ASIAN ARCHIVES OF PATHOLOGY

THE OFFICIAL JOURNAL OF THE ROYAL COLLEGE OF PATHOLOGISTS OF THAILAND

Volume 5
Number 3
July – September 2023

INDEX COPERNICUS INTERNATIONAL

Print ISSN: 1905-9183
Online ISSN: 2673-0499

EDITORIAL BOARD

Editor-in-Chief

Assistant Professor Dr Chetana Ruangpratheep
MD, FRCPath (Thailand), MSc, PhD
Phramongkutklao College of Medicine, Bangkok, Thailand

Associate Editors

- Associate Professor Dr Mongkol Kunakorn
  MD, FRCPath (Thailand)
  Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
- Associate Professor Dr Theerapong Krajaejun
  MD, FRCPath (Thailand)
  Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
- Assistant Professor Dr Thirayost Nimmanon
  MD, FRCPath (Thailand), MRes, PhD
  Phramongkutklao College of Medicine, Bangkok, Thailand
- Assistant Professor Dr Wisarn Worasuwannarak
  MD, FRCPath (Thailand)
  Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
- Dr Anirut Worawat
  MD, FRCPath (Thailand)
  Siriraj Hospital, Mahidol University, Bangkok, Thailand
- Dr Panuwat Chutivongse
  MD, FRCPath (Thailand)
  Chulalongkorn University, Bangkok, Thailand

Editorial Consultant

Professor Dr Vorachai Sirikulchayanonta
MD, FRCPath (Thailand)
Rangsit University, Pathumtani, Thailand

ABOUT THE JOURNAL

Aims and Scope

Asian Archives of Pathology (AAP) is an open access, peer-reviewed journal. The journal was first published in 2002 under the Thai name "วารสารราชวิทยาลัยพยาธิแพทย์แห่งประเทศไทย" and English name "Journal of the Royal College of Pathologists of Thailand". The journal is a publication for workers in all disciplines of pathology and forensic medicine. In the first 3 years (volumes), the journal was published every 4 months. Until 2005, the journal has changed its name to be "Asian Archives of Pathology: The Official Journal of the Royal College of Pathologists of Thailand", published quarterly to expand the collaboration among people in the fields of pathology and forensic medicine in the Asia-Pacific regions and the Western countries.

The full articles of the journal are appeared in either Thai or English. However, the abstracts of all Thai articles are published in both Thai and English languages. The journal features letters to the editor, original articles, review articles, case reports, case illustrations, and technical notes. Diagnostic and research areas covered consist of (1) **Anatomical Pathology** (including cellular pathology, cytopathology, haematopathology, histopathology, immunopathology, and surgical pathology); (2) **Clinical Pathology (Laboratory Medicine)** [including blood banking and transfusion medicine, clinical chemistry (chemical pathology or clinical biochemistry), clinical immunology, clinical microbiology, clinical toxicology, cytogenetics, parasitology, and point-of-care testing]; (3) **Forensic Medicine (Legal Medicine or Medical Jurisprudence)** (including forensic science and forensic pathology); (4) **Molecular Medicine** (including molecular genetics, molecular oncology, and molecular pathology); (5) **Pathobiology**; and (6) **Pathophysiology**.

All issues of our journal have been printed in hard copy since the beginning. Around the late 2014, we developed our website (www.asianarchpath.com) in order to increase our visibility. We would like to acknowledge that our journal has been sponsored by the Royal College of Pathologists of Thailand. We have the policy to disseminate the verified scientific knowledge to the public on a non-profit basis. Hence, we have not charged the authors whose manuscripts have been submitted or accepted for publication in our journal.

On the other hand, if any authors request a printed copy of the journal issue containing the articles, each of the copied journals costs 450 bahts for Thai authors and 30 United States dollars (USD) for international authors.

Publication Frequency

Four issues per year

Disclaimer

The Royal College of Pathologists of Thailand and Editorial Board cannot be held responsible for errors or any consequences arising from the use of information contained in Asian Archives of Pathology. It should also be noted that the views and opinions expressed in this journal do not necessarily reflect those of The Royal College of Pathologists of Thailand and Editorial Board.

MANUSCRIPT REVIEWERS

- **Professor Dr Aileen Wee**
  MBBS, FRCPath, FRCPA
  National University Hospital, Singapore
- **Professor Dr Eiichi Morii**
  MD, PhD
  Osaka University Hospital, Osaka, Japan
- **Professor Dr Jasvir Khurana**
  MBBS, FCAP
  Temple University, Lewis Katz School of Medicine, Pennsylvania, The United States of America
- **Professor Dr Paisit Paueksakon**
  MD, FRCPath (Thailand), FCAP
  Vanderbilt University School of Medicine, Tennessee, The United States of America
- **Professor Dr Nidhi Chongchitnant**
  MD, FRCPath (Thailand)
  Bangkok Hospital, Bangkok, Thailand
- **Professor Dr Vorachai Sirikulchayanonta**
  MD, FRCPath (Thailand)
  Rangsit University, Pathumtani, Thailand
- **Professor Dr Oytip Na-thalang**
  PhD
  Thammasat University Rangsit Campus, Pathumtani, Thailand
- **Associate Professor Dr Phaibul Punyarit**
  MD, FCAP, FRCPath (Thailand)
  Bumrungrad International Hospital, Bangkok, Thailand
- **Associate Professor Dr Mongkon Charoenpitakchai**
  MD, FRCPath (Thailand)
  Phramongkutklao College of Medicine, Bangkok, Thailand
- **Assistant Professor Dr Yingluck Visessiri**
  MD, FRCPath (Thailand)
  Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
- **Assistant Professor Dr Pasra Arnutti**
  PhD
  Phramongkutklao College of Medicine, Bangkok, Thailand

- **Dr Jutatip Kintarak**
  MD, FRCPath (Thailand)
  Thammasat University Rangsit Campus, Pathumtani, Thailand
- **Dr Kantang Satayasoontorn**
  MD, FRCPath (Thailand)
  Army Institute of Pathology, Bangkok, Thailand
- **Dr Sivinee Charoenthammaraksa**
  MD, FRCPath (Thailand)
  Bumrungrad International Hospital, Bangkok, Thailand
- **Dr Sorranart Muangsomboon**
  MD, FRCPath (Thailand)
  Siriraj Hospital, Mahidol University, Bangkok, Thailand

CONTENTS

ORIGINAL ARTICLE

A comparison of age estimation accuracy in Thai aged 13 to 18 years by wrist radiography and dental radiography

Pan Jantamattukarn, M.D.[1], Jitta Udnoon, M.D.[1*], Praman Fuangfa ,M.D.[2] and Sitra Sankanjanavanit, D.D.S., M.S., Ph.D.[3]

1 *Forensic Medicine, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University*

2 *Diagnostic Radiology, Department of Diagnostic and Therapeutic Radiology, Faculty of Medicine Ramathibodi Hospital, Mahidol University*

3 *Dentistry Unit, Faculty of Medicine Ramathibodi Hospital, Mahidol University*

* Correspondence to: Jitta Udnoon, M.D., Forensic Medicine, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Email: sleep_poon@yahoo.com

Conflict of interest: The authors declare that they have no conflicts of interest with the contents of this article.

Submitted: 4 March 2022
Accepted: 21 April 2022
Published: 1 September 2023

Abstract

Background: For trials in children and adolescents, acknowledging their ages in lawsuits is necessary. Radiography of wrist bone growth by Greulich & Pyle and dental growth by Demirjian are the standard methods used for age assessment worldwide. However, there are conflicts in age estimation.

Objective: To compare the accuracy of age estimation between wrist and dental radiographs.

Materials and Methods: The 2-year Ramathibodi Hospital database (Aug 2019 – Jul 2021) of wrist bone and dental radiography of 13-18 years old patients was analyzed and compared the estimated age with chronological age. The data from 4 volunteers who participated by taking both radiographs were included.

Results: There were a total of 60 films of wrist radiography and 56 films of dental radiography. According to estimation by Cohen's kappa statistic, it demonstrated that wrist radiography was slightly more accurate. However, both methods had relatively low reliability (Fair agreement) and estimated ages tended to be older than the chronological ages.

Conclusions: The age estimation is not suitable to use only one of the methods, or to use the mean. The estimation methods should rather be improved. Or other reliable methods should be added or substituted.

Keywords: age estimation, Thai adolescent, wrist radiography, dental radiography

Introduction

Age estimation is essential in several situations, such as growth monitoring, refugee registration, legal judgment, and personal identification. Calendar age is frequently compared or confirmed with the bone formation and maturity on the radiographic image. The most suitable and accurate age estimation method for living individuals is depended on their skeleton development [1]. For example, deciduous tooth eruption and maturation are suggested in young children while third molar development is available after 5 years of age [1]. As for teenagers and adolescents, age assessment by wrist radiography (Greulich and Pyle method) [2] and dental radiography (Demirjian's method) [3] are the majority uses because these two methods are simple, inexpensive, and non-intrusive. However, the accuracy of age estimation results remains doubtful due to the key issue of ethnic difference [4]. According to the law in Thailand, the ages of offenders and victims are crucial to determining punishment, particularly sexual guilts. The milestone ages include 13, 15, and 18 years. This minor difference in ages consequently causes slightly different physical appearances among these ages. Uncertain age acknowledgment might cause errors and injustice of prosecution in child and juvenile cases.

The accuracy of age estimation using wrist and dental radiographs in the previous literature displayed the conflict results. On the contrary, the majority of the research agreed that age assessment was more accurate in males. In India, left wrist bone radiography (Greulich and Pyle method) is compared with dental radiography (Demirjian's method). It was found that dental radiography is as reliable as wrist bone radiography and can be a substitute for each other as the age estimation tool [5]. The research result of the bone age by dental radiography in 547 individuals who were in aged 7-25 years in India revealed that the mean error of age was 1.29 years [6]. According to wrist radiography in 406 participants in the age range of 0-20 years in Scotland, it was found that the mean error in females was 14.97 months and in males was 14.16 months [7]. For wrist radiography in 767 participants in the age group of 7-17 years in Turkey, the mean error in females was 1.1 years and in males was 0.6 years[8]. There are still a few research papers on the experimental comparison of age estimation methods by radiography of the different positions. Although there is comparative research on age estimation by the wrist and dental radiography in Italy, the groups of participants were different. The concluded standard deviation (S.D.) presented that wrist radiography was more accurate than dental radiography with 0.522 versus 0.76[9]. However, the comparison of radiography in a Finnish body caused by a Tsunami in Thailand displayed a different outcome, the result showed that dental radiography was better at 5.2 months less than the chronological age while wrist radiography was 9.7 months less than the real age [10].

According to the book "Guidelines on Child Age Assessment in Lawsuits, Ministry of Public Health (Revised Edition, 2021) [11], the assessment methods in lawsuits are stated in the diagram on page 42. This flowchart demonstrates Thai standard guideline which mentions that the uncertain age children will undergo a physical examination, weighing, height measurement, and investigation of dental age and bone age. The external examination will be compared with the database of Thai weight and height ages. The bone age is estimated by wrist radiograph using the standard Greulich and Pyle method. As for dental age, the assessment is applied by combining methods of standard Demirjian and 3rd permanent molar estimation by Gleiser and Hunt [12]. The second one was brought from the research published in 2009 by Thevissen et al. who studied 1,199 panoramic dental films of the Thai population (613 females and 686 males). All of this information will be used for age estimation, however, in the case of children suspected age over 13 years, the guideline recommends relying mainly on dental and bone ages. The intersection of different age range results obtained by more than one method is suggested. For instance, if the result of a physical examination represents an age below 15 years and the result of a dental examination reveals an age range of 13-17 years, the final age range should be 13-15 years. Even the result interpretation of the age assessment might come out in ranges, for conclusions, physicians are supported to write only one digit based on a single number with the highest probability, or still possible to write in an age range. In case the physicians report the estimated age as one digit, the legal officers should be aware that children might be aged in age ranges. Although there is a guideline generated by the government, the incongruent results were encountered in the real practice which caused problems determining age estimation. The authors doubted which method of bone age assessment is the most accurate for Thai teenagers.

