma and had undergone emergency splenectomy (patient No. 1) and the rest were chronic ITP patients. All 5 patients were classified as 'no response' to treatment based on the criteria of the International Working Group (a platelet count <30 x 10^9/L or less than 2-fold increase in platelet count from baseline or the presence of bleeding).[5] Patient characteristics, clinical information, image acquisitions/ findings, and pathological results, are listed in Table 1.

DRBCS image quality was excellent in 5 scans. A patient (No. 3) showed an abnormal homogeneous increased tracer uptake in the liver and bone marrow. Specifically, the uptake in the residual spleen was less than in the liver. In this case, planar imaging gave a false negative result, but SPECT/CT imaging could demonstrate the residual spleen (Fig 2). Another patient (No. 2) had uptake in splenosis less than in the liver, but no marrow uptake.

The scan was negative in a patient with refractory ITP after an emergency splenectomy for acute ITP with subdural hematoma (Fig 3). The rest 6 scans were positive for residual spleen or accessory spleen. A single positive lesion was detected in all patients. Median lesion size was 2.8 cm (range 1.4-7.4 cm). Location of the all residual spleens was in the left upper quadrant of the abdomen (Fig 4). The accessory spleen was in the pelvic cavity along with the lobulated-shaped native spleen in the splenic bed. In this patient, the accessory spleen was subsequently removed and pathologically confirmed splenic tissue (Fig 5).

SPECT and SPECT/CT imaging were subsequently obtained after the routine multi-planar views in 2 and 4 patients, respectively. SPECT/CT could identify the residual spleen in a patient with negative multi-planar scan (patient No. 3). This patient had crescent-shaped residual spleen in the location that almost attached to the left liver lobe, which was misinterpreted as the elongated left liver lobes in the planar images. However, the lesion was clearly demonstrated in the 3-D imaging by SPECT/CT scanning.

Two out of 7 patients had undergone resection of the residual/accessory spleen following the positive DRBCS (patient No. 4 and 7). The pathological results confirmed splenic tissue in both cases. However, there was no rising of the platelet level after the surgery in patient No. 4 and she needed long-term treatment with corticosteroid. Patient No. 7 required resection of the accessory pelvic spleen due to compression effect to the bladder. His urinary incontinence subsided after the surgery. DRBCS prevented futile investigation/ surgery in patient No. 5, who was confirmed to have a residual spleen, not a focus of metastatic colon cancer.

Removal of the residual spleen was not undertaken in 3 patients with refractory ITP despite the positive DRBCS results (patient No. 2, 3 and 6). One of them (patient No. 6) had a spontaneous rising of the platelets to the normal range without any specific treatment. Patient No. 3 responded to corticosteroid and Eltrombopag. Nonetheless, patient No. 2 as well as patient No. 1 (whose DRBCS result was negative), had no response to the medical treatment.

Comparison of DRBCS and other imaging modalities results was summarized in Table 2. The detection sensitivity of DRBCS is better than US. DRBCS is more specific to spleen tissue, compared with US and CT.

DISCUSSION

Splenosis is the acquired benign condition. It is estimated to occur in 26%-67% of the patients after traumatic or iatrogenic splenic parenchymal rupture.[2] The mechanism is the autotransplantation of the spillage cells to the nearby body cavity or hematogenously spreading of the cells and implanting to the remote sites.[6] The most common location of splenosis is in the left upper quadrant

TABLE 1. Baseline patient characteristics and main results.

No.	Sex/ Age (years)	Splenectomized	Indication	Planar	SPECT or SPECT/CT[1]	Intensity[2]	Lesion number	Lesion size (cm)[3]	Location	Pathology
1	M/31	+	Refractory ITP	-	-	0	0	NA	NA	NA
2	F/5	+	Refractory ITP	+	+	1	1	2	Splenic bed	NA
3	F/54	+	Refractory ITP	-	+	1	1	4.7	Splenic bed	NA
4	F/44	+	Refractory ITP	+	+	3	1	1.4	Splenic bed	Confirmed
5	F/70	+	DDx. Metastasis vs. splenosis	+	+	3	1	2.8	Splenic bed	NA
6	M/8	+	Refractory ITP	+	NA	3	1	NA	Splenic bed	NA
7	M/7	-	Pelvic mass	+	+	3	1	7.4	Pelvic	Confirmed

Abbreviations: M: Male; F: Female; ITP: Immune thrombocytopenia; DDx: Differential diagnosis; SPECT: Single photon emission computed tomography; CT: Computed tomography; NA: Not available.

[1] SPECT only in patient No. 1 and 2; SPECT/CT in patient no. 3-5 and 7

[2] Intensity of uptake, compared to the liver: 0 = no uptake, 1 = less than the liver, 2 = equal to the liver, 3 = greater than the liver

[3] Maximal diameter; measured on SPECT/CT images. Patient No. 2 was measured on ultrasonography (performed SPECT without CT)

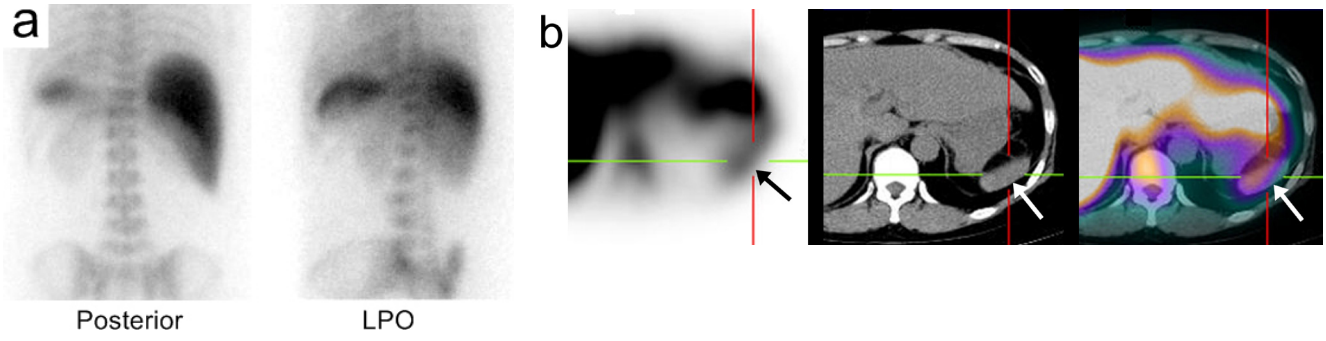


Fig 2. Abnormal distribution and false-negative planar scan. A 54-year old female with HCV-related refractory ITP status post splenectomy 33 years ago (Patient No. 3). DRBCS planar images (a) show abnormally intense uptake in the liver and diffused marrow uptake. Planar images are negative. DRBCS SPECT/CT (b) images reveal a faint uptake corresponding with a curvilinear soft tissue locates beneath the posterior aspect of the left hemidiaphragm, measured about 1.5 x 4.7 x 1.6 cm. that suggestive of splenosis.
Abbreviation: LPO: Left posterior oblique.

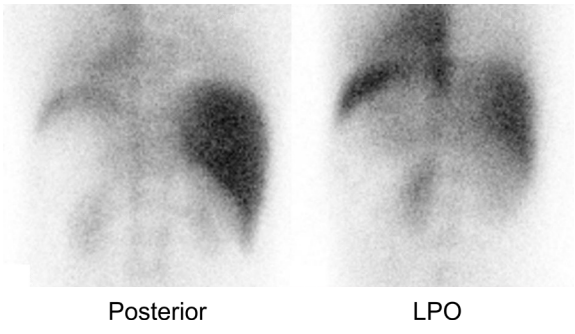


Fig 3. Negative study. A 31-year old man with acute ITP with subdural hematoma status post emergency splenectomy (Patient No. 1). DRBCS was sent due to no platelet response after splenectomy. DRBCS images show no evidence of residual splenic tissue. He has been treated with high-dose prednisolone, but no platelet response.
Abbreviation: LPO: Left posterior oblique.

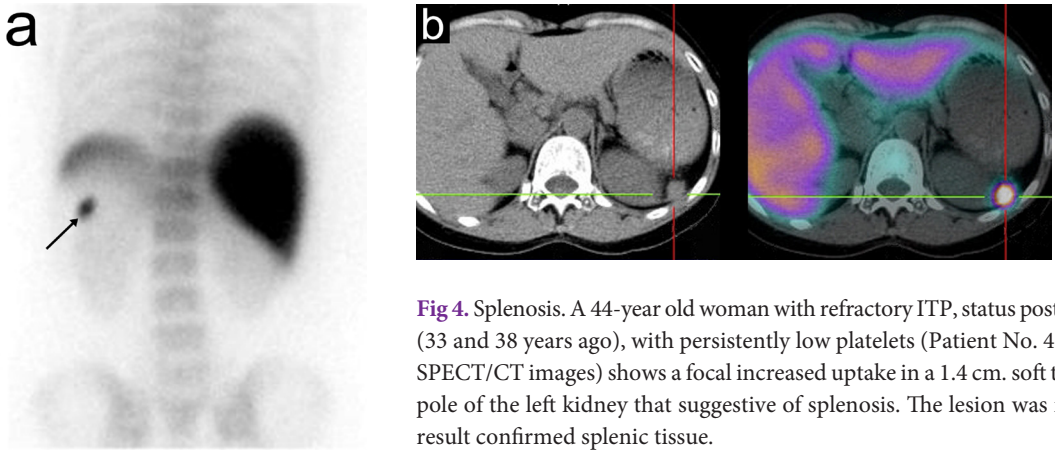


Fig 4. Splenosis. A 44-year old woman with refractory ITP, status post 2 sessions of splenectomy (33 and 38 years ago), with persistently low platelets (Patient No. 4). DRBCS (a Planar and b SPECT/CT images) shows a focal increased uptake in a 1.4 cm. soft tissue mass near the upper pole of the left kidney that suggestive of splenosis. The lesion was resected and pathological result confirmed splenic tissue.

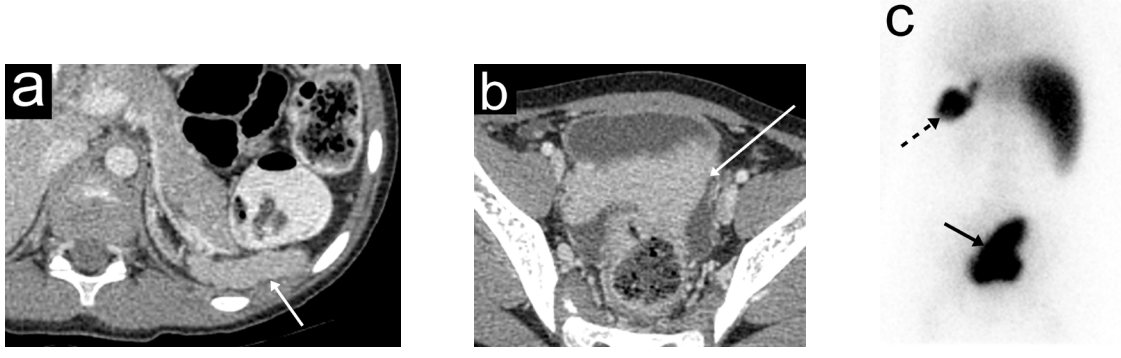


Fig 5. Accessory spleen in the pelvic cavity. A 7-year old boy presented with urinary incontinence (Patient No. 7). The CT shows the small spleen (a) and a lobulated homogeneous enhancing soft tissue mass in the pelvic cavity (b). The differential diagnosis includes lymphoma, germ cell tumor and other mesenchymal tumors. The pelvic mass was biopsied and revealed splenic tissue. DRBCS (c) was sent for pre-operative evaluation. There is increased uptake in the pelvic mass, size about 7.4 x 2.6 cm. (arrow) as well as in the lobulated shaped spleen in the left upper quadrant (dot arrow). He had undergone removal of the accessory spleen and the pathological result confirmed the diagnosis.

TABLE 2. Comparison of DRBCS and other imaging modalities.

No.	DRBCS	Other imaging modalities	
4	Positive	US	Negative
5	Positive	US	Negative
7	Positive	US	Positive with concordant result
8	Positive	US	Positive with inconclusive nature of the lesion
6	Positive	CECT	Positive with inconclusive nature of the lesion

Abbreviations: US: ultrasonography; CECT: contrast enhanced computed tomography.

of the abdomen. Usually, it is asymptomatic, incidentally detected and requires no treatment, because it may have some immunologic value and microorganism filtering function.[3] However, in patients with ITP, splenosis leads to failure of the platelet response after splenectomy, owing to the presence of functioning splenic tissue. Detection and removal of the residual functioning splenic tissue are essential for the treatment in this patient group.

DRBCS is the recommended imaging modality for the detection of splenosis.[6] The uptake mechanism is spleen-specific, based on the sequestration of the DRBC by the pulp region of splenic tissue[7]. The sensitivity of DRBCS is higher than that of US, CT[6,8], Tc-99m labeled sulfur colloid scintigraphy[6,9,8], and laparoscopy.[10] Moreover, DRBCS is non-invasive, technically simple and able to obtain whole-body imaging within a single injection. Despite the superiority of DRBCS, it is not frequently reported and studied. In our institution, only 8 patients and 1 healthy volunteer performed DRBCS since we established this test in 2007.

DRBCSs are able to detect both splenosis and accessory spleen. In the literature, splenosis is usually observed in multiple[11], but we detected a solitary lesion in every case. Our findings confirmed that the most common location of splenosis is in the left upper quadrant. We found a patient with pelvic accessory spleen, which is a very rare condition. In literature, accessory spleens were found in splenic hilum in 75% of cases, and less in the gastrosplenic ligament, splenorenal ligament and pancreatic tail. The development of an accessory spleen presumably involves multifocal embryonic origins. In most reports, spleens detected in pelvic regions are from a different origin, the wandering spleens, which are the abnormal position of the spleen caused by splenic ligament laxity.[12]

The sensitivity of DRBCS was confirmed to be higher than other modalities such as US, CT, and Tc-99m sulfur colloid scan, especially for the small lesion and in the unusual location.[9,8,13,4] The differential diagnosis for intraabdominal soft tissue nodule seen on CT images including peritoneal metastasis, lymphadenopathy, lymphoma, pancreatic mass, endometriosis, uterine mass, teratoma and splenosis.[6,14] DRBCS was more specific to splenic tissue than CT. In our patients, DRBCS ruled out the metastatic nodule and teratoma, so futile biopsy or surgery could be avoided.

In the current study, DRBCSs were positive in 6 (85.7%) patients. Of these, 1 was negative on the planar scan but was clearly positive on the SPECT/CT scan. The planar scan was negative due to the superimposition of splenic tissue by the liver tail. The problem was eliminated by SPECT/CT which provided attenuation correction, three-dimensional view, and higher contrast resolution. Moreover, lesion characterization and anatomical localization using CT technique are important for the patients who planned for pre-surgery. The superior sensitivity of SPECT or SPECT/CT imaging was in concordance with the previous study.[15] However, another study showed no contribution of SPECT to the planar imaging.[16]

In this study, only 1 out of 4 patients with refractory ITP and positive DRBCS results had undergone removal of the splenic tissue. However, that patient still suffered from persistently low platelets after the surgery. On the other hand, another patient had spontaneously increased platelet level to the normal range without any surgical or medical treatment. Because DRBCS is spleen-specific, the false-positive result is not possible and have never been reported in the literatures.[16] Post-surgical failure could be described by several factors such as rapid regeneration of the residual splenic tissue, the presence of IgG against platelet and other sites of platelet sequestration.[17]

There were limitations to this study. Firstly, this was retrospective which could have many uncontrolled factors. Secondly, it contained a small sample size. Thirdly, we

used different gamma cameras with different scanning parameters. And lastly, pathological confirmation was not done in every case.

CONCLUSION

DRBCS is useful for the detection of splenosis and the accessory spleen. The detection sensitivity of DRBCS is better than US. DRBCS is more specific to spleen tissue, compared with US and CT. Furthermore, SPECT/CT imaging increases sensitivity, localization and lesion characterization. Clinical impact of DRBCS is apparent including pre-surgical localization, elimination of additional follow-up imaging and invasive procedure.

ACKNOWLEDGMENTS

The research authors received a Chalermprakiat grant from the Faculty of Medicine, Siriraj Hospital, Mahidol University.

Conflict of Interest: The research authors (Apichaya Claimon and Shanigarn Thiravit) received a Chalermprakiat grant from the Faculty of Medicine, Siriraj Hospital, Mahidol University. All other authors (Napaporn Tojinda, Ninmanee Taweewatanasopon, Pornphit Boonkhon, Boontham Amornkitticharoen, and Sirilak Wiriyaakradecha) declare that they have no conflict of interest.

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

REFERENCES

1. de Porto AP, Lammers AJ, Bennink RJ, ten Berge IJ, Speelman P, Hoekstra JB. Assessment of splenic function. Eur J Clin Microbiol Infect Dis 2010;29:1465-73.
2. Wu C, Zhang B, Chen L, Zhang B, Chen X. Solitary perihepatic splenosis mimicking liver lesion: a case report and literature review. Medicine 2015;94:e586.
3. Fan B, Shepherd JA, Levine MA, Steinberg D, Wacker W, Barden HS et al. National Health and Nutrition Examination Survey whole-body dual-energy X-ray absorptiometry reference data for GE Lunar systems. J Clin Densitom 2014;17:344-77.
4. Ehrlich CP, Papanicolaou N, Treves S, Hurwitz RA, Richards P. Splenic scintigraphy using Tc-99m-labeled heat-denatured red blood cells in pediatric patients: concise communication. J Nucl Med 1982;23:209-13.
5. Neunert C, Lim W, Crowther M, Cohen A, Solberg L, Jr., Crowther MA. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood 2011;117:4190-207.
6. Lake ST, Johnson PT, Kawamoto S, Hruban RH, Fishman EK. CT of splenosis: patterns and pitfalls. AJR Am J Roentgenol 2012;199: W686-93.
7. Owunwanne A, Halkar R, Al-Rasheed A, Abubacker KC, Abdel-Dayem H. Radionuclide imaging of the spleen with heat denatured technetium-99m RBC when the splenic reticuloendothelial system seems impaired. J Nucl Med 1988;29:320-3.
8. Hagan I, Hopkins R, Lyburn I. Superior demonstration of splenosis by heat-denatured Tc-99m red blood cell scintigraphy compared with Tc-99m sulfur colloid scintigraphy. Clin Nucl Med 2006;31: 463-6.
9. Massey MD, Stevens JS. Residual spleen found on denatured red blood cell scan following negative colloid scans. J Nucl Med 1991;32:2286-7.
10. Gigot JF, Jamar F, Ferrant A, van Beers BE, Lengele B, Pauwels S, et al. Inadequate detection of accessory spleens and splenosis with laparoscopic splenectomy. A shortcoming of the laparoscopic approach in hematologic diseases. Surg Endosc 1998;12:101-6.
11. Muñoz-Bellvis L, Quiñones-Sampedro JE, Iglesias M, Blanco O, Fernandez LMG, Velasco CE et al. Contribution of the single photon emission computed tomography with 99mTc red blood cells in splenosis. Intern J Res Med Sci 2017;4:2464-7.
12. Varga I, Babala J, Kachlik D. Anatomic variations of the spleen: current state of terminology, classification, and embryological background. Surg Radiol Anat. 2018;40:21-29.
13. Hagman TF, Winer-Muram HT, Meyer CA, Jennings SG. Intrathoracic splenosis: superiority of technetium Tc 99m heat-damaged RBC imaging. Chest 2001;120:2097-8.
14. Mortelé KJ, Mortelé B, Silverman SG. CT Features of the Accessory Spleen. AJR Am J Roentgenol 2004;183:1653-7.
15. Horger M, Eschmann S, Lengerke C, Claussen C, Pfannenberg C, Bares R. Improved detection of splenosis in patients with haematological disorders: the role of combined transmission-emission tomography. Eur J Nucl Med Mol Imaging 2003;30: 316-9.
16. Ekmekci S, Diz-Kucukkaya R, Turkmen C, Adalet I. Selective Spleen Scintigraphy in the Evaluation of Accessory Spleen/ Splenosis in Splenectomized/Nonsplenectomized Patients and the Contribution of SPECT Imaging. Mol Imaging Radionucl Ther 2015;24:1-7.
17. Supe A, Parikh M, Prabhu R, Kantharia C, Farah J. Post-splenectomy response in adult patients with immune thrombocytopenic purpura. Asian J Transfus Sci 2009;3:6-9.

Does Acute Kidney Injury Condition Affect Revised BAUX Score in Predicting Mortality in Major Burn Patients?

Kusuma Chinaroonchai, M.D., Pornprom Muangman, M.D., Jatuporn Sirikun, M.D.

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

ABSTRACT

Objective: This study aimed to assess the accuracy of the revised BAUX score for predicting mortality among the major burn patients with acute kidney injury (AKI) compared with non-AKI group. The epidemiologic information and risk factors of AKI in major burn patients were also the point of interest.

Methods: This study was a retrospective cohort study. The medical records of 144 major burn patients admitted at the burns unit of Siriraj Hospital from 2010-2016 were reviewed and important data were retrieved.

Results: Age, hypertension, diabetes mellitus, severity of the burn injuries, and inhalation injuries were the factors related to AKI in major burn patients. The mortality rate due to AKI in burn patients was high (44.4%). The accuracy of the revised BAUX score in predicting the mortality among the major burn patients from our series was only fair (66.7%).

Conclusion: AKI affected on mortality of the major burn patients. Until the better predictor comes up, the revised BAUX score should be considered as a predictor of mortality in these patients.

Keywords: Acute kidney injury; acute renal failure; major burns; mortality rate; revised BAUX score (Siriraj Med J 2019;71: 150-157)

INTRODUCTION

In 2016, of the 447 burn patients treated at Siriraj Hospital, 62 were admitted to the burns unit. The length of stay per case averaged 23 days, costing about US$11,000 per admission. In addition, many hundreds of thousands of Thais in the wider population receive burns every year. This data suggests that millions of dollars are being spent by the government every year to pay for medical treatment related to burns. Better burn treatment can save money, but the most important objective for improving care is to enhance the quality of life of burn victims, which is priceless.

A systemic response after a burn injury occurs after at least 15% of the total body surface area is involved.[1,2] Proinflammatory cytokines are secreted and stimulate the body into a hypermetabolic phase in preparation for stress. Copious intravascular fluid leakage, the signature phenomenal response of endothelial cells to the proinflammatory cytokines, is one of the crucial phases that can result in edema formation in non-burned tissues. This can progress to burn shock if a burn patient receives inadequate fluid resuscitation treatment. An acute kidney injury in a burn victim can happen from many pathways, such as a poor resuscitation process or a hyper-response of the body to the kidney itself due to the secretion of pro-inflammatory cytokines. This condition can result in an increased mortality rate for burn victims, especially among major burn patients.

Acute kidney injury was defined by the Acute Dialysis Quality Initiative Group, which was founded

Corresponding author: Jatuporn Sirikun
E-mail: j_sirikun@hotmail.com
Received 21 August 2018 Revised 13 November 2018 Accepted 4 February 2019
ORCID ID: http://orcid.org/0000-0001-5514-6355
http://dx.doi.org/10.33192/Smj.2019.23

by nephrologists and intensivists. It was classified into 3 stages: risk (R), injury (I), and failure (F). The consequences of this condition are divided into 2 groups: sustained loss of kidney function (L), and end-stage kidney disease (E). The 3 stages and 2 groups combine to form the acronym, RIFLE. The RIFLE criteria[3] are used with critically-ill patients to form an early diagnosis of acute kidney injury conditions in order to decrease the mortality rate of this group of patients.[4,5] However, the criteria are limited by the availability of the baseline creatinine value of a patient for use as part of the basic data in the criteria. Consequently, the Acute Kidney Injury Network criteria (AKIN criteria) were developed by the Acute Kidney Injury Network (AKIN) to provide a more accurate tool than the RIFLE criteria for the diagnosis of acute kidney injuries. The AKIN criteria classifies acute kidney injuries into 3 stages, based on the method of treatment.[6,7]

It has been reported that the mortality rate of critically-ill patients with acute kidney injuries varies from 10%–100%, depending on the type of illness.[8] Another study reported a 34.9% (mean: 41.9%) mortality rate for burn patients with acute kidney injuries, which is 5–6 times higher than the mortality rate of non-AKI burn patients.[9] Even when a sub-group of AKI patients in that study received renal replacement therapy (RRT) for the treatment of renal failure, their mortality rate was still very high at 80%.[9]

The revised BAUX score has been reported as the most accurate score for predicting mortality due to burns. This score's factors are age, percentage of total body surface area (%TBSA) burn, and inhalation injuries.[10] A revised BAUX score of ≥ 100 is equivalent to a prediction of a 100% mortality rate.

Revised BAUX score = Age + %TBSA + 17x

(x as inhalation injury: 1 = yes, 0 = no)

To make it more convenient to calculate the score, it was developed into nomogram form.[11] Nevertheless, an acute kidney injury condition is still not one of the factors used to calculate the score.

MATERIALS AND METHODS

This studied was conducted with the approval of the Ethics Committee of Siriraj Hospital (Si 690/2016). The subjects were ≥ 20% total body surface area burn injury patients who had been admitted to the burns unit of Siriraj Hospital from 2010–2016. Those patients aged < 18 years or with a history of diagnosed chronic kidney disease were excluded, as were patients who had incomplete medical records. Of the 190 patients screened for the study, 46 were excluded due to having incomplete data, leaving 144 burn patients to be analyzed. The RIFLE and AKIN criterias were used to define the AKI group and to classify the severity of their burns. The qualitative data comprised of sex, underlying diseases, type of burn injury, with-inhalation injury, and any episode of hypotension in the first 24 hours post-injury (hypotension was defined as a systolic blood pressure of < 90 mmHg). These were presented as mean ± SD or median (IQR: percentile 25, percentile 75) and frequency (%). The continuous data comprised of age, length of hospital stay (days), %TBSA burn, length of ventilator use (days), and the volume of intravenous fluids for resuscitation in the first 24 hours post-injury and the second 24 hours post-injury; these were presented as frequency (%).

The program Statistical Package for Social Science (SPSS) version 18.0 was used to analyze the data. The independent t-test was used to analyze continuous data, while the Mann–Whitney test, Chi-square test and Fisher's exact test were used for the categorical data. A p-value less than 0.05 was considered to be statistically significant.

Factors associated with acute kidney injury were presented as odds ratio (95% confidence interval). The p-value corresponds to the logistic regression analysis. The association between the revised BAUX score and the AKI-with-mortality rate were analyzed by logistic regression analysis and presented as odds ratio (OR) with a 95% confidence interval (95% CI). The accuracy of the revised BAUX score in predicting mortality in the AKI group of burn patients was calculated from AUC.

RESULTS

The demographic data are in Table 1. The majority of the burn patients were male (75.7%), and the mean age of all burn victims was 44.09 ± 18.58 years. Essential hypertension was the most common underlying disease, followed by diabetes mellitus. The average size of the burn injuries was 41.75%TBSA, and most injuries had been caused by flame burn. The AKI group was older than the non-AKI group (49.97 ± 20.2 vs. 39.52 ± 15.88 years, respectively, p = 0.001). The average injury of the AKI group was more severe than that of the non-AKI group (%TBSA 55% vs. 35%, respectively, p < 0.001, with an incidence of > 60%TBSA 25 vs. 9, respectively, p < 0.001). Inhalation injuries were mostly found in the AKI group (27 vs. 19, respectively, p = 0.019). Most patients in the AKI group required ventilator support (80.9% vs. 30.9%, respectively, p < 0.001), and the length of ventilator use was longer for the AKI than the non-AKI group (19 vs. 7 days, respectively, p = 0.001). The

TABLE 1. Demographic data.