Materials and Methods

This study is mixed-method research between a cross-sectional prospective study and a retrospective study as follows.

1. **Prospective group:** Patients in the age range of 13-18 years who received dental radiographic study at Dental Care Center, Faculty of Dentistry, Ramathibodi Hospital, and took an additional left wrist radiograph on the same day for age comparative estimation. The data of the patients had been collected for 10 months from October 2020 to July 2021.
2. **Retrospective group:** Dental and left wrist radiography of the patients aged between 13 and 18 years old were searched from the 2-year database of Ramathibodi Hospital (Aug 2019 – Jul 2021).

The inclusion criteria in both groups were Thai nationality and the age range of 13-18 years with acknowledgment of their exact dates of birth. For the prospective group, the participants and their parents consented to join the experiment and accepted taking additional wrist radiography. The exclusion criteria were the participants with problems related to bone and dental growth and the participants who underwent severe accidents around the assessed area of the dental and left wrist radiographs that disturbed age estimation. This study was approved by the human research ethics committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (COA. MURA2021/25).

All of the dental and left wrist radiographic data were coded for blinding and sent to the co-researchers for further age estimation by the experts using Demirjian's combined with Gleiser and Hunt methods for dental radiography and Greulich and Pyle method for left wrist radiography. The chronological age and estimated age were classified into three groups, 13-less than 15 years, 15-less than 18 years, and 18 years or above. As for the dates later than a month, if over or equal to 15 days, they were rounded up to 1 month. The data were recorded in Microsoft Excel and the correlation was analyzed by Stata version 17. The second program also calculated the proper sample size for each procedure at above 50 samples. Because it was the comparison of statistical reliability between the two independent groups, inter-rater reliability, called Cohen's kappa statistic was commonly used [13]. The results would be compared and interpreted according to Cohen's kappa level of agreement in Table 1.

$$K = (po - pe) / (1 - pe)$$

po referred to the proportion of k raters with a slight – perfect agreement.

pe referred to the proportion of k raters with an accidental agreement

Table 1: Interpretation of Cohen's Kappa statistic values.

Cohen's Kappa	Interpretation
0	No agreement
0.10-0.20	Slight agreement
0.21-0.40	Fair agreement
0.41-0.60	Moderate agreement
0.61-0.80	Substantial agreement
0.81-0.99	Near perfect agreement
1	Perfect agreement

Results

There were 4 participants in the experiment who underwent both dental and left wrist radiography. All of them were female in the age range of 15-less than 18 years. The 2-year retrospective database provided 56 left wrist radiography, plus 4 participants, there were a total of 60 films. The 52 dental radiographs were found during the same period, add with 4 participants, there were 56 samples in total.

For wrist radiography, there were 34 males (56.67%) and 26 females (43.33%) and for dental radiography, there were 22 males (39.29%) and 34 females (60.71%). The chronological ages in both methods were mainly in the age range of 15-less than 18 years which involved 82 from 116 samples (70.69%) as presented in Table 2. Ages estimated by radiography showed similar data with the majority of the age range being 15-less than 18 years. There were 62 from 116 samples (53.45%) in this age group.

Table 2: The number of both radiographies, classified by sex and chronological age groups.

	Male	Female	Total
Wrist radiography	34 (56.67%)	26 (43.33%)	60
Dental radiography	22 (39.29%)	34 (60.71%)	56

Real age range	Number	Percentage
13-<15	32	27.59
15-<18	82	70.69
>=18	2	1.27
total	116	100.00

The overview interpretation of both dental and left wrist radiography displayed that the correctly estimated age from a radiograph of the chronological age group 13- less than 15 years was 18 from 32 (56.25%), calendar age group of 15- less than 18 years was 48 out of 82 (58.54%), and the real age group 18 years or above was 2 from 2 (100%). The calculated reliability by applying Cohen's kappa statistic was 0.2735, implying a fair agreement as seen in Table 3.

Table 3: Comparison between chronological ages and estimated ages by both radiography and the reliability of estimated ages when compared with chronological ages by Cohen's kappa statistic.

Estimated age / Chronological age	13 - <15	15 - <18	>=18	Total
13 - <15	**18 (56.25%)**	14 (43.75%)	0 (0%)	32 (100%)
15 - <18	2 (2.44%)	**48 (58.54%)**	32 (39.02%)	82 (100%)

>=18	0 (0%)	0 (0%)	**2 (100%)**	2 (100%)
Total	20 (17.24%)	62 (53.45%)	34 (29.31%)	116 (100%)

Agreement	agreement	**Kappa**	Std. err	Z	Prop>Z
58.62%	43.04%	**0.2735**	0.0574	4.77	0.0000

According to the data demonstration of wrist radiography, the estimated ages of the chronological age group 13- less than 15 years were correct at 9 from 18 (50.00%), and the real age group of 15- less than 18 years was 29 out of 42 (69.05%). No participant who was 18 years old or above was found in wrist radiographic samples. The calculated reliability by using Cohen's kappa statistic was 0.2834, implying fair agreement as displayed in Table 4.

Table 4: Comparison between chronological ages and estimated ages by wrist radiography and comparison between estimated ages and chronological ages by Cohen's kappa statistic.

Estimated age (Wrist) / Chronological age	13 - <15	15 - <18	>=18	Total
13 - <15	**9 (50.00%)**	9 (50.00%)	0 (0%)	18 (100%)
15 - <18	0 (0%)	**29 (69.05%)**	13 (30.95%)	42 (100%)
Total	9 (15.00%)	38 (63.33%)	13 (21.67%)	60 (100%)

Agreement	agreement	**Kappa**	Std. err	Z	Prop>Z
63.33%	48.83%	**0.2834**	0.0857	3.31	0.0005

For dental radiography, the correctly assessed age from a radiograph of the chronological age group 13- less than 15 years was found in 9 of 14 participants (64.29%), calendar age group of 15- less than 18 years was 19 from 40 (47.50%), and the actual age group of 18 years or above was 2 out of 2 (100%). The reliability calculated by Cohen's kappa statistic was 0.2646, implying a fair agreement as seen in Table 5.

Table 5: Comparison between chronological ages and estimated ages by dental radiography and comparison between estimated ages and chronological ages by Cohen's kappa statistic.

Estimated age (Dental) / Chronological age	13 - <15	15 - <18	>=18	Total
13 - <15	**9 (64.29%)**	5 (35.71%)	0 (0%)	14 (100%)
15 - <18	2 (5.00%)	**19 (47.50%)**	19 (47.50%)	40 (100%)
>=18	0 (0%)	0 (0%)	**2 (100%)**	2 (100%)
Total	11 (19.64%)	24 (42.86%)	21 (37.50%)	56 (100%)

Agreement	agreement	**Kappa**	Std. err	Z	Prop>Z
53.57%	36.86%	**0.2646**	0.0755	3.51	0.0002

According to the analysis of age estimation of those 4 participants who received both methods of radiography, it was found that all of them (100%) had the correct estimated age of 15- less than 18 years by wrist radiography, and only 1 participant (25%) received the correct age assessment by dental radiography as presented in Table 6.

Table 6: Comparison between chronological ages and estimated ages of participants who underwent both methods of radiography.

Chronological age	Age by wrist radiography	Age by dental radiography
15.42	**16**	**15.6**
15.58	**15**	18.24
15.58	**15**	18.03
15.58	**16**	18.03

Discussion

According to age estimation in the 4 participants taking both methods of radiography, the wrist radiograph was more accurate than the dental radiograph, however, due to the limited sample size, further study with enough participants is recommended. To evaluate the children suspected of age between 13 and 15 years, dental radiography seems to be the preferable method with an accuracy of 64.29% compared to 50.00% of wrist radiography. On the contrary, if the individuals are likely to be in the age range of 15-18 years old, a wrist radiograph tends to provide a better result with 69.05% accuracy compared with 47.50% by a dental radiograph.

In the samples with the chronological age range of 13-18 years at Ramathibodi Hospital in the past 2 years (Aug 2019 – Jul 2021), age estimation by wrist radiography displayed slightly more accuracy than that of dental radiography by Cohen's kappa statistic of 0.2834 versus 0.2646. Nevertheless, both wrist and dental radiographic methods are considered in the same level of reliability as fair agreement (Cohen's kappa 0.21-0.40) to be used as the substitutes for real ages. The majority of age assessment results that came from both methods of radiography tended to overestimate the chronological age (bone age > chronological age). These findings from this study were consistent with the results of previous literature. Some of the researchers noticed that age estimation by wrist radiography or dental radiography in many recent research papers had broader S.D. values with lower accuracy. In addition, most of the ages obtained by radiography were older than chronological ages [(6-7,9,14)]. There might be several factors influencing current populations to have faster bone and dental growth than in the past, for instance, race, lifestyle, and food. Several dental care units have tried to improve Demirjian's method to be following their populations by changing coefficients. However, there have not been any new standards of age estimation by dental radiography were established [(5-6,12)]. The authors consider if these age assessment methods are still suitable to apply for determining the ages of offenders or victims involved in juvenile cases since age is an indispensable factor to settle punishment and detention places in lawsuits. Many textbooks recommended inter-rater agreement (Cohen’s Kappa) above 0.80 (substantial agreement) for the clinical laboratory while any methods that had Cohen’s Kappa below 0.60 were indicated as an inadequate agreement for laboratory testing [(13)]. Cohen himself suggested that the lowest acceptable level for the health-related study should be 0.41 or at least moderate agreement [(13)].

Because age estimation in this research contained different groups of data, the researcher viewed that it would be more beneficial for age assessment if wrist radiography is directly compared with dental radiography based on the data of the same participants and the same dates of radiography. For future research implementation, contacts for research cooperation with educational institutions to search for participants at desirable age ranges from various domiciles should be very useful.

Conclusion

Both dental and left wrist radiography for age estimation in teenagers between 13 and 18 years old had relatively low accuracy (fair agreement) which was below the minimum acceptable level of reliability in general. Therefore, it seemed inappropriate to apply only one of the methods, even use both radiographs and try to intersect their results, or use the means of both outcomes, they might still generate low accurate estimated age.

The researcher perceived the necessity to search for additional high reliable age estimation methods or to improve the current/original methods of assessment to be following the chronological ages of the current populations.

Limitation

Due to the pandemic of covid-19 infection during the study period, almost outpatient appointments at Dental Care Center, Ramathibodi Hospital were canceled and indefinitely postponed which dramatically affect the numbers of the volunteers. There was also an asymmetrical distribution of numbers in each age group which was possibly influenced by the age that teenagers got braces or sustained motorcycle accidents (the two majority reasons for imaging that used in this research).

Acknowledgements

This research project is supported by the Faculty of Medicine Ramathibodi Hospital, Mahidol University, and the authors would like to thank Ms.Saowanee Jitprasong and Ms.Kornpira Sirivej for their generous assistance in this study.

References

(1). Black S, Ferguson E, editors. Forensic anthropology: 2000 To 2010. Boca Raton, FL: CRC Press; 2016.

(2). Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist, 2nd ed. Stanford, CA: Stanford University Press; 1959.

(3). Demirjian A, Goldstein H, Tanner JM. A new system of dental age assessment. Hum biol 1973; 1: 211 - 27.

(4). Duangto P, Janhom A, Prasitwattanaseree S, et al. Age estimation methods in forensic odontology. J Dent Indones 2016; 23: 74 - 80.

(5). Srisuwannachit K, Wechphanich S, Brohmwitak C. Age assessment of child laborer. Tha J Public Health 2020; 50: 99 - 110.

(6). Acharya AB. Age estimation in Indians using Demirjian's 8-teeth method. J Forensic Sci 2011; 56: 124 - 7.

(7). Hackman L, Black S. The reliability of the Greulich and Pyle atlas when applied to a modern Scottish population. J Forensic Sci 2013; 58: 114 - 9.

(8). Cantekin K, Celikoglu M, Miloglu O, et al. Bone age assessment: the applicability of the Greulich-Pyle method in eastern Turkish children. J Forensic Sci 2012; 57: 679 - 82.

(9). Pechnikova M, Gibelli D, De Angelis D, et al. The "blind age assessment": applicability of Greulich and Pyle, Demirjian, and Mincer aging methods to a population of unknown ethnic origin. La radiologia medica. 2011; 116: 1105 - 14.

(10). Varkkola O, Ranta H, Metsäniitty M, et al. Age assessment by the Greulich and Pyle method compared to other skeletal X-ray and dental methods in data from Finnish child victims of the Southeast Asian Tsunami. Forensic Sci Med Pathol 2011; 7: 311 - 6.

(11). Ministry of Public Health. Guidelines on child age assessment in lawsuits, 2nd ed. ISBN 978-616-11-4522-4. Samut Sakhon: Born to Be Publishing; 2020.

(12). Thevissen PW, Pittayapat P, Fieuws S, et al. Estimating age of majority on third molars developmental stages in young adults from Thailand using a modified scoring technique. J Forensic Sci 2009; 54: 428 - 32.

(13). McHugh ML. Interrater reliability: the kappa statistic. Biochem Med 2012; 22: 276 - 82.

(14). Shaikh AH, Rikhasor RM, Qureshi AM. Determination of skeletal age in children aged 8-18 years. J Pak Med Assoc 1998; 48: 104 - 6.

ORIGINAL ARTICLE

Decomposition scoring system and decomposition process in Thailand

Kitti Krailasratanasiri M.D.[1*], Vijarn Vachirawongsakorn M.D.[1] and Nitikorn Poriswanish M.D.[1]

1 *Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

* Correspondence to: Kitti Krailasratanasiri M.D Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. Telephone: +6689-119-1555 Email: kitti.kra@mahidol.ac.th

Conflict of interest: The authors declare that they have no conflicts of interest with the contents of this article.

Submitted: *12 March 2022*
Accepted: *20 April 2022*
Published: *1 September 2023*

Abstract

Background: Decomposition is a complex process that can be used to estimate the time since death (TSD). The decomposition of human remains in terrestrial and water environments have been investigated, but this process varies due to many factors

Objective: To investigate tropical decomposition scoring system (TDS) and factors affecting decomposition process in Thailand

Materials and methods: A total of 203 cases of terrestrial and submerged bodies were used to investigate decomposition process. The photographs were scored using the TDS. Postmortem interval was estimated and using the accumulated degree hours (ADH). Different postures and environments were compared using TDS.

Results: The moderate correlation between TDS and ADH was found ($r = 0.423$, $p < 0.001$), meaning this decomposition scoring system could be used to estimate time since death. The TDS showed no statistical significance of decomposition process between submerged and each posture of terrestrial environments ($p = 0.273$). This study also found a significant difference of decomposition rate between supine and prone posture ($t=2.677$, $p=0.008$), but not for the other posture ($p>0.05$).

Conclusions: This study indicates that decomposition is dependent on temperature and body posture.

Keywords: Accumulated degree hours, Decomposition, Forensic taphonomy, Postmortem interval, Time since death

Introduction

Estimation of the time since death (TSD) is one of the most significant issues in a medicolegal investigations as it is able to affirm witness's words, downscope a potential list of assailants and also help determine a possible causes of death[1]. Development of a reliable method of TSD estimation has been so far conducted by several forensic pathologists and taphonomists over the past few decades, including decomposition [2-6], decomposition combined with accumulated degree days [7-9] and bacterial growth [10].

The process of decomposition is initiated immediately after death and continues until the body has been reduced to a dried skeleton [11-13]. A conventional TSD estimation is based on a gross observation of degree of soft tissue decomposition and scoring with several methods [7-9]. Galloway et al. were the first to attempt to quantify human decomposition stage by dividing decomposition process into five broad stages: fresh (no visible discoloration); early decomposition (bloating with pink-green-black discoloration); advanced decomposition (sagging of the flesh, some bone exposure, mummification or adipocere formation); skeletonization (bones with a significant loss of soft tissue coverage); and extreme decomposition (skeletons with bleaching or exfoliation) [3]. Several proposed TSD estimation methods of the decomposition stages are mainly calculated from potential variables affecting the process i.e. temperature, moisture, seasonal time, insect activity, and animal scavenging [2-5].

So far, temperature shows the most impact over a decomposition rate [8,11-15]. Vass et al.[4] and Edwards et al.[16] similarly introduced the concept of accumulated degree days (ADD) which is the sum of the average daily temperature in degrees Celsius above 0°C for the duration that the body has been decomposing. Therefore, ADD represents a combination of chronological time and temperature. Likewise, accumulated degree hours (ADH) are the continuous addition of 'energy hours' from a starting point. ADH is normally used for shorter period than ADD such as the development of insect larvae [17]. In 2005, a hallmark retrospective-study conducted by Megyesi et al. was the first to propose that a combined calculation of ADD and a numerical representation of the degree of decomposition stage, known as a Total Body Score (TBS), could quantitatively speculate TSD. In their study, a whole body was divided into three parts: head and neck, trunk, and limbs – owing to different decomposition manner to create TBS from a sum of scores of all body parts then putting in the TSD equation together with ADD[8]. However, Megyesi et al. emphasized that other potential factors apart from accumulated time and environmental temperature such as moisture, sunlight, insect access, and clothing might not involve in their study[8].

Though numerous novel methods have been developed to demonstrate more potential in accurate TSD estimation that is also able to use universally [15,18-19], none is tailored for specific geographic regions considering unique climate and environmental setting [5,9,12]. Recently, there have been studies emerged to tackle this issue such as ones that are created for the specific regions of the United State [6,8], Australia [9,18], and South Africa [19]. Unfortunately, no specific method is suitable for tropical zone. In 2014, Meewuttisom and Poriswanish proposed TSD estimation from 818 Tsunami victims in Thailand that the time of death and time of postmortem examination are believed to be accurate [20]. This study reveals that the method coined Tropical Decomposition Score (TDS) based on soft tissue decomposition would probably be pragmatic [20]. However, all of the cases were submerged without comparison with decomposition on land.

A differentiation of decomposition rate in different terrestrial body posture is made in very few study. Vasquez (2012) analyzed an effect of upright posture on the decomposition process and found that the body sitting on the chair had different patterns of decomposition when compared with the body sitting against a wall and the body lying on the ground [21]. Shalaby et al. detailed a delayed progression of decomposition process in hanging pig carcasses [22]. In addition, a difference in decomposition process between terrestrial and freshwater submerged bodies is important to consider. The early processes of freshwater decomposition are very similar to terrestrial decomposition [23-24]. However, freshwater environment affects decomposition rate, making human remains in freshwater decompose much more quickly than those on land [14,24]. As in terrestrial studies, research into freshwater decomposition is separated into distinct stage of decomposition, and large variations occur in decomposition stages used and the analytical methods of each stage amongst each literature [7,25]. Developments in the ability to estimate the TSD in terrestrial and freshwater environment can provide more accurate estimation of the postmortem interval.

This study aimed to address two important issues. The first issue was to investigate the use of tropical decomposition scoring system to demonstrate its potential in accurately determining TSD of decomposed remains. The second was to compare different types of terrestrial and freshwater environments, to observe the differences in decomposition rate. These findings will give further information to help forensic pathologists to determine TSD of bodies found in Bangkok, Thailand.

Materials and Methods

The study sample

The present study was carried out in Bangkok, Thailand from January 2016 to September 2021. The study site is comprised of a metropolitan area of Bangkok, the capital city of Thailand. The region experiences tropical climate, with average daily temperature of

28.0°C. It is classified as Aw according to Köppen-Geiger climate classification system [26]. This classification means Bangkok has an equatorial savannah with dry winter.

Ethical approval was granted by Human Research Unit of Faculty of Medicine Siriraj Hospital, Mahidol University (SIRB Protocol No.1071/2563 (IRB2)). From 2016 to 2021, there were a total of 8900 postmortem investigations at the unit of forensic pathology, department of forensic medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. To be included in this study, the following data had to be available: personal identification data, date and time of last sighting, date and time of human remains recovery, the posture of the remains, terrestrial or aquatic temperature, and good quality of postmortem photographs of the face, trunk and the limbs. The human remains with missing parts were also excluded from this study, in order that the state of decomposition could be scored from the entire body. Children younger than 18 years of age had to be excluded from this study due to their larger body surface area compared with body content [9]. After the selection, 203 cases were used for this study.

In this study, human remains were separated into two groups: submerged and terrestrial groups. Submerged cases include bodies recovered from Chaophraya River with the history of witness to their drowning while the other terrestrial deaths were divided into five subgroups depending on their posture: hanging, sit, lying on the lateral side, prone, and supine posture.

Measuring decomposition

The TSD was calculated for all cases in this study as the time between the last sighting and recovery of the body. The state of decomposition was evaluated and scored from postmortem photographs taken during the death scene investigation and medico-legal data provided in forensic reports. The level of decomposition was evaluated using the decomposition score adapted from Galloway et al.[3], Megyesi et al.[8] and Meewuttisom and Poriswanish[20] (Tables 1-3). Because the aim of this study is to investigate the method using for estimating early postmortem interval in tropical country, the new scoring system was developed. Eleven different features from the three main areas of the body (the head, trunk, and extremities) were given a score between zero to four based on the most advanced decomposition stage showed at the death scene. This system has been assigned to each descriptive and sequential changes of skin discoloration and bloating as well as skin and hair slippage showed by three regions separately, as each area do not necessary show the same state of decomposition. The scores from all areas were combined to produce a tropical decomposition score (TDS). The lowest score a case would receive was 0 (fresh in all areas) and the highest score was 40 (dark green-black skin discoloration, total skin slippage and deflated corpse).

Table 1: Decomposition score for the head and neck

TABLE 1 - Categories and stages of decomposition for head

Skin discoloration (Chromatic phase)
(0 pt) Fresh, no discoloration
(1 pt) Pink skin discoloration
(2 pts) Skin marbling
(3 pts) Greenish skin discoloration
(4 pts) Dark green-black skin discoloration

Bloating (Gaseous phase)
- Eye
(0 pt) No eyelid swelling
(1 pt) Eyelid swelling, closed eyes
(2 pts) Eyelid swelling, partial eyes bulging
(3 pts) Full eyes bulging
(4 pts) Eyes deflated
- Tongue
(0 pt) No lip swelling
(1 pt) Lip swelling
(2 pts) Tongue protruding
(3 pts) Tongue deflated

Skin blisters and skin slippage
(0 pt) No skin blister
(1 pt) few skin blisters, no skin slippage
(2 pts) generalized skin blisters (<25% BSA of the head), mild skin slippage
(3 pts) Moderate skin slippage (25-75% BSA of the head)
(4 pts) Total skin slippage

Hair
(0 pt) No hair loss
(1 pt) Detachable hair loss

Table 2: Decomposition score for the trunk

TABLE 2 - Categories and stages of decomposition for Trunk

Skin discoloration (Chromatic phase)
(0 pt) Fresh, no discoloration
(1 pt) Pink skin discoloration
(2 pts) Skin marbling
(3 pts) Greenish skin discoloration
(4 pts) Dark green-black skin discoloration

Bloating (Gaseous phase)
(0 pt) No change
(1 pt) Partial bloating
(2 pts) Full bloat
(3 pts) Partial deflation
(4 pts) Total deflated

Skin blisters and skin slippage
(0 pt) No skin blister
(1 pt) few skin blisters, no skin slippage
(2 pts) generalized skin blisters (<25% BSA of the trunk), mild skin slippage
(3 pts) Moderate skin slippage (25-75% BSA of the trunk)
(4 pts) Total skin slippage

Table 3: Decomposition score for the extremities

TABLE 3 - Categories and stages of decomposition for Extremities	
Skin discoloration (Chromatic phase)	
(0 pt)	Fresh, no discoloration
(1 pt)	Pink skin discoloration
(2 pts)	Skin marbling
(3 pts)	Greenish skin discoloration
(4 pts)	Dark green-black skin discoloration
Bloating (Gaseous phase)	
(0 pt)	No change
(1 pt)	Partial bloating
(2 pts)	Full bloat
(3 pts)	Partial deflation
(4 pts)	Total deflated
Skin blisters and skin slippage	
(0 pt)	No skin blister
(1 pt)	few skin blisters, no skin slippage
(2 pts)	generalized skin blisters, mild skin slippage
(3 pts)	Moderate skin slippage
(4 pts)	Total skin degloved

Calculated accumulated degree hours (ADH)

Throughout the study, temperature data was obtained for each case from the nearest weather station. The terrestrial temperature was obtained from Thai Meteorological Department, Bangkok, Thailand, while hourly aquatic temperature was obtained from the Metropolitan Waterworks Authority (MWA) in Bangkok, Thailand. Due to short period of study, all temperature data was calculated in the form of hourly averages as the accumulated degree hours (ADH). This was measured as follows: ADH = time [hours] × the average of the maximum and minimum terrestrial or aquatic temperature (°C) for the hour.