Factors	AKI group (n=63)	Non-AKI group (n=81)	Total (n=144)	P-value
Mean age (years)	49.97 ± 20.2	39.52 ± 15.88	44.09 ± 18.58	0.001*
Sex (Male:female)	48(76.2%):15(23.8%)	61(75.3%):20(24.7%)	109(75.7%):35(24.3%)	
Underlying diseases				
– HT	15(23.8%)	6(7.4%)	21(14.6%)	0.008*
– DM	6(9.5%)	1(1.2%)	7(4.9%)	0.043*
– DLP	5(7.9%)	2(2.5%)	7(4.9%)	0.24
– Coronary artery disease	2(3.2%)	1(1.2%)	3(2.1%)	0.581
– Gout	0(0%)	1(1.2%)	1(0.7%)	1
– Cirrhosis	1(1.6%)	0(0%)	1(0.7%)	0.438
– Asthma	0(0%)	3(3.7%)	3(2.1%)	0.257
– COPD	1(1.6%)	0(0%)	1(0.7%)	0.438
– Other eg. CVA, Parkinson disease, etc.	10(15.9%)	11(13.6%)	21(14.6%)	0.813
Type of burn injury				
– Flame burn	51(81%)	53(65.4%)	104(72.2%)	0.042*
– Scald burn	6(9.5%)	10(12.3%)	16(11.1%)	0.79
– Electrical burn	5(7.9%)	18(22.2%)	23(16%)	0.023*
– Other eg. contact hot objects.	1(1.6%)	0(0%)	1(0.7%)	0.438
%TBSA (IQR)	55(38.5,73)	35(25,46)	41.75(30,60)	<0.001*
Depth burn wound				
– 2nd degree (IQR)	49(30,63)	35(25,50)	38(28,58)	0.015*
– 3rd degree (IQR)	36.5(15,43.5)	15(10,37.5)	28(10,40)	0.225
Inhalation injury	27(42.9%)	19(23.5%)	46(31.9%)	0.019*
Length of hospital stay, day (LOS) (IQR)	36(17,60)	31(20,54)	34.5(18.5,56.5)	0.622
Ventilator used	51(80.9%)	25(30.9%)	76(52.8%)	<0.001*
Total ventilator days (IQR)	19 (10,38.5)	7 (5,11)	13 (7,26)	<0.001*
1st 24 h fluid (mL) (IQR)	12,967 (9,800,19,000)	9,656 (6,630,13,982)	11,679 (7,180,17,236)	0.001*
1st 24 h fluid (mL/kg/%TBSA) (IQR)	4.05 (2.93,5.29)	4.21 (3.07,5.45)	4.07 (2.98,5.33)	0.413
2nd 24 h fluid (mL) (IQR)	8,640 (7,100,10,513)	5,950 (4,800,7860)	7,378.5 (5,360,8,985)	<0.001*
2nd 24 h fluid (mL/kg/%TBSA) (IQR)	2.44 (1.73,3.08)	2.48 (1.79,3.29)	2.47 (1.75,3.2)	0.417
Hx of Hypotension in 1st 24 h (SBP < 90 mmHg)	16(25.4%)	4(4.9%)	20(13.9%)	0.001*

* P-value < 0.05 **Abbreviations:** HT = essential hypertension; DM = diabetes mellitus; DLP = dyslipidemia; COPD = chronic obstructive pulmonary disease; CVA = cerebrovascular disease; IQR = interquartile range (25th percentile, 75th percentile); h = hours; %TBSA = % total body surface area burn; SBP = systolic blood pressure.

AKI group required more fluid resuscitation in the first and second 24 hours post-injury, and also developed hypotension more often than the non-AKI group in the first 24 hours post-injury.

The APACHE II scores calculated for both groups are in Table 2. The APACHE II score for the AKI group was significantly higher than that for the non-AKI group (16.86 ± 8.16 vs. 6.49 ± 4.39, p < 0.001). Parameters for the two groups (such as GCS, MAP, RR, FiO_2, arterial pH, Hct, serum HCO3 and serum creatinine) differed significantly.

A logistic regression was performed to calculate the odds ratio of the factors (Table 3). The odds ratio of patients needing to use a ventilator was very high at 11.23. The DM and HT odds ratios were 8.42 and 3.91, respectively. The odds ratio for a history of hypotension in the first 24 hours post injury was 6.55. Extensive injury > 60%TBSA showed an odds ratio of 5.26, while inhalation injury had an odds ratio of 2.45.

63 patients were diagnosed with AKI (Table 4). The highest incidence of AKI (29) occurred with RIFLE-Risk and AKIN stage 1; all of the patients in those two categories were successfully treated using conservative treatment with medication. A total of 13 other patients received renal replacement therapy; 12 of those patients were RIFLE-Failure, while the 13th was RIFLE-Loss; and all 13 were in AKIN stage 3.

The incidences of mortality at 28 days and at 6 months were significantly higher for the AKI group, with p <0.001 (Table 5). Revised BAUX scores were calculated for each group. The score for the AKI group (115.59 ± 27.73) was higher than that for the non-AKI group (82.56 ± 24.41), with p < 0.001 (a revised BAUX score ≥ 100 predicts 100% mortality rate[10,12]). The revised BAUX scores were also significantly higher for the dead patients in both groups (Table 6).

Both the revised BAUX scores and AKI in Table 7 are also related to the mortality rate from multivariate analysis, with OR 1.04, p < 0.001 and OR 8.69, p = 0.002, respectively.

DISCUSSION

In the past, AKI in burns was considered to be caused by hypovolemia resulting from inadequate volume resuscitation in the first 24 hours, post injury.[13,14] Historically, Parkland's formula was used to determine

TABLE 2. APACHE II scores.

Factors	AKI group (n=63)	Non-AKI group (n=81)	Total (n=144)	P-value
APACHE II score	16.86 ± 8.16	6.49 ± 4.39	11.03 ± 8.14	<0.001*
– GCS	10.87 ± 2.72	13.89 ± 2.09	12.57 ± 2.81	<0.001*
– BT (*C)	37.92 ± 0.84	37.89 ± 0.64	37.9 ± 0.74	0.833
– MAP (mmHg)	86.35 ± 11.39	90.8 ± 10.5	88.85 ± 11.08	0.016*
– HR (beat/min)	131.43 ± 126.17	107.64 ± 16.28	118.05 ± 84.79	0.095
– RR (per min)	21.84 ± 4.86	20.17 ± 2.08	20.9 ± 3.66	0.013*
– FiO_2	0.5 ± 0.2	0.42 ± 0.09	0.45 ± 0.15	0.004*
– PaO_2 (mmHg)	134.07 ± 80.28	116.43 ± 52.36	124.15 ± 66.37	0.134
– Arterial pH	7.32 ± 0.11	7.38 ± 0.05	7.34 ± 0.1	0.048*
– Serum HCO_3 (mmol/L)	19.87 ± 5.01	23.9 ± 3.75	22.14 ± 4.78	<0.001*
– Na (mmol/L)	134.54 ± 25.03	137.85 ± 4.14	136.4 ± 16.85	0.303
– K (mmol/L)	4.21 ± 0.76	4.09 ± 0.59	4.14 ± 0.67	0.28
– Serum Cr (mg/dL)	2.01 ± 1.58	0.77 ± 0.25	1.31 ± 1.23	<0.001*
– Hct (%)	35.93 ± 11.19	40.71 ± 8.6	38.62 ± 10.06	0.006*
– WBC (cells/mm^3) (IQR)	14,110 (5,960, 21,860)	10,920 (6,800, 16,010)	11,645 (6,560, 18,025)	0.232

Data is presented as mean ± SD, *p-value < 0.05. **Abbreviations:** APACHE II = Acute Physiology and Chronic Health Evaluation version II; GCS = Glasgow Coma Score; BT = body temperature; MAP = mean arterial pressure; HR = heart rate, RR = respiratory rate, IQR = interquartile range (25^{th} percentile, 75^{th} percentile)

TABLE 3. Associated factors for AKI.

Factor	Univariate Crude OR (95%CI)	P-value	Factor	Univariate Crude OR (95%CI)	P-value
Age	1.03 (1.01, 1.05)	0.001*	APACHE II score	1.29 (1.18, 1.41)	<0.001*
HT	3.91 (1.31, 13.05)	0.008*	GCS	0.64 (0.55, 0.74)	<0.001*
DM	8.42 (0.97, 392.01)	0.043*	BT	1.05 (0.67, 1.65)	0.832
Electrical Injury	0.3 (0.08, 0.92)	0.023*	MAP	0.96 (0.93, 0.99)	0.019*
Total %TBSA injury	1.04 (1.02, 1.06)	<0.001*	HR	1.03 (1.01, 1.06)	0.004*
– <20%TBSA	1 (0, 1)	0.438	RR	1.15 (1.03, 1.28)	0.011*
– 20%–40%TBSA	0.24 (0.11, 0.5)	<0.001*	FiO_2	56.26 (3.18, 995.95)	0.006*
– 41%–60%TBSA	1.33 (0.6, 2.93)	0.462	PaO_2	1 (1, 1.01)	0.123
– >60%TBSA	5.26 (2.09, 14)	<0.001*	Arterial pH	0 (0, 1.07)	0.052
Type of burn injury			Serum HCO_3	0.81 (0.74, 0.88)	<0.001*
– 2nd degree	1.02 (1.01, 1.04)	0.01*	Na	0.99 (0.96, 1.01)	0.294
– 3rd degree	1.03 (0.99, 1.08)	0.134	K	1.32 (0.8, 2.17)	0.28
Inhalation Injury	2.45 (1.13, 5.35)	0.019*	Cr	195.62 (26, 1471.65)	<0.001*
Ventilator used	11.23 (4.71, 27.65)	<0.001*	Hct	0.95 (0.92, 0.99)	0.005*
Event of hypotension in 1st 24 h (SBP<90 mmHg)	6.55 (1.93, 28.2)	0.001*	WBC	1 (1, 1)	0.083

*P-value < 0.05 **Abbreviations:** HT = essential hypertension; DM = diabetes mellitus; %TBSA = % total body surface area burn; h = hours; SBP = systolic blood pressure; GCS = Glasgow Coma Score; BT = body temperature; MAP = mean arterial pressure; HR = heart rate; RR = respiratory rate; Cr = serum creatinine; Hct = hematocrit; WBC = white blood cell

TABLE 4. Type of AKI (total AKI group = 63 patients).

RIFLE	Incidence	AKIN	Incidence	Dead in 28 days	Dead in 6 months
Risk	29 (46%)	Stage 1	29 (46%)	3 (16.7%)	6 (21.4%)
Injury	21 (33.3%)	Stage 2	21 (33.3%)	8 (44.4%)	12 (42.9%)
Failure	12 (19%)	Stage 3	13 (20.6%)	7 (38.9%)	10 (35.7%)
Loss	1 (1.59%)		Total	18	28
ESKD	0				

Abbreviations: RIFLE = Risk-Injury-Failure-Loss of Kidney Function-End-stage Kidney Disease; AKIN =Acute Kidney Injury Network; ESKD = end stage kidney disease

TABLE 5. Mortality rates for both groups.

Factors	AKI group (n=63)	Non-AKI group (n=81)	Total (n=144)	P-value
Mortality at 28 days post injury	18 (28.6%)	2 (2.5%)	20 (13.9%)	<0.001*
Mortality at 6 months post injury	28 (44.4%)	3 (3.7%)	31 (21.5%)	<0.001*

*P-value < 0.05

TABLE 6. The revised BAUX scores for each group.

Total (n=144)	AKI group (n=63)			Non-AKI group		P-value
97.01 ± 30.61	115.59 ± 27.73			82.56 ± 24.41		<0.001*
	Dead (n=28)	Survive (n=35)	P-value	Dead (n=3)	Survive (n=78)	P-value
Revised BAUX score	127.23 ± 27.72	106.27 ± 24.31	0.002*	125.33 ± 44.06	80.91 ± 22.23	0.036*

Data presented as mean ± SD, *p-value < 0.05

TABLE 7. The relationship of the revised BAUX scores and AKI with the mortality rate.

	Univariate analysis		Multivariate analysis	
	Crude OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value
Revised BAUX	1.06 (1.03, 1.08)	<0.001*	1.04 (1.02, 1.06)	<0.001*
AKI	20.8 (5.93, 73.01)	<0.001*	8.69 (2.23, 33.84)	0.002*

*P-value < 0.05, CI = confidence interval

the fluid resuscitation volume required daily. However, this standard formula was often found to calculate more fluid than patients needed in practice. The current trend is to use the same formula to estimate the fluid volume, but then to adjust the fluid rate every 2 hours, based on the patient's clinical response. This technique better balances the fluid needs of the patient, thereby ensuring that there is adequate fluid resuscitation. The trend to keep a dryer status has been proven to decrease the incidence of acute complications such as compartment syndrome, both in the extremities and abdomen.[15,16]

Late complications such as infection, which is the biggest killer for late death burns, have been reported to relate to the hyper-resuscitation volume that had been given.[17] Even in the case of patients who received adequate fluid resuscitation, AKI can still happen from other pathways. For example, the direct-injury effect of pro-inflammatory cytokines acts on the stress response pathway, which stimulates the Renin-angiotensin-system (RAS); the RAS hormones then enhance the kidney's vascular tone, which boosts its glomerular filtration rate. Moreover, sometimes cytokines themselves can create a direct injury to the kidneys and the myocardial muscle; this worsens the perfusion to the kidney, which results in additional and indirect kidney damage.[13]

The stress response to burn injuries in the elderly is not the same as that for younger people.[18] Due to the elderly's poor body reserves resulting from the aging process, they are prone to develop more complications after burn injuries, such as AKI, as found in the study by Wu et al.,[19] and our study. Essential hypertension and diabetes mellitus are diseases that affect systemic vascular quality. The poor quality vessels affect end-organ perfusion of the heart and kidney. Low renal perfusion,

especially in a stressed condition, creates further damage to kidneys, which results in the acute kidney injury condition.

Most patients in this series received their injuries from flame burns, thus sharing a common etiology with burn victims in other countries all around the world. However, in the present study, the AKI condition was found to be more common for the electrical-injury group than had been reported in other studies. This may be explained by the fact that this kind of injury causes more severe damage to the tissue than a flame injury, and it can develop the rhabdomyolysis condition, which burdens the kidneys and makes them prone to further injury. Pro-inflammatory cytokines play a major role in the systemic response of the body after major burn injuries. AKI occurs more frequently when patients have larger areas of burn injury. The severity of the body's systemic response to a burn injury is related to the inflammatory cytokine level, which varies with how large the injured area is.[20] Second-degree burns play a significant role in the development of AKI complications. This may be explained by the level of cytokines that are stimulated and secreted. Most of these are triggered by second-degree burns of the skin[21], which create more hemodynamic changes from fluid shifts than from third degree wounds, in which all cells are dead.[22,23]

In this study, the presence of inhalation injuries, ventilator use, episodes of hypotension in the first 24 hours, and arterial pH were related to AKI, which was also found in the study by Schneider et al.[24] The AKI group had a longer average hospital stay and more complications, consistent with other studies.[9,13,14,19,23] Moreover, the mortality in the AKI group in our study was 44.4%, which is in the range of 4%–90% from other studies.[13,14,23]

The average fluid resuscitation volume in the AKI group was larger than that for the non-AKI group, although after calculating the volume per kg per %TBSA, the difference proved to be non-significant. Currently, there is no valid, fixed formula available for calculating resuscitation fluids for all types of burn patients. The best thing that we can do is to closely monitor and titrate the fluid rate based on a patient's hemodynamic status to reach the optimum and adequate resuscitation endpoint. During the first 24 hours, the resuscitation-fluid rate for both groups was about 4 mL/kg/%TBSA, calculated using Parkland's formula; the need for fluid decreased by a half during the second 24-hour period.

The data in Table 2 shows that APACHE II is a good scoring system for evaluating the severity of a burn injury with a coexistent AKI condition. The AKI group showed significantly higher APACHE II scores than the non-AKI group ($p < 0.001$). As for the parameters of APACHE II, it was found that GCS, MAP, RR, FiO2, arterial pH, serum HCO_3, Hct and serum creatinine differed significantly between the AKI and non-AKI groups.

Most of the AKI patients were RIFLE-R and AKIN stage I, which can be treated conservatively with medication. A total of 13 patients in RIFLE-F, RIFLE-L and AKIN stage 3 received renal replacement therapy (RRT) treatment. Even though effective RRT treatment was initiated in these groups, the mortality rates were still high (38% for RIFLE-I and AKIN stage 2, 58.3% for RIFLE-L, and 53.8% for AKIN stage 3). Detection at an earlier stage may help to decrease patient morbidity from the necessary intervention treatment (RRT) and may lessen mortality.

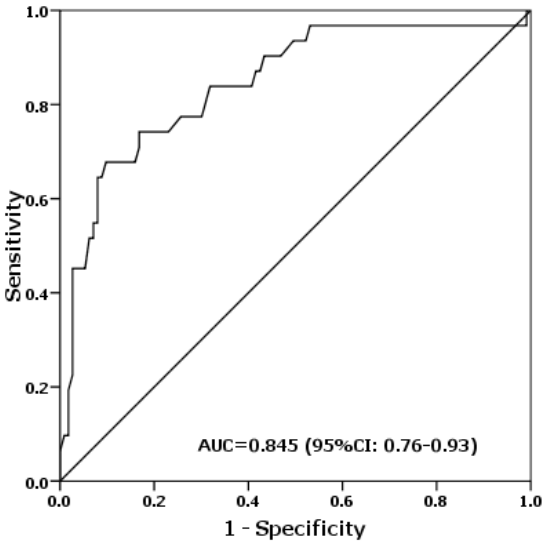
From a multivariate analysis of the revised BAUX score and AKI with the mortality rate, we found that both factors relate with mortality rate with a statistically significant adjusted OR. This means that both factors heavily affect the mortality rate. Consequently, the AKI factor should be considered in order to improve the accuracy of the calculation formula used to predict burn mortality rates.

Using the revised BAUX score cut-off at 100 for prediction of mortality in the AKI group (Osler T et al. defined a score ≥ 100 as a prediction of a 100% mortality rate[10]) as shown in Fig 1, the sensitivity is 83.9%, with 61.9% specificity. The positive predictive value is 37.7%, and the negative predictive value is 93.3%. The accuracy is 66.7%.

At 66.7%, the accuracy of the revised BAUX score to predict the mortality rate in our study was quite low compared with other studies.[24,25] The accuracy of the calculated score may have been impacted by the fact that nearly half of our study population (43.7%) were in the AKI group.

This study had added another supporting evidence that AKI is one of the important factors in predicting the outcome of the burn patients. The total number of the patients and the fact that nearly half of the population has developed AKI might be the main factor which using BAUX score in predicting mortality rate was not as accurate as shown the previous studies.

AKI is one of the significant conditions that increase the mortality rate of major burn patients. The future calculation scoring system should recognize it as an important factor affecting mortality. The revised BAUX score is not accurate enough to use to predict mortality of major burn patients with AKI.



Sensitivity	83.9%
Specificity	61.9%
PPV	37.7%
NPV	93.3%
Accuracy	66.7%

Fig 1. AUC of revised BAUX score at cut off value of 100.

ACKNOWLEDGMENTS

This research project was supported by the Faculty of Medicine Siriraj Hospital, Mahidol University (Grant Number: [IO] R016031018). We thank Mr.Tanut Sornmanapong for facilitating the research coordinative process.

Conflicts of interest: It is hereby declared that there are no conflicts of interest.

REFERENCES

1. Kao CC, Gamer WL. Acute burns. Plast Reconstr Surg 2000; 105:2482-92.
2. Kramer GC. Pathophysiology of burn shock and burn edema. In: Herndon DN. Total burn care. 4th ed. Edinburgh; New York: Saunders Elsevier; 2012.p.103-13.
3. Bellow R, Ronco C, Kellum JA, Mehta RL, Palevsky P, Acute Dialysis Quality Initiative workgroup. Acute renal failure – definition, outcome measures, animal models, International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004;8:R204-12.
4. Abosaif NY, Tolba YA, Heap M, Russell J, El Nahas AM. The outcome of acute renal failure in the intensive care unit according to RIFLE: Medel application, sensitivity, and predictability. Am J Kidney Dis 2005;46:1038-48.
5. Uchino S, Bellow R, Goldsmith D, Bates S, Ronco C. An assessment of the RIFLE criteria for acute renal failure in hospitalized patients. Brit Care Med 2006;34:1913-17.
6. Molitoris BA, Levin A, Warnock DG, Joannidis M, Mehta RL, Kellum JA, et al. Acute Kidney Injury Network Working Group. Improving outcomes of acute kidney injury: report of an initiative. Nat Clin Pract Nephrol 2007;3:439-42.
7. Mehta RL, Vellum Ja, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al. Acute Kidney Injury Network. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care 2007;11:R31.
8. Hoste EA, Schurgers M. Epidermiology of acute kidney injury: How big is the problem? Crit Care Med.2008;36:S146-51.
9. Brusselaers N, Monstrey S, Colpaert K, Decruyenaere J, Blot SI, Hoste EA. Outcome of acute kidney injury in severe burns: a systematic review and meta-analysis. Intensive Care Med 2010;36:915-25.
10. Osler T, Glance LG, Hosmer DW. Simplified estimates of the probability of death after burn injuries: extending and updating the baux score. J Trauma 2010;68:690-7.
11. Williams DJ, Walker JD. A nomogram for calculation of the Revised Baux Score. Burns 2015;41:85-90.
12. Woods JF, Quinlan CS, Shelley OP. Predicting Mortality in Severe Burns – What Is the Score?: Evaluation and Comparison of 4 Mortality Prediction Scores in an Irish Population. Plast Reconstr Surg Glob Open 2016;4:e606.
13. Emara SS, Alzaylai AA. Renal failure in burn patients: a review. Ann Burns Fire Disasters 2013;26:12-15.
14. Ibrahim AE, Sarhane KA, Fagan SP, Goverman J. Renal dysfunction in burns: a review. Ann Burns Fire Disasters 2013;26:16-25.
15. Cartotto R, Zhou A. Fluid creep: the pendulum hasn't swung back yet! J Burn Care Res 2010;31:551-8.
16. Strang SG, Van Lieshout EM, Breederveld RS, Van Waes OJ. A systematic review on intra-abdominal pressure in severely burned patients. Burns 2014;40:9-16.
17. Klein MB, Hayden D, Elson C, Nathens AB, Gamelli RL, Gibran NS, et al. The association between fluid administration and outcome following major burn: a multicenter study. Ann Surg 2007;245:622-8.
18. Jeschke MG, Patsouris D, Stanojcic M, Abdullahi A, Rehou S, Pinto R, et al. Pathophysiologic Response to Burns in the Elderly. EBioMedicine 2015;2:1536-48.
19. Wu G, Xiao Y, Wang C, Hong X, Sun Y, Ma B, et al. Risk Factors for Acute Kidney Injury in Patients With Burn Injury: A Meta-Analysis and Systematic Review. J Burn Care Res 2017; 38:271-82.
20. Sood RF, Gibran NS, Arnoldo BD, Gamelli RL, Herndon DN, Tompkins RG, et al. Early leukocyte gene expression associated with age, burn size, and inhalation injury in severely burned adults. J Trauma Acute Care Surg 2016;80:250-7.
21. Sakallioglu AE, Basaran O, Karakayali H, Ozdemir BH, Yucel M, Arat Z, et al. Interactions of systemic immune response and local wound healing in different burn depths: an experimental study on rats. J Burn Care Res 2006;27:357-66.
22. Farina JA, Jr., Rosique MJ, Rosique RG. Curbing inflammation in burn patients. Int J Inflam 2013;2013:715645.
23. Schneider DF, Dobrowolsky A, Shakir IA, Sinacore JM, Mosier MJ, Gamelli RL. Predicting acute kidney injury among burn patients in the 21st century: a classification and regression tree analysis. J Burn Care Res 2012;33:242-51.
24. Pantet O, Faouzi M, Brusselaers N, Vernay A, Berger MM. Comparison of mortality prediction models and validation of SAPS II in critically ill burns patients. Ann Burns Fire Disasters 2016;29:123-9.
25. Salehi SH, As'adi K, Abbaszadeh-Kasbi A, Isfeedvajani MS, Khodaei N. Comparison of six outcome prediction models in an adult burn population in a developing country. Ann Burns Fire Disasters 2017;30:13-17.

An Association of *Cryptococcus neoformans/ C. gattii* Genotype and HIV Status in Asia: A Systematic Review

Popchai Ngamskulrungroj, M.D.,Ph.D.*, Patcharee Kammarnjassadakul, M.D.,Ph.D., Kantarawee Khayhan, M.D. ,Ph.D.***, Poramate Pitak-Arnnop, M.D.D.D.S.,Ph.D.****, Pathompong Ungprasert, M.D.*****, Sujiraphong Pharkjaksu, M.D.,M.Sc.***

**Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, **Department of Clinical Microbiology, Faculty of Medical Technology, Huachiew Chalermprakiet University, Samut Prakan 10270, ***Department of Microbiology and Parasitology, Faculty of Medical Sciences, University of Phayao, Phayao 56000, Thailand, ****Department of Oral and Maxillofacial Surgery, University Hospital of Marburg UKGM, Faculty of Medicine, Philips University, Marburg, Germany, *****Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: It has been known that VNI molecular type of *Cryptococcus neoformans/C. gattii* is strongly associated with HIV patients. However, this paradigm has recently been challenged because of the high prevalence of VNI molecular type among non-HIV patients with cryptococcosis in East Asia. The purpose of this study was to answer the question: "Among cryptococcosis in Asia, is there an association between the genotype and the patient's HIV status?"

Methods: Using a systematic review and meta-analysis study design, we included all relevant published data, which were any type of study designs, mainly studied clinical *Cryptococcus neoformans/C. gattii* strains isolated in Asia and had available molecular typing data. The primary study variables were *Cryptococcus neoformans* molecular type (VNI/non-VNI or ST5/non-ST5) and the HIV status of the patients at the time of diagnosis. We used a random-effects meta-analysis model to estimate the prevalence of HIV infection.

Results: Sixteen retrospective descriptive studies during 2005 – 2018 (1,584 isolates) were included. Most of the cryptococcosis cases in East Asian countries were in non-HIV patients (72.4-81.8%), which differed from non-East Asian countries (2.6-28.3% associated with non-HIV patients). In East Asia, the HIV prevalence among VNI and ST5 infected patients ranged from 7.5% - 46.7% with the pooled prevalence of 19.8% (95% CI, 12.2% - 30.4%) and 5.3% - 52.4% with the pooled prevalence of 19.9% (95% CI, 6.9% - 45.3%), respectively. In non-East Asia, the HIV prevalence among VNI and ST5 infected patients ranged from 48.3% - 98.8% with the pooled prevalence of 81.9% (95% CI, 73.3% - 88.2%) and 52.3% - 88.0% with the pooled prevalence of 74.9% (95% CI, 40.7% - 92.8%), respectively. Statistical heterogeneity was high in both analyses with the I^2 of 79-89% in all analyses.

Conclusion: Our results confirmed the low prevalence of HIV prevalence among VNI and ST5 strains in East Asian countries. The emergence of high virulence genotype causing disease in non-HIV patient is highly unlikely, because the VNI and ST5 were associated with HIV patients in other Asian countries. It can be hypothesized that the low HIV prevalence among VNI and ST5 strains in East Asian is due to the high susceptibility to cryptococcosis of people living in this region. This requires further investigation.

Keywords: Cryptococcosis; VNI; *Cryptococcus neoformans*; HIV; Asia (Siriraj Med J 2019;71: 158-164)

Corresponding author: Popchai Ngamskulrungroj
E-mail: popchai.nga@mahidol.ac.th
Received 21 August 2018 Revised 12 November 2018 Accepted 4 February 2019
ORCID ID: http://orcid.org/0000-0001-5162-5498
http://dx.doi.org/10.33192/Smj.2019.24

INTRODUCTION

Cryptococcus neoformans and *C. gattii* play an important role in today's medical practice and have received intensive attention from researchers/clinicians because they cause not only meningitis but also pneumonia in human worldwide.[1] The two species are different in many aspects. This includes environmental niche, molecular epidemiology, pathogenesis and clinical manifestations once they cause the disease, the so-called "cryptococcosis".[1]

It is known that *C. neoformans* is associated mainly with immunosuppression, especially among HIV-infected patients, while *C. gattii* generally causes cryptococcosis in immunocompetent patients.[2] However, some reports have demonstrated contradictory results. Recent studies from China and South Korea showed that most of cryptococcosis patients caused by *C. neoformans* were HIV-negative patients without other immunocompromised conditions.[3,4] Especially in China, cryptococcosis frequently occurred in immunocompetent patients.[4,5]

C. neoformans is composed of 4 major molecular types, VNI, VNII, VNIII, and VNIV and *C. gattii* is composed of 4 major molecular types, VGI, VGII, VGIII and VGIV.[6] The VNI-molecular type of *C. neoformans* is the most common cryptococcal molecular type and typically associated with HIV patients.[7] However, VNI in China was associated with non-HIV patients.[4] Further molecular typing among the VNI strains by the Multi Locus Sequence Typing (MLST)[8] revealed the non-HIV-associated VNI strains was almost exclusively belonged to the sequence type 5 (ST5) genotype.[4] This phenomenon has also been found in Korea[3] and Japan[9] Therefore, the association of this VNI-ST5 genotype and non-HIV status may be possible. However, although Thais are a close sibling of Chinese, there was no association between VNI-ST5 and non-HIV status.[10] The East Asian race is highly susceptible to VNI-ST5 or is a high virulent genotype capable of causing infection in immunocompetent individuals.

A search of the published data revealed no evidence of any randomized trials or systematic reviews addressing the association of the VNI-ST5 and HIV status in Asian patients with cryptococcosis. The purpose of the present report was to complete a systematic review and meta-analysis to answer the following research question: "Among cryptococcosis in Asia, is there an association between the genotype and the patient's HIV status?" We hypothesized that such association is limited only to patients in East Asia – China and neighboring. Our specific aims were to perform a systematic review of the published data and identify studies for analysis and to execute a meta-analysis to determine whether the VNI-ST5 causes diseases mainly in non-HIV patients across all Asian countries.

MATERIALS AND METHODS

Study design

To address our research question, we designed and implemented a systematic literature review and meta-analysis.

Samples

Our review of the published data was designed and conducted in concordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.[11] Three authors (P. N., P. K. and K. K.) searched the published studies from January 1999 (The year that cryptococcal molecular typing system was firstly established[12]) to May 2018, using the National Library of Medicine (PubMed, available from http://www.pubmed.gov), the Web of Science (available from https://webofknowledge.com), and the Cochrane database (available from http://www.cochranelibrary.com/) using specific medical subject headings and key words, including (Cryptococ*) AND (molecular typ* OR molecular epidemiology). Because each *Cryptococcus* species comprises 4 major molecular types: *C. neoformans*, VNI (AFLP1), VNII (AFLP1A of AFLP1B), VNIII (AFLP3) and VNIV (AFLP2); and *C. gattii*, VGI (AFLP4), VGII (AFLP6), VGIII (AFLP5) and VGIV (AFLP7) (REF), we also used the specific terms for all major molecular types (VNI OR VNII OR VNIII OR VNIV OR VGI OR VGII OR VGIII OR VGIV OR AFLP1 OR AFLP1A OR AFLP1B OR AFLP2 OR AFLP3 OR AFLP4 OR AFLP6 OR AFLP5 OR AFLP7) AND (Cryptococ*) to identified all publications investigating cryptococcal molecular typing. Citations in the accessed articles were further analyzed for additional published data.

In addition, the available post-meeting online materials of relevant meetings including American Society for Microbiology (ASM) 2010-2015, 48th-55th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), ASM microbe 2016-2017, International Society for Human & Animal Mycology (ISHAM) 2000-2015 and International Conference on *Cryptococcus* and Cryptococcosis (ICCC) 4th-10th were searched for additional eligible studies.

Study inclusion and exclusion criteria

Studies were included if they were mainly studied Asian isolates, provided data on statistics, patient's HIV status and major molecular type data. Searching methodology was mentioned above. We excluded studies that, selectively included only one type of immune status of patients or genotype of the strains, were case-reports or case-series, did not identify the correlation between HIV status with individual isolates or contained 100% duplicated cryptococcal isolates.

Study variables

The primary predictor variable was the genotype (i.e., major molecular type or sequence type). The main outcome variable was the patient's HIV status. The primary analysis of interest was the prevalence of the patient's HIV positivity across study groups (i.e., VNI and ST5 in East Asia [China, Japan, Taiwan, and Korea] and Non-East Asia [Thailand, Malaysia, Singapore, Vietnam, Indonesia, India, Kuwait, and Qatar] countries). The other study variables were classified as demographic, anatomic, environmental and genetic. The demographic data were total sample size, number of subjects in both genotype groups, and mean patient's age. The anatomic location of infection, environmental niche and genetic profiles were also recorded.

Data extraction and statistical analysis

Using a standardized data extraction form, two authors (P. Ng. and K. K.) extracted and tabulated all data. Discrepancies were resolved by group discussion of the investigator authors. As standard quality assessment of descriptive study for meta-analysis was not available, a quality assessment for case-control studies was used according to a previous published method.[13] A quality scale for observational study was used. A narrative overview is provided summarizing the data gathering from included published data. The pooled prevalence of HIV positivity and 95% CI for ST5 and VNI were calculated using DerSimonian-Laird random-effect model with double arcsine transformation.[14] The pooled prevalence and 95% CI were calculated for each region (East Asia and non-East Asia). Random-effect model, rather than fixed-effect, was used because of the high likelihood of between-study heterogeneity. Cochran's Q test and I^2 statistic were used to determine the between-study heterogeneity. This I^2 statistic quantifies the proportion of total variation across studies that is due to heterogeneity rather than chance. A value of I^2 of 0% to 25% represents insignificant heterogeneity, greater than 25% but less than or equal to 50% represents low heterogeneity, greater than 50% but less than or equal to 75% represents moderate heterogeneity, and greater than 75% represents high heterogeneity.[15] The software Comprehensive meta-analysis (Englewood, NJ, USA) and IBM SPSS statistics 18.0 (IBM (Thailand), Bangkok, Thailand) were used for statistical analysis.

Research ethics

The recommendations of the Helsinki declaration were thoroughly maintained during this study. Since this study did not involve human subjects or records directly, ethical approval by an ethics committee and consent from the authors of the articles studied were not required.

RESULTS

Search results

The literature searches initially yielded 1,630 potentially relevant studies; titles and abstracts were screened, and full papers were obtained for relevant articles (Fig 1). A total of 36 full-text articles were reviewed, of which 20 were excluded for various reasons. The remaining 16 studies were finally included in this meta-analysis and yielded a total of 1,584 isolates.[3-5,7,10,16-26] There was no additional published data from citations in the accessed articles.

Characteristics and quality of included studies

The characteristics of the 16 included studies are shown in Table 1. All were retrospective descriptive studies. Quality of the included studies was scored 4.5 to 8.5 out of 14.5. All included studies reported the data on HIV status in at least some of the isolates but the availability of this data varied considerably across the studies, ranging from 13.5% to 100% (median 80.2%). Only strains with HIV status data were used in this current analysis. Only 7 studies had information of ST5 genotype with HIV data (Table 1).

Prevalence of HIV and genotype across all studies of cryptococcosis

Cryptococcosis in Asia was not common in non-HIV patients (2.6-28.3%), except in East Asia (72.4-81.8%) and Northwest Asia (60-70%). Interestingly, VNI molecular type was predominant across all Asian countries, regardless of HIV status of the patients. Among the VNI strains, ST5 was more prevalent than non-ST5 only in East Asia. The results were summarized in Fig 2.

HIV prevalence in East Asian countries

The HIV prevalence among VNI-infected cryptococcosis patients from the included 8 studies ranged from 7.5% - 46.7% with the pooled prevalence of 19.8% (95% CI, 12.2% - 30.4%) (Fig 3). The HIV prevalence among ST5 infected patients from the 5 studies ranged from 5.3% - 52.4% with the pooled prevalence of 19.9% (95% CI, 6.9% - 45.3%) (Fig 4). Statistical heterogeneity was high in both analyses with the I^2 of 89% in both analyses.

HIV prevalence in non-East Asian countries

The HIV prevalence among VNI-infected cryptococcosis patients from the 9 studies ranged from 48.3% - 98.8% with the pooled prevalence of 81.9% (95% CI, 73.3% -

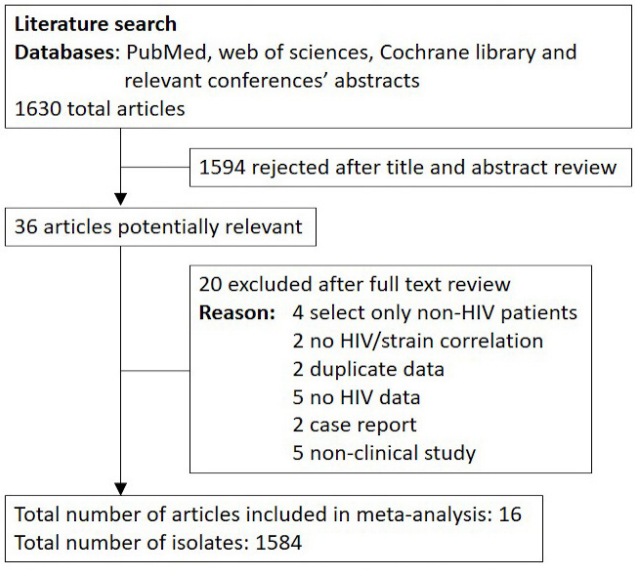


Fig 1. Study selection process.

88.2%) (Fig 3). The HIV prevalence among ST5-infected patients from the 3 studies ranged from 52.3% - 88.0% with the pooled prevalence of 74.9% (95% CI, 40.7% - 92.8%) (Fig 4). Statistical heterogeneity was high in both analyses with the I^2 of 79% in both analyses.

DISCUSSION

The study's principal aim was to identify the particular association between the cryptococcosis genotypes VNI/ST5 and the non-HIV status of patients in Asia. We hypothesized that this association would be seen only in East Asia countries. Our specific aims were to conduct a systematic review and meta-analysis to identify the relationship between the VNI/ST5 strain and the patient's HIV status.

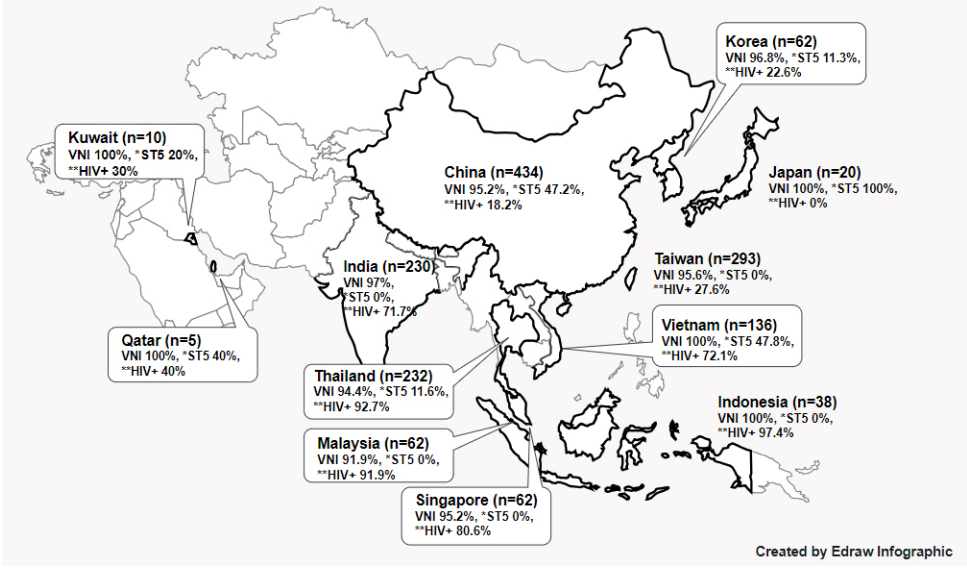


Fig 2. Summary of the collected data; n=a total number of strains with the major molecular type information (*Availability of the ST data was varied: China = 234, Korea = 13, Japan = 20, Taiwan = 0, Thailand = 195, Malaysia = 0, Singapore = 0, Vietnam = 136, Indonesia = 38, India = 58, Kuwait = 10, and Qatar = 5; **Availability of HIV data was varied: China = 349, Korea = 59, Japan = 20, Taiwan = 293, Thailand = 87, Malaysia = 62, Singapore = 62, Vietnam = 136, Indonesia = 38, India = 172, Kuwait = 10, and Qatar = 5).

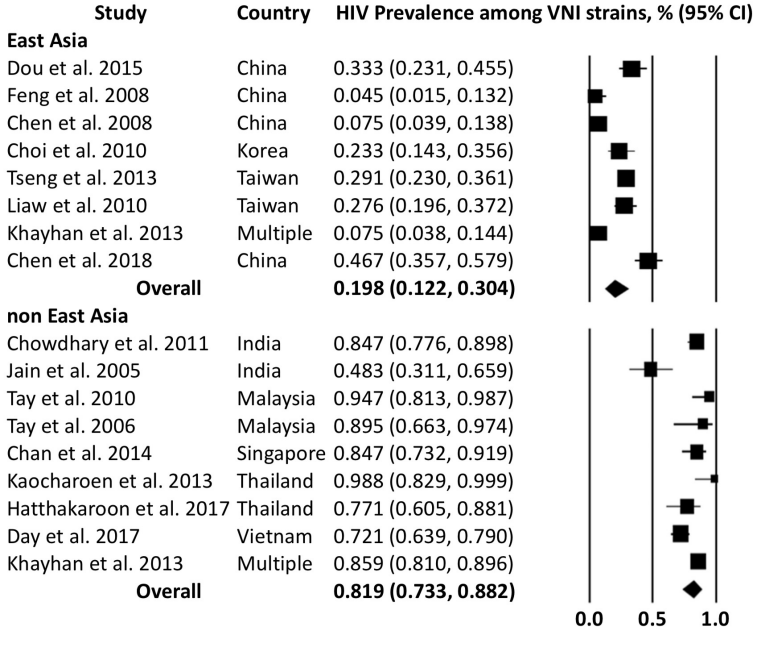


Fig 3. Meta-analysis of HIV prevalence in VNI infected patients. Khayhan *et al.* contained data of China and Japan for East Asia, and India, Indonesia, Kuwait, Qatar and Thailand for non-East Asia.

TABLE 1. Characteristics of the included studies.

Study	Country of origin	No. of isolates	Study design	Years	Study center types	HIV data coverage (%)	HIV prevalence (%)	Primary typing method
Dou *et al*. 2015*	China	83	Descriptive, retrospective	2007-2013	Multicenter	80.7	32.8	MLST
Feng *et al*. 2008	China	115	Descriptive, retrospective	1976-2007	Multicenter	67.8	3.8	M13
Chen *et al*. 2008*	China	129	Descriptive, retrospective	1980-2006	Multicenter	100.0	8.6	M13
Chowdhary *et al*. 2011	India	160	Descriptive, retrospective	2002-2009	Multicenter	85.6	84.7	M13
Jain *et al*. 2005	India	57	Descriptive, retrospective	before 2005	Single center	61.4	40	URA5
Choi *et al*. 2010*	Korea	78	Descriptive, retrospective	1990-2008	Multicenter	79.5	22.6	M13
Tay *et al*. 2010	Malaysia	96	Descriptive, retrospective	2003-2004	Multicenter	39.6	94.7	URA5
Tay *et al*. 2006	Malaysia	78	Descriptive, retrospective	1980-2003	Multicenter	30.8	87.5	URA5
Chan *et al*. 2014	Singapore	62	Descriptive, retrospective	1999-2007	Single center	100.0	80.6	URA5
Tseng *et al*. 2013	Taiwan	219	Descriptive, retrospective	1997-2010	Multicenter	89.0	27.7	M13
Liaw *et al*. 2010	Taiwan	100	Descriptive, retrospective	1994-2004	Single center	98.0	27.6	M13
Kaocharoen *et al*. 2013	Thailand	386	Descriptive, retrospective	1993-2005	Multicenter	13.5	88.5	M13
Hatthakaroon *et al*. 2017*	Thailand	51	Descriptive, retrospective	2012-2014	Single center	68.6	77.1	MLST
Day *et al*. 2017*	Vietnam	151	Descriptive, retrospective	1996-2010	Single center	90.1	72.1	MLST
Khayhan *et al*. 2013*	Multiple**	476	Descriptive, retrospective	1983-2009	Multicenter	76.1	62.7	MLST
Chen *et al*. 2018*	China	86	Descriptive, retrospective	2016-2017	Single center	87.2	46.7	MLST

*ST5/HIV data available, **China, India, Indonesia, Japan, Kuwait, Qatar, Thailand

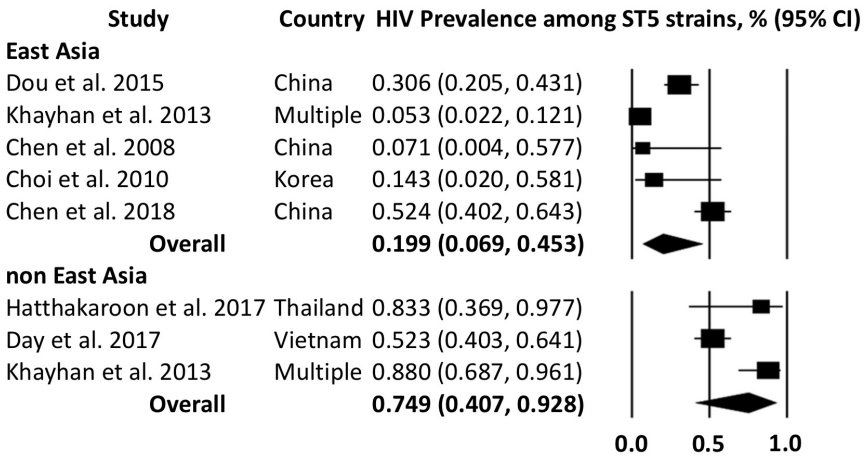


Fig 4. Meta-analysis of HIV prevalence in ST5 infected patients. Khayhan *et al.* contained data of China for East Asia, and Qatar and Thailand for non-East Asia.

Cryptococcosis, especially from *C. neoformans* VNI molecular type, has been found to be linked to HIV infection since the HIV pandemic in 1980s.[1,6] Our study confirmed this association. The pooled HIV prevalence among non-East Asia patients with VNI infection was >80%. However, contradictory observations from China and other East Asian countries have been reported since 2008.[3,4,21] Our analysis included the results of those studies and demonstrated a strong link between the VNI molecular type and non-HIV patients in this region with the low pooled prevalence of HIV among VNI-infected cryptococcosis patients (only 19.8%). Although the exact mechanisms behind this observation are not known, few possible explanations include the highly virulent VNI strain of *C. neoformans* and/or the high susceptibility of individuals living in those areas.

The MLST has been introduced and used to study the association between the VNI molecular type and non-HIV patient's status.[8] This technique provides more genotypic details of microorganisms. With this particular technique, ST5 was found to be highly prevalent (86.9%) among the VNI molecular type strains in East Asia.[4,5] This might be implied that ST5 is a highly virulent genotype among VNI molecular type strains and specifically infects non-HIV individuals. In fact, our analysis demonstrated the similar finding that the pooled HIV prevalence among ST5 strains was low in East Asia (19.9%). High pooled HIV prevalence was found in other Asia countries only (74.9%). As one would expect that if the ST5 strains is high virulent, it should be able to infect non-HIV individual regardless of their countries. Therefore, the assumption about the highly virulent genotype causing infection in non-HIV individuals should be omitted.

Another assumption is that people in East Asia might be susceptible to cryptococcosis more than those in other Asian region. A recent environmental study in China revealed that ST5 prevalence in nature was low (6.2%); thereby, one must be highly susceptible to the causal microorganism to be infected. In contrast, ST31 was commonly found in China (66.7%)[27], but rarely caused any diseases (only 1.3% among cryptococcosis in East Asian countries, data not shown). However, it is still unclear whether this high susceptibility is due to genetic background or other factors. Molecular epidemiological studies focused on patient's genetics are still warrant.

Our study suffers from many limitations. First, not all published data provided underlying diseases other than HIV. The non-HIV patients may be immunocompromised because of other conditions. This can be considered as the common pitfall and limitation in evidence-based medicine[28] and may help to explain the extraordinary relationship between the non-HIV patient and cryptococcosis in East Asia. Second, high statistical heterogeneity in this study may arise from many factors, such as difference in methodology and quality of study. Lastly, all of the included studies were descriptive. The overall numbers of isolates/patients in some countries were relatively small and may not be representative to the whole nation of the country. Taken all together, the results of this study should be interpreted with caution.

CONCLUSION

Our results revealed the low HIV prevalence in cryptococcosis patients with VNI and ST5 strains in East Asia. The emergence of high virulence genotype causing disease in non-HIV patient seems to be impossible, because the VNI and ST5 strains were mostly associated with HIV patients in other Asian regions. Therefore, the high

susceptibility to cryptococcosis of people living in East Asia may be caused by the low HIV prevalence among VNI and ST5 strains in this geographic area. However, mechanism of this possibly high susceptibility requires further investigations.

Disclosure of potential conflicts of interest

The authors indicate full freedom of manuscript preparation and no potential conflicts of interest.

REFERENCES

1. Kwon-Chung KJ, Fraser JA, Doering TL, Wang Z, Janbon G, Idnurm A, et al. *Cryptococcus neoformans* and *Cryptococcus gattii*, the etiologic agents of cryptococcosis. Cold Spring Harb Perspect Med 2014;4:a019760.
2. Kwon-Chung KJ, Saijo T. Is *Cryptococcus gattii* a Primary Pathogen? J Fungi (Basel) 2015;1:154-67.
3. Choi YH, Ngamskulrungroj P, Varma A, Sionov E, Hwang SM, Carriconde F, et al. Prevalence of the VNIc genotype of *Cryptococcus neoformans* in non-HIV-associated cryptococcosis in the Republic of Korea. FEMS Yeast Res 2010;10:769-78.
4. Chen J, Varma A, Diaz MR, Litvintseva AP, Wollenberg KK, Kwon-Chung KJ. *Cryptococcus neoformans* strains and infection in apparently immunocompetent patients, China. Emerg Infect Dis 2008;14:755-62.
5. Chen YH, Yu F, Bian ZY, Hong JM, Zhang N, Zhong QS, et al. Multilocus Sequence Typing Reveals both Shared and Unique Genotypes of *Cryptococcus neoformans* in Jiangxi Province, China. Sci Rep 2018;8:1495.
6. Meyer W, Castaneda A, Jackson S, Huynh M, Castaneda E, IberoAmerican Cryptococcal Study G. Molecular typing of IberoAmerican *Cryptococcus neoformans* isolates. Emerg Infect Dis 2003;9:189-95.
7. Khayhan K, Hagen F, Pan W, Simwami S, Fisher MC, Wahyuningsih R, et al. Geographically structured populations of *Cryptococcus neoformans* Variety *grubii* in Asia correlate with HIV status and show a clonal population structure. PLoS One 2013;8:e72222.
8. Meyer W, Aanensen DM, Boekhout T, Cogliati M, Diaz MR, Esposto MC, et al. Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii*. Med Mycol 2009;47:561-70.
9. Mihara T, Izumikawa K, Kakeya H, Ngamskulrungroj P, Umeyama T, Takazono T, et al. Multilocus sequence typing of *Cryptococcus neoformans* in non-HIV associated cryptococcosis in Nagasaki, Japan. Med Mycol 2013;51:252-60.
10. Hatthakaroon C, Pharkjaksu S, Chongtrakool P, Suwannakarn K, Kiratisin P, Ngamskulrungroj P. Molecular epidemiology of cryptococcal genotype VNIc/ST5 in Siriraj Hospital, Thailand. PLoS One 2017;12:e0173744.
11. Swartz MK. The PRISMA statement: a guideline for systematic reviews and meta-analyses. J Pediatr Health Care 2011;25:1-2.
12. Meyer W, Marszewska K, Amirmostofian M, Igreja RP, Hardtke C, Methling K, et al. Molecular typing of global isolates of *Cryptococcus neoformans* var. *neoformans* by polymerase chain reaction fingerprinting and randomly amplified polymorphic DNA-a pilot study to standardize techniques on which to base a detailed epidemiological survey. Electrophoresis 1999;20:1790-9.
13. La Torre G, Chiaradia G, Gianfagna F, De Laurentis A, Boccia S, Ricciardi W. Quality assessment in meta-analysis. Ital J Public Health 2006;3:44-50.
14. Mathes T, Kuss O. A comparison of methods for meta-analysis of a small number of studies with binary outcomes. Res Synth Methods 2018;9:366-81.
15. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557-60.
16. Chowdhary A, Randhawa HS, Sundar G, Kathuria S, Prakash A, Khan Z, et al. In vitro antifungal susceptibility profiles and genotypes of 308 clinical and environmental isolates of *Cryptococcus neoformans* var. *grubii* and *Cryptococcus gattii* serotype B from north-western India. J Med Microbiol 2011;60:961-7.
17. Dou HT, Xu YC, Wang HZ, Li TS. Molecular epidemiology of *Cryptococcus neoformans* and *Cryptococcus gattii* in China between 2007 and 2013 using multilocus sequence typing and the DiversiLab system. Eur J Clin Microbiol Infect Dis 2015;34:753-62.
18. Feng X, Yao Z, Ren D, Liao W, Wu J. Genotype and mating type analysis of *Cryptococcus neoformans* and *Cryptococcus gattii* isolates from China that mainly originated from non-HIV-infected patients. FEMS Yeast Res 2008;8:930-8.
19. Tay ST, Lim HC, Tajuddin TH, Rohani MY, Hamimah H, Thong KL. Determination of molecular types and genetic heterogeneity of *Cryptococcus neoformans* and *C. gattii* in Malaysia. Med Mycol 2006;44:617-22.
20. Tay ST, Rohani MY, Hoo TS, Hamimah H. Epidemiology of cryptococcosis in Malaysia. Mycoses 2010;53:509-14.
21. Kaocharoen S, Ngamskulrungroj P, Firacative C, Trilles L, Piyabongkarn D, Banlunara W, et al. Molecular epidemiology reveals genetic diversity amongst isolates of the *Cryptococcus neoformans/C. gattii* species complex in Thailand. PLoS Negl Trop Dis 2013;7:e2297.
22. Jain N, Wickes BL, Keller SM, Fu J, Casadevall A, Jain P, et al. Molecular epidemiology of clinical *Cryptococcus neoformans* strains from India. J Clin Microbiol 2005;43:5733-42.
23. Chan M, Lye D, Win MK, Chow A, Barkham T. Clinical and microbiological characteristics of cryptococcosis in Singapore: predominance of *Cryptococcus neoformans* compared with *Cryptococcus gattii*. Int J Infect Dis 2014;26:110-5.
24. Tseng HK, Liu CP, Ho MW, Lu PL, Lo HJ, Lin YH, et al. Microbiological, epidemiological, and clinical characteristics and outcomes of patients with cryptococcosis in Taiwan, 1997-2010. PLoS One 2013;8:e61921.
25. Liaw SJ, Wu HC, Hsueh PR. Microbiological characteristics of clinical isolates of *Cryptococcus neoformans* in Taiwan: serotypes, mating types, molecular types, virulence factors, and antifungal susceptibility. Clin Microbiol Infect 2010;16:696-703.
26. Day JN, Qihui S, Thanh LT, Trieu PH, Van AD, Thu NH, et al. Comparative genomics of *Cryptococcus neoformans* var. *grubii* associated with meningitis in HIV infected and uninfected patients in Vietnam. PLoS Negl Trop Dis 2017;11:e0005628.
27. Dou H, Wang H, Xie S, Chen X, Xu Z, Xu Y. Molecular characterization of *Cryptococcus neoformans* isolated from the environment in Beijing, China. Med Mycol 2017;55:737-47.
28. Pitak-Arnnop P, Hemprich A, Pausch NC. Evidence-based oral and maxillofacial surgery: some pitfalls and limitations. J Oral Maxillofac Surg 2011;69:252-7.

Clinical Application of Toothbrush Technique for Specimen Collection of Tinea Capitis

Lalita Matthapan, B.Sc.*, Sumanas Bunyaratavej, M.D.*, Charussri Leeyaphan, M.D.*, Chuda Rujitharanawong, M.D.*, Pichaya Limphoka, M.D.*, Waranyoo Prasong, B.Sc.*, Chulaluk Komoltri, DrPh.**

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

ABSTRACT

Objective: To demonstrate the efficacy of toothbrush culture test. Moreover, influential factors which affected the success of these methods were analyzed.

Methods: To retrospectively analyze clinical presentation and investigation data of 59 Buddhist novices who diagnosed TC. The laboratory data of the toothbrush technique was reviewed and the efficacy of this technique was investigated.

Results: The efficacy of toothbrush technique was demonstrated as 49.2% by comparing to the standard method. The analysis proved that more than 15% area TC involvement of scalp group achieved higher positive fungal culture outcome to be 65.5 % (p-value = 0.013). In the aspect of the clinical presentation, the effectiveness of the hairbrush technique significantly increased in the gray patch TC group (p-value 0.024). For the reason that the toothbrush technique demonstrated statistically higher efficacy in a group of patients with > 15 % gray patch area involvement (p-value = 0.007).

Conclusion: The toothbrush technique was a good cooperation method that was recommended to apply in TC patients who presented as gray-patch type and more than 15% scalp area involvement.

Keywords: Tinea capitis; ectothrix dermatophyte infection; gray-patch type; toothbrush technique; hair-plucking technique (Siriraj Med J 2019;71: 165-167)

INTRODUCTION

Tinea capitis (TC) is a contagious condition that commonly diagnosed in children due to immature sebum production.[1] Outbreaks of TC usually especially occurred in poor personal hygiene and low socioeconomic area. TC is reported in several areas in the world. Interestingly, the difference of causative organisms is demonstrated in the geographic region.[2] For example, *Trichophyton tonsurans* is the most reported species in the United States.[3] The variety of etiologic organisms in different countries in Asia is demonstrated. *Trichophyton violaceum* is commonly found in India,[4] whereas, *Microsporum canis* is the main causative organism in Thailand.[5] Moreover, the crossover organism is detected in each outbreak. Asymptomatic carriers are assumed as a major role in the spread of infection.[6] The carrier is defined as an individual who has dermatophyte-positive scalp culture without any appearance of TC.[7] Clinical presentations of TC depend on the degree of hair invasion and inflammation that varies from a scaly noninflamed lesion to erythematous lesions with permanent alopecia. Ectothrix remains a dermatophyte infection that confined to hair surface. In contrast, endothrix refers to the invasion of dermatophyte into the hair shaft and internalize into the hair cell. Practically, clinical presentations are categorized into 4 types as follows: (1) gray-patch type: single or multiple scaly patches with lack of inflammation, (2) black dot type: broken hair at the level of the scalp on nonscarring

Corresponding author: Chulaluk Komoltri
E-mail: chulaluk.kom@mahidol.ac.th
Received 15 November 2017 Revised 3 August 2018 Accepted 11 September 2018
ORCID ID: http://orcid.org/0000-0001-8620-1126
http://dx.doi.org/10.33192/Smj.2019.25

alopecia (3) kerion: intense inflamed plaques on scarring alopecia and (4) favus: chronic inflammation with thick yellow crusts on scarring alopecia.[4] In the mycological laboratory, standard investigation of TC is a microbial culture from a specimen obtained by hair-plucking technique. This technique was collecting infected hairs and scraping scale by a sharp blade.[8] However, pain usually was the major complaint of patients of the standard technique, furthermore, the panic of the blade was observed in children. Owing to disadvantages, non-invasive techniques such as cotton swab and toothbrush were developed to collect infected scalp scales.[9,10]

Toothbrush technique was gently performed on the suspected area by sterile toothbrush for scale scratching and then pressed onto the surface of sabouraud dextrose agar (SDA) culture medium, consequently. However, studies about the toothbrush technique were limited in number. Accordingly, the main purpose of the study is to determine the efficacy of the toothbrush technique, comparing with standard technique focusing on specimen collection in TC patients. Duration and satisfaction on specimen collection were also analyzed.