Statistical analysis

The statistical analysis was carried out using SPSS for Window version 18.0. The normality was tested and the TDSs were analyzed using Two-way ANOVA analysis to study the differences of the decomposition process between each body posture and also between each group of different environmental setting. The spearman correlation coefficient was used to test the correlation between the TDS and TSD. Also, linear models were used to explore the relationship between ADH and measures of decomposition. Lastly, to test inter-observer reliability, a set of photograph of 20 remains was randomized and re-evaluated.

Results

A total of 203 human remains cases have been selected from the case files. For terrestrial environment, 158 cases met the study criteria, while 45 submerged cases from the Chaophraya River were included. There were 171 males (84.2%) and 32 females (15.8%). The mean age was 50 years with a minimum of 18 and a maximum of 83. Mean TSD was approximately 64.3 hours (range 10-144, standard deviation (SD) ± 27.1). Mean ADH data was 1894 (range 304 - 4284, SD ± 819.9). Bodies were identified either submerged, hanging, sit, lying on the lateral side, prone or supine posture. The majority of cases were found to be supine posture (90 cases; 44.3%) while only 7 cases (3.5%) were found to die in sitting posture. In addition, 45 cases (22.2%) were recovered from the freshwater surface. All of the terrestrial cases died indoors. Certainly, it was unknown that the temperature of indoor environment was. Thus, the authors decided to use a temperature close to the outside temperature. Bangkok experiences hot temperatures, with an average daily temperature of 29.6°C (range 23.4-33.2, SD ± 1.7) on land and 29.5°C (range 25.7-32.3, SD ± 1.6) in the water.

The bodies were in relatively good condition. In terrestrial group, score 2 and 3 of skin discoloration were assigned mostly to the head and trunk because of skin marbling and greenish discoloration. In submerged bodies, partial or complete skin slippage was observed mainly all the regions

Table 4: Tropical decomposition score (TDS) for each study groups

Environment	n	TSD (hours)		ADH		Score			
		Mean	SD	Mean	SD	Mean	SD	Min	Max
Drowning	45	42.4	13.3	1232	419	26.6	9.5	6	40
Land									
Hanging	11	58.8	23.5	1758	684	26.0	10.7	6	38
Lateral	17	70.2	25.3	2113	785	28.1	5.3	12	36
Prone	33	69.0	27.7	2029	856	30.1	6.0	5	39
Sit	7	61.7	18.9	1738	405	27.3	3.4	21	32
Supine	90	73.3	27.5	2167	827	27.0	7.9	5	40

The average values of TDS assigned to the study group are demonstrated in Table 4. The TDS varied from score 5 to score 40, meaning the degree of decomposition in the study group differed from a fresh state to advanced decomposition. Because this study was interested in predicting ADH from an observation of decomposition process (TDS), the linear regression was compared to give the resulting equation as follow:

$$\text{Log ADH} = 0.05(\text{TDS}) + 2.68 \pm 45.8$$

Where 45.8 is the standard error of the regression. The correlation between ADH and TDS was significant, but in a moderate positive linear relationship ($r = 0.423$, $p < 0.001$). The moderate correlation was also found between TSD and TDS ($r = 0.437$, $p < 0.001$). In addition, TDS showed there was no statistical significance of decomposition process between freshwater and each posture of terrestrial environments ($p = 0.273$). Therefore, the bodies recovered from terrestrial and freshwater environments went through the same stage of decomposition. In the study group, adipocere was not able to appear on submerges bodies.

Using posture variables, results showed a significant difference between supine and prone posture ($t=2.677$, $p=0.008$). This study also showed no significant difference between supine posture and the hanging, lateral and sitting posture ($p > 0.05$).

The reliability of this study, especially intra-observer variation, was also investigated. Using Cohen's Kappa coefficient, the average intraclass correlation coefficient (ICC) was 0.92 for inter-observer reliability. To conclude, the evaluation of TDS system between two authors was excellent reliability [(27)].

Discussion

At the crime scene, a morphological evaluation of decomposition process and pattern is the most common method to estimate TSD; however, it may vary depending on the inspector's experience and crime scene environment. The TDS scoring system in this study can be applied to score the decomposition of human remains in all tropical regions with a similar climate as in Thailand (an equatorial savannah with dry winter (Köppen-Geiger climate classification 'Aw'))[26]. This method is a simple, practical and user-friendly technique to estimate TSD. An autopsy is not required, so that it would be an obvious advantage for a forensic pathologist to give correct post-mortem interval in the death scene.

The authors attempted to use the TDS to estimate soft tissue decomposition. This scoring system uses the new criteria and terminology derived from the scoring system introduced by Meewuttisom and Poriswanish, who developed the decomposition score from Tsunami victims in Thailand [20]. The authors noted hair loss in scoring system which is not accounted for in the original decomposition scoring system of Megyesi et al. [8]. There were also remains with skin slippage and degloving in this study. One reason is that this TDS system in this study is applicable to use for the early to late stage of soft tissue decomposition, which is the most common decomposition stage encountered in metropolitan city of Bangkok. No skeletonization was represented in this sample. According to Mann et al., human remains under warm to hot environment usually takes between 2-4 weeks to become nearly or completely skeletonized[14] , but the exact time is not known in Thailand.

In this study, the moderate correlation was also found between ADH and TDS. This may suggest that ADH was not fully successful to predict TSD of human remains with early decomposition process. It is important to take into consideration that there were possible temperature errors as well as micro-environmental differences affecting the decomposition process. All of the human remains in this study are indoor bodies. This causes a major problem because when the bodies were found, the authors could not know when the individuals died. As a result, the authors could not precisely measure the exact indoor temperature of the death scene during the time a deposited body was decomposing. In this study, the temperature for the indoor bodies was estimated to have the same temperature as outdoor environment. It is likely that this estimation is not accurate in all the terrestrial cases and this creates some margin of error. Therefore, the distinction between outdoor and indoor environments was not made in this study and this could be different in several variables e.g. sun exposure and insect succession [13-16].

It is widely accepted that terrestrial decomposition differs from aquatic decomposition [7,11-12,14]. Although the most logical reason of this finding was unanticipated, this study showed that freshwater bodies decomposed at the same rate as terrestrial bodies. There are some possible explanations for this finding. The early decomposition process of freshwater decomposition is very similar to bodies decomposing on land [12]. It was assumed to be associated with temperature, as the temperature data in this study was very similar between terrestrial and freshwater environments. Heaton et al. mentioned that time in the water and temperature were the factors that affected substantially the decomposition process [7]. Some different patterns of decomposition were observed in this study. Submerged bodies were likely to have early skin slippage and degloving of their hands, which is common with prolonged aqueous exposure [28]. At the same postmortem period, bodies dying on land showed much more skin discoloration resulting from denaturation of hemoglobin and its relation with putrefactive gases [11-12].

The second aim of this study was to find an effect of body posture on decomposition process. The effect of posture on the decomposition rate and pattern was analysed in the previous study [21]. In this study, comparative analysis between each terrestrial group showed that a statistical significance was noted between supine and prone posture. Human remains in the prone position, which the majority of the body lying horizontally with the face and torso facing down toward the ground, were likely to decompose faster than those in the supine position. This finding may be explained by the position of livor mortis. Livor mortis develops over the front in the bodies who remain in the prone position, leading to blood accumulation in the gastrointestinal tract especially large intestine. As a result, postmortem bacterial overgrowth from blood pooling of livor and acceleration of soft tissue breakdown would be increased in prone bodies. This study gives a source of information for future study into the ways in which posture may have an effect on the decomposition process.

Some limitations occurred in this study. First, not every part of the human remains could be visible on the photographs. Therefore, the TDS could be further along than is visible on the photographs. Morphological scoring of the human decomposition can be more accurate when the remains can be fully exposed. Second, it is not possible to control over all variables in decomposition research. Some bodies were exposed to more insect activity than others and not all bodies exposed to the exact same environmental settings such as temperature and precipitation. Third, this study did not include human remains in the advanced stage of decomposition. Fourth, this study showed a lack of ambient temperature measurement. Further studies would benefit from more cases with outdoor environment and include the later stage decomposition to validate the method.

Conclusion

This study produced preliminary outcomes in a tropical region that had not previously been investigated for decomposition research. This study showed no statistical significance of decomposition process between submerged and terrestrial environments. A significant difference of decomposition rate between supine and prone posture was observed. This study indicates that decomposition, when scored quantitatively and used to predict ADH, can provide forensic physicians with a reliable and accurate method to estimate TSD. In addition, this study indicates that decomposition is dependent on temperature and body posture.

Acknowledgements

We would like to give special thanks to Assist.Prof.Dr. Chulaluk Komoltri for her assistance with the statistical analysis.

References

(1). Cockle DL, Bell LS. Human decomposition and the reliability of a 'Universal' model for post mortem interval estimations. Forensic Sci Inter 2015; 253: 136.e1 – 9.

(2). Rodriquez WC, Bass WM. Insect activity and its relationship to decay rates of human cadavers in East Tennessee. J Forensic Sci 1983; 28: 423 – 32.

(3). Galloway A, Birkby WH, Jones AM, at al. Decay rates of human remains in an arid environment. J Forensic Sci 1989; 34: 607 - 16.

(4). Vass AA, Bass WM, Wolt JD, et al. Time since death determinations of human cadavers using soil solution. J Forensic Sci 1992; 37: 1236 – 53.

(5). Bass W, Outdoor decomposition rates in Tennessee, in: Haglund WD, Sorg MH (eds.) Forensic taphonomy: The postmortem fate of human remains. Boca Raton, FL: CRC Press Taylor & Francis Group; 1997: 181 – 6.

(6). Rhine S, Dawson JE. Estimation of time since death in the Southwestern United States, in: Reichs KJ (ed.) Forensic osteology: Advances in the identification of human remains. Springfield, Illinois: Charles C Thomas; 1998: 145 – 59.

(7). Heaton V, Lagden A, Moffaat C, et al. Predicting the postmortem submersion interval for human remains recovered from U.K. waterways. J Forensic Sci 2010; 55: 302 – 7.

(8). Megyesi MS, Nawrocki SP, Haskell NH. Using accumulated degree-days to estimate the post mortem interval from decomposed human remains. J Forensic Sci 2005; 50: 618 – 26.