MATERIALS AND METHODS

Clinical and laboratory data of 59 Buddhist novices who confirmed TC diagnosis by the standard technique were reviewed in this retrospective study. This study was approved by Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital (Si 624/2014) and consent from the Royal Thai Monastery in India. Demographic and clinical data of novices whom toothbrush technique was used to collect specimens concurrently with standard method were included in the analysis. Efficacy of toothbrush techniques was reviewed and applicable situations were explored.

In this study, statistical analysis was achieved by Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA) version 22. To compare the efficacy of the toothbrush technique with the standard technique was analyzed by Mann-Whitney U test. Additionally, Fisher's exact test was applied to identify significant factors that influence the efficacy of the toothbrush technique. The reliability of all statistical significance was demonstrated with a p-value that was less than 0.05.

Specimen collection

All participants have performed scalp examination to detect broken hairs and scaling. All the lesions were cleansed with 70% alcohol. Then, both of standard technique and toothbrush technique was applied to collect a specimen from all area of the scalp with the addition of a suspected area such as an area having the scale or broken hair until obtaining an adequate amount of specimen.[10] After that, the specimen was placed onto the medium and continue the incubation process. The evidence of growth was recorded in the patient's medical history. The laboratory data were enrolled in this study to further analysis.

RESULTS

All of Fifty-nine of Buddhist novices with TC, the mean age (SD) of patients was 11.6 (1.9) years. All cases had positive fungal culture from standard technique including hair-plucking technique and scraping scale by the sharp blade to confirmed diagnosis TC. In the aspect of epidemiology, *Trichophyton violaceum* was the major isolated TC pathogen (33 cases, 55.9%). Additionally, *T. mentagrophytes* (27 cases, 45.8%), *Microsporum canis* (21 cases, 35.6%), and *T. tonsurans* (8 cases, 13.6%) were reported respectively. Mixed dermatophyte infection was noticeable in 34 cases (57.6%).

To compare outcomes between toothbrush technique and standard technique, the efficacy of the toothbrush technique to collect a specimen for culture was 49.2% (29 cases). Adjacent areas of scalp including face, neck, ear, and shoulder areas were defined as extended scalp areas. Extended area involvement was associated with more efficacy of toothbrush technique. (p-value = 0.036). In this study, median scalp area involvement in patients with successful toothbrush techniques was 20%, compared with patients whose toothbrush failed to collect a specimen (11.5%). More than 15% area involvement was significantly higher efficacy of this technique that demonstrated by the increased efficacy to be 65.5 % (19 cases, p-value = 0.013).

Clinical presentations were considered and described as follows: (1) gray-patch type (51 cases, 86.4%) (2) black dot type (2 cases, 3.4%) (3) kerion (37 cases, 62.7%). No favus type was reported in this study. Focus on the gray-patch type, scalp area involvement was related with positive toothbrush technique. To demonstrate in the gray-patch group, the median scalp area involvement of positive toothbrush technique patients was 25% while other types of TC were 11.5% (p-value 0.024).

Patients with >15 % gray patch area involvement had a statistically higher probability to obtain a positive fungal culture from the toothbrush technique (p-value = 0.007). Efficacy of toothbrush technique through the patient selection with gray-patch type and more than 15% of scalp area involvement was increasing from 49.2% to 66.7%.

DISCUSSION

Outbreaks of TC were still reported as global health care problems and economic burden. TC remained a common disease in childhood. To confirm the diagnosis of TC, positive fungal infection in hair or scalp was performed by hair-plucking technique and scraping scale by a sharp blade which was the standard technique. However, most children are not well-cooperated due to pain and panic. Previous studies reported noninvasive techniques including hairbrush, cytobrush and toothbrush techniques. Toothbrush technique was performed by sterile toothbrush and rub it in gently on the affected area. This technique was preferred to apply in asymptomatic carrier patients.[11] According to this study, the toothbrush technique was also practical in patients with symptomatic TC, however, the patient selection by clinical presentation is considered as the important points. This study showed that patients with gray patch and extended scalp area involvement were most suitable for the toothbrush technique.

The clinical presentations were categorized into 2 groups by the severity of inflammation. The gray-patch and black dot type were classified to be a non-inflammatory group of infection. Owing to the severe inflammation, the kerion and favus were labeled as an inflammatory group. Gray-patch type predominantly demonstrated scaling with lack of inflammation that was consistent with ectothrix TC. For this reason, it was appropriate to apply with the toothbrush technique for collecting scale. More than that this type is the most common presentation of TC, therefore it is practicable to use in screening and in outbreaks period. Moreover, this technique was simple and painless therefore it may easy to apply in children. To compare with other endothrix types including black dot and kerion types, the toothbrush technique results in unfavorable outcomes due to the inability to detect the fungal penetration in the hair shaft. Owing to the fact that the hair-plucking and scraping scale by sharp blade technique was more recommended to be performed for endothrix TC investigation.

Other important factor in the toothbrush technique was scalp area involvement. The previous study mentioned that the greater surface area involves, the higher efficacy of hairbrush technique significantly increases.[10] However, the experiment to set suitable conditions for the toothbrush technique has been never established. As stated in the results of the toothbrush technique in this study, more than 15% scalp area involvement of TC was suitable for practice. Accordingly, the efficacy of the toothbrush technique depended on patient selection in the aspect of clinical presentation and scalp area involvement. Thus the patients with gray-patch presentation and more than 15% scalp area involvement were suggested to confirm TC diagnosis by toothbrush technique, especially in outbreaks. Furthermore, laboratory technicians mostly agreed with the toothbrush technique was more appreciative and comfortable in practice than the standard technique. Time-saving on specimen collection and good patient's cooperation were reasonable to be the advantages of this technique. Furthermore, time for fungal culture process was not different between the toothbrush technique and standard technique.

In conclusion, the toothbrush technique was recommended to practice with "gray-patch" TC patients who presented more than 15% scalp area involvement due to the time-saving and well-cooperated method.

ACKNOWLEDGMENTS

We would like to express our deepest appreciation to Assistant Professor Dr.Orawan Supapueng who provided us the advice about statistical analysis to complete this report. Moreover, we would also like to show our gratitude to Mr. Chanai Muanprasat for data collection assistance during this research. With respect to the study, all authors report no conflict of interest.

REFERENCES

1. Hay RJ. Tinea capitis: Current status. Mycopathologia 2017;182:87-93.
2. Seebacher C, Bouchara JP, Mignon B. Updates on the epidemiology of dermatophyte infections. Mycopathologia 2008;166:335-52.
3. Price H, Taylor DR. Trichophyton tonsurans (crateriforme) infection of the scalp. Calif Med 1952;76:283-8.
4. Bunyaratavej S, Leeyaphan C, Rujitharanawong C, Muanprasat C, Matthapan L. Clinical and Laboratory Characteristics of a Tinea Capitis Outbreak Among Novice Buddhist Monks. Pediatr Dermatol 2017;34:371-3.
5. Pomeranz AJ, Sabnis SS, McGrath GJ, Esterly NB. Asymptomatic dermatophyte carriers in the households of children with tinea capitis. Arch Pediatr Adolesc Med 1999;153:483-6.
6. Ilkit M, Demirhindi H. Asymptomatic dermatophyte scalp carriage: laboratory diagnosis, epidemiology and management. Mycopathologia 2008;165:61-71.
7. Ali S, Graham TA, Forgie SE. The assessment and management of tinea capitis in children. Pediatr Emerg Care 2007;23:662-5.
8. Williams JV, Honig PJ, McGinley KJ, Leyden JJ. Semiquantitative study of tinea capitis and the asymptomatic carrier state in inner-city school children. Pediatr 1995;96:265-7.
9. Thomas WH, John MT. Brush-Culture Method for Diagnosing Tinea Capitis. Pediatr 1992;90:416-8.
10. Akbaba M, Ilkit M, Sutoluk Z, Ates A, Zorba H. Comparison of hairbrush, toothbrush and cotton swab methods for diagnosing asymptomatic dermatophyte scalp carriage. J Eur Acad Dermatol Venereol 2008;22:356-62.
11. Ilkit M, Demirhindi H. Asymptomatic dermatophyte scalp carriage: laboratory diagnosis, epidemiology and management. Mycopathologia 2008;165:61-71.

Gummy Smile: A Review of Etiology, Manifestations, and Treatment

Basel Mahardawi, B.D.S.*, Teeranut Chaisamut, D.D.S.*, Natthamet Wongsirichat, B.Sc., D.D.S.*,**

**Department of Oral Maxillofacial Surgery, Faculty of Dentistry, Mahidol University, Bangkok 10400, **Walailak University International College of Dentistry, Bangkok 10400, Thailand.*

ABSTRACT

Excessive gingival display, or what is known as Gummy Smile, is a clinical condition which is attracting a great attention and focus recently. Having knowledge and background about the cause and the treatment of each case of excessive gingival display is essential for making the accurate diagnosis and performing the correct procedure. This article reviews the most common causes of excessive gingival display, manifestations of this condition, and some of the important treatment options for Gummy Smile. In addition, the article also puts the light on some points regarding the accepted measurement in several studies, and considers the prediction planning for Gummy Smile associated with a skeletal etiology.

Keywords: Excessive gingival display; gummy smile; vertical maxillary excess (Siriraj Med J 2019;71: 168-174)

INTRODUCTION

A wonderful smile is an essential feature of beauty to which society gives an increasing importance currently. The concept of a nice smile basically depends on the entity of three anatomic components: gum, teeth, and lips.[1] In the meantime, an increasing awareness regarding beauty and physical appearance has become a motivation for every clinician to evaluate the important aspects of patient's smile and link the dynamic relationship between the teeth, gingiva, and lips together when smiling.[2]

The gingiva is a crucial aspect to be considered in the esthetics of smiling[1], in which the upper lip should be elevated around 80% of its normal length, exposing teeth and gingiva.[3] Sharma et al.,[2] defined the normal gingival display as the gum exposure between the inferior border of upper lip and gingival margin of anterior central incisors when smiling. An exposure of gingiva around 0-2 mm when smiling, and 2-4 mm of the maxillary incisor edges when the lips are at the rest state are considered as acceptable. While more than 2 mm of gingival exposure when a person smiles is stated by experts as an excessive gingival display, or what is known as "Gummy Smile",[4] which is more of a descriptive term than a diagnosed condition, and affects a notable proportion of the population, especially women who are affected more than men.[5] Maxillary excess both vertically and anterioposteriorly (Bimaxillary protrusion), hyper mobility of the upper lip due to hyper function of labial elevator muscles, and excessive gingival display associated with altered passive eruption are the three major causes of this condition.[6] In addition to the stated reasons, medications, dental plaque, hereditary and incompetent lip can also result in abnormal gingival display.[5]

For adults, improved aesthetic outcomes are becoming increasingly essential in these procedures to the point where some patients are pursuing only the esthetic side,

Corresponding author: Natthamet Wongsirichat
E-mail: natthamet.won@mahidol.ac.th
Received 25 August 2018 Revised 6 December 2018 Accepted 24 December 2018
ORCID ID: http://orcid.org/0000-0003-3005-2680
http://dx.doi.org/10.33192/Smj.2019.26

not a functional benefit.[7] Therefore, finding an effective treatment for each cause of the gummy smile is a must.

Etiologies of gummy smile:

Many studies have stated the main causes of excessive gingival display, presenting the most important factors which may lead to having a Gummy Smile. The study of Roe et al.,[8] described that lip length and the upper lip mobility rate are the main contributing factors. The previous research of Peck et al.,[9] stated that the exposure of teeth and gingiva depends on the integrated effects of a number of variables (increased muscle capacity, vertical maxillary excess, greater interlabial gap at resting position, and the amount of overjet and overbite). Pausch et al.,[4] mentioned that abnormal gingival and maxillary anterior teeth display may take place due to numerous anatomic or functional factors, either hereditary or inborn. A narrow upper lip, an irregular eruption of teeth, excessive protuberance or vertical maxillary growth, and hypermobility of the maxillary lip and elevator muscle are common reasons for a Gummy Smile.

In fact, several contributing factors are affecting individuals to have a Gummy Smile. Sometimes one of them is presented, although in some cases more than only one cause can be seen. Correct diagnosis of the reason leads to a proper treatment plan. The most common and discussed factors associated with Gummy Smile are:

Altered passive eruption:

Also known as impaired passive eruption,[1] is defined as a condition in which the relationship between teeth, alveolar bone in the maxilla, and the soft tissues displays an excessive gingiva. This, in turn, reveals the clinical characteristic of Gummy Smile.[10] In other words, altered passive eruption (APE) is characterized by excessive gingival exposure in relation to the crowns of the maxillary teeth.[11] In this case, the gingiva fails to migrate in the apical direction during the eruption of teeth, thus, it remains in a coronal position in relation with the cementoenamel junction (CEJ), which results in having an unacceptable gingival exposure and unfavorable small size of the teeth when smiling.[5] Miskinyar et al.,[12] found that the prevalence of this condition is about 12% of the population. Altered passive eruption was first identified by the study of Coslet et al.,[13] and according to Rossi et al.,[10] it was classified into two types and two subtypes (Fig 1):

1. *Type I* : the vertical length of keratinized gingiva is greater than normal, the mucogingival junction (MGJ) is located in an apical position to the level of the cementoenamel junction (CEJ), and clinical crowns appear shorter.

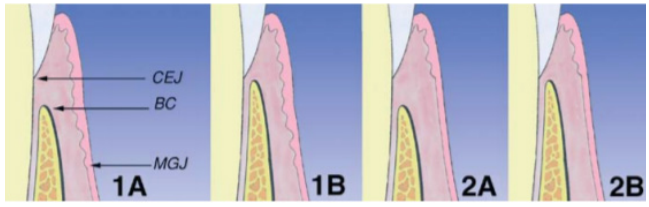


Fig 1. Classification of altered passive eruption (APE)[14]

2. *Type II* : the vertical dimension of the keratinized gingiva is normal, although the mucogingival junction is positioned at the level of the CEJ.

2.1 Subtype A : the measurement between the maxillary alveolar crest and the CEJ is around 1.5 mm, and in this case a regular attachment can be found.

2.2 Subtype B : the level of maxillary alveolar crest is at the level of the CEJ, or occlusal to the CEJ in some cases.

Bony maxillary excess:

This includes vertical maxillary excess and bimaxillary protrusion.

Vertical maxillary excess:

Wolford et al.,[15] defined Maxillary vertical hyperplasia or vertical maxillary excess as an excessive vertical growth of the maxilla which may or may not lead to an anterior open bite. In his study Robbins[16] explained that to make the correct diagnosis, the face must be divided into three equal thirds or parts for the critical evaluation of the height of the face. Vertical maxillary excess is noticed when the length of the lower third of the face is more than the other two thirds, causing an excessive gingival display.[5]

In the analysis of the face, vertical maxillary excess has the following features: longer lower third of the face, in relation with the upper and middle thirds, a greater display of maxillary incisors at rest, an incompetent lip, inclination towards class II malocclusion with or without open bite, and a noticeable Gummy Smile. The nose is longer as well, the alar bases are small and the zygoma appears to be generally flat. The lower third of the face is long, leading to a retrognathic shape of the jaw.[17] Furthermore, the incisal edge of the upper anterior teeth might be covered by the lower lip because of the extravagant downward growth of the maxilla.[16] Peck et al.,[9] and Mackley[18] found that a Gummy Smile is highly related to anterior vertical maxillary excess (about 2-3 mm). Moreover, Ezquerra and Berrazueta[19] discuss that excessive maxilla associated with the protrusion of anterior alveolar bone consequently produce a Gummy

Smile. Whilst Wu et al.,[6] in their study also stated that anterior maxillary height (upper incisor to the palatal plane) was considerably more in both male (+1.03 mm) and female (+2.13 mm) groups who have Gummy Smile.

Bimaxillary protrusion:

Bimaxillary protrusion is a frequently diagnosed deformity in African Americans[20] and Asian populations as well. This deformity is described regarding the protrusive and buccally positioned maxillary and mandibular incisors, as well as the enlarged prostration of the lips. It can be found with an incompetent lip, excessive gingival display, mentalis strain with an anterior open bite. Bimaxillary protrusion refers to an abnormally protruded maxillary and mandibular dentoalveolus. Generally, this presents with malocclusion and dentoalveolar flaring of both the maxillary and mandibular anterior teeth, which cause the lips to be protruded, thus, producing an additional convexity of the facial profile. Bimaxillary protrusion is mainly accompanied by several degrees of lip deficiency (defined as more than 4 mm of lip detachment at the rest state). The tendency of the anteroposterior correlation is to be a class II malocclusion with a deficient mandible, although it may also range from severe class II to class III.[21]

The etiology behind bimaxillary protrusion is associated with various reasons between genetics and environment, such as mouth breathing, lip biting habit and large size of the tongue.[22]

Keating et al.,[23] discovered that in Caucasians with bimaxillary protrusion, it is likely to notice a posterior cranial base which is shorter than normal, a prognathic maxilla with vertical excess, mild class II skeletal occlusion, and a prostrate profile.

Conditions causing gingival enlargement

Gingival enlargement might be the outcome of bacterial plaque-enhanced chronic inflammation and medications.[5] Narwal et al.,[24] showed in their case report an abnormal unilateral palatal soft tissue enlargement in a 61-year-old hypertensive female using amlodipine, which is a safe antihypertensive drug. Ritchhart et al.,[25] stated a process behind uncontrolled cell proliferation in drug-induced gingival overgrowth which is the suppression of apoptotic pathways, with the following effect on the normal gingival formation by cell turnover. Hormonal differences which take place in pregnancy and puberty, in addition to the use of oral contraceptives have been associated with gingival overgrowth.[5] Other effects such as age, demographic and pharmacokinetic changes, genetic predisposition, oral hygiene condition, as well as molecular and cellular variables may impact the mechanism of gingival enlargement.[25, 26] Orthodontic treatment using orthodontic appliances can also participate in the presence of gingival enlargement.[27] In the case of leukemia, leukemic cells might infiltrate to the gingiva,[28] and this will cause some manifestations to be present such as gingival enlargement and bleeding.[29]

Short upper lip

The upper lip length is defined as the length from the base of the nose (subnasale point) to the inferior part of the upper lip (upper lip stomion point),[8] which is normally around 23 mm in males and 20 mm in females.[3] If an individual with excessive gingival display has a short upper lip as well, the esthetic compromise will be affected.[16] The diagnosis of an excessive gingival display can be done regarding the clinical measurement of the upper lip length with an excessive amount of tooth display at rest.[5] Conversely, Roe et al.,[8] found in their study that in maximum smile, there is no notable difference in the gingival display between an individual with a short or normal upper lip, and they concluded that the association of some factors such as higher muscle capacity, vertical maxillary excess, excessive interlabial gap at rest, and the amount of overjet and overbite have a greater effect on the gingival display than the upper lip length itself. Moreover, Sarver et al.,[30] explained that what is crucial is the correspondence between the upper lip length, the maxilliary incisors, and the commissure of the mouth. In other words, the lip length should be almost equal to the commissure of the mouth.

Hypermobility of the upper lip:

In the case of normal face height, gingival levels, lip length and length of central incisors in a patient who has an excessive gingival display, the possible etiology is hypermobility of the maxillary upper lip.[16] Upper lip mobility was explained as the volume of lip movement that exists when an individual smiles. This was measured by deducting the incisal shown at rest from the dentogingival display during a full smile.[8] Hypermobility of the upper lip is associated with a hyper function of the lip elevator muscles and basically leads to excessive gingival display.[19]

Peck and Peck[31] reported an average lip movement of 5.2 mm (23% decrease) from a measured lip length of 22.3 mm during a full smile. Furthermore, in a study evaluating spontaneous smiles, Tarantili et al.,[32] identified a 28% decrease in the initial upper lip length, while Roe et al.,[8] concluded the overall means of lip mobility for females with normal lip length, females with short lip length, males with normal lip length and males with

short lip length were 5.8 ± 1.7 mm, 5.0 ± 1.3 mm, 6.7 ± 1.5 mm, and 5.7 ± 1.1 mm, respectively. Moreover, Sabri et al.,[3] stated that in smiling, the upper lip is elevated by about 80% of its original length. Bhola et al.[5], as well as Robbins[16] stated that the upper lip is generally elevated around 6-8 mm from the rest position to the position reached when a full smile takes place.

Treatment of gummy smile:

Excessive gingival display can be managed by a variety of procedures. These procedures include non-surgical and surgical methods. The underlying cause of excessive gingival display or Gummy Smile has the main effect on the type of procedure that will be performed.[33] Non-surgical procedures may include Botulinum toxin type A injection as well as orthodontics, While surgical procedures might include lip repositioning or orthognathic surgery following orthodontics.

Robbins[16] defined the healthy gingiva as having a minimal length of approximately 3 mm from the gingival crest to the alveolar crest. In the case of altered passive eruption, a higher amount will exist, and the treatment of choice is normally gingivectomy following by aesthetic crown lengthening to attain the desired dimension and morphology of teeth. However, when the diagnosis shows bone levels close to the CEJ, a gingival flap with ostectomy is performed, or what is also known as an apically positioned flap.[34] Orthodontic eruption or intrusion can also be done when having gingival asymmetry on one or multiple anterior teeth[34]

In the case of vertical maxillary excess, the only treatment option to consider is orthognathic surgery.[11] This is applied to impact the maxilla considering the amount of gingival exposure diagnosed. Maxillary impaction allows correction of the Gummy Smile, long face syndrome, specific types of open bite from a skeletal origin and labial sealing.[35] The Le Fort I osteotomy of the maxilla allows reduction of bone between the nasal floor and apices of maxillary teeth which permits superior repositioning (impaction) of the maxilla.[17]

When having bimaxillary protrusion, the treatment option may be composed of first premolar extractions followed by the osteotomy through the extraction sites to mobilize the anterior segment of the maxilla. The aim is to setback the segment in addition to lessening the labial flaring of the incisors. Maxillary setback alone can sometimes provide a substitute for the treatment of anterioposterior maxillary excess without any need for tooth extraction or segmental osteotomy in these cases:

(1) it is contraindicated to perform extractions in an orthodontic diagnosis (no dental crowning, adequate curve of Spee, etc.),

(2) the proclination of maxillary incisors can be adjusted to an acceptable position with a Le Fort I osteotomy in addition to clockwise rotation.[21]

In the presence of external factors causing gingival overgrowth, the treatment plan should be focused on the exact cause of the enlarged gingiva. Meticulous history taking, in addition to an excisional/incisional biopsy and/or hematologic/histologic inspection might be performed generally to make the correct diagnosis of the uncommon conditions of gingival enlargement. Plaque control is an important aspect of treatment in all the patients. Some of these cases may resolve when the external element that is inducing the gingival enlargement is adjusted or suspended, For example, gingival overgrowth during pregnancy and puberty might need the elimination of all local irritants followed by surgical treatment for the removal of any fibrotic residuals.[36]

For the excessive gingival display resulted from short upper lip and/or hypermobility of the upper lip, a surgical procedure known as lip repositioning surgery is preferred for a minimally invasive surgery. It is composed of an oval mucosal excision followed by coronally advanced flap. This procedure is done to reduce the hyperactivity of the elevator muscles and reform the depth of the vestibule.[37] It was first described by Rubinstein and Kostianovsky[38] and the aim was the treatment of excessive gingival display associated with hypermobility of the lip, and was then modified by Litton and Fournier[39] to include also the treatment of Gummy Smile caused by short upper lip, by separating the muscles from the underlying bony structures to place the upper lip in a coronal position. This surgical procedure was accompanied with no complications, although there were some incidences of relapse,[40] consequently, some attempts were done to improve it by Miskinyar.[12] Recently, Bhola et al.,[5] described a technique similar to the one which was described by the article of Rubinstein and Kostianovsky under the name of Lip Stabilization Technique (LipStaT). The only obvious difference between the two techniques is that in LipStaT, a vertical incision is done posteriorly to connect the inferior incision (at the mucogingival junction) and the superior incision (into the vestibule). The ratio of this incision is the height being double of gingival exposure during a full smile. While in Rubinstein and Kostianovsky technique, the two incisions were approximated till they meet posteriorly.

Currently, another nonsurgical treatment option is the injection of Botulinum toxin type A which has been suggested for treatment of hypermobility of the upper lip, but this may only provide temporary advantages.[41]

Polo[41] reported a relapse of around 2 mm after 24 weeks of following up. This was similar for Indra et al.,[42] by reporting a relapse of treatment in the third month. Yet it is still a viable option for those who do not prefer undergoing surgeries, and need only a minimal treatment procedure.

DISCUSSION

It was stated recently that the dimension and visibility of teeth, and upper lip position were crucial effects when judging smile attractiveness.[43] Many studies described the acceptable gingival exposure and the concept of Gummy Smile (table below). Having an acceptable smile in the eyes of other people is, in fact, a subjective view, because the perception of a nice smile has differed from country to country, from study to study, and even when males or females are evaluating the smile. In other words, a study showed that the male raters differed in respect of gender specificity when judging the attractiveness of the smile, while female raters rated males less gender specific.[4] Therefore, no reliable or unified measurements were stated in the literature regarding a clear description of having an attractive smile or when it is diagnosed as a Gummy Smile. Although the different visions of an accepted gingival exposure were evaluated in different countries, they should be identical for the same ethnic origins. Sabri et al[3] concluded that an ideal smile has the features of an upper lip that will reach the gingival edges when smiling, in addition to an upward or straight curvature through the philtrum and commissures; with maxillary incisal margins parallel to the lower lip line; with no lateral negative space or minimal if it presents; and a commissural line, as well as an occlusal frontal plane parallel to the line drawn through the pupils of the eyes; and attractive integration of gingiva and teeth. The acceptable amount of gingival exposure when smiling was mentioned in several studies. For instance, Kokich et al.,[44] reported noticeably unattractive lip to gingiva distance when the exposure is 4 mm by laypersons and when it is 2 mm by orthodontists, while Geron and Atalia[45] accepted only 1 mm of gingival display when smiling. Pausch et al.,[4] concluded in their study that a 0- to 2-mm exposure of the maxillary gingiva was acceptable when an individual smiles. In addition to that, they found that although the Gummy Smile is unattractive, it is much better than invisible or minimally visible maxillary teeth when smiling. Bhola et al.,[5] explained that affected by the increased aesthetic concerns, a gingival exposure of more than 1 mm during smiling might be diagnosed as excessive gingival display (EGD). Van der Geld et al.[43] concluded in their study that a lip line height that exposed more than 4 mm of gingival display was considered as a Gummy Smile line. Jannani et al.[46] stated that a smile is introduced as attractive when the maxillary teeth are entirely shown in addition to approximately 1 mm of gingival display. However, a gingival display not more than 2-3 mm is also contemplated as acceptable, while a disproportionate exposure (>3 mm) is basically stated as not pleasant. Pinto et al.,[11] mentioned three kinds of a smile: high, medium and low, and the high smile is considered acceptable when accompanied with exposed gingiva of 1 to 3 mm.

Moreover, treatment of Gummy Smile was being practiced many years ago. Several techniques and procedures were developed to reduce the excessive gingival display and change it into an aesthetic, acceptable appearance. Taking the bony maxillary excess into consideration, a review of the literature may conclude that although the treatment of Gummy Smile caused by vertical maxillary excess and/or bimaxillary protrusion is not a new procedure, no studies have made a clear treatment plan, and no one has described a step by step way to predict the treatment according to a certain prediction plan before performing orthognathic surgery, based on clear measurements of moving the maxilla. Thus, a thorough prediction and movement plan of the maxilla for Gummy Smile treatment might be of a great value to help surgeons establish an accurate treatment plan based on scientific data, not only experience. The acceptable gingival exposure and the diagnosis of Gummy Smile in some previous studies is shown in Table 1.[4,5,11,43-46]

CONCLUSION

Gummy Smile is a condition in which an increasing awareness has been noticed recently. Therefore, it is attracting more attention from all the clinicians to find the best solutions with the least complications and relapse, as well as the most satisfying results for every patient. Additional unified standards of accepting and not accepting the amount of gingival exposure might be better to help with diagnosing and deciding whether to perform surgery or consider the gingival exposure as acceptable. This should be decided considering the ethnic origin and trying to collect a wider range of opinions, not only taking experts' view because they will judge more critically. This could be done by performing a greater statistical analysis regarding this topic, including more people to evaluate and judge.