(9). Marhoff SJ, Fahey P, Forbes SL, Green H. Estimating post-mortem interval using accumulated degree-days and a degree of decomposition index in Australia: A validation study. Aus J Forensic Sci 2016; 48: 24 - 36.

(10). Benbow ME, Pechal JL, Lang JM, et al. The potential of high-throughput metagenomic sequencing of aquatic bacterial communities to estimate the postmortem submersion interval. J Forensic Sci 2015; 60: 1500 – 10.

(11). Madea B. Estimation of the time since death. 3rd ed. Boca Raton, FL: CRC Press Taylor & Francis Group; 2016.

(12). Hayman J, Oxenham M. Estimation of the time since death: Current research and future trends. London, UK: Academic Press; 2020.

(13). Lee Goff M. Early post-mortem changes and stages of decomposition in exposed cadavers. Exp. Appl. Acarol. 2009; 49: 21 - 36.

(14). Mann RW, Bass WM, Meadows L. Time since death and decomposition of the human body: Variables and observations in case and experimental field studies. J Forensic Sci. 1990; 35: 103 – 11.

(15). Simmons T, Adlam RE, Moffatt C. Debugging decomposition data- comparative taphonomic studies and the influence of insects and carcass size on decomposition rate. J Forensic Sci. 2010; 55: 8 - 13.

(16). Edwards R, Chaney B, Bergman M. Temperature developmental units for insects. Perdue University Pest & Crop Newsletter 1987; 2: 5 - 6.

(17). Sharma R, Kumar Garg R, Gaur JR. Various methods for the estimation of the postmortem interval from Calliphoridae: A review. Egyp J Forensic Sci. 2015; 5: 1 - 12.

(18). Fitzgerald C, Oxenham M. Modeling time-since-death in Australian temperate conditions. Aus J Forensic Sci. 2009; 41: 27 - 41.

(19). Myburgh J, L'Abbé EN, Steyn M, et al. Estimating the postmortem interval (PMI) using accumulated degree-days (ADD) in a temperate region of South Africa. Forensic Sci Inter. 2013; 229: 165.e1 - 6.

(20). Meewuttisom K, Poriswanish N. Decomposition score for estimation the time of death in tropical zone. J Forensic Physician Assoc Thai. 2014; 8: 112 - 26.

(21). Vasquez BJ. The effect of upright posture on decomposition. Chancellor's Honors Program Projects [Internet]. 2012. [cited 2022 Feb 5]. Available from: https://trace.tennessee.edu/utk_chanhonoproj/ 1565

(22). Shalaby OA, deCarvalho LML, Goff ML. Comparison of patterns of decomposition in a hanging carcass and a carcass in contact with soil in xerophytic habitat on the Island of Oahu, Hawaii. J Forensic Sci. 2000; 45: 1267 - 73.

(23). Haglund WD. Disappearance of soft tissue and the disarticulation of human remains from aqueous environments. J Forensic Sci. 1993; 38: 806 - 15.

(24). Sorg MH, Dearborn JH, Monahan EI, et al. Forensic Taphonomy in Marine Contexts. In Haglund WD, Sorg MH (eds.) Forensic Taphonomy: The Postmortem Fate of Human Remains. CRC Press, Boca Raton. 1993: 567 - 604.

(25). Payne JA, King EW. Insect succession and decomposition of pig carcasses in water. Journal of the Georgia Entomological Society. 1972; 7: 153 - 62.

(26). Kottek M, Grieser J, Beck C, et al. World map of the Köppen-Geiger climate classification updated. Meteorol Z. 2006; 15: 259 - 63.

(27). Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med. 2016; 15: 155 - 63.

(28). Caruso JL. Decomposition changes in bodies recovered from water. Acad Forensic Pathol. 2016; 6: 19 - 27.

REVIEW ARTICLE

ดีเอ็นเอที่ถูกปล่อยออกจากเซลล์เข้าสู่กระแสโลหิต [Circulating Cell-Free Deoxyribonucleic Acid (ccfDNA)]

กันตพงศ์ อุทัยวรรณ, อมรรัตน์ ลาชมภู และ อาทิตยา บุญนาค

นักศึกษาชั้นปีที่ 3 ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ
จังหวัดกรุงเทพมหานคร ประเทศไทย
โทรศัพท์: +66 (0) 95 307 0619 Email: Kantapong.kiims@g.swu.ac.th

ส่งต้นฉบับ: *วันที่ 31 เดือนมีนาคม พ.ศ. 2565*
รับลงตีพิมพ์: *วันที่ 25 เดือนเมษายน พ.ศ. 2565*
ตีพิมพ์เผยแพร่: *วันที่ 1 เดือนกันยายน พ.ศ. 2566*

บทคัดย่อ

Circulating cell free DNA (ccfDNA) คือชิ้นส่วนของ DNA ที่อยู่ภายนอกเซลล์ จากการถูกปล่อยออกมาจากเซลล์ด้วยขบวนการ necrosis หรือ apoptosis โดยสามารถพบได้ทั้ง single strand DNA หรือ double strand DNA ccfDNA จะถูกปล่อยเข้าสู่กระแสโลหิต ทำให้สามารถตรวจหาความผิดปรกติทางพันธุกรรมต่างๆ ได้แก่ copy number variation, mutation, methylation, การตรวจหามะเร็ง และการตรวจความผิดปรกติของทารกในครรภ์ การตรวจหา ccfDNA สามารถตรวจพบได้ด้วยวิธีการที่เรียกว่า liquid biopsy ซึ่งทำได้ง่าย รวดเร็ว และสามารถตรวจหาความผิดปรกติทางพันธุกรรมได้ในระยะเริ่มต้น

คำสำคัญ: Circulating cell free DNA, apoptosis, necrosis

บทนำ

Circulating cell free DNA (ccfDNA) หมายถึง ดีเอ็นเอที่อยู่ภายนอกเซลล์แล้วถูกปล่อยออกจากเซลล์เข้าสู่ของเหลวในร่างกายด้วยกระบวนการตายของเซลล์ (cell death) คือการเกิด Necrosis และ Apoptosis โดยเซลล์นั้นอาจจะเป็นเซลล์ปกติหรืออาจจะเป็นเซลล์ที่เป็นโรคแล้วปล่อยชิ้นส่วนดีเอ็นเอออกมา ซึ่งชิ้นส่วนดีเอ็นเอนั้นอาจจะเป็นได้ทั้งดีเอ็นเอสายเดี่ยว (single stranded DNA) หรือดีเอ็นเอสายคู่ (double stranded DNA) [1-2]

ในปี ค.ศ. 1948 Mandel และ Metais มีการพิสูจน์เพื่อหากรดนิวคลีอิคจากพลาสมาในเลือด โดยใช้วิธีตกตะกอนของกรดเปอร์คลอริก (perchloric acid precipitation) ได้พบ RNA และ DNA ในพลาสมาของคนปกติและคนที่มีโรคต่าง ๆ และสตรีมีครรภ์ นอกจากนี้ยังพบ cfDNA เป็นครั้งแรก จนกระทั่งปีค.ศ. 1966 ได้มีความสนใจใน cfDNA อีกครั้ง เนื่องจากมีการค้นพบ cfDNA ในซีรัมของผู้ป่วยโรคแพ้ภูมิตัวเอง (SLE) โรคข้ออักเสบรูมาตอยด์ โรคไตอักเสบ โรคตับอ่อนอักเสบ โรคนิ่วในถุงน้ำดี โรคลำไส้อักเสบ โรคแผลในกระเพาะอาหาร โรคตับอักเสบและโรคหลอดอาหารอักเสบ ต่อมาในปี ค.ศ. 1977 Leon S.A. และคณะ พบว่าระดับ cfDNA ในผู้ป่วยโรคมะเร็งมีแนวโน้มสูงกว่าคนปกติ และพบ cfDNA ในซีรัมของผู้ป่วยที่มีการลุกลามของเนื้อร้ายไปตามร่างกายสูงกว่าผู้ป่วยทั่วไป และในปี ค.ศ. 1997 Dennis Lo และคณะได้ตรวจพบ cfDNA ปริมาณน้อยที่หลั่งออกจากรกในครรภ์แล้วมาไหลเวียนอยู่ในเลือดของมารดาที่ตั้งครรภ์ ซึ่ง cfDNA จะถูกนำมาใช้ในการตรวจคัดกรองความผิดปกติของทารกในครรภ์จากเลือดมารดา (Noninvasive prenatal testing : NIPT)[1,3-4]

ในปัจจุบันนิยมตรวจหาโรคหรือความผิดปกติของร่างกายจากการตรวจ cfDNA โดยวิธีการตรวจเลือด (liquid biopsy) เนื่องจากเป็นวิธีที่สามารถทำได้สะดวกและรวดเร็ว สามารถวินิจฉัยโรคได้ตั้งแต่ระยะเริ่มต้นของการเกิดโรค สามารถวิเคราะห์ความเสี่ยงหรือโอกาสในการแพร่กระจายของโรคได้ และสามารถนำผลการรักษามาใช้เพื่อการพยากรณ์การเกิดโรคได้ ซึ่งจะสามารถติดตามผลการตรวจได้แบบ real time[1]

1. วัฏจักรของ ccfDNA

วัฏจักรของ ccfDNA ประกอบด้วย 3 กระบวนการหลักคือ การปลดปล่อยชิ้นส่วนดีเอ็นเอ (Release) การออกฤทธิ์ (Biological activity) และการกำจัด (Clearance)[5] ซึ่งเมื่อเซลล์เกิดการบาดเจ็บเสียหายหรือเกิดความผิดปกติของร่างกายจนไม่สามารถทำการรักษาหรือฟื้นฟูได้ (irreversible injury) ส่งผลให้ร่างกายเกิดการสูญเสียการรักษาสมดุล (homeostasis) จึงนำไปสู่การตายของเซลล์ (cell death) โดยกระบวนการตายของเซลล์นั้นสามารถแบ่งเป็น 2 รูปแบบหลัก คือ

1. Necrosis เป็นการตายของเซลล์ทั่วไปที่ไม่สามารถคาดการณ์ได้ (unpredicable) ซึ่งอาจจะเกิดจากปัจจัยภายนอกต่าง ๆ เช่น อุณหภูมิ สารเคมี รังสี ภาวะขาดเลือด ภาวะขาดออกซิเจน การติดเชื้อ ทำให้เซลล์ไม่สามารถรักษาสมดุลได้อย่างมีประสิทธิภาพจึงสูญเสียความสามารถในการควบคุมไอออน น้ำจึงเกิดการออสโมซิส (osmosis) เข้ามาในเซลล์ทำให้เกิดการบวมของเซลล์ (cell swells) จนนำไปสู่การแตกของเซลล์แล้วปลดปล่อยสารต่าง ๆ ที่อยู่ภายในเซลล์ออกมาไปรบกวนเซลล์อื่น ๆ ที่อยู่ใกล้เคียงและจะมีการกระตุ้นให้สร้างเอนไซม์ cyclooxygenases ทำให้เกิดการอักเสบของเซลล์ (inflammation) ส่งผลให้เซลล์ที่อยู่ใกล้เคียงเกิดการอักเสบและเกิดการตายของเซลล์ตาม โดยเซลล์ที่ตายจะเกิดการย่อยสลาย (phagocytosis) แบบไม่จำเพาะและจะไม่สามารถสร้าง ATP ได้ตามเดิม
2. Apoptosis เป็นการตายของเซลล์ที่เกิดจากการควบคุมและดำเนินการอย่างมีระบบแบบแผน เพื่อให้ร่างกายสามารถมีการทำงานได้อย่างปกติ และเพื่อให้เกิดการพัฒนาของเอ็มบริโอได้อย่างปกติ โดยจะเกิดการหดตัวของเซลล์ (cell shrinks) และจะต้องมีการใช้เอนไซม์ในการไปตัดโปรตีนอื่นให้เล็กลง เช่น

caspase ซึ่งจะมีการตัดอย่างมีแบบแผนทำให้เซลล์เกิดการแตกตัว (budding) ได้เป็นถุงเวสิเคิลเล็ก ๆ เรียกว่า apoptosis bodies ทำให้สะดวกต่อการย่อยสลาย (phagocytosis) ซึ่งเซลล์จะไม่มีการปลดปล่อยสารต่าง ๆ ออกมารบกวนเซลล์อื่น ๆ ที่อยู่ใกล้เคียงจึงทำให้ไม่เกิดการอักเสบ ดังนั้นจะเกิดการตายของเซลล์เพียงหนึ่งเซลล์ โดยที่เซลล์ที่อยู่ใกล้เคียงจะไม่เกิดการตายของเซลล์และเซลล์ที่ตายยังคงสามารถสร้าง ATP ได้ปกติ