In addition, clear prediction planning of moving the maxilla to improve the condition of Gummy Smile, taking into account if it is resulted from vertical maxillary excess alone or with bimaxillary protrusion, would be of a great benefit to perform a well-planned surgery. This

TABLE 1. Acceptable gingival exposure and the diagnosing of Gummy Smile in some studies in the literature.

Authors	Year	Acceptable gingival exposure	Having a Gummy Smile
Kokich et al[44]	1999	Less than 4 mm by laypersons Less than 2 mm by orthodontists	4 mm or more by laypersons 2 mm or more by orthodontists
Geron and Atalia[45]	2005	Untill 1 mm	More than 1 mm
Van der Geld et al.[43]	2011	Untill 4 mm	More than 4 mm
Jannani et al.[46]	2014	2-3 mm	More than 3 mm
Pinto et al.[11]	2015	1-3 mm	More than 3 mm
Bhola et al.[5]	2015	Untill 1 mm	More than 1 mm
Pausch et al.[4]	2017	0-2 mm	More than 2 mm

surgery should be done according to certain principles and criteria, not depending upon each surgeon's experience or preference. To reach this stratified surgical plan, this literature review suggests further studies to design a prediction formula of moving the maxilla, following certain calculations regarding the amount of vertical maxillary excess, bimaxillary protrusion, x-rays, and considering opinions of experienced surgeons to get the best aesthetic results.

ACKNOWLEDGMENTS

The authors would like to acknowledge the staff, dental assistants, and our colleagues in the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Mahidol University.

Funding: The authors received no funding
Conflict of interest: The authors declared that they have no conflicts of interest.
Ethics approval: Not required

REFERENCES

1. Izraelewicz-Djebali E, Chabre C. Gummy smile: orthodontic or surgical treatment? Dentofacial Anom Orthod 2015;18:102. Published online: 04 September 2015.
2. Sharma A, Sharma S, Garg H, Singhal V, Mishra P. Lip repositioning: A boon in smile enhancement. J Cutan Aesthet Surg 2017;10:219-22.
3. Sabri R. The eight components of a balanced smile. J Clin Orthod 2005;39:155-67.
4. Pausch NC, Katsoulis D. Gender-specific evaluation of variation of maxillary exposure when smiling. J Craniomaxillofac Surg 2017;45:913-20.
5. Bhola M, Fairbairn PJ, Kolhatkar S, Chu SJ, Morris T, de Campos M. LipStaT: The Lip Stabilization Technique- Indications and Guidelines for Case Selection and Classification of Excessive Gingival Display. Int J Periodontics Restorative Dent 2015;35:549-59.
6. Wu H, Lin J, Zhou L, Bai D. Classification and craniofacial features of gummy smile in adolescents. J Craniofac Surg 2010;21:1474-9.
7. Charrier JB. Orthognathic surgery of adults and facial aesthetics. J Dentofacial Anom Orthod 2012;15:302.
8. Roe P, Rungcharassaeng K, Kan JYK, Patel RD, Campagni WV, Brudvik JS. The Influence of Upper Lip Length and Lip Mobility on Maxillary Incisal Exposure. Am J Esthet Dent 2012;2:116-125.
9. Peck S, Peck L, Kataja M. The gingival smile line. Angle Orthod 1992;62:91-100.
10. Rossi R, Brunelli G, Piras V, Pilloni A. Altered passive eruption and familial trait: a preliminary investigation. Int J Dent 2014;2014:874092.
11. Pinto SCS, Higashi C, Bonafé E, Pilatti GL, Santos FA, Tonetto MR, et al. Crown Lengthening as Treatment for Altered Passive Eruption: Review and Case Report. World J Dent 2015;6:178-83.
12. Miskinyar SA. A new method for correcting a gummy smile. Plast Reconstr Surg 1983;72:397-400.
13. Coslet JG, Vanarsdall R, Weisgold A. Diagnosis and classification of delayed passive eruption of the dentogingival junction in the adult. Alpha Omegan 1977;7:24-8.
14. Alpiste-Illueca F. Altered passive eruption (APE): a little-known clinical situation. Med Oral Patol Oral Cir Bucal 2011;16:e100-4.
15. Wolford LM, Karras SC, Mehra P.Considerations for orthognathic surgery during growth, part 2: maxillary deformities. Am J Orthod Dentofacial Orthop 2001;119:102-5.
16. Robbins JW.Differential diagnosis and treatment of excess gingival display. Pract Periodontics Aesthet Dent 1999;11:265-72.
17. Nascimento Meger M, Tiboni F, dos Santos FS, Verbicaro T, Deliberador TM, Scariot R, et al. Surgical correction of

vertical maxillary excess associated with mandibular self-rotation. RSBO 201714:56-61.
18. Mackley RJ. An evaluation of smiles before and after orthodontic treatment. Angle Orthod 1993;63:183-90.
19. Ezquerra F, Berrazueta MJ, Ruiz-Capillas A, Arregui JS. New approach to the gummy smile. Plast Reconstr Surg 1999;104:1143-50.
20. Scott SH, Johnston LE. The perceived impact of extraction. and nonextraction treatments on matched samples of. African American patients. Am J Orthod Dentofacial Orthop 1999;116: 352-8.
21. Chu YM, Bergeron L, Chen YR. Bimaxillary protrusion: an overview of the surgical-orthodontic treatment. Semin Plast Surg 2009;23:32-9.
22. Lamberton CM, Reichart PA, Triratananimit P. Bimaxillary protrusion as a pathologic problem in the Thai. Am J Orthod 1980;77:320-9.
23. Keating PJ. Bimaxillary protrusion in the Caucasian: a cephalometric study of the morphological features. Br J Orthod 1985;12:193-201.
24. Narwal A, Singh V, Bala S. Drug-induced atypical hyperplasia enveloping salivary gland malignancy. J Indian Soc Periodontol 2017;21:409-11.
25. Ritchhart C, Joy A. Reversal of drug-induced gingival overgrowth by UV-mediated apoptosis of gingival fibroblasts - an in vitro study. Ann Anat 2018;217:7-11.
26. Nakib N, Ashrafi SS. Drug-induced gingival overgrowth. Dis Mon 2011;57:225-30.
27. Trossello VK, Gianelly AA. Orthodontic treatment and periodontal status. J Periodontol 1979;50:665-71.
28. Savona M, Talpaz M. Chronic myeloid leukemia: changing the treatment paradigms. Oncology (Williston Park) 2006;20: 707-11.
29. Chowdhri K, Tandon S, Lamba AK, Faraz F. Leukemic gingival enlargement: A case report and review of literature. J Oral Maxillofac Pathol 2018;22(Suppl 1):S77-S81.
30. Sarver DM, Ackerman MB.Dynamic smile visualization and quantification: Part 2. Smile analysis and treatment strategies. Am J Orthod Dentofacial Orthop 2003;124:116-27.
31. McNamara JA. Jr, Brust EW, Riolo ML. Soft tissue evaluation of individuals with an ideal occlusion and a well-balanced face. In: McNamara JA Jr. (Ed.) Esthetics and the Treatment of Facial Form. Monograph 28. Craniofacial Growth Series. Center for Human Growth and Development, Ann Arbor; 1993.
32. Tarantili VV, Halazonetis DJ, Spyropoulos MN.The spontaneous smile in dynamic motion. Am J Orthod Dentofacial Orthop 2005;128:8-15.
33. Abdullah WA, Khalil HS, Alhindi MM, Marzook H. Modifying gummy smile: a minimally invasive approach. J Contemp Dent Pract 2014;15:821-6.
34. Dolt AH 3rd, Robbins JW. Altered passive eruption: an etiology of short clinical crowns. Quintessence Int 1997;28:363-72.
35. Steinhäuser S, Richter U, Richter F, Bill J, Rudzki-Janson I. Profile changes following maxillary impaction and autorotation of the mandible. J Orofac Orthop 2008;69:31-41.
36. Agrawal AA. Gingival enlargements: Differential diagnosis and review of literature. World J Clin Cases 2015;3:779-88.
37. Humayun N, Kolhatkar S, Souiyas J, Bhola M. Mucosal coronally positioned flap for the management of excessive gingival display in the presence of hypermobility of the upper lip and vertical maxillary excess: a case report. J Periodontol 2010;81: 1858-63.
38. Rubinstein AM, Kostianovsky AS. Cosmetic surgery for the malformation of the laugh: Original technique in Spanish. Prensa Med Argent 1973;60:952.
39. Litton C, Fournier P. Simple surgical correction of the gummy smile. Plast Reconstr Surg 1979;63:372-3.
40. Grover HS, Gupta A, Luthra S. Lip repositioning surgery: A pioneering technique for perio-esthetics. Contemp Clin Dent 2014;5:142-45.
41. Polo M. Botulinum toxin type A (Botox) for the neuromuscular correction of excessive gingival display on smiling (gummy smile). Am J Orthod Dentofacial Orthop 2008;133:195-203.
42. Aly LA, Hammouda NI. Botox as an adjunct to lip repositioning for the management of excessive gingival display in the presence of hypermobility of upper lip and vertical maxillary excess. Dent Res J (Isfahan) 2016;13:478-83.
43. Van der Geld P, Oosterveld P, Schols J, Kuijpers-Jagtman AM. Smile line assessment comparing quantitative measurement and visual estimation. Am J Orthod Dentofacial Orthop 2011;139: 174-80.
44. Kokich Jr VO, Kiyak HA, Shapiro PA. Comparing the Perception of Dentists and Lay People to Altered Dental Esthetics. J Esthetic Dentistry 1999;11:311-24.
45. Geron S, Atalia W. Influence of sex on the perception of oral and smile esthetics with different gingival display and incisal plane inclination. Angle Orthod 2005;75:778-84.
46. Jananni M, Sivaramakrishnan M, Libby TJ. Surgical correction of excessive gingival display in class I vertical maxillary excess: Mucosal strip technique. J Nat Sci Biol Med 2014;5:494-8.

A Comparison of the Bacterial Culture Results of Maxillary Sinus Mucosa and Pus Collections for Chronic Maxillary Rhinosinusitis

Anan Bedavanija, M.D.*, Pongsakorn Tantilipikorn, M.D, Ph.D.*, Chetta Banditsing, M.D., Pattarachai Kiratisin, M.D, Ph.D.***, Chaweewan Bunnag, M.D.***

**Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, **Department of Otorhinolaryngology, Bangkok Hospital Rayong, Rayong 21000, ***Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: Although maxillary antral taps are the standard for collecting pus for bacterial culture, they sometimes reveal no growth. Intraoperative mucosal cultures are another method to collect pathogen samples. This study compared aerobic bacterial cultures from maxillary sinus mucosa and pus collected from chronic maxillary rhinosinusitis patients.

Methods: A prospective study of 22 chronic maxillary rhinosinusitis patients was conducted. Antral pus and mucosa collected during endoscopic sinus surgery were immediately sent to a microbiological laboratory. The degree of concordance between maxillary sinus mucosa aerobic bacterial cultures and pus cultures was then analyzed.

Results: Twenty-seven specimens were obtained for the cultures. The proportions of positive mucosal and pus cultures were 40.74% and 51.85%, respectively. The common aerobic pathogens from the two culture techniques were Pseudomonas aeruginosa and Staphylococcus aureus. A concordance between the pus and mucosal cultures was demonstrated by 19 out of 27 specimens (70.37%). Compared with the pus cultures, the mucosal cultures had a specificity of 84.62% (95% CI, 54.55%-98.08%), a sensitivity of 57.14% (95% CI, 28.86%-82.34%), a predictive value of a positive result of 80% (95% CI, 50.83%-93.93%), and a predictive value of a negative result of 64.71% (95% CI, 48.96%-77.80%).

Conclusion: Similar pathogenic bacteria were recovered from the mucosa and pus. Given the high degree of similarity of the bacteria found, the good concordance rate, and the high specificity and positive predictive value of the mucosal cultures compared with the pus cultures, mucosal cultures should be a reference standard and an option when pus is unavailable, especially with immunocompromised patients.

Keywords: Bacterial culture; concordance; chronic rhinosinusitis; maxillary sinus mucosa; pus (Siriraj Med J 2019;71: 95-101)

INTRODUCTION

Rhinosinusitis is a common disease in Thailand. In 2017, the incidence among otolaryngologic outpatients at Siriraj Hospital was about 5%. Obtaining a culture might be useful with patients who have not responded to conventional medical treatment, with immunocompromised patients, and in cases of rhinosinusitis with orbital and intracranial complications. Pus collection from the maxillary sinus by an antral puncture or open sinus surgery is usually used in clinical practice.[1-6] Although a maxillary antral

Corresponding author: Pongsakorn Tantilipikorn
E-mail: ptantili@yahoo.com
Received 12 December 2018 Revised 5 February 2019 Accepted 12 February 2019
ORCID ID: http://orcid.org/0000-0003-1995-4798
http://dx.doi.org/10.33192/Smj.2019.15

tap is the standard method of obtaining a sinus culture, it is invasive and difficult to perform,[1–6] and sometimes reveals no growth. In addition, otolaryngologists cannot always get pus from a maxillary antral tap because of the small amount of pus, or the absence of pus, in the maxillary antrum. In 1979, Karma et al.[7] concluded that an intraoperative mucosal culture is a better method to get true pathogens than collecting pus from the maxillary antrum; that conclusion has since been supported by the findings of other studies.[8-9] The objectives of the present study were to compare aerobic bacterial cultures obtained from the sinus mucosa with those from pus from the antrum of chronic maxillary rhinosinusitis patients.

MATERIALS AND METHODS

A prospective study was conducted at Siriraj Hospital between May 2010 and January 2011. All patients, which considered for inclusion, were more than 18 years old and diagnosed with chronic maxillary rhinosinusitis with medical treatment failure. Before undergoing endoscopic sinus surgery, they had to stop taking any antibiotics for at least one week. The diagnosis of chronic rhinosinusitis was based on the guidelines of the European Position Paper on Rhinosinusitis and Nasal Polyps,[10] i.e., an inflammation of the nose and the paranasal sinuses, characterized by two or more symptoms of nasal obstruction, discolored nasal discharge or postnasal drip, frontal pain/headache, and smell disturbance (one of which should be nasal obstruction or discolored discharge) for more than 12 weeks; and either a positive finding on a nasal endoscopy (nasal polyps and/or mucopurulent discharge from the middle meatus, and/or edema/mucosal obstruction primarily in the middle meatus); and/or mucosal changes within the ostiomeatal complex and/or sinuses on preoperative computed tomography.[10] The study protocol was approved by the Institutional Review Board Committee of the Faculty of Medicine Siriraj Hospital, and informed consent was obtained from every patient. During the endoscopic sinus surgery, immediately after opening the maxillary sinus ostium, antral pus was collected in a sterile bottle by curved suction; 1-2 small pieces of maxillary sinus mucosa was also randomized collected in a sterile bottle using cutting forceps. All specimens were sent to the microbiological laboratory within 10 minutes and immediately incubated on chocolate, blood, and MacConkey agar plates. Bacteria that grew on the culture were reported as semiquantitative identification (rare, few, moderate, and numerous growth). The results of both cultures were compared. Samples were considered to have a strong concordance if the same bacterial organisms were recovered, or if there was no growth and/or commensal flora on both the mucosal and pus cultures. Samples were considered to have a moderate concordance if the predominant organisms recovered from the mucosa were also recovered from the pus, but an additional organism was recovered in small quantities by one of the two methods. Samples were considered to have no concordance if the predominant organisms recovered by the two techniques differed.[11-13]

Statistical analysis

The data were presented as numbers and percentages. The difference in the culture rates for the maxillary sinus mucosa and pus was calculated with McNemar's Chi-square test. Comparisons were made of the results of the bacterial cultures of the maxillary sinus mucosa and pus collections from the chronic maxillary rhinosinusitis patients. The sensitivity, specificity, and positive and negative predictive values of the mucosal cultures were calculated and compared with those of the pus cultures, as the reference standard.

RESULTS

The ages of the 22 adult patients included in this study ranged from 26 to 83 years, with a mean of 53.36 years. There were 11 males and 11 females. Five patients had a bilateral maxillary sinus operation, resulting in 27 paired samples. The positive rates of the mucosal and pus cultures for aerobic pathogenic bacteria represented 40.74% and 51.85% of samples, respectively, as shown in Table 1. The difference in the culture rates for the maxillary sinus mucosa and pus samples was statistically insignificant ($p = 0.4531$, 95% CI: -0.292204 to 0.084671). The aerobic bacterial growths common to both groups were *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Methicillin-susceptible), as shown in Table 2. The maxillary sinus mucosal cultures were strongly concordant with the cultures from pus from the maxillary sinus in 19 out of the 27 aerobic samples (70.37%; Table 1). Two of the 27 samples were considered to have a moderate concordance (7.41%); the additional organism was noted in small quantities in those 2 samples. The mucosal cultures provided a specificity of 84.62% (95% CI, 54.55%–98.08%), a sensitivity of 57.14% (95% CI, 28.86%–82.34%), a predictive value of a positive result of 80% (95% CI, 50.83%–93.93%), a predictive value of a negative result of 64.71% (95% CI, 48.96%–77.80%), a positive likelihood ratio of 3.71 (95% CI, 0.96–14.37), and a negative likelihood ratio of 0.51 (95% CI, 0.27–0.97) compared with the pus cultures from the maxillary sinus (Table 3).

TABLE 1. Types of aerobic bacteria isolated from pus and mucosal cultures from the same chronic maxillary rhinosinusitis patients.

No.	Sex	Age	Diagnosis	Pus culture	Mucosal culture	Concordance
1	Male	64	CRSwNP	-MSSA (moderate) -*Morganella morganii* (few)	-MSSA (few)	+
2	Male (Rt.)	31	CRSwNP	-*P. aeruginosa* strain 1 (moderate) -*P. aeruginosa* strain 2 (few)	-*K. pneumoniae* ESBL-negative (few)	-
3	Male (Lt.)	31	CRSwNP	-*P. aeruginosa* (numerous) -*K. pneumoniae* (rare)	-*P. aeruginosa* (few)	+
4	Female	62	CRSsNP	-Non-fermentative GNR (few)	-NG	-
5	Female	43	CRSsNP	-NG	-NG	++
6	Female	57	CRSsNP	-*P. aeruginosa* (moderate)	-NG	-
7	Male (Rt.)	54	CRSwNP	-*K. pneumoniae* ESBL-negative (few)	-NG	-
8	Male (Lt.)	54	CRSwNP	-*E. coli* ESBL-negative (few)	-*E. coli* ESBL-negative (few)	++
9	Female	75	CRSsNP	-MSSA (moderate)	-MSSA (few)	++
10	Female	83	CRSsNP	-*P. aeruginosa* (few) -Commensal flora (few)	-NG	-
11	Male (Rt.)	62	CRSwNP	-NG	-Commensal flora (few)	++
12	Male (Lt.)	62	CRSwNP	-NG	-NG	++
13	Female	45	CRSsNP	-*P. aeruginosa* (moderate) -Non-fermentative GNR (moderate)	-*P. aeruginosa* (few) -Non-fermentative GNR (few)	++
14	Female	70	CRSsNP	-*P. aeruginosa* (numerous)	-*P. aeruginosa* (rare)	++
15	Female	74	CRSsNP	-*P. aeruginosa* (moderate)	-*P. aeruginosa* (few)	++
16	Male (Rt.)	26	CRSwNP	-NG	-Commensal flora (rare)	++
17	Male (Lt.)	26	CRSwNP	-NG	-NG	++
18	Female	58	CRSsNP	-NG	-NG	++
19	Male (Rt.)	27	CRSsNP	-*Enterobacter aerogenes* (rare)	-NG	-
20	Male (Lt.)	27	CRSsNP	-NG	-*Enterobacter aerogenes* (few)	-
21	Male	61	CRSsNP	-NG	-NG	++
22	Male	46	CRSsNP	-*Pasteurella multocida* -MSSA (few)	-*Pasteurella multocida* -MSSA (few)	++
23	Male	49	CRSsNP	-NG	-NG	++
24	Male	32	CRSsNP	-Commensal flora (few)	-NG	++
25	Female	80	CRSsNP	-NG	-*S. agalactiae* (few) -MSSA (few)	-
26	Male	49	CRSsNP	-NG	-NG	++
27	Female	26	CRSsNP	-NG	-NG	++

N.B.: CRSwNP = chronic rhinosinusitis with nasal polyps; CRSsNP = chronic rhinosinusitis without nasal polyps; NG = no growth; MSSA = Methicillin-susceptible *Staphylococcus aureus*; GNR = Gram-negative rods; *P.aeruginosa* = *Pseudomonas aeruginosa; K. pneumoniae* = *Klebsiella pneumoniae; E. coli* = *Escherichia coli;* ESBL = Extended-Spectrum Beta-Lactamase; *S.agalactiae* = *Streptococcus agalactiae*; Rt. = right; Lt. = left; - = no concordance; + = moderate concordance; ++ = strong concordance

TABLE 2. Common aerobic bacteria isolated from pus and mucosal cultures.

Pathogens	Pus	Mucosa	Pus (Kirtsreesakul V et al.)[12]
Coagulase negative *Staphylococcus aureus*	-	-	8
Pseudomonas aeruginosa	*7*	*4*	*2*
Staphylococcus aureus (Methicillin-susceptible)	3	4	3
Commensal flora	2	2	-
Klebsiella pneumoniae	2	1	2
Non-fermentative gram-negative rods	2	1	-
Escherichia coli	*1*	*1*	*2*
Enterobacter aerogenes	*1*	*1*	*1*
Pasteurella multocida	*1*	*1*	-
Morganella morganii	*1*	*0*	-
Citrobacter diversus	-	-	*1*
Streptococcus agalactiae	0	1	-
Alpha-hemolytic *Streptococcus* not group D	-	-	3
Beta-hemolytic *Streptococcus* not group A, B, D	-	-	1
Micrococcus spp.	-	-	1
No growth	12	14	1

TABLE 3. Comparison of pus and mucosal culture methods.

Culture methods	Positive pus cultures	Negative pus cultures	Total
Positive mucosal cultures	8	2	10
Negative mucosal cultures	6	11	17
Total	14	13	27

Specificity = 84.62%; sensitivity = 57.14%; positive predictive value = 80%; negative predictive value = 64.71%
N.B.: Negative mucosal cultures = no pathogenic bacterial growth or different pathogenic bacterial growth from pus culture

DISCUSSION

The 40.74% rate for the mucosal cultures was not very different from the 51.85% rate for the pus cultures (p = 0.4531, 95% CI: -0.292204 to 0.084671), as shown in Table 1. Similar isolated pathogenic bacteria were recovered from the mucosa and pus, as shown in Table 2. A good concordance rate of 70.37% was achieved for the two aerobic bacterial culture techniques. The mucosal cultures provided a specificity of 84.62, a sensitivity of 57.14%, a predictive value of a positive result of 80%, a predictive value of a negative result of 64.71%, a positive likelihood ratio of 3.71, and a negative likelihood ratio of 0.51 compared with the pus cultures collected by the maxillary antral tap (Table 3).

Antibiotic treatment is essential in patients with chronic rhinosinusitis with acute exacerbation. Otolaryngologists usually treat rhinosinusitis patients by empirical therapy. If patients do not respond to antibiotics, then correct identification of the bacterial organisms is very important. The standard sampling method for sinus cultures is a maxillary sinus tap through the canine fossa or inferior meatus, or open sinus surgery utilizing the Caldwell–Luc approach.[1-6] However, pus collection from the maxillary sinus during surgery is sometimes unsuccessful because

of small pus amounts, an absence of pus in the sinus, or the accidental removal of all pus. The study by Karma et al.[7] found that intraoperative mucosal tissue cultures can be used as one method to get true pathogens and yielded a higher proportion of positive bacterial cultures than positive pus cultures, but the researchers did not compare the types of organisms obtained from the tissue and pus cultures. The current study is probably the first, at least in Thailand, to compare the outcomes of cultures derived from tissue biopsies and pus collected from the maxillary sinus. A good concordance of the bacterial organisms of both groups, as well as a high specificity, and a high positive predictive value in the mucosal group compared to the pus cultures, were found in the present study. Therefore, if we cannot collect pus, a mucosal culture in chronic maxillary rhinosinusitis can be used to identify the pathogenic organisms.

However, the interpretation of mucosal culture data should be made cautiously if a negative culture is obtained because of its low sensitivity and low negative predictive value. The proportion of positive cultures from the sinus mucosa in this study was slightly lower than that from the pus cultures, which contrasts with the earlier study by Karma et al.[7-9] This might be because bacterial pathogens are hidden in the biofilm on the surface of the mucosa and are difficult to culture. Biofilm bacteria are usually found on the surface of the mucosa, but planktonic bacteria are found in pus content. Biofilms of *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae*, and *Haemophilus influenzae* are commonly found in patients with chronic rhinosinusitis that is resisting antibiotic treatment.[14-18] Biofilm bacteria metabolize more slowly, reproduce less frequently, and show different phenotypes than planktonic bacteria, and standard microbiological culture techniques are incapable of identifying all species present in pus cultures.[19] Nevertheless, since the mucosal cultures in the present study showed a good specificity and positive predictive value, mucosal cultures may be used as a guide to the prescribing of target antibiotics for chronic rhinosinusitis patients who do not have any pus in their maxillary sinuses.

We have applied this finding in our department, especially with immunocompromised patients, e.g., those with hematologic malignancy who have chronic rhinosinusitis with acute exacerbation. With such cases, the active sinus infection tends to progress rapidly with a high risk of serious complications. Therefore, the need for sinus surgery is usually indicated early in order to restore drainage and obtain pus for culture because correct antibiotics selection is very important. However, as such patients sometimes have no, or only a small amount of, pus in the maxillary sinus, especially during chemotherapy, a mucosal culture can be used instead. Moreover, we can use this method with other chronic rhinosinusitis patients with acute exacerbation if a pus specimen is not available.

The common pathogens were the same in both groups, as shown in Table 1&2. This finding is another reason why a mucosal culture is a good alternative method for identifying causative pathogens. In a comparison with other studies in Thailand as well as with a recent systematic review by Thanasumpun and Batra[23] (Table 4), the majority of causative aerobic bacteria are usually gram-negative rods, such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and *Staphylococcus aureus*. This data should be used as a guide to the prescribing of the correct antibiotics for chronic rhinosinusitis patients in Thailand.

Although novel molecular methods can be used to identify bacteria, pus cultures from maxillary sinuses are a reference standard. Culture methods from pus or tissue are cheap, easy to perform, reliable to identify pathogenic bacteria, and suitable to use in our country. The drawback of this study is that it did not include anaerobic bacterial cultures. Because anaerobic bacteria are also common organisms in chronic rhinosinusitis, further studies that include anaerobic bacterial cultures are needed to develop more complete data.

CONCLUSION

The types of pathogenic bacteria recovered from the mucosa and pus from the chronic maxillary rhinosinusitis patients were similar. The mucosal cultures should be a reference standard and can be used as an alternative method when pus is unavailable, especially with immunocompromised patients. This is because of, firstly, the high degree of similarity between the types of bacteria found in the mucosal and pus cultures; secondly, the good concordance rate of the two aerobic bacterial culture techniques; and finally, the high specificity and positive predictive value of the mucosal cultures, compared with the pus cultures.

ACKNOWLEDGMENTS

This study was supported by a grant from the Faculty of Medicine Siriraj Hospital, Bangkok, Thailand. The authors thank Mr. Suthipol Udompunturak for his help with the statistical analyses, Prof.Dr.med.Dr.hc.mult. Wolf J. Mann for his very useful and valuable advice in the manuscript, and Ms. Ngamrat Treerassapanich for her support compiling the data.

TABLE 4. Results of aerobic bacteriological cultures from chronic maxillary rhinosinusitis patients from several studies in Thailand and a systematic review.

Study authors	Year of study	Participants (Number of cases)	Results (Positive pus cultures)	
Jareoncharsri P, et al.[20]	1997–1999	31	74.19% (23/31)	
			H. influenzae	10.77%
			Non-fermentative gram-negative rod	7.69%
			K. pneumoniae	4.62%
Tantilipikorn P, et al.[21]	2004	162	50.61% (82/162)	
			P. aeruginosa	16.2%
			Non-fermentative gram-negative rod	10.8%
			CNS	9.9%
			K. pneumoniae	9.9%
This study	2010–2011	27	51.85% (14/27)	
			P. aeruginosa	21.88%
			S. aureus	9.38%
			K. pneumoniae	6.25%
			Non-fermentative gram-negative rod	6.25%
Udomsiri N and Bedavanija A, et al.[22]	2011–2012	85	51.76% (44/85)	
			P. aeruginosa	18.82%
			S. aureus	5.88%
			K. pneumoniae	4.71%
			Non-fermentative gram-negative rod	2.35%
Thanasumpun T and Batra PS[23]	2015 (systematic review)	6005 CNS	63.71% (3826/6005) 10.24%	
			S. aureus	7.82%
			H. influenzae	3.98%
			Viridans streptococci	3.79%
			P. aeruginosa	3.22%

N.B.: *H. influenzae=Haemophilus influenzae, K. pneumoniae= Klebsiella pneumoniae*, CNS= Coagulase negative *Staphylococcus aureus, S. aureus=Staphylococcus aureus*

REFERENCES

1. Carenfelt C, Lundberg C, Nord CE, Wretlind B. Bacteriology of maxillary sinusitis in relation to quality of the retained secretion. Acta Otolaryngol. 1978; 86:298-302.
2. Gwaltney JM Jr, Sydnor A Jr, Sande MA. Etiology and antimicrobial treatment of acute sinusitis. Ann Otol Rhinol Laryngol Suppl. 1981;90:68-71.
3. Evans FO Jr, Sydnor JB, Moore WE, Moore GR, Manwaring JL, Brill AH, et al. Sinusitis of the maxillary antrum. N Engl J Med. 1975;273:735-9.
4. Frederick J, Braude AI. Anaerobic infection of the paranasal sinuses. N Engl J Med. 1974; 290:135-7.
5. Bjorkwall T. Bacteriological examinations in maxillary sinusitis. Acta Otolaryngol Suppl. 1950; 83:9-58.
6. Benninger MS, Appelbaum PC, Denneny JC, Osguthorpe DJ, Stankiewicz JA. Maxillary sinus puncture and culture in

the diagnosis of acute rhinosinusitis: the case for pursuing alternative culture methods. Otolaryngol Head Neck Surg. 2002;127:7-12.
7. Karma P, Jokipii L, Sipila P, Luotonen J, Jokipii AM. Bacteria in chronic maxillary sinusitis. Arch Otolaryngol. 1979;105:386-90.
8. Jiang RS, Liang KL, Jang JW, Hsu CY. Bacteriology of endoscopically normal maxillary sinuses. J Laryngol Otol. 1999;113:825-8.
9. Su WY, Liu C, Hung SY, Tsai WF. Bacteriological study in chronic maxillary sinusitis. Laryngoscope. 1983;93:931-4.
10. Fokkens W, Lund V, Mullol J. European position paper on rhinosinusitis and nasal polyps 2007. Rhinol Suppl. 2007;20:1-136.
11. Fokkens W, Lund V, Mullol J, Bachert C, Alobid I, Baroody F, et al. European position paper on rhinosinusitis and nasal polyps 2012. Rhinol Suppl. 2012;23:1-298.
12. Kirtsreesakul V, Chatwiwat Y, Laohaprertthisan V. A comparison between endoscopically middle meatal aspiration culture using modified aspiration instrument and direct maxillary antral tap culture in chronic rhinosinusitis. J Med Assoc Thai. 2005;88:1591-7.
13. Vogan JC, Bolger WE, Ketes AS. Endoscopically guided sinonasal cultures: a direct comparison with maxillary sinus aspirate cultures. Otolaryngol Head Neck Surg. 2000; 122:370-3.
14. Desrosiers M, Bendouah Z, Barbeau J. Effectiveness of topical antibiotics on Staphylococcus aureus biofilm in vitro. Am J Rhinol. 2007;21:149-53.
15. Kilty SJ, Desrosiers MY. The role of bacterial biofilms and the pathophysiology of chronic rhinosinusitis. Curr Allergy Asthma Resp. 2008;8:227-33.
16. Costerton JW, Stewart PS, Greenbreg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284:1318-22.
17. Dlugaszewska J, Leszczynska M, Lenkowski M, Tatarska A, Pastusiak T, Szyfter W. The pathophysiological role of bacterial biofilms in chronic sinusitis. Eur Arch Otorhinolaryngol. 2016;273:1989-94.
18. Fastenberg JH, Hsueh WD, Mustafa A, Akbar NA, Abuzeid WM. Biofilms in chronic rhinosinusitis:pathophysiology and therapeutic strategies. World J Otorhinolaryngol Head Neck Surg. 2016;2:219-29.
19. Metcalf DG, Bowler PG, Hurlow J. A clinical algorithm for wound biofilm identification. J Wound Care. 2014;23:137-8, 140-2.
20. Jareoncharsri P, Bunnag C, Tunsuriyawong P, Voraprayoon S, Srifuengfung S, Bedavanija A. Bacteriologic profile of acute and chronic maxillary sinusitis. J Infect Dis Antimicrob Agents. 2001;18:96-102.
21. Tantilipikorn P, Bunnag C, Srifuengfung S, Dhiraputra C, Tiensasitorn C, Jareoncharsri P, et al. A surveillance study of bacteriologic profile in rhinosinusitis. Siriraj Med J. 2007;59:177-80.
22. Udomsiri N. Prevalence study of Anaerobic bacteria in chronic rhinosinusitis [dissertation]. Bangkok: Mahidol University; 2012.
23. Thanasumpun T, Batra PS. Endoscopic-derived bacterial cultures in chronic rhinosinusitis: a systematic review. Amer J Otolaryngol Head Neck Med Surg. 2015;36:686-91.

Impact Assessment of Smell and Taste Disorders on Quality of Life in Thais Using the SF-36 Health Survey (Thai version)

Bannapuch Pinkaew, M.Sc.*, Paraya Assanasen, M.D.*, Olaf Michel, M.D., Ph.D., Kanokporn Talek, B.Sc.*, Tharatham Phonmanee, B.Ed.*, Jeerapa Kerdnoppakhun, B.A.***

**Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, **Department of Otorhinolaryngology, University Hospital Brussels, Vrije Universiteit Brussel, Brussels, Belgium.*

ABSTRACT

Objective: Smell and taste defects adversely affect both physical and mental health. The Short Form (SF)-36 Health Survey is a widely used tool for the quality of life (QoL) assessment. The aim of this study was to investigate the impact of smell and taste disorders on QoL in Thais using the SF-36 Health Survey (Thai version).

Methods: This retrospective chart review included the patients with smell and taste disorders that attended our clinic during 2011 to 2016. Smell ability was evaluated by phenyl ethyl alcohol odor detection threshold, and smell discrimination and identification tests. Taste ability was evaluated by electrogustometry, regional testing, and modified taste strips. SF-36 was used to assess QoL.

Results: Three hundred fifty-five patients were included in the final analysis. The mean age was 50.8±15.5 years, and 64.2% were female. Most patients (78.59%) had smell disorder only, 15.78% had taste disorder only, and 5.63% had both disorders. Specific to taste disorders, QoL was significantly lower in the patient group than in healthy population for the following 6 domains: physical function, role-physical, bodily pain, general health, vitality, and role-emotional (all $p<0.05$).

Conclusion: The four major causes of smell and taste disorders are nasal/sinonasal diseases, idiopathic causes, post-URI, and head trauma. Women are more often affected than men. Although smell and taste disorders both adversely affect physical and mental health, the taste disorders cause more adverse effect. An assessment tool that is specific to smell and taste disorders may facilitate more detailed elucidation of the effect of these conditions on QoL.

Keywords: Impact assessment; smell and taste disorders; quality of life; QoL; Thai population; SF-36 Health Survey (Thai version) (Siriraj Med J 2019;71: 102-109)

INTRODUCTION

Smell and taste sensations are important neurosensory functions that are known to be closely related. Disorder of one, the other, or both sensations can adversely affect mental health and quality of life[1,2] due to the loss of ability to enjoy food and drink, as well as to enjoy and appreciate pleasant aromas.[3,4] In addition, loss of ability to notice toxic chemicals and gases also affects life safety. Moreover, these disorders have been associated with aging[5] and a broad range of diseases, including Alzheimer's disease and Parkinsonism.[6,7] Causes of olfactory dysfunction include head/surgical trauma, chronic rhinosinusitis, iatrogenic

Corresponding author: Bannapuch Pinkaew
E-mail: bangon.pin@mahidol.ac.th
Received 24 July 2018 Revised 17 January 2019 Accepted 4 February 2019
ORCID ID: http://orcid.org/0000-0002-2304-9507
http://dx.doi.org/10.33192/Smj.2019.16

causes, viral infection, nasal obstruction, neurologic disorders, medications, endocrine disturbances, and normal aging.[8,9]

However, despite being common symptoms, smell and taste problems are usually underestimated by patients and overlooked by physicians.[10-12] Interestingly, few studies have investigated the effect of olfactory and gustation disorders on quality of life (QoL). Temmel, *et al.*,[13] reported that a high percentage of patients with olfactory disorders complained about daily life problems, such as cooking, eating, detecting their own body odor, and detecting contaminated or spoiled food. A study in 750 patients with abnormal smell or taste perception conducted at the University of Pennsylvania Smell and Taste Center reported that 68% of participants experienced poor QoL, 46% had decreased appetite and body weight, and 56% had worse daily living and psychological well-being.[8] The impact on QoL, including safety issues and interpersonal relations, as well as eating habits and nutritional intake, may be severely altered in a large proportion of patients with olfactory disorders.[13-14] Furthermore, complaints of decreased taste function actually reflect decreased smell function.[8]

The Short Form (SF)-36 Health Survey questionnaire is a widely used tool for the assessment of QoL in various diseases.[15-16] Our review of the literature revealed very few studies that investigated the impact of smell disorder on QoL using the SF-36.[17-19] All 3 of those studies reported a markedly lower QoL in patients with smell problems, especially in the domains of general health (GH) and vitality (VT). The SF-36 was translated into Thai with permission from the original developer in 2000[20], and the SF-36 (Thai version) has since been evaluated several times[21]. To our knowledge, no study has evaluated the effect of smell and taste disorders on QoL in Thai population. Accordingly, the aim of this study was to investigate the impact of smell and taste disorders on QoL in Thai population using the SF-36 Health Survey questionnaire (Thai version 1.0).

MATERIALS AND METHODS

Subjects

This retrospective chart review included patients with smell and taste disorders that attended the Smell and Taste Clinic of the Division of Allergy and Rhinology, Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand during the August 2011 to June 2016 study period. Siriraj Hospital is Thailand's largest national tertiary referral center. The protocol for this study was approved by the Siriraj Institutional Review Board (Si 387/2013).

Patient data were collected, including age, gender, smoking habits, medication history, and possible causes of smell and/or taste disorders. Smell and/or taste tests and ENT examination were then performed. The validated Thai version (version 1.0) of the SF-36 Health Survey was used (with permission) to assess patient QoL.[20] The smell tests included phenyl ethyl alcohol (PEA) odor detection threshold, and smell discrimination and identification tests. The taste tests included electrogustometry (EGM), regional (spatial) testing of taste function, and modified taste strips.

Smell testing

The following 3 smell tests were performed: smell detection, discrimination, and identification. The smell detection threshold test consisted of a forced-choice single-staircase method using PEA, which is a rose-like odorant in different diluents in a concentration series of light mineral oil. PEA has been proven to activate the olfactory nerve, but relatively low activation of the trigeminal nerve.[22] The objective of the smell detection threshold test was to identify the lowest concentration of PEA that the patient could detect.[23,24] The smell discrimination test (SDT) is used to determine a person's ability to distinguish between odorous substances (e.g., coffee) and water, which is an odorless substance.[25] For the smell identification test (SIT), a total of 10 common odorous substances concentrated to suprathreshold concentration levels were selected. The aim of the SIT is to determine a person's ability to correctly identify and name odorants.[25] Since smell identification is strongly affected by culture and eating behavior, the tested odorants must appropriately match the culture and dietary habits of the person being tested.

Taste testing

Electrogustometry (EGM) is a measurement of taste threshold that was performed using a commercially available electrogustometer (Hortmann Neuro-Otometric Myoton, Alberti Technikhandel GmbH, Bad Neuenahr-Ahrweiler, Germany). Regional (spatial) taste function testing was performed using a test developed by the Connecticut Chemosensory Clinic Research Center (CCCRC) (Farmington, New Mexico, USA).[26] The aim of the regional taste function test is to examine taste ability at areas innervated by the greater superficial petrosal branch (right and left soft palate) and the chorda tympani branch (right and left anterior tongue) of cranial nerve VII and cranial nerve IX (right and left posterior tongue). The 4 suprathreshold tastants used for testing included 1.0 mol sodium chloride (NaCl) for salty flavor, 1.0 mol

sucrose for sweet flavor, 0.03 mol citric acid for sour flavor, and 0.001 mol quinine hydrochloride (QHCl) for bitter flavor. Patients were asked not to smoke, brush their teeth, or eat or drink anything other than water for 1 hour before the test. Each tastant was applied to each area of the palate (areas 1-2) and tongue (areas 3, 4, 5, and 6) (Fig 1) with a sterile cotton bud according to a randomized order, for a total of 24 tests (4 tastants x 6 tested areas). The patients had to identify the taste as either salty, sweet, sour, or bitter. If the answer was correct, a score of 1 was given. Patients were also asked to rate the intensity of each tastant on a visual analogue scale (VAS: 0-10), with a higher score indicating a stronger level of taste intensity. After each of the 24 tests, the patient was asked to rinse his/her mouth prior to the next test.

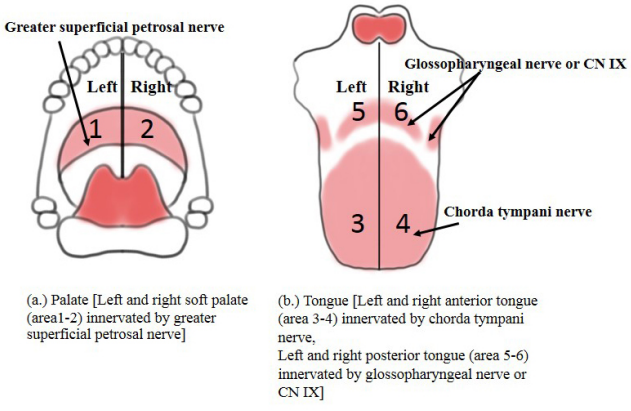


Fig 1. Images showing the palate and tongue areas that are swabbed during regional testing. (Drawing by Miss Boonyisa Pinkaew)

Modified taste strips were applied using a sterile cotton bud (length: 15 cm) with a tip area of 1 cm in length (small size). Each of the 16 cotton buds was impregnated with one of the 4 tastants (salty, sweet, sour, or bitter). Each type of tastant was tested at 4 different concentrations, as follows: *salty* – 1.0, 0.75, 0.50, and 0.25 mol/L sodium chloride; *sweet* – 1.0, 0.75, 0.50, and 0.25 mol/L sucrose; *sour* – 0.03, 0.0225, 0.015, and 0.0075 mol/L citric acid; and, *bitter* – 0.001, 0.00075, 0.0005, and 0.00025 mol/L quinine hydrochloride. These tests were performed at all areas of both the right side and left side of the tongue (Fig 2). The patient had to identify the taste as salty, sweet, sour, or bitter. A correct answer was given a score of 1. The test score ranged from 0 to 16 for each side of the tongue.

Quality of life (QoL) questionnaire

The Thai version (version 1.0) of the SF-36 Health Survey is a 36-item patient-reported survey of health that consists of scaled scores for the following 8 domains:

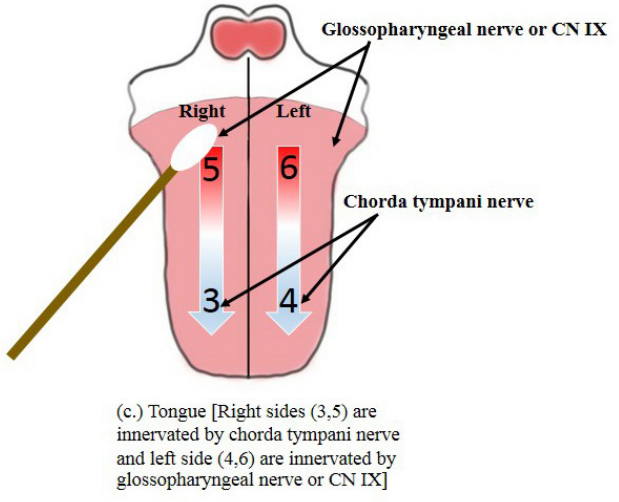


Fig 2. Image showing the areas of the tongue that are swabbed during modified taste strip testing. (Drawing by Miss Boonyisa Pinkaew)

physical functioning (PF); role-physical (RP); bodily pain (BP); general health (GH), which measures physical health; vitality (VT); social functioning (SF); role-emotional (RE); and, mental health (MH), which measures mental health. The SF-36 (Thai version) was adapted from English, and was shown to have proven validity and reliability. [20-21] Raw scores are transformed to scores ranging from 0 to 100, with higher scores indicating better health status.

Data analysis

All statistical analyses were performed using SPSS Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics were used to summarize participant demographic and lifestyle characteristics, and the causes of smell and/or taste disorders. Data are reported as percentage, number and percentage, or mean ± standard deviation (SD). Normally distributed continuous data were evaluated by One-Sample Kolmogorov-Smirnov Test, and are reported as mean ± SD. Demographic data, including age and duration of disorder, were compared between groups using one-way analysis of variance (ANOVA). The number of cases was compared between groups using Pearson's chi-square test. QoL scores were analyzed using Kruskal-Wallis test for non-normally distributed data (PF, RP, BP, VT, SF, RE, and MH), and by ANOVA for normally distributed data (GH). A *p*-value less than 0.05 was considered statistically significant for all tests.

RESULTS

Of 372 patients with complaints of smell and/or taste disorders who were seen at our clinic during the study period, 17 (4.57%) patients were excluded for

having either incomplete data or normal smell and/or taste test result. The remaining 355 patients were enrolled and included in the final analysis. We categorized three groups of disorders for further evaluation smell only, taste only and combined smell and taste disorder determined by phenyl ethyl alcohol (PEA) detection threshold, smell discrimination and identification tests and taste ability including electrogustometry (EGM), regional (spatial) testing of taste function and modified taste strips. Two hundred and seventy-nine patients (78.6%) had problems with smell only (mean age: 49.9 ±16.0 years, range: 8-87; 102 males and 177 females), 56 patients (15.8%) had problems with taste only (mean age: 56.5±13.9 years, range: 15-81; 16 males and 40 females), and 20 patients (5.6%) had problems with both smell and taste (mean age: 48.7±12.1, range: 30-68; 5 males and 15 females). Demographic characteristics, and causes of smell and/or taste disorders are shown in Table 1. More women had smell and/or taste problems than men (64.2% vs. 35.8%, respectively). Almost all patients (82.3%) were non-smokers, with 9.6% being ex-smokers and 8.2% being current smokers. The diagnosed cause of smell and/or taste disorders was nasal/sinonasal diseases (SND; 50.7%), unknown (idiopathic) cause (21.7%), post-URI (10.1%), head/surgical trauma (10.1%), chemical exposure (1.7%), and other causes (5.6%).

SF-36 (Thai version) scoring compared among Thai patients with smell and/or taste disorders and healthy Thai population is shown in Table 2. There was no significant difference among the smell only, taste only, and smell and taste disorder groups, except for the Physical function (PF) dimension (p=0.013), and the significant difference was between the smell only and the smell and taste defect groups. A significant difference was found between smell only and taste only, and also between both smell and taste and taste only. However, patients with taste disorders tended to have poorer QoL among the 3 groups followed by patients with smell disorders and patients with smell and taste disorders, respectively. Nevertheless, in comparison between each group of this study and the data of a healthy population[21], QoL was significantly lower in the domain of general health (GH) which evaluates overall health, including current health, health outlook and resistance to illness in all groups. In addition, there was also significantly lower score in some domains especially, vitality (VT) which evaluates feeling energetic and full of pep versus feeling tired and worn out (Table 2).

TABLE 1. Demographic characteristics, and causes of smell and/or taste disorders.

Characteristics	Smell disorder (N=279)	Taste disorder (N=56)	Smell and taste disorders (N=20)	All groups (N=355)
Age (yr), (mean±SD)	49.9±16.0	56.5±13.9	48.7±12.1	50.8±15.5
Age range (yr)	8-87	15-81	30-68	8-87
Gender, n (%)				
Male	102 (36.6%)	16 (28.6%)	5 (25.0%)	127 (35.8%)
Female	177 (36.4%)	40 (71.4%)	15 (75.0%)	228 (64.2%)
Smoking status, n (%)				
Never smoked	228 (81.7%)	48 (85.7%)	16 (80.0%)	292 (82.3%)
Current smoker	23 (8.2%)	5 (8.9%)	1 (5.0%)	29 (8.2%)
Stopped smoking	28 (10.0%)	3 (5.4%)	3 (15.0%)	34 (9.6%)
Duration of disorder (mo), (mean±SD)	34.7±54.4	17.9±34.5	19.7±24.7	33.2±52.6
Causes, n (%)				
Nasal/sinonasal diseases	162 (58.1%)	8 (14.3%)	10 (50.0%)	180 (50.7%)
Post-URI	34 (12.2%)	2 (3.6%)	0 (0.0%)	36 (10.1%)
Head/surgical trauma	33 (11.8%)	0 (0.0%)	3 (15.0%)	36 (10.1%)
Chemical exposure	5 (1.8%)	0 (0.0%)	1 (5.0%)	6 (1.7%)
Idiopathic causes	34 (12.2%)	37 (66.1%)	6 (30.0%)	77 (21.7%)
Others	11 (3.9%)	9 (16.1%)	0 (0.0%)	20 (5.6%)

TABLE 2. SF-36 score compared among Thai patients with smell and/or taste disorders and healthy Thai population.

SF-36 scores	Smell disorder (mean±SD) (N=279)	Taste disorder (mean±SD) (N=56)	Smell and taste disorders (mean±SD) (N=20)	Healthy population (mean±SD) (N=1,345)[21]
Physical function (PF)	75.53±20.17	67.23±21.55#	77.50±17.43	77.50±17.40
Role-physical (RP)	71.14±38.94*	63.84±43.93#	72.50±37.96	82.20±28.50
Bodily pain (BP)	68.85±19.56*	66.27±19.23#	69.75±21.79	74.90±18.20
General health (GH)	50.37±16.35*	44.93±19.50#	50.05±15.99*#	65.20±17.40
Vitality (VT)	57.75±16.38*	51.88±19.44#	58.50±13.48	61.80±13.50
Social functioning (SF)	72.76±22.88*	73.88±21.22	71.88±15.64	78.30±18.50
Role-emotional (RE)	64.16±42.29*	60.12±44.69#	81.67±35.00	80.20±31.80
Mental health (MH)	65.02±18.04	62.64±20.36	67.60±14.90	65.50±13.00

A P-value<0.05 indicates statistical significance

**Statistically significant by unpaired t-test between smell disorder and healthy Thai population*

#Statistically significant by unpaired t-test between taste disorder and healthy Thai population

**#Statistically significant by unpaired t-test between smell and taste disorders and healthy Thai population.*

DISCUSSION

Few studies have investigated the impact of smell and/or taste impairment on QoL. Moreover, the study of smell and taste disorders in Thai patients are rather new and rare. We are able to report the results from a large study population because our clinic is the first Smell and Taste Clinic to be established in Thailand. Clinical investigations have shown poor general QoL, depression and mood changes, and difficulties in daily life in this patient population. This is the first investigation of QoL impairment due to smell and/or taste disorders in Thai patients using the SF-36 Health Survey (Thai version).

Our findings revealed the most common etiologies to be nasal/sinonasal diseases (SND) (50.7%), idiopathic cause (21.7%), post-URI (10.1%), head/surgical trauma (10.1%), chemical exposure (1.7%), and other causes (5.6%), including aging, diabetes, hypertension, genetic disorders (Kallmann syndrome), Parkinsonism, and other neurologic disorders. These results are similar to those reported from other studies (Table 3). Kaolawanich, *et al.*,[27] reported possible causes of smell disorder in a clinical population of Thais to be SND (66.7%), head injury (12.1%), idiopathic cause (10.6%), post-viral upper respiratory infection (URI) (6.8%), congenital cause (3%), and other (0.8%). Damm, *et al.*, found inflammatory diseases of the nose/paranasal sinus (53%), postviral conditions (11%), idiopathic causes (6%), head trauma (5%), chemical exposure (2%), and other (23%) to be the most common causes of smell defect.[28] Miwa, *et al.*,[4] reported different proportions of the same causes reported by Damm, *et al.* Nordin and Bramerson[29] reported the most common etiologies of smell loss to be URI (18-45% of the clinical population) and nasal/sinus disease (7-56%), followed by head trauma (8-20%), exposure to toxins/drugs (2-6%), and congenital anosmia (0-4%). Amongst the range of etiologies that have been reported from numerous studies[8,13], the most commonly reported cause of smell disorders was URI, followed by idiopathic cause and head injury. These variations in reported incidences among etiologies may be due to differences in race, environment, socioeconomic status, lifestyle, and culture- all of which may play an important role in smell and taste disorders.[27] As a result, the causes reported from various studies are very similar, with differences only in the proportions of patients affected within each study.

The data from this study revealed taste disorders as having the most impact on QoL in all domains of the SF-36 Health Survey (Thai version). Significantly lower scores were found in 6 domains when compared to healthy population, including physical function (p<0.0001), role-physical (p<0.0001), bodily pain (p=0.0005), general health (p<0.0001), vitality (p<0.0001), and role-emotional (p<0.0001). Smell only and combined smell and taste

TABLE 3. Etiology of olfactory disorders from this study and prior studies.

Etiology	Pinkaew, *et al.*(2019) %	Miwa, *et al.* (2011) %	Kaolawanich, *et al.* (2009) %	Damm, *et al.* (2004) %
Nasal/sinonasal diseases	50.7%	21.4%	66.7%	53.0%
Idiopathic causes	21.7%	28.4%	10.6%	6.0%
Post-URI	10.1%	17.1%	6.8%	11.0%
Head/surgical trauma	10.1%	17.1%	12.1%	5.0%
Chemical exposure	1.7%	3.2%	0.0%	2.0%
Other causes	5.6%	11.6%	3.8%	23.0%

Abbreviation: URI =upper respiratory infection

disorders resulted in a higher score. This may be due to the Thai cultural habit of consuming hot and spicy food. Trachootham, *et al.*,[30] reported that Thai people have a strong preference for spicy food, with a preference for mild-moderate spicy food in 70%, and very spicy food in 10% of respondents. That study also found that 70% of Thai people consumed spicy food on a weekly basis. These findings suggest that populations with a preference for hot and spicy foods, such as Thailand, may have much poorer taste sensitivity and perception.[30] Another reason that SF-36 (Thai version) scores were significantly lower in patients with taste defect may be due to the small number of patients in the taste disorder group. Future study in a larger study population is, therefore, recommended-especially since problems with taste were found to have the most adverse effect on QoL.

In groups of taste only and combined, higher scores were observed. This was striking, but could also a bias due to the small-sized number of patients in both groups.

Moreover, in a comparison among the 3 study groups, the lowest scores were observed in the General health (GH) and Vitality (VT) domains. Additionally, when compared with 2019 data from healthy Thai population[21], the scores were significantly lower in all domains and in all groups. Prior studies in the impact of smell disorders on QoL using the SF-36 Health Survey are shown in Table 4 and Fig 3. These studies show a similar pattern in each domain. Consistent with the findings of the present study, the lowest scores were found in the General health (GH) and Vitality (VT) domains.[17-19] The GH domain assesses QoL relative to general physical health, and the VT domain assesses QoL relative to the patient's level of feeling drowsy or sedated.[16] Interestingly, the findings of the present study are lower than those reported in all previous reports[17-19], which may signify the impact of differences in race, behavior, culture, environment, socioeconomic status, and lifestyle among different cultures.

This study has some mentionable limitations. First and consistent with the retrospective nature of this study, some patient data may have been missing or incomplete. Second, the size of two of our three study groups was relatively small. As a result, our study may have lacked sufficient power to identify all significant differences and associations. Third, the patients enrolled in this study were from a single center, which is located within a large urban metropolis. Fourth, our center is Thailand's largest tertiary referral hospital, which means that we are often referred patients with complicated and intransigent conditions. As such, it is possible that our findings may not be generalizable to patients with the same condition in other settings. Importantly, the strength of this study is that this data is representative of treatment outcomes in a real-world setting from the first Smell and Taste Clinic to be established in Thailand.

CONCLUSION

The present study revealed that Thai women suffer from smell and taste disorders more than Thai men. The four major causes of smell and taste disorders are nasal/sinonasal diseases (50.7%), idiopathic causes (21.7%), post-URI (10.1%), and head trauma (10.1%). Smell and taste disorders adversely affect both physical and mental health, with taste disorders adversely effectuating more causation than smell disorders. Although the SF-36 is the most widely used assessment for evaluating QoL, a questionnaire that is specific to smell and taste disorders may facilitate more detailed elucidation of the effect of these conditions on QoL.

TABLE 4. Mean SF-36 scores by domain in patients with smell disorders from this study and prior studies.