หลังจากที่เกิดการตายของเซลล์แล้วจะส่งผลให้ชิ้นดีเอ็นเอขนาดเล็กที่อยู่ภายนอกเซลล์ถูกปล่อยออกจากเซลล์เข้ามาแล้วล่องลอยอย่างอิสระอยู่ภายในของเหลวในร่างกาย เช่น เลือด เสมหะ น้ำปัสสาวะ น้ำไขสันหลัง หรือน้ำคั่งในช่องท้อง แล้วจะเกิดการแพร่กระจายไปอวัยวะส่วนต่าง ๆ[1,4,6-7]

2. ลักษณะของ cfDNA

cfDNA คือ ชิ้นส่วนของดีเอ็นเอที่มีขนาดเล็กอาจจะเป็นดีเอ็นเอสายเดี่ยว (single-stranded DNA) หรือดีเอ็นเอสายคู่ (double-stranded DNA)[1] ที่มีความยาวประมาณ 40-200 bp[5] และมีความเข้มข้นอยู่ที่ประมาณ 30 ng/ml[1] ในขณะที่สายดีเอ็นเอของมนุษย์ปกติจะมีความยาวอยู่ที่ประมาณ 3×10^9 bp[9] และมีความเข้มข้นอยู่ที่ประมาณ 50 μg/ml[10] ซึ่ง cfDNA นั้นจะมีช่วงชีวิตที่สั้นมากสามารถอยู่ได้ประมาณ 15 นาทีถึง 2 ชั่วโมง[11] ดังนั้นจึงมีการเก็บรักษา cfDNA ไว้ในหลอด cell free DNA blood collection tube ที่มีสารช่วยรักษาสภาพเซลล์ และยับยั้งเอนไซม์ไม่ให้ cfDNA และ ccfDNA ถูกทำลาย ทำให้สามารถเก็บรักษาตัวอย่างได้นานอย่างน้อย 14 วัน ที่อุณหภูมิ 4-37°C[12]

3. การเกิดความแปรผันทางพันธุกรรมของ cfDNA

ความแปรผันทางพันธุกรรม (genetic variations) อาจเกิดขึ้นมาจากความผิดปกติของจำนวนชุดดีเอ็นเอ (copy number aberrations), การเปลี่ยนตำแหน่งนิวคลีโอไทด์เพียงหนึ่งตำแหน่ง (single nucleotide polymorphisms), กระบวนการเกิด methylation เป็นต้น ซึ่งในการตรวจพบ cfDNA ที่เกิดความแปรผันทางพันธุกรรมในพลาสมานั้นจะเป็นตัวบ่งชี้ที่แสดงถึงการเกิดโรคหรือการเกิดความผิดปกติของผู้ป่วยได้[1]

3.1 ความแปรผันของจำนวนชุดดีเอ็นเอ

ความแปรผันของจำนวนชุดดีเอ็นเอ (copy number variations of cfDNA) เป็นการเปลี่ยนแปลงลำดับเบสซ้ำโดยการเพิ่ม (insertion) หรือลด (deletion) จำนวนเบสมากกว่าหนึ่งตำแหน่ง เช่น การเพิ่มจำนวนของเบสจาก AGC เป็น AAAAGC เป็นต้น(13-15)

3.2. การเปลี่ยนตำแหน่งนิวคลีโอไทด์เพียงหนึ่งตำแหน่ง

การเปลี่ยนตำแหน่งนิวคลีโอไทด์เพียงหนึ่งตำแหน่ง (single nucleotide polymorphisms) หรือเรียกว่า สนิปส์ (SNPs) เช่น การเปลี่ยนแปลงแทนที่เบสแล้วได้ลำดับกรดอะมิโนเดิม (silent mutation) ซึ่งส่วนใหญ่จะเกิดกับเบสตัวที่ 3 ของรหัสพันธุกรรมที่เรียกว่า wobble base, การเปลี่ยนแปลงแทนที่เบสจากเบสภายในกลุ่มเดียวกัน (transition) หรือการแทนที่เบสต่างกลุ่มกัน (transversion) แล้วทำให้ได้ลำดับกรดอะมิโนต่างไปจากเดิม (missense mutation), การเปลี่ยนแปลงแทนที่เบสแล้วทำให้กรดอะมิโนกลายเป็นรหัสหยุด ส่งผลให้สายพอลิเพปไทด์มีขนาดสั้นลง (nonsense mutation)(14,16-17)

3.3 กระบวนการเกิด methylation

DNA methylation เป็นการควบคุมเหนือพันธุกรรม โดยจะเติมหมู่เมทิล (CH_3) ที่คาร์บอนตำแหน่งที่ 5 ของเบสไซโทซีน (cytosine) ในดีเอ็นเอทำให้ได้ 5-เมทิลไซโทซีน (5-methyl cytosine) ซึ่งจะอาศัยการทำงานของเอนไซม์ดีเอ็นเอเมทิลทรานเฟอร์เรส (DNA methyltransferase) กระบวนการ DNA methylation มี 2 รูปแบบ คือ การเติมหมู่เมทิลเข้าจะทำให้ยับยั้งการแสดงออกของยีน และการดึงหมู่เมทิลออกจะทำให้เกิดการแสดงออกของยีน (4,8,17-18)

4. การนำ ccfDNA มาใช้ประโยชน์ในทางการแพทย์

การเกิดความแปรผันทางพันธุกรรมของ cfDNA จะเป็นตัวบ่งชี้ที่แสดงถึงการเกิดโรคหรือการเกิดความผิดปกติต่าง ๆ ของร่างกายได้ เช่น เนื้องอกร้ายหรือมะเร็ง (malignancies)(1) ความผิดปกติของทารกในครรภ์ เป็นต้น ซึ่งในอดีตการที่เราจะพบความผิดปกติของยีนหรือพบว่าเป็นโรคต่าง ๆ จะใช้เวลานานในการตรวจพบทำให้อาจเกิดการลุกลามของโรคไปมาก ส่งผลให้ยากต่อการทำการรักษา ในการผ่าตัดเพื่อทำการตรวจนั้นจะทำให้ผู้ป่วยเกิดความเจ็บปวดและจะเกิดรอยแผลเป็นจากการผ่าตัด อีกทั้งยังมีโอกาสในการเกิดการติดเชื้อได้สูง(19) แต่ในปัจจุบันนี้จะใช้การตรวจโดยวิธี liquid biopsy ซึ่งเป็นเทคนิคในการตรวจหาชิ้นเนื้อเยื่อหรือ ดีเอ็นเอที่ถูกปล่อยออกจากเซลล์เข้าสู่ของเหลวในร่างกาย โดยสามารถให้การวินิจฉัยโรคจากเซลล์เนื้องอกในกระแสเลือด (CTCs), ชิ้นส่วนดีเอ็นเอในกระแสเลือด (cfDNA), exosomes และอื่น ๆ ซึ่งเทคนิคนี้จะช่วยทำให้ผู้ป่วยเจ็บตัวน้อยลง (noninvasive technique) และมีประโยชน์อย่างมากในการนำไปใช้เพื่อการวินิจฉัยโรคในระยะเริ่มต้น สามารถติดตามผลการได้แบบ real time วิเคราะห์ความเสี่ยงในการลุกลามของโรคได้และสามารถนำผลการรักษามาใช้เพื่อการพยากรณ์การเกิดโรคได้(1,3)

4.1 การวินิจฉัยโรคมะเร็ง (cancer)

จากการศึกษาของ Leon S.A. และคณะพบระดับ cfDNA ที่สูงผิดปกติในตัวอย่างซีรัมผู้ป่วยโรคมะเร็งเมื่อเปรียบเทียบกับในซีรัมของผู้ป่วยที่ไม่เป็นมะเร็ง โดยได้ทำการศึกษาในซีรัมผู้ป่วยมะเร็งต่าง ๆ จำนวน 173 ตัวอย่าง และซีรัมผู้ป่วยที่ไม่เป็นมะเร็งจำนวน 55 ตัวอย่าง โดยอาศัยเทคนิค radioimmunoassay พบว่าในซีรัมของผู้ป่วยมะเร็งและซีรัมของผู้ป่วยที่ไม่เป็นมะเร็งจะพบ cfDNA มีค่าเฉลี่ยเท่ากับ 180± 38 ng/ml และ

13± 3 ng/ml ตามลำดับ จากการรายงานค่าข้างต้นพบว่าค่าเฉลี่ยความเข้มข้นของ cfDNA ในเลือดผู้ป่วยมะเร็งมีค่าสูงกว่าเลือดคนปกติมากกว่า 10 เท่า จึงเป็นที่น่าสนใจว่า cfDNA อาจสามารถนำมาใช้เป็นตัวบ่งชี้ทางชีวภาพสำหรับวินิจฉัยโรคหรือพยากรณ์โรคได้ ปัจจุบันมีการพยายามศึกษาเพื่อนำ cfDNA มาใช้เป็นตัวบ่งชี้ทางชีวภาพ ทั้งในแง่เกี่ยวกับการเกิด mutation หรือ epigenetic ของยีนต่าง ๆ ในหลายโรคมะเร็ง

มีรายงานอย่างต่อเนื่องว่าโปรตีน 14-3-3σ เกี่ยวข้องกับการเกิดมะเร็ง และหลายการศึกษาแสดงให้เห็นว่าการสูญเสียการแสดงออกของโปรตีนชนิดนี้เกิดจากกระบวนการ DNA hypermethylation ของยีน 14-3-3σ ที่เกิดขึ้นบริเวณ CpG island ของ promoter หรือบริเวณใกล้เคียง นอกจากนี้การเกิด 14-3-3σ hypermethylation ยังสามารถตรวจวัดได้ในซีรัม ดังนั้นจึงมีความเป็นไปได้ว่าการเกิด 14-3-3σ hypermethylation สามารถนำมาใช้เป็นตัวบ่งชี้การเกิดมะเร็ง (tumor marker) เพื่อช่วยวินิจฉัยในเลือดได้

การเกิด 14-3-3σ methylation มีระดับสูงในซีรัมผู้ป่วยมะเร็งปอดชนิดเซลล์ไม่เล็ก (non small cell lung cancer : NSCLC) อาจเป็นผลมาจากการปนเปื้อน DNA จากเซลล์เม็ดเลือดขาวภายในตัวอย่างซีรัม โดยมีรายงานว่าการแข็งตัวของเลือดอาจทำให้เซลล์เม็ดเลือดขาวมีการแตกตัวและปลดปล่อยดีเอ็นเอออกมา ส่งผลให้มีปริมาณของ cfDNA ภายในตัวอย่างซีรัมสูง จากการศึกษาตัวอย่างซีรัมในผู้บริจาคโลหิตที่มีร่างกายแข็งแรงเป็นกลุ่มควบคุมพบว่าการเกิด 14-3-3σ methylation ในซีรัมของผู้ป่วยสูงกว่าในกลุ่มควบคุม ดังนั้นจึงอาจเป็นไปได้ว่าระดับที่สูงในซีรัมของกลุ่มผู้ป่วย NSCLC เกิดจากเซลล์มะเร็งที่มีการปลดปล่อย DNA เข้าสู่กระแสเลือดมากกว่าปกติ แม้ว่าในปัจจุบันกลไกของการปลดปล่อย cfDNA ออกมาภายในกระแสเลือดยังไม่เป็นที่ทราบชัดเจน แต่ก็มีความเป็นไปได้ว่าปริมาณที่มากขึ้นของ cfDNA ในตัวอย่างซีรัมของผู้ป่วยนั้น อาจมีสาเหตุจากร่างกายมีกลไกในการกำจัดเซลล์มะเร็ง เช่น การเกิด apoptosis และ necrosis ส่งผลให้เซลล์มะเร็งเกิดความเสียหายและทำการปลดปล่อย cfDNA ออกมาในที่สุด(4,20)