SF-36 scores	Pinkaew, *et al.* (2019) (mean±SD) (N=279)	Katotomichelakis, *et al.* (2013)[17] (mean±SD) (N=89)	Neuland, *et al.* (2011)[18] (mean±SD) (N=280)	Seems, *et al.* (2009)[19] (mean±SD) (N>82)
Physical function (PF)	75.53±20.17	80.00±19.42	71.53±27.50	81.44±22.78
Role-physical (RP)	71.14±38.94	69.66±28.52	56.52±42.77	75.00±37.55
Bodily pain (BP)	68.85±19.56	87.67±16.54	61.51±30.31	84.38±20.63
General health (GH)	50.37±16.35	60.07±19.43	52.73±20.08	66.44±20.73
Vitality (VT)	57.75±16.38	68.09±21.88	52.43±20.69	64.43±17.74
Social functioning (SF)	72.76±22.88	83.57±18.42	74.07±25.72	91.52±21.44
Role-emotional (RE)	64.16±42.29	74.53±28.34	63.10±42.84	84.67±29.99
Mental health (MH)	65.02±18.04	68.40±19.46	66.36±19.77	73.24±16.13

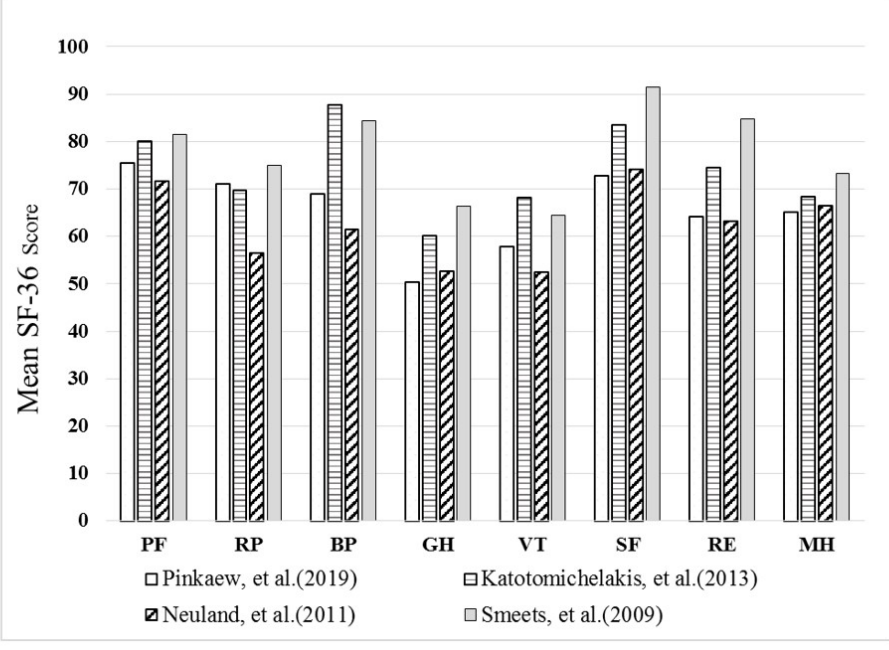


Fig 3. Comparison of the mean SF36 scores for each domain among 4 studies.

Physical function domain (PF)	Role-physical domain (RP)
Bodily pain domain (BP)	General health domain (GH)
Vitality domain (VT)	Social functioning domain (SF)
Role-emotional domain (RE)	Mental health domain (MH)

ACKNOWLEDGMENTS

The authors gratefully acknowledge Assoc. Prof. Dr. Watcharee Leurmarnkul of the Department of Pharmacology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand for granting us permission to use the SF-36 Health Survey (Thai version 1.0); Miss Booyisa Pinkaew for her assistance with anatomical illustrations; Mr. Thitiphol Chomsook for assistance with data acquisition; and Mr. Suthipol Udompunthurak of the Division of Clinical Epidemiology, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University for assistance with statistical analysis.

Conflict of interest declaration: All authors declare no personal or professional conflicts of interest relating to any aspect of this report.

Funding disclosure: This was an unfunded study.

REFERENCES

1. Lombion-Pouthier S, Vandel P, Nezelof S, Haffen E, Millot JL. Odor perception in patients with mood disorders. J Affect Disord 2006;90:187-91.
2. Sergerie K, Chochol C, Armony JL. The role of the amygdala in emotional processing: A quantitative meta-analysis of functional neuroimaging studies. Neurosci Biobehav Rev 2008;32:811-30.
3. Mattes RD, Cowart BJ. Dietary assessment of patients with chemosensotyr disorders. J Am Diet Assoc 1994;94:50-56.
4. Miwa T, Furukawa M, Tsukatani T, Costanzo RM, DiNardo LJ, Reiter ER. Impact of olfactory impairment on quality of life and disability. Arch Otolaryngol Head Neck Surg 2001;127: 497-503.
5. Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, Rosenberg L. Smell identification ability: changes with age. Science 1984;226:1441-3.
6. Schiffman SS. Taste and smell in disease (first of two parts). N Engl J Med. 1983;308:1275-9.
7. Chalke HD, Dewhurst JR. Accidental coal-gas poisoning; loss of sense of smell as a possible contributory factor with old people. Br Med J. 1957;2:915-7.
8. Deems DA, Doty RL, Settle RG, Moore-Gillon V, Shaman P, Mester AF, et al. Smell and taste disorders, a study of 750 patients from the University of Pennsylvania Smell and Taste Center. Arch Otolaryngol Head Neck Surg 1991;117:519-28.
9. Schiffman SS. Taste and smell losses in normal aging and disease. JAMA 1997;278:1357-62
10. Marschner H, Gudziol H, Guntinas-Lichius O. Olfactory dysfunctions are substantially more frequent than they are complained. Laryngorhinootologie 2010;89:654-9.
11. Landis BN, Hummel T, Hugentobler M, Giger R, Lacroix JS. Ratings of overall olfactory function. Chem Senses 2003;28: 691-4.
12. Welge-Luessen A, Hummel T, Stojan T, Wolfensberger M. What is the correlation between ratings and measures of olfactory function in patients with olfactory loss? Am J Rhinol 2005;19:567-71.
13. Temmel AF, Quint C, Schickinger-Fischer B, Klimek L, Stoller E, Hummel T. Characteristics of olfactory disorders in relation to major causes of olfactory loss. Arch Otolaryngol Head Neck Surg 2002;128:635-41.
14. Hummel T, Nordin S. Olfactory disorders and their consequences for quality of life. Acta Otolaryngol 2005;125:116-21.
15. Stewart AL, Hays RD, Ware JE, Jr. The MOS short-form general health survey. Reliability and validity in a patient population. Med Care 1988;26:724-35.
16. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473-83.
17. Katotomichelakis M, Simopoulos E, Tripsianis G, Prokopakis E, Danielides G, Velegrakis SG, et al. Improvement of olfactory function for quality of life recovery. Laryngoscope. 2013;123: E10-6.
18. Neuland C, Bitter T, Marschner H, Gudziol H, Guntinas-Lichius O. Health-related and specific olfaction-related quality of life in patients with chronic functional anosmia or severe hyposmia. Laryngoscope. 2011;121(4):867-72.
19. Smeets MA, Veldhuizen MG, Galle S, Gouweloos J, de Haan AM, Vernooij J, et al. Sense of smell disorder and health-related quality of life. Rehabil Psychol 2009;54:404-12.
20. Leurmankul W. Meetam P. Development of a quality of life questionnaire: SF-36 (Thai version). Thai J Pharm Sci 2000;24: 92-111.
21. Lim LLY, Seubsman S-a, Sleigh A. Thai SF-36 health survey: tests of data quality, scaling assumptions, reliability and validity in healthy men and women. Health Qual Life Outcomes 2008;6:52.
22. Doty RL, Gregor TP, Settle RGJCS. Influence of intertrial interval and sniff-bottle volume on phenyl ethyl alcohol odor detection thresholds. Chem Senses. 1986;11(2):259-64.
23. Doty RL. Practical approaches to clinical olfactory testing. In: Seiden A, ed. Taste and smell disorders. New York: Thieme; 1996.p.38-51.
24. Assanasen P, Tunsuriyawong P, Pholpornphisit W, Chatameteekul M, Bunnag C. Smell detection threshold in Thai adults. J Med Assoc Thai 2009;92:813-6.
25. Pinkaew B, Assanasen P, Bunnag C. Smell discrimination and identification scores in Thai adults with normosmia. Asian Biomedicine.2017;9:789.
26. Bartoshuk L. Clinical evaluation of the sense of taste. Ear Nose Throat J 1989;68:331-7.
27. Kaolawanich A, Assanasen P, Tunsuriyawong P, Bunnag C, Tantilipikorn P. Smell disorders: a study of 132 patients from the first Smell and Taste Clinic of Thailand. J Med Assoc Thai 2009;92:1057-62.
28. Damm M, Temmel A, Welge-Lussen A, Eckel HE, Kreft MP, Klussmann JP, et al. Olfactory dysfunctions. Epidemiology and therapy in Germany, Austria and Switzerland. HNO 2004;52: 112-20.
29. Nordin S, Bramerson A. Complaints of olfactory disorders: epidemiology, assessment and clinical implications. Curr Opin Allergy Clin Immunol 2008;8:10-15.
30. Trachootham D, Satoh-Kuriwada S, Lam-Ubol A, Promkam C, Chotechuang N, Sasano T, et al. Differences in Taste Perception and Spicy Preference: A Thai-Japanese Cross-cultural Study. Chem Senses 2017;43:65-74.

Risk Scoring System for the Prediction of Postpartum Blood Loss over 300 mL at Chiang Rai Regional Hospital

Natakorn I-Tuporn, M.N.S.*, Amornrat Ratanasiri, Ph.D., Thitima Nutravong, Ph.D.***, Kultalee Boonprasert, M.D.****, Tasanee Na Pikul, M.N.S.******

**Faculty of Nursing, Chiang Rai College, Chiang Rai 57000, **Department of Community Medicine, ***Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, ****Chiang Rai Regional Hospital, Chiang Rai 57000, Thailand.*

ABSTRACT

Objective: To develop an assessment tool for the prediction of postpartum blood loss over 300 mL and 500 mL after vaginal delivery.

Methods: A retrospective case-control study of 504 (72 cases: 432 controls) pregnant women with gestational age of 28 weeks or above who underwent vaginal delivery at Chiang Rai Regional Hospital between 1st October 2015 and 30th September 2016 was conducted. The predictors were selected using "Sign OK" selection. The risk scoring system was developed according to the seven steps of clinical prediction model development of Steyerberg.

Results: This form included eight predictors: maternal age of 35 years old or above, gestational age over 40 weeks, being nulliparous, history of curettage in prior pregnancy, gestational hypertensive disorder, hemoglobin level equal to or less than 10 g/dL, fundal height of 38 centimeters or above, and the use of pethidine for pain relief in the 1st stage of labor. The sensitivity of predicting postpartum blood loss over 300 mL after vaginal delivery by the risk scoring system was 80.7%, specificity of 60.8%, and the ROC curve was equal to 0.71 at the optimal cut-off score of four points or above. To predict the postpartum blood loss of 500 mL or above after vaginal delivery, the sensitivity was 88.1%, specificity of 54.6%, and the ROC curve was equal to 0.71 at the optimal cut-off score of three points or above.

Conclusion: This form had an acceptable performance in predicting postpartum blood loss over 300 mL and 500 mL at the optimal cut-off scores of four points or above and three points or above, respectively.

Keywords: Risk score; postpartum blood loss; vaginal delivery; Chiang Rai Regional Hospital (Siriraj Med J 2019;71: 110-116)

INTRODUCTION

In Thailand, the incidence of postpartum hemorrhage (PPH) was 2.30%, 2.37%, 2.44%, 2.40%, 2.39%, 2.54%, and 2.65%, from 2009 to 2015.[1] Similarly, in Chiang Rai province, the incidence of PPH increases by 1.12%, 1.15%, 1.34%, and 2.07%; the pregnant women who had PPH and hysterectomy were 3.05%, 4.58%, 1.82%, and 1.23%, from 2012 to 2015.[2] Moreover, in fiscal year 2014 and 2015, the pregnant women who died from PPH numbered 2 cases, and 1 case, respectively.[2]

For early diagnosis of PPH, the healthcare providers in Chiang Rai Regional Hospital used a collector bag to measure postpartum blood loss over 300 mL after vaginal delivery following the LABOR strategy. Similarly,

Corresponding author: Thitima Nutravong
E-mail: thicha@kku.ac.th
Received 21 November 2018 Revised 14 January 2019 Accepted 13 February 2019
ORCID ID: http://orcid.org/0000-0001-8039-6401
http://dx.doi.org/10.33192/Smj.2019.17

many settings used collector bags as an effective tool for measuring postpartum blood loss after vaginal delivery in the third stage of labor.[3-6] However, the risk of PPH could be assessed in the antepartum or the first stage of labor as indicated in the study of Sittiparn and Siwadune that identified the risk factors associated with PPH and incorporated those risk factors into a risk score to predict PPH in normal labor. It could predict PPH in normal labor with sensitivity of 81.30% and specificity of 50.80% at the optimal cut-off score of four points or above.[7]

The purpose of this study was to set a tool for use in the first stage of labor for the early detection of postpartum blood loss over 300 mL after vaginal delivery compared with using a collector bag for measuring postpartum blood loss after vaginal delivery in the third stage of labor. This study was part of the risk prediction model development for postpartum hemorrhage at the hospitals in Chiang Rai province.

MATERIALS AND METHODS

Before the investigation of the retrospective case-control study, twenty articles from PubMed, Science Direct, Pro Quest, and EBSCO host related to risk factors for PPH in studies from 2005 to 2017, in Thailand and globally, were analyzed by MedCalc Version 18.2.1 (Free Trail Version 15 days)[12] to create the data collection form. This study was approved by the Khon Kaen University Ethics Committee (HE601234) and the Chiang Rai Regional Hospital Ethics Committee on 21st July 2017.

Study design and population

The retrospective case-control study included 2,957 pregnant women with gestational age of 28 weeks or above who underwent vaginal delivery between 1st October 2015 and 30th September 2016 at Chiang Rai Regional Hospital. Five hundred and four of them who had blood loss in the range of 50-3,000 ml were enrolled in this study. The case study included 72 pregnant women with PPH and the control was 432 women with no PPH. The control groups were selected on maternal age, gestational age, gravida, and parity similar to the cases. The ratio of case : control was 1 : 6.

Identification of risk factors for postpartum blood loss after vaginal delivery

The following seven steps for development and an ABCD for validation of the clinical prediction model[8,9] include:

Step 1: problem definition and data inspection; step 2: coding of predictors, candidate risk factors for postpartum blood loss of 500 mL or above after vaginal delivery were identified from the secondary analysis of twenty articles from PubMed, Science Direct, Pro Quest, and EBSCO host related to the risk factors for PPH in studies from 2005 to 2017, in Thailand and globally. They categorized that individual factors, obstetric factors, and placental factors followed the 4T's causes of PPH[10,11] to create the data collection form.

Step 3: model specification; step 4: model estimation, the data collection form created in steps 1-2 was used in the investigation of the retrospective case-control study of 72 pregnant women with PPH and 432 pregnant women with no PPH. They had gestational age of 28 weeks or above and underwent vaginal delivery between 1st October 2015 and 30th September 2016 at Chiang Rai Regional Hospital. First, the samples were categorized to 93 pregnant women with postpartum blood loss over 300 mL and 411 pregnant women with postpartum blood loss equal to or less than 300 mL. Second, the association between the factors and postpartum blood loss over 300 mL was determined by univariate logistic regression. Then, the factors associated with blood loss over 300 mL, statically significant at a p-value < 0.05, were selected for analysis by multiple logistic regression. There were eight predictors for postpartum blood loss over 300 mL after vaginal delivery. Lastly, these predictors were confirmed by experts' opinion agreement, namely two obstetricians, three registered nurses, and five nursing instructors. This predictor identification method is called "Sign OK" selection and included statistical analysis and experts' opinion agreement.

Step 5: model performance; step 6: model validity, eight predictors approved by "Sign OK" selection were incorporated into a risk scoring system for the prediction of postpartum blood loss over 300 mL and 500 mL after vaginal delivery, with calculation of sensitivity, specificity, and a receiver operating characteristic (ROC) curve by the bootstrap resampling technique. However, this step had only 415 samples for data analysis (missing data 89 records).

Step 7: model presentation; the model was presented in the Postpartum Hemorrhage Risk Score Record Form.

Data analysis

Data were analyzed using MedCalc Version 18.2.1 (Free Trail Version 15 days)[12] in steps 1-3 and IBM SPSS software version 22 in steps 4-6. The variables that were significantly associated with postpartum blood loss over 300 mL after vaginal delivery according to the Chi-square test were entered into logistic regression model and used to calculate the index of item objective congruence (IOC). A risk scoring system was developed from the sum of

each significant adjusted odds ratio value. The ROC curve was calculated to determine the optimal score for the prediction of postpartum blood loss over 300 mL and 500 mL after vaginal delivery. Sensitivity and specificity with an associated 95% confidence interval (CI) of each cut-off point were presented. The statistically significant difference was set at a p-value < 0.05.

RESULTS

In steps 1-2, candidate risk factors for postpartum blood loss of 500 mL or above after vaginal delivery from hospitalization at the labor room unit to the first stage of labor were as follows: individual factors as teenage pregnancy, advanced age pregnancy, body mass index (OR > 1.0-1.5), and hemoglobin level equal to or less than 10 g/dL (OR > 2.0); obstetric factors as nulliparous, primiparous, grand-multiparous, pre-term gestational age, gestational diabetes meatus (OR > 1.0-1.5), post-term gestation age, gestational hypertensive disorder, abnormal presentation (OR > 1.5-2.0), prior PPH, previous abortion, and large gestational age / multiple pregnancy / fibroid / hydramnios (OR > 2.0); placental factors as placenta previa, placenta abruption, and placenta accrete (OR > 2.0); complications and procedures of the first stage of labor as induction or augmentation of labor, use of anesthetic drugs (OR > 1.0-1.5), and chorioamnionitis (OR > 1.5-2.0) (data not shown).

In steps 3 – 4, eight predictors for postpartum blood loss over 300 mL after vaginal delivery were selected using "Sign OK" selection as being maternal age of 35 years old or above, hemoglobin level equal to or less than 10 g/dL, being nulliparous, gestational age over 40 weeks, curettage in prior pregnancy, gestational hypertensive disorder, fundal height of 38 centimeters or above, and having received pethidine for pain relief in the first stage of labor. In addition, their adjusted odds ratio was transformed to a score that is presented in Table 1.

In steps 5-6, the total of the risk score ranged from 0 to 30. The sensitivity and specificity with an associated 95% CI for the risk score at different cut-off values are presented in Tables 2 and 3. The ROC curve of a risk score for the prediction of postpartum blood loss over 300 mL after vaginal delivery demonstrated overall an area under the curve (AUC) of 0.71 (95% CI 0.65 to 0.77) (Table 2; Fig 1). The cut-off score was three points or greater, which had a sensitivity of 85.5% and specificity of 56.0%. In addition, the cut-off score of four points or greater had a sensitivity of 80.7% and specificity of 60.8%. On the other hand, the ROC curve of the risk score for the prediction of postpartum blood loss of 500 mL or above after vaginal delivery demonstrated overall an AUC of 0.71 (95% CI 0.65 to 0.77) (Table 3; Fig 2). The cut-off score was three points or greater, which had a sensitivity of 88.1% and specificity of 54.6%.

In step 7, the model was presented in the Postpartum Hemorrhage Risk Score Record Form (Fig 3).

TABLE 1. Risk factors associated with postpartum blood loss over 300 mL after vaginal delivery were identified by "Sign OK" selection.

Risk factors	Statistical analysis (multiple logistic regression) Adjusted odds ratio [95% CI], P-value	Expert's opinion agreement Item objective congruence	Score
Maternal age ≥ 35 years old	3.33 [1.44 – 7,67], .005	0.60	3
Hemoglobin ≤ 10 gram per deciliters	8.19 [3.05 – 22.00], <.001	0.90	8
Nulliparous	2.42 [1.26 – 4.63], .008	0.60	2
Gestational age >40 weeks	2.25 [1.19 – 4.24], .012	1.00	2
Curettage in prior pregnancy	3.81 [1.58 – 9.22], .003	1.00	4
Gestational hypertensive disorder	7.59 [2.49 – 23.08], <.001	0.90	7
Fundal high ≥ 38 centimeters	2.67 [1.29 – 5.35], .008	0.80	2
Having received pethidine for relief pain in the 1st of labor	2.11 [1.16 – 3.82], .014	0.60	2

Statistically significant at p-value <.05

TABLE 2. Performances of a risk score at different cut-off values for the prediction of postpartum blood loss over 300 mL after vaginal delivery.

Cut-off value	Number of women with score at cut-off level	ROC curve	% sensitivity	% specificity	95% CI	P-value
0	415	0.50	100.00	NA	0.43 – 0.57	1.000
1	336	0.61	98.80	23.50	0.55 – 0.67	.002
2	336	0.61	98.80	23.50	0.55 – 0.67	.002
3	217	0.71	85.50	56.00	0.65 – 0.77	<.001
4	197	0.71	80.70	60.80	0.65 – 0.77	<.001
5	96	0.68	51.80	84.00	0.60 – 0.75	<.001
6	85	0.67	47.00	86.10	0.60 – 074	<.001
7	60	0.63	34.90	90.70	0.56 – 0.70	<.001
8	50	0.64	33.70	93.40	0.56 – 0.71	<.001
9	35	0.61	26.50	96.10	0.54 – 0.69	.001
10	28	0.59	21.70	97.00	0.52 – 0.67	.008
11	19	0.58	16.90	98.50	0.50 – 0.65	.030
12	14	0.55	12.00	98.80	0.48 – 0.63	.126
13	5	0.52	4.80	99.70	0.45 – 0.59	.524
14	5	0.52	4.80	99.70	0.45 – 0.59	.524
15	3	0.52	3.60	100.00	0.45 – 0.59	.610
16	3	0.52	3.60	100.00	0.45 – 0.59	.610
17	1	0.51	1.20	100.00	0.44 – 0.58	.865
18	0	0.50	NA	100.00	0.43 – 0.57	1.000

Abbreviations: ROC = receiver operating characteristic, NA = not analysis

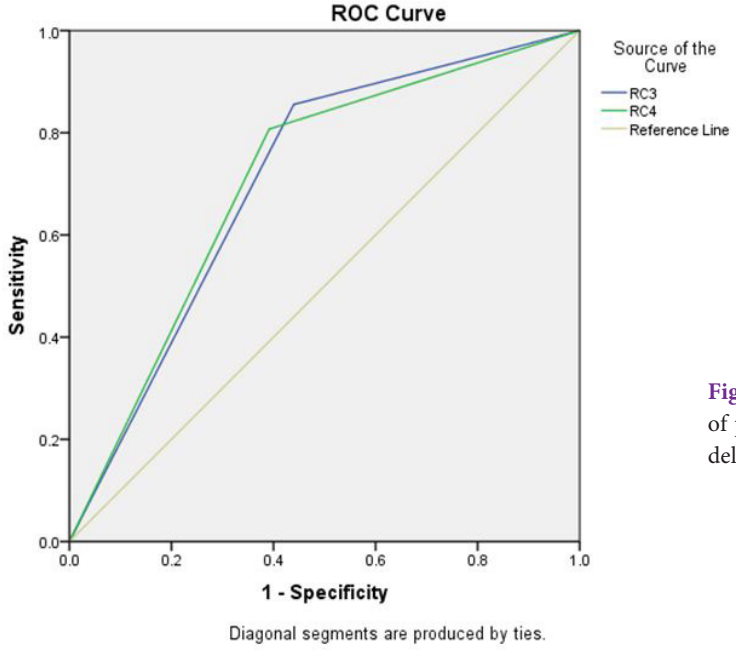


Fig 1. The ROC curve of a risk score for the prediction of postpartum blood loss over 300 mL after vaginal delivery.

TABLE 3. Performances of a risk score at different cut-off values for the prediction of postpartum blood loss of 500 ml or above after vaginal delivery.

Cut-off value	Number of women with score at cut-off level	ROC curve	% sensitivity	% specificity	95% CI	P-value
0	415	0.50	100.00	NA	0.42 – 0.58	1.000
1	336	0.61	98.50	22.40	0.54 – 0.67	.007
2	336	0.61	98.50	22.40	0.54 – 0.67	.007
3	217	0.71	88.10	54.60	0.65 – 0.77	<.001
4	197	0.71	82.10	59.20	0.64 – 0.77	<.001
5	96	0.69	55.20	83.00	0.62 – 0.77	<.001
6	85	0.67	49.30	85.10	0.59 – 0.75	<.001
7	60	0.64	37.30	89.90	0.56 – 0.72	<.001
8	50	0.64	35.80	92.50	0.56 – 0.72	<.001
9	35	0.63	29.90	95.70	0.55 – 0.71	.001
10	28	0.60	23.90	96.60	0.52 – 0.68	.008
11	19	0.58	17.90	98.00	0.50 – 0.66	.039
12	14	0.55	11.90	98.30	0.47 – 0.63	.185
13	5	0.53	6.00	99.70	0.45 – 0.61	.461
14	5	0.53	6.00	99.70	0.45 – 0.61	.461
15	3	0.52	4.50	100.00	0.45 – 0.60	.562
16	3	0.52	4.50	100.00	0.45 – 0.60	.562
17	1	0.51	1.50	100.00	0.43 – 0.58	.847
18	0	0.50	NA	100.00	0.42 – 0.58	1.000

Abbreviations: ROC = receiver operating characteristic, NA = not analysis

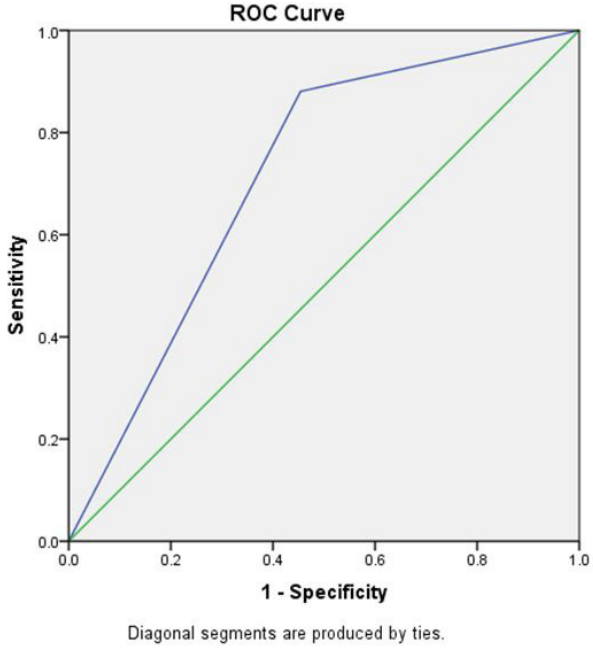


Fig 2. The ROC curve of a risk score for the prediction of postpartum blood loss of 500 mL or above after vaginal delivery.

Postpartum Hemorrhage Risk Score Record Form			
Predictors	Score	Pt. Score	Criteria
1. Age ≥ 35 years old	3		[ ] Low risk
2. Gestational age > 40 weeks	2		Score < 4
3. Nulliparous	2		(EBL ≤ 300 mL)
4. Curettage in prior pregnancy	4		
5. Gestational hypertensive disorder	7		
6. Hemoglobin ≤ 10 gram per deciliters	8		[ ] High risk
7. Fundal height ≥ 38 centimeters	2		Score ≥ 4
8. Having received pethidine for pain relief in the 1st of labor	2		(EBL > 300 mL)
Total score	30		

Type of delivery [ ] ND [ ] V/E [ ] F/E [ ] BA [ ] VBAC
Total blood loss from collector bag_____ml
Others medication for prevent or treat PPH_____.
_____.
_____.
_____.

Fig 3. The Postpartum Hemorrhage Risk Score Record Form for prediction of postpartum blood loss over 300 mL after vaginal delivery.

DISCUSSION

Eight predictors incorporated into the Postpartum Hemorrhage Risk Score Record form are the risk factors of 4T's causing PPH. Being of maternal age of 35 years old or above, gestational age over 40 weeks, fundal height of 38 centimeters or above, and having received pethidine for pain relief in the first stage of labor may affect uterine atony, failure of contractions, and retraction of myometrial muscle fibers.[10,11] Curettage in prior pregnancy may affect retained placenta.[10,11] Being nulliparous may affect uterine atony and cause damage to the birth canal or the genital tract.[10,11] Hemoglobin level equal to or less than 10 g/dL may lead to dysfunction of the coagulation system.[10,11] All of these may lead to excessive bleeding and PPH. Prior studies found that being of maternal age of 35 years old or above,[7] hemoglobin level equal to or less than 10 d/dL,[13] and gestational hypertensive disorder[7,14-18] were risk factors associated with PPH. On the other hand, being nulliparous, gestational age over 40 weeks, curettage in prior pregnancy, and fundal height of 38 centimeters or above were predictors of PPH in this study which are different from those of the previous studies. These findings may result from demographic differences. However, they were similar to results in the secondary analysis of the twenty articles in steps 1 – 2.

For performance on the Postpartum Hemorrhage Risk Score Record Form, it had sensitivity (80.7%) lower than the sensitivity (88.1%) of the tool of Sittiparn and Siwadune,[7] but it had specificity (60.8%) and the ROC curve (0.71) higher than the specificity (54.6%) and the ROC curve (0.66) of the tool of Sittiparn and Siwadune.[7] However, the Postpartum Hemorrhage Risk Score Record Form incorporated many predictors (eight predictors) and covered all of the causes of PPH (4T's), while the tool of Sittiparn and Siwadune[7] included only three risk factors, which are being of maternal age of 35 years old or above, gestational hypertensive disorder, and gestational diabetes meatus. These three factors cover only one of the four causes of PPH, specifically tone or uterine atony.

Although the cut-off scores were three points or greater and four points or greater to predict postpartum blood loss over 300 mL after vaginal delivery had the same ROC curve (0.71) and summation of sensitivity and specificity (Table 2), the cut-off score of four points or greater had higher accuracy (64.82%) and positive predictive value (PPV) (34.01%) than the cut-off score of three points or greater (61.93% and 32.72%, respectively). Therefore, the cut-off score of three points or greater had a high sensitivity (85.5%) and low specificity (56.0%) and maybe resulted in false positives leading to overtreatment. Early detection or diagnosis, theoretically, must have a high specificity and screening must have a high sensitivity.[9] The cut-off score of four points or greater, which had 80.7% sensitivity, 60.8% specificity, and 64.82% accuracy,

was appropriate for early detection or diagnosis in the first stage of labor of postpartum blood loss over 300 mL compared to the cut-off score of three points or greater.

In practice, early diagnosis of PPH in the third stage of labor used a collector bag to measure postpartum blood loss over 300 mL and the healthcare providers used a high dose uterotonic drug to prevent excessive bleeding to 500 mL when it occurred.[1] Due to the circumstances whereby some settings in Chiang Rai province are far from Chiang Rai Regional Hospital, it is hard to refer the pregnant women with PPH. Thus, the cut-off score of three points or greater to predict postpartum blood loss of 500 mL or above after vaginal delivery is inappropriate for patients who are far from Chiang Rai Regional Hospital but appropriate for those who are nearby. So, the cut-off score of four points or greater to predict postpartum blood loss over 300 mL after vaginal delivery is more appropriate for the early detection of PPH. The pregnant women with PPH are immediately referred from remote areas to Chiang Rai Regional Hospital.

To conclude, the Postpartum Hemorrhage Risk Score Record Form is the first useful clinical assessment tool for the early detection or diagnosis of PPH. It has high sensitivity and specificity for the prediction of postpartum blood loss over 300 mL after vaginal delivery according to the LABOR strategy for the early detection or diagnosis of PPH. However, it had the limitation that some factors had a high score. For application, the healthcare providers should take into consideration the setting and characteristics of the pregnant women. Moreover, there should be confirmation of the score of each predictor and the total score by a retrospective or prospective cohort study.

ACKNOWLEDGMENTS

The authors thank the Thai Society of Maternal and Fetal Medicine for funding this study.

REFERENCES

1. Limpanyalert P. How to reduce morbidity and mortality of postpartum hemorrhage? Paper presented at Workshop Proceeding of Community Practice for Patient Safety in Labor Room; 2015 Aug 7; Bangkok, Thailand.
2. Ajalapong J. Problem solving for reduce maternal death from postpartum hemorrhage [Presentation]. Chiangrai: Chiangrai Prachanukroh Hospital; 2015.
3. Abbaspoor Z, Vaziri L. The effectiveness of a collector bag for measurement of postpartum hemorrhage. Afr J Health 2017; 21:99-103.
4. Bamberg C, Niepraschk-von Dollen K, Mickley L, Henkelmann A, Hinkson L, Kaufner L, et al. Evaluation of measured postpartum blood loss after vaginal delivery using a collector bag in relation to postpartum hemorrhage management strategies: a prospective observational study. J Perinat Med 2016;44:433-9.
5. Jandapradit R, Anusornteerakul S. The effectiveness of using plastic film collector bag for the measurement postpartum blood loss. Srinagarind Med J 2015;30:270-5.
6. Zhang WH, Deneux-Tharaux C, Brocklehurst P, Juszczak E, Joslin M, Alexander S, et al. Effect of a collector bag for measurement of postpartum blood loss after vaginal delivery: cluster randomized trial in 13 European countries. BMJ 2010;340:c293.
7. Sittiparn W, Siwadune T. Risk score for prediction of postpartum Hemorrhages in normal labor at Chonburi Hospital. J Med Assoc Thai 2017;100:382-8.
8. Steyerberg EW, Vergouwe Y. Towards better clinical prediction model: Seven steps for development and an ABCD for validation. EUR Heart J 2014;35:1925-31.
9. Steyerberg EW. Clinical prediction models: a practical approach to development, validation, and updating. NY: Springer; 2009.
10. Smith JR. Postpartum hemorrhage [Internet] 2017 Jul 21. Available from: URL: http://goo.gl/YuRp4B [Cited 2018 Jan 30]
11. Cunningham FG, Williams JW, editors. Obstetrical hemorrhage. In: Williams Obstetrics. 23rd ed. NY: McGraw-Hill Medical; 2010.p.757-803.
12. MedCalc Version 18.2.1 (Free Trail Version 15 days). Available from: URL: https://www.medcalc.org/download.php [Cited 2017 May 3]
13. Frass KA. Postpartum hemorrhage is related to the hemoglobin levels at Labor: observational study. Alex J Med 2015;51:33-7.
14. Durmaz A, Komurcu N. Relationship between maternal characteristics and postpartum hemorrhage: a meta-analysis study. The journal of nursing research: JNR. 2017 Dec.
15. Lertbunnaphong T, Leetheeragul J, Thitadilok W. Risk factors of primary postpartum hemorrhage in Siriraj Hospital. Siriraj Med J 2017;62:195-8.
16. Ngwenya S. Postpartum hemorrhage: incidence, risk factors, and outcome in low-resource setting. Int J Womens Health 2016;8:647-50.
17. Puccilli P. The risk factors of postpartum hemorrhage during labor. Dissertation for the degree of Doctorate of Nursing Practice. Durham, NH: University of New Hampshire Graduate School; 2015.
18. Sheiner E, Sarid L, Levy A, Seidman DS, Hallak M. Obstetric risk factors and outcome of pregnancies complicated with early postpartum hemorrhage: a population-based study. J Matern Fetal Neonatal Med 2005;18:149-54.

The Evaluation of Posterior Cingulate Gyrus by Diffusion Tensor Imaging in Alzheimer's Disease Patients Compared with Normal Control Subjects

Chanon Ngamsombat, M.D.*, Thanapat Dechasasawat, M.D.*, Jitsupa Wongsripuemtet, M.D.*, Panida Charnchaowanish, B.Sc.*, Weerasak Muangpaisan, M.D., Orasa Chawalparit, M.D.***

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

ABSTRACT

Objective: Posterior cingulate gyrus atrophy is found in early clinical stage of Alzheimer's disease (AD) patients.[1] Diffusion tensor imaging (DTI) can be used for evaluating microstructure change in brain parenchyma.[2] Our objective was to compare the microstructural change at posterior cingulate gyrus between AD patients and normal control subjects by using DTI.

Methods: The retrospective review of 23 AD patients, diagnosed by NINCDS-ADRDA with available MRI data including DTI, and 19 normal control subjects was performed. The DTI parameters of posterior cingulate gyrus of each group were analyzed and compared.

Results: The mean diffusivity (MD), axial diffusivity and radial diffusivity (RD) of posterior cingulate gyrus were significantly increased in AD patients compared with normal control subjects (p value <0.001, <0.001, <0.001, respectively). The fractional anisotropy (FA) was slightly decreased in AD patients compared with normal control subjects but did not reach statistical significance (p value=0.71).

Conclusion: Microstructural change at posterior cingulate gyrus demonstrated by DTI parameters including MD, axial diffusivity and RD were significantly different between AD patients and normal control subjects. These results were probably helpful for early diagnosis, evaluation, and follow up of the AD patients as correlate with clinical findings.

Keywords: Diffusion tensor imaging; Posterior cingulate gyrus; Alzheimer's disease (Siriraj Med J 2019;71: 117-122)

Abbreviations: DTI: Diffusion tensor imaging; FA: Fractional anisotropy; ROI: Region of interest; MD: Mean diffusivity; RD: Radial diffusivity; AD: Alzheimer's disease

INTRODUCTION

Alzheimer's disease (AD) is a major cause of dementia which brings disability to older adults worldwide. Early detection of AD can provide early treatment for this disease and thus delay progression and disability. Many investigations are now emerging to fulfill this purpose such as CSF tau level, genetic test, FDG or amyloid PET. However, AD patients are still diagnosed late in disease course.[1-6]

Furthermore, other causes of dementia such as dementia with Lewy bodies (DLB), frontotemporal dementia (FTD) and idiopathic normal pressure hydrocephalus

Corresponding author: Orasa Chawalparit
E-mail: oak_art@yahoo.com, orasa.cha@gmail.com
Received 25 July 2017 Revised 1 November 2017 Accepted 20 November 2017
ORCID ID: http://orcid.org/0000-0001-7073-3750
http://dx.doi.org/10.33192/Smj.2019.18

(iNPH) also share common symptoms and signs with AD, but need different specific treatments. A test that can detect AD early and also provide differentiation from other causes will bring many benefits to these patients.[7-9]

The previous study by Pengas et al.,[10] showed that atrophy of posterior cingulate cortex and hippocampus was a feature of early AD. The studies by Minoshima et al., and Nestor et al., also showed hypometabolism in both areas.[11-13]

Diffusion tensor imaging (DTI) is one of the imaging tools for studying microstructural change of white matter which is believed to account for early AD. Previous studies[14-16] demonstrated abnormal FA and MD in posterior cingulate gyrus in AD patients. Our objective was to compare microstructural change at posterior cingulate gyrus between AD patients and normal control subjects by using DTI.

MATERIALS AND METHODS

The study was approved by Siriraj Institutional Review Board (Si 651/2557). Twenty-three AD patients (11 males and 12 females, mean age 78.82 years; range 66-90 years) diagnosed according to NINCDS-ADRDA criteria 2011[17,18,19] with available MRI scan including DTI data were included in the disease group (Table 1). Nineteen normal cognitive subjects (10 males and 9 females, mean age 59.52 years old; range 47-80 years) were enrolled as the control group and recruited from the patients who underwent the MRI study due to non-specific symptoms such as headache or vertigo with normal cognitive function, no abnormal neurological examination and no detectable gross MRI abnormality such as infarction, hemorrhage or mass lesion.

MR Imaging Data Acquisition

MRI acquisitions were done on two machines; the first one was a 3.0 tesla MR system (Archieva, Philips, The Netherlands) with an 8-channel head coil and DTI protocol was a single shot, spin echo EPI; 32 diffusion encoding directions; b value = 0 and 800 s/mm^2; acquisition matrix 112x112; FOV 22.4 cm; voxel size = 2 mm (RL) x 2 mm (AP) with 60 contiguous slices, slice thickness 2.3 mm, and acquisition time was 12:39 min. The other machine was a 3.0 tesla MR system (Ingenia, Philips Medical System, Best, the Netherlands) with 16-channel head coil and DTI protocol was a single shot, spin echo EPI; 32 diffusion encoding directions; b-value = 0 and 800 s/mm^2; acquisition matrix 112x112; FOV 22.4 cm; voxel size = 2 mm (RL) x 2 mm (AP) with 60 contiguous slices, slice thickness = 2.0 mm, and acquisition time was 12:14 min. The 3D-T1 weighted image and fluid-attenuated inversion recovery images were performed in all patients. In AD group, coronal oblique T1W at right angles to the longitudinal axis of the hippocampus was also obtained.

DTI Processing

Processing of the diffusion data including brain extraction and correction for eddy current distortions by using FSL (FMRIB Software Library, version 5.0.8) were done.[20] Then dtifit for local fitting of diffusion tensors was performed. The standard template for ROI measurement (Harvard-Oxford Cortical Structural Atlas) of the posterior cingulate gyrus was placed. Finally, the diffusion tensor parameters including FA, MD, axial diffusivity, and RD were derived and compared between the AD group and control group. (Fig 1)

Statistical analysis

Statistical analysis was performed using PASW (SPSS) version 18. Continuous data analysis was measured and presented as means, standard deviations (SD), min and max parameters. Categorical data analysis was divided by each group and converted into percentage. Sex, education and Scheltens score between AD patients and normal control subjects were compared using Chi-square test. Age at scanning between each group was compared using unpaired t-test.

The diffusion tensor parameters (FA, MD, Axial diffusivity, and RD) were compared between the AD group and control group using Mann-Whitney U test. P-value less than 0.05 was considered statistically significant.

RESULTS

We investigated 23 AD patients (11 males, 12 females, mean age at scanning: 78.8±6.1 years) and 19 normal control subjects (10 males, 9 females, mean age at scanning: 55.0±9.0 years). The age of scan in AD group was higher than that in control group ($p< 0.001$). No gender difference between the two groups ($p= 0.757$) was observed.

Regarding education in AD group, there were 2 patients (8.7%) with no study, 11 patients (47.8%) with primary school graduation, 5 patients (21%) with secondary school graduation and 5 patients (21%) with college degree. The mean age of onset was 77.6±7.0 years and mean TMSE scores (0-30) was 20.83±4 .5 points in AD group.

Scheltens score (range from 0-4 points) in AD group were 2 points in six patients (26.1%), 3 points in fifteen patients (65.2%) and 4 points in two patients (8.7%) whereas the Scheltens score in control group was 1 point

TABLE 1. Shows demographic data as sex, age, TMSE score, education, Scheltens score, age at MRI scanning and underlying diseases of AD patient group.

Number	Sex	Age (years)	TMSE (0-30)	Education	Scheltens score (0-4)	Age at MRI scanning (years)	Underlying diseases
AD1	Male	87	24	Primary school	3	87	DM, HT, DLP
AD2	Female	78	21	Primary school	3	77	DM, HT, DLP
AD3	Male	66	19	Secondary school	3	66	None
AD4	Female	87	22	Primary school	3	87	HT
AD5	Male	82	21	Secondary school	3	82	HT, DLP
AD6	Female	79	25	Primary school	3	79	HT
AD7	Female	84	19	Primary school	3	84	HT, DLP
AD8	Female	90	19	No study	3	90	DM, HT
AD9	Male	83	14	University	3	83	DM
AD10	Male	71	23	No study	2	71	HT, DLP
AD11	Male	72	23	University	2	73	HT, DLP
AD12	Female	80	18	Secondary school	3	81	HT, DLP
AD13	Female	84	21	Primary school	3	84	DM, HT, DLP
AD14	Female	79	26	University	3	79	HT, DLP
AD15	Male	67	22	Secondary school	2	70	DM, HT, DLP
AD16	Male	74	8	Secondary school	2	74	HT, DLP
AD17	Female	76	16	Primary school	3	78	HT, DLP
AD18	Female	81	15	Primary school	4	82	DM, HT
AD19	Female	67	23	Primary school	2	70	HT, DLP
AD20	Female	79	27	Primary school	3	80	HT, DLP
AD21	Male	71	24	Primary school	3	79	HT, DLP
AD22	Male	81	27	University	4	82	None
AD23	Male	68	22	University	2	75	None

Abbreviations: DM=diabetes mellitus, HT=hypertension, DLP= dyslipidemia

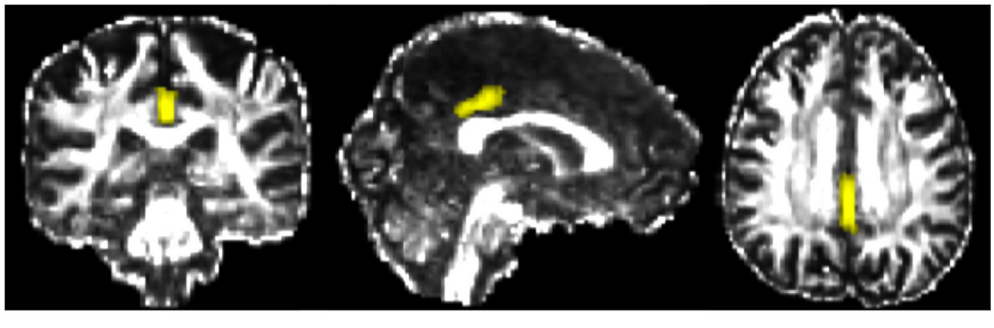


Fig 1. Showed region of interest analysis of the posterior cingulate gyrus demonstrated in axial, sagittal and coronal views in FA map in AD patient.

in eighteen patients (94.7%), and 2 points in one patient (5.3%). Mean Scheltens score in AD group was significantly higher than that in control group ($p < 0.001$). (Table 1)

FA

The mean FA at posterior cingulate gyrus in AD group was slightly lower than that in control group (0.216 ± 0.035 vs 0.218 ± 0.027, respectively). However, there was no statistically significant difference between the two groups (p-value is 0.71). (Table 2)

MD

MD at posterior cingulate gyrus in AD group (1.396 ± 0.195 x 10^{-3} mm^2/s) was statistically significantly higher than that in control group (1.178 ± 0.137 x 10^{-3} mm^2/s), p value <0.001. (Table 2)

Axial diffusivity

Axial diffusivity at posterior cingulate gyrus in AD group (1.659 ± 0.194 x 10^{-3} mm^2/s) was significantly higher than that in control group (1.459 ± 0.263 x 10^{-3} mm^2/s), p value <0.001. (Table 2)

Radial diffusivity (RD)

RD at posterior cingulate gyrus in AD group (1.267 ± 0.200 x 10^{-3} mm^2/s) was significantly higher than that in control group (1.064 ± 0.143 x 10^{-3} mm^2/s), p value <0.001. (Table 2)

TABLE 2. Shows mean FA, mean MD, mean axial diffusivity, and mean RD of AD patients and normal control subjects.

Parameters	AD patients (±SD and range)	Normal control subjects (±SD and range)	P value
Fraction anisotropy (FA)	0.216 ± 0.035 (0.181-0.251)	0.218 ± 0.027 (0.191-0.245)	0.71
Mean diffusivity (MD, 10^{-3} mm^2/s)	1.396 ± 0.195 (1.201-1.591)	1.178 ± 0.137 (1.041-1.315)	<0.0001
Axial diffusivity (AD, 10^{-3} mm^2/s)	1.659 ± 0.194 (1.465-1.853)	1.459 ± 0.263 (1.196-1.722)	<0.0001
Radial diffusivity (RD, 10^{-3} mm^2/s)	1.267 ± 0.200 (1.067-1.467)	1.064 ± 0.143 (0.921-1.207)	<0.0001

DISCUSSION

In early AD, atrophy of hippocampus and posterior cingulate gyrus is a distinct imaging feature.[13] Several studies[10-12] have found that the earliest hypometabolic (F18 FDG-PET) region in AD was the posterior cingulate cortex which showed more significantly decreased metabolism than the mesial temporal structure.

Additional study revealed that there was decreased FA coupled with increased diffusivity (MD) in cingulum fiber which connects the hippocampus and posterior cingulate gyrus reflecting axonal loss or demyelinating process.[16,21-25] Moreover, Salat D et al., revealed that diffusion measurement was related to indices of disease severity and cognitive disability and specifically associated with episodic memory impairment. These findings represented a potential clinical role for DTI to index white matter degeneration and track AD symptoms.[26]

Several previous studies[26-29,31,33] have widely used post-processing analysis software consisting of FSL for DTI analysis at posterior cingulate gyrus. All of these studies supported association of abnormal DTI value at posterior cingulate cortex with early AD. In our study, there was decreased FA with increased mean diffusivity (MD), axial and radial diffusivity in AD patients compared with control subjects. However, only MD, axial diffusivity and RD were significantly statistically different between the two groups. Our result was consistent with the studies by Yoshiura et al., Nakata, et al., and Fellgiebel, et al., which found that increased mean, axial and radial diffusivity in posterior cingulate correlated with AD.

Yoshiura T et al.,[14] found that mean axial and radial diffusivity, but not FA, in the posterior cingulate white matter correlated with MMSE which reflect progression of AD-related histopathological changes. Fellgiebel A et al.,[15]

found significantly decreased FA and increased MD at posterior cingulate white matter in AD compared to normal controls. Nakata Y et al.,[27] found that MD in the posterior cingulum significantly correlated with the MMSE score. However, no significant correlation was seen between FA and MMSE score. The measuring methods were probably different in detail in each study, such as the method of ROI measurement, and also DTI scanning protocol that probably effected the measurement of DTI values.

For correlation with pathophysiology of disease, explanation of the association of increased diffusivity, hypometabolism and atrophy at posterior cingulate gyrus in AD patients was established. MD is a measurement of translational diffusion which increases in the presence of tissue damage.[30] Therefore, MD is expected to increase at the posterior cingulate gyrus in AD patients which indicates pathology in limbic system contributing to memory loss.

In addition, the dissociation between radial diffusivity and axial diffusivity was concordant with the previous study by Song SK et al.,[31-32] which pointed out that significantly increased radial diffusivity reflected myelin injury in AD. However, increased axial diffusivity was not consistent with axonal injury in AD.

In the aspect of FA, there was no significant difference between AD patients and normal control subjects in our study. The FA demonstrated directionality of diffusion and in the white matter, high FA can be found in highly organized tissue with parallel structure. Damage to white matter disrupts the organized structure leading to a decrease in FA. Three decreased FA regions involving medial temporal lobe, temporal lobe proper, and posterior cingulate gyrus were reported to be relatively consistent with AD.[30]

FA in our study was not significantly lower in the AD group which was similar to a study by Yoshiura et al. This can be contributed to rotationally variant during three orthogonal directions measurement of FA. Therefore, the relative orientation of the patient's head concerning the fixed geometry of the MR imaging system gradients can affect FA parameters.[14]

A Study by Nakata et al., in 2008 using tract-specific analysis of posterior cingulate fiber tracts showed significant lower FA in AD patients.[28] However, another study by Nakata et al., in 2009[27] showed no significant correlation between FA and MMSE which used MMSE as an indicator of disease progression in AD. His conclusion suggested that MD in the posterior cingulum is a more sensitive indicator of progression of AD than FA at the posterior cingulum and hippocampal volume.

All of the aforementioned studies reflected the variability of FA and implied that FA is not a reproducible parameter. Thus, factors that can alter FA values are detectable by DTI technique such as parameter, ROI placement,[26,33] voxel size and number of collinear gradients.

Limitations of our study

1. Abnormal DTI findings could be related to other pathology such as dementia other than AD and/or cerebral vascular disease due to non specific in nature of DTI metrics.
2. Mean age at scanning of control group were lower than the AD group. The age-related degeneration of white matter may affect the DTI parameters.

CONCLUSION

The microstructural change at the posterior cingulate gyrus demonstrated by DTI parameters including MD, AD and RD were significantly different between AD patients and normal control subjects. Our findings suggested that DTI parameters including MD, AD and RD at posterior cingulate gyrus are non-invasive markers of AD pathology. Therefore, these are probably helpful for early diagnosis, evaluation, and follow-up of AD patients to correlate with clinical findings. For further studies, advanced imaging techniques that can differentiate between AD and mild cognitive impairment (MCI) will be helpful in clinical practice.

ACKNOWLEDGMENT

The authors thank Mrs. Angkana Jongsawaddipatana and all staff members of the primary care unit of Siriraj Hospital for their contribution to data collection.

REFERENCES

1. Hong YJ, Yoon B, Shim YS, Cho AH, Lim SC, Ahn KJ, Yang DW. Differences in microstructural Alterations of the hippocampus in Alzheimer disease and idiopathic normal pressure hydrocephalus: A diffusion tensor imaging study. AJNR Am J Neuroradiol 2010;31:1867-72.
2. Alzheimer's Association. 2012 Alzheimer's disease facts and figures. Alzheimers Dement 2012;8:131-68.
3. Kumman P, Srijittra B. Alzheimer's disease: Age or disease, Bangkok, 2000.
4. Feldman HH, Jacova C, Robillard A Garcia A, Chow T, Borrie M, et al . Diagnosis and treatment of dementia: 2.Diagnosis. CMAJ 2008;178: 825-36.
5. Alves L, Correia AS, Miguel R, Alegria P, Bugalho P. Alzheimer's disease: a clinical practice-oriented review. Front Neurol 2012;3:63.
6. Trojanowski JQ, Vandeerstichele H, Korecka M, Clark CM, Aisen PS, Petersen RC, et al. Update on the biomarker core of the Alzheimer's disease neuroimaging initiative subjects. Alzheimers Dement 2010;6:230-8.

7. Hattori T1, Sato R, Aoki S, Yuasa T, Mizusawa H. Different patterns of fornix damage in idiopathic normal pressure hydrocephalus and Alzheimer disease. AJNR Am J Neuroradiol 2012;33:274-9.
8. Daouk J, Chaarani B, Zmudka J, Capel C, Fichten A, Bouzerar R, et al. Relationship between cerebrospinal fluid flow, ventricles morphology, and DTI properties in internal capsules: Differences between Alzheimer's disease and normal-pressure hydrocephalus. Acta Radiol 2014;55:992-9.
9. Kilimann I1, Likitjaroen Y, Hampel H, Teipel S. Diffusion tensor imaging to determine effects of antidementive treatment on cerebral structural connectivity in Alzheimer's disease. Curr Pharm Des 2013;19:6416-25.
10. Pengas G, Hodges JR, Watson P, Nestor PJ. Focal posterior cingulate atrophy in incipient Alzheimer's disease. Neurobiology Aging 2010;31:25-33.
11. Minoshima S, Giordani B, Berent S, Frey KA, Foster NL, Kuhl DE. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. Ann Neurol 1997;42(1):85-94.
12. Nestor PJ, Fryer TD, Smielewski P, Hodges JR. Limbic hypometabolism in Alzheimer's disease and mild cognitive impairment. Ann Neurol 2003;54:343-51.
13. Nestor PJ, Fryer TD, Hodges JR. Declarative memory impairments in Alzheimer's disease and semantic dementia. Neuroimage 2006;30:1010-20.
14. Yoshiura T, Mihara F, Ogomori K, Tanaka A, Kaneko K, Masuda K. Diffusion tensor in posterior cingulate gyrus: correlation with cognitive decline in Alzheimer's disease. Neuroreport 2002;13: 2299-302.
15. Fellgiebel A, Muller MJ, Wille P, Dellani PR, Scheurich A, Schmidt LG, et al. Color-coded diffusion-tensor-imaging of posterior cingulate fiber tracts in mild cognitive impairment. Neurobiol Aging 2005;26:1193-8.
16. Takahashi S, Yonezawa H, Takahashi J, Kudo M, Inoue T, Tohgi H. Selective reduction of diffusion anisotropy in white matter of Alzheimer disease brains measured by 3.0 Tesla magnetic resonance imaging. Neurosci Lett 2002;332:45-48.
17. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision. Washington, DC, American Psychiatric Association, 2000.p. 157-8.
18. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263-9.
19. Scheltens P, Leys D, Barkhof F, Huglo D, Weinstein HC, Vermersch P, et al. Atrophy of medial temporal lobes on MRI in Probable Alzheimer's disease and normal ageing: diagnostic clue and neuropsychological correlates. J Neurol Neurosurg Psychiatry 1992;55:967-72.
20. Jenkinson M, Beckmann CF, Behrens TE, Woolrich MW, Smith SM. FSL. Neuroimage 2012;62:782-90.
21. Zhang Y, Schuff N, Jahng GH, Bayne W, Mori S, Schad L, et al. Diffusion tensor imaging of cingulum fibers in mild cognitive impairment and Alzheimer disease, Neurology 2007;68:13-19.
22. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. J Magn Reson B 1996;111:209-19.
23. Sullivan EV, Pfefferbaum A. Diffusion tensor imaging in normal aging and neuropsychiatric disorders. Eur J Radiol 2003;45: 244-55.
24. Wakana S, Jiang H, Nagae-Poetscher LM, van Zijl PC, Mori S. Fiber tract-based atlas of human white matter anatomy. Radiology 2004;230:77-87.
25. Catani M, Howard RJ, Pajevic S, Jones DK. Virtual in vivo interactive dissection of white matter fasciculi in the human brain. Neuroimage 2002;17:77-94.
26. Salat DH, Tuch DS, van der Kowe AJW, Greve DN, Pappu V, Lee SY, et al. White matter pathology isolates the hippocampal formation in Alzheimer's disease. Neurobiol Aging 2010;31: 244-56.
27. Nakata Y, Sato N, Nemoto K, Abe O, Shikakura S, Arima K, et al. Diffusion abnormality in the posterior cingulum and hippocampal volume: correlation with disease progression in Alzheimer's disease. Magn Reson Imaging 2009;27:347-54.
28. Nakata Y, Sato N, Abe O, Shikakura S, Arima K, Furuta N. Diffusion abnormality in posterior cingulate fiber tracts in Alzheimer's disease: tract-specific analysis. Radiat Med 2008;26:466-73.
29. Zhou Y, Dougherty JH Jr, Hubner KF, Bai B, Cannon RL, Hutson RK. Abnormal connectivity in the posterior cingulate and hippocampus in early Alzheimer's disease and mild cognitive impairment. Alzheimers Dement 2008;4:265-70.
30. Stebbins GT, Murphy CM. Diffusion tensor imaging in Alzheimer's disease and mild cognitive impairment. Behav Neurol 2009;21: 39-49.
31. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 2002;17: 1429-36.
32. Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufield AH. Diffusion tensor imaging detects and differentiates axon and myelin degeration in mouse optic nerve after retinal ischemia. Neuroimage 2003;20:1714-22.
33. Kalus P, Slotboom J, Gallinat J, Mahlber R, Cattapan-Ludewig K, Weist R, et al. Examining the gateway to the limbic system with diffusion tensor imaging: the perforant pathway in dementia. Neuroimage 2006;30:713-20.