4.2. การวินิจฉัยความผิดปกติของทารกในครรภ์

รกเป็นแหล่งของ fetal DNA ในพลาสมามารดาที่สำคัญ โดยอาศัยความรู้ทางด้าน epigenetics ทำให้สามารถแยก fetal DNA ออกจาก cfDNA ของมารดา จากการศึกษาพบว่า fetal DNA ส่วนใหญ่มาจากรก เนื่องจากรกมีภาวะ apoptosis และ necrosis จะเกิดขึ้นบ่อยโดยเฉพาะในบางภาวะเช่นภาวะโลหิตสูงในสตรีตั้งครรภ์ (preeclampsia) สำหรับการนำเอาความรู้เรื่อง cell free fetal DNA (cffDNA) มาใช้ในทางคลินิกจะใช้วิธี quatitative analysis จากการศึกษาพบว่าปริมาณของ cffDNA ในเลือดมารดามีความสัมพันธ์กับภาวะครรภ์เป็นพิษ คลอดก่อนกำหนด และ fetal chromosomal aneuploidy แต่เนื่องจากพบว่ามีความคาบเกี่ยวกันของปริมาณเฉลี่ยของ cffDNA ในครรภ์ปกติและในครรภ์ที่มีภาวะแทรกซ้อนทำให้ sensitivity specificity ของการตรวจกรองนี้ลดลงจึงได้มีความพยายามที่จะหา fetal nucleic acid markers ที่มีความจำเพาะของทารกในภาวะความผิดปกตินั้น ๆ เนื่องจาก cffDNA มีปริมาณน้อยมากเมื่อเทียบกับ maternal DNA โดยพบว่ามีสัดส่วนน้อยกว่า 1:20 จึงได้มีการทำ fetal DNA enrichment ได้แก่ elective enrichment of fatal DNA และ suppression of maternal เพื่อเพิ่มปริมาณ cffDNA ให้มากพอที่จะนำไปศึกษาวิจัยต่อไป(21)

มีการทดลองทำ fetal DNA enrichment โดยเปรียบเทียบกลุ่มตัวอย่างของเลือดมารดาที่เติมสารละลาย formaldehyde กับกลุ่มตัวอย่างที่ไม่เติมสารละลาย formaldehyde พบว่าการเติมสารละลาย formaldehyde จะทำให้เยื่อหุ้มเซลล์มีความเสถียรขึ้น และช่วยยับยั้งการสลายของ maternal cell เพื่อไม่ให้ maternal DNA ปล่อยออกมาปนกับ cffDNA จากการทดลองพบว่ากลุ่มตัวอย่างของเลือดมารดาที่เติม

formaldehyde จะมีค่าเฉลี่ย 66.1 fetal genomes/mL ที่จากเดิมมีค่าเฉลี่ย 25.4 fetal genomes/mL ซึ่งเพิ่มขึ้น 2.6 เท่า

นอกจากนี้ยังมีปัจจัยอื่น ๆ ที่ส่งผลต่อการเพิ่มปริมาณของ cffDNA เช่น ในการเจาะเลือดโดยปกติแล้วจะพบ maternal DNA ในเลือดมารดามีปริมาณน้อย แต่อาจจะพบว่ามีปริมาณสูงได้เนื่องจากการย่อยสลายของเซลล์เม็ดเลือดขาวและเซลล์เกล็ดเลือดในเลือดของมารดา ขณะขนส่งเลือดตัวอย่างที่ถูกเจาะออกมา หรือขณะตรวจวิเคราะห์เลือดตัวอย่างนั้น การใส่สารละลาย formaldehyde ลงไปจึงสามารถลดการเกิดการแตกสลายของเซลล์ดังกล่าวได้[22]

สรุป

Circulating cell free DNA (ccfDNA) คือ ชิ้นส่วนของดีเอ็นเอที่มีขนาดเล็กที่ถูกปลดปล่อยออกมาในกระแสเลือดด้วยกระบวนการตายของเซลล์ (cell death) คือการเกิด Necrosis หรือ Apoptosis ซึ่งในปัจจุบันนิยมนำมาใช้ตรวจโรคหรือหาความผิดปกติของร่างกายด้วยการตรวจเลือด (liquid biopsy) เนื่องจากจะช่วยทำให้ผู้ป่วยเจ็บตัวน้อยลง (noninvasive technique) สามารถให้ผลตรวจที่รวดเร็ว และเป็นประโยชน์ในการวินิจฉัยโรคได้ตั้งแต่ระยะเริ่มต้นของการเกิดโรค วิเคราะห์ความเสี่ยงในการลุกลามของโรค และสามารถนำผลการรักษามาใช้เพื่อพยากรณ์การเกิดโรคได้

<u>เอกสารอ้างอิง</u>

(1). Yingli Sun, Ke An, Caiyun Yang. IntechOpen [Internet]. United kingdom: IntechOpen; 2019 [cited 2021 Jun 22]. Available from: https://www.intechopen.com/books/liquid-biopsy/circulating-cell-free-dna

(2). Hendy O, Tawfik Abdel Motalib, El MA, et al. Circulating cell free DNA as a predictor of systemic lupus erythematosus severity and monitoring of therapy [Internet]. Egypt: Egypt J Med Hum Genet. 2016 [cited 2021 Jun 22]. Available from: https://www.sciencedirect.com/science/article/pii/S1110863015000816

(3). ปานเทพ สุทธินนท์, มานพ พิทักษ์ภากร, สมชัย บวรกิตติ. การเก็บของเหลวเพื่อชันสูตร. วารสารธรรรมศาสตร์เวชสาร [อินเทอร์เน็ต]. ก.ค. – ก.ย. 2560 [เข้าถึงเมื่อ 22 ก.ย. 2564]; 17(3): 456-7. เข้าถึงได้จาก file:///C:/Users/Administrator/Downloads/99531-Article%20Text-249447-1-10-20170919%20(4).pdf

(4). พิงค์พร เพ็ชรเจริญ. การเกิดดีเอ็นเอเมทิลเลชั่นของยีน 14-3-3σ ในผู้ป่วยคนไทยด้วยโรคมะเร็งปอดชนิดเซลล์ไม่เล็ก [อินเทอร์เน็ต]. [ปริญญานิพนธ์ ปริญญาวิทยาศาสตร์มหาบัณฑิต]. สงขลา: มหาวิทยาลัยสงขลานครินทร์; 2558 [เข้าถึงเมื่อ 4 ก.ค. 2564]. เข้าถึงได้จาก https://kb.psu.ac.th/psukb/bitstream/2016/10461/1/404588.pdf

(5). Anatoli Kustanovich, Ruth Schwartz, Tamar Peretz, et al. Taylor & Francis Group [Internet]. Israel: Hadassah-Hebrew University Medical Center; 2019 [cited 2021 Jun 22]. Available from: https://www.tandfonline.com/doi/full/10.1080/15384047.2019.1598759

(6). วุฒิพงษ์ ทองใบ. cell injury and cell death. กรุงเทพฯ: มหาวิทยาลัยศรีนครินทรวิโรฒ, คณะวิทยาศาสตร์, ภาควิชาชีววิทยา; 2562.; 2 - 28

(7). วุฒิพงษ์ ทองใบ. cancer. กรุงเทพฯ: มหาวิทยาลัยศรีนครินทรวิโรฒ, คณะวิทยาศาสตร์, ภาควิชาชีววิทยา; 2562.

(8). วุฒิพงษ์ ทองใบ. The origin of animal diversity. กรุงเทพฯ: มหาวิทยาลัยศรีนครินทรวิโรฒ, คณะวิทยาศาสตร์, ภาควิชาชีววิทยา; 2564.

(9). อัจฉริยา รังษิรุจิ. Gene regulation in eukaryotes. กรุงเทพฯ: มหาวิทยาลัยศรีนครินทรวิโรฒ, คณะวิทยาศาสตร์, ภาควิชาชีววิทยา; 2563.

(10). ศุภกาญจน์ รัตนกร. Nucleic acids. กรุงเทพฯ: มหาวิทยาลัยศรีนครินทรวิโรฒ, คณะวิทยาศาสตร์, ภาควิชาเคมี; 2563.

(11). Blog Staff. Circulating Cell-Free DNA (cfDNA): How Can You Utilize it to its Full Potential? Part: 1 [Internet]. 2018 [cited 2 July 2021]. Available from: https://sequencing.roche.com/en/blog/circulating-cell-free-dna-part-1.html

(12). พริมา ไซเอ็นติฟิค. [อินเตอร์เน็ต]. กรุงเทพฯ: บริษัท; 2564 [เข้าถึงเมื่อ 9 ก.ค. 2564]. เข้าถึงได้จาก: https://www.prima-sci.com/17130670/cf-dna-tube?%20fbclid=IwAR2zu_%20%20by pxocyPZ8ROrDUHutilm8_VALcIXfPIZvhpKhVoGS4oqJ4xZl1MQ

(13). รักชนก โคโต. Chromosome Aberrations. กรุงเทพฯ: มหาวิทยาลัยศรีนครินทรวิโรฒคณะวิทยาศาสตร์, ภาควิชาชีววิทยา; 2563.

(14). ศุภณัฐ ไพโรหกุล. BIOLOGY. พิมพ์ครั้งที่ 12. กรุงเทพฯ: แอคทีฟ พริ้นท์; 2561.

(15). อัจฉริยา รังษิรุจิ. Genetic variability & Species concepts. กรุงเทพฯ: มหาวิทยาลัยศรีนครินทรวิโรฒ; 2564.

(16). อัจฉริยา รังษิรุจิ. Principles of evolution. กรุงเทพฯ: มหาวิทยาลัยศรีนครินทรวิโรฒ; 2563.

(17). รักชนก โคโต. Mutation. กรุงเทพฯ: มหาวิทยาลัยศรีนครินทรวิโรฒ, คณะวิทยาศาสตร์, ภาควิชาชีววิทยา; 2563.

(18). ธมลวรรณ วรฤทัย, ชินดนัย หงสประภาส, สิทธิศักดิ์ หรรษาเวก, กฤษณ์ เจริญลาภ, อภิวัฒน์ มุทิรางกูร. ระดับ LINE-1 methylation ต่ำลงในผู้ป่วยโรคเนื้องอกเนื้อเยื่ออ่อน. จุฬาลงกรณ์เวชสาร [อินเทอร์เน็ต]. ม.ค.-ก.พ. 2561 [เข้าถึงเมื่อ 29 มิ.ย. 2564];62(1):79-90. เข้าถึงได้จาก: http://clmjournal.org/_fileupload/journal/327-5-8.pdf

(19). วรรณพงษ์ เตรียมโพธิ์, สเตฟาน ไชย์อิเออร์. ศูนย์ความเป็นเลิศด้านฟิสิกส์ [อินเทอร์เน็ต]. กรุงเทพฯ: สาขาวิชาชีวนวัตกรรมและผลิตภัณฑ์ฐานชีวภาพอัจฉริยะ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล; 2562

[เข้าถึงเมื่อ 29 มิ.ย. 2564]. เข้าถึงได้จาก: http://thep-center.org/src2/views/network-academic-news.php?news_id=274

(20). Fiala C, Diamandis EP. New approaches for detecting cancer with circulating cell-free DNA. [Internet]. BMC Medicine. 2019 [cited 11 July 2021]. Available from: https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-019-1400-z?fbclid=IwAR3wC3fQr2fh3Eq4AgET0BY3-dQV71wOrxDYvrdWkxsvFb9ma0tTF9r1Uww

(21). พัญญู พันธ์บูรณะ. การวินิจฉัยทารกในครรภ์ด้วย cell free fetal DNA ในเลือดมารดา (Prenatal diagnosis by cell free fetal DNA in maternal blood). รายงานประจำปี 2554 โครงการพัฒนาศักยภาพประชากรไทย คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล [อินเทอร์เน็ต]. 2554 [เข้าถึงเมื่อ 29 มิ.ย. 2564]; 14-7. เข้าถึงได้จาก: https://med.mahidol.ac.th/sdmc/sites/default/files/public/pdf/annual2554.pdf

(22). Dhallan R. Methods to Increase the Percentage of Free Fetal DNA Recovered From the Maternal Circulation [Internet]. JAMA. 2004 [cited 11 July 2021]; 1117-9. Available from: https://jamanetwork.com/journals/jama/fullarticle/198312

APPENDIX 1
INFORMATION FOR AUTHORS

All authors listed in a paper submitted to Asian Archives of Pathology (AAP) must have contributed substantially to the work. It is the corresponding author who takes responsibility for obtaining permission from all co-authors for the submission. When submitting the paper, the corresponding author is encouraged to indicate the specific contributions of all authors (the author statement, with signatures from all authors and percentage of each contribution can be accepted). Examples of contributions include: designed research, performed research, contributed vital new reagents or analytical tools, analysed data, and wrote the paper. An author may list more than one type of contribution, and more than one author may have contributed to the same aspect of the work.

Authors should take care to exclude overlap and duplication in papers dealing with related materials. See also paragraph on Redundant or Duplicate Publication in “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” at http://www.icmje.org/index.html.

The submitted manuscripts will be reviewed by the members of the Editorial Board or the expert reviewers. At the discretion of the Editorial Board, the manuscripts may be returned immediately without full review, if deemed not competitive or outside the realm of interests of the majority of the readership of the Journal. The decision (reject, invite revision, and accept) letter will be coming from the Editorial Board who has assumed responsibility for the manuscript’s review. The editor’s decision is based not just on technical merit of the work, but also on other factors such as the priority for publication and the relevance to the Journal’s general readership. All papers are judged in relation to other submissions currently under consideration.

Categories of Manuscripts

1. **Letters to the Editor**

The letters to the editor are the reactions to any papers published in AAP. These letters will be reviewed by the Editorial Board and sent to the authors of the original paper with an invitation to respond. Letters and eventual responses will be published together, when appropriate.

- *Word Count: 300 – 500 words (excluding references and figure or table legends)*
- *Abstract: Not required*
- *References: Maximum of 10*
- *Figure or Table: Maximum of 1 (if needed)*

2. **Original Articles**

The original articles are the researches describing the novel understanding of anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. Systematic reviews, meta-analyses and clinical trials are classified as articles. The articles should be clearly and concisely written in the well-organised form (see ***Organisation of Manuscripts***): abstract; introduction; materials and methods; results; discussion; and conclusions. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two or more experts in the field.

- *Word Count: 3,000 – 5,000 words (excluding abstract, references, and figure or table legends)*
- *Structured Abstract (see* ***Organisation of Manuscripts****): 150 – 200 words*
- *References: Maximum of 150*
- *Figures or Tables: Maximum of 6*

3. Review Articles

The review articles are generally invited by the Editor-in-Chief. They should focus on a topic of broad scientific interest and on recent advances. These articles are peer-reviewed before the final decision to accept or reject the manuscript for publication. Therefore, revisions may be required.

- *Word Count: 3,000 – 5,000 words (excluding abstract, references, and figure or table legends)*
- *Unstructured Abstract: 150 – 200 words*
- *References: Maximum of 150*
- *Figures or Tables: Maximum of 4*

4. Case Reports

AAP limits publication of case reports to those that are truly novel, unexpected or unusual, provide new information about anatomical pathology, clinical pathology (laboratory medicine) or forensic medicine (legal medicine or medical jurisprudence). In addition, they must have educational value for the aforementioned fields. The journal will not consider case reports describing preventive or therapeutic interventions, as these generally require stronger evidence. Case reports that involve a substantial literature review should be submitted as a review article. The submitted case reports will undergo the usual peer-reviewed process.

- *Word Count: 1,200 – 2,000 words (excluding abstract, references, and figure or table legends)*
- *Unstructured Abstract: 150 – 200 words*
- *References: Maximum of 20*
- *Figures or Tables: Maximum of 4*

5. **Case Illustrations**

    Case illustrations are aimed to provide education to readers through multidisciplinary clinicopathological discussions of interesting cases. The manuscript consists of a clinical presentation or description, laboratory investigations, discussion, final diagnosis, and up to 5 take-home messages (learning points). Regarding continuous learning through self-assessment, each of the case illustrations will contain 3 – 5 multiple choice questions (MCQs) with 4 – 5 suggested answers for each question. These MCQs are placed after the final diagnosis and the correct answers should be revealed after the references. The questions and take-home messages (learning points) are included in the total word count. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two experts in the field.

    - *Word Count: 1,000 – 2,000 words (excluding references and figure or table legends)*
    - *Abstract: Not required*
    - *References: Maximum of 10*
    - *Figures: Maximum of 2*
    - *Tables: Maximum of 5*

6. **Technical Notes**

    The technical notes are brief descriptions of scientific techniques used in the anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. The submitted manuscripts are usually peer-reviewed.

    - *Word Count: Maximum of 1,000 words (excluding references and figure or table legends)*
    - *Abstract: Not required*
    - *References: Maximum of 5*
    - *Figures or Tables: Maximum of 2*

Organisation of Manuscripts

1. **General Format**

    The manuscripts written in English language are preferable. However, Thai papers are also acceptable, but their title pages, abstracts, and keywords must contain both Thai and English. These English and Thai manuscripts are prepared in A4-sized Microsoft Word documents with leaving 2.54-cm (1-inch) margins on all sides. All documents are required to be aligned left and double-spaced throughout the entire manuscript. The text should be typed in 12-point regular Times New Roman font for English manuscript and 16-point regular TH SarabunPSK font for Thai manuscript.

The running titles of English and Thai manuscripts are placed in the top left-hand corner of each page. They cannot exceed 50 characters, including spaces between words and punctuation. For the header of English paper, the running title will be typed in all capital letters. The page number goes on the top right-hand corner.

Footnotes are not used in the manuscripts, but parenthetical statements within text are applied instead and sparingly. Abbreviations should be defined at first mention and thereafter used consistently throughout the article. The standard abbreviations for units of measure must be used in conjunction with numbers.

All studies that involve human subjects should not mention subjects' identifying information (e.g. initials) unless the information is essential for scientific purposes and the patients (or parents or guardians) give written informed consent for publication.

2. Title Page

The title page is the first page of the manuscripts and must contain the following:

- The title of the paper (not more than 150 characters, including spaces between words)
- The full names, institutional addresses, and email addresses for all authors (If authors regard it as essential to indicate that two or more co-authors are equal in status, they may be identified by an asterisk symbol with the caption "These authors contributed equally to this work" immediately under the address list.)
- The name, surname, full postal address, telephone number, facsimile number, and email address of the corresponding author who will take primary responsibility for communication with AAP.
- Conflict of interest statement (If there are no conflicts of interest for any author, the following statement should be inserted: "The authors declare that they have no conflicts of interest with the contents of this article.")

3. Abstract

A structured form of abstract is used in all Original Article manuscripts and must include the following separate sections:

- *Background: The main context of the study*
- *Objective: The main purpose of the study*
- *Materials and Methods: How the study was performed*
- *Results: The main findings*

- *<u>Conclusions</u>: Brief summary and potential implications*
- *<u>Keywords</u>: 3 – 5 words or phrases (listed in alphabetical order) representing the main content of the article*

4. Introduction

The Introduction section should clearly explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

5. Materials and Methods

The Materials and Methods section must be described in sufficient detail to allow the experiments or data collection to be reproduced by others. Common routine methods that have been published in detail elsewhere should not be described in detail. They need only be described in outline with an appropriate reference to a full description. Authors should provide the names of the manufacturers and their locations for any specifically named medical equipment and instruments, and all chemicals and drugs should be identified by their systematic and pharmaceutical names, and by their trivial and trade names if relevant, respectively. Calculations and the statistical methods employed must be described in this section.

All studies involving animal or human subjects must abide by the rules of the appropriate Internal Review Board and the tenets of the recently revised Helsinki protocol. Hence, the manuscripts must include the name of the ethics committee that approved the study and the committee's reference number if appropriate.

6. Results

The Results section should concisely describe the findings of the study including, if appropriate, results of statistical analysis which must be presented either in the text or as tables and figures. It should follow a logical sequence. However, the description of results should not simply repeat the data that appear in tables and figures and, likewise, the same data should not be displayed in both tables and figures. Any chemical equations, structural formulas or mathematical equations should be placed between successive lines of text. The authors do not discuss the results or draw any conclusions in this section.

7. Discussion

The Discussion section should focus on the interpretation and the significance of the findings against the background of existing knowledge. The discussion should not repeat information in the results. The authors will clearly identify any aspects that are novel. In addition, there is the relation between the results and other work in the area.

8. **Conclusions**

The Conclusions section should state clearly the main summaries and provide an explanation of the importance and relevance of the study reported. The author will also describe some indication of the direction future research should take.

9. **Acknowledgements**

The Acknowledgements section should be any brief notes of thanks to the following:

- *Funding sources*
- *A person who provided purely technical help or writing assistance*
- *A department chair who provided only general support*
- *Sources of material (e.g. novel drugs) not available commercially*

Thanks to anonymous reviewers are not allowed. If you do not have anyone to acknowledge, please write "Not applicable" in this section.

10. **References**

The Vancouver system of referencing should be used in the manuscripts. References should be cited numerically in the order they appear in the text. The authors should identify references in text, tables, and legends by Arabic numerals in parentheses or as superscripts. Please give names of all authors and editors. The references should be numbered and listed in order of appearance in the text. The names of all authors are cited when there are six or fewer. When there are seven or more, only the first three followed by "et al." should be given. The names of journals should be abbreviated in the style used in Index Medicus (see examples below). Reference to unpublished data and personal communications should not appear in the list but should be cited in the text only (e.g. A Smith, unpubl. Data, 2000).

- *Journal article*
  1. Sibai BM. Magnesium sulfate is the ideal anticonvulsant in preeclampsia – eclampsia. Am J Obstet Gynecol 1990; 162: 1141 – 5.
- *Books*
  2. Remington JS, Swartz MN. Current Topics in Infectious Diseases, Vol 21. Boston: Blackwell Science Publication, 2001.
- *Chapter in a book*
  3. Cunningham FG, Hauth JC, Leveno KJ, Gilstrap L III, Bloom SL, Wenstrom KD. Hypertensive disorders in pregnancy. In: Cunningham FG, Hauth JC, Leveno KJ, Gilstrap L III, Brom SL, Wenstrom KD, eds. Williams Obstetrics, 22nd ed. New York: McGraw-Hill, 2005: 761 – 808.

11. Tables

The tables should be self-contained and complement, but without duplication, information contained in the text. They should be numbered consecutively in Arabic numerals (Table 1, Table 2, etc.). Each table should be presented on a separate page with a comprehensive but concise legend above the table. The tables should be double-spaced and vertical lines should not be used to separate the columns. The column headings should be brief, with units of measurement in parentheses. All abbreviations should be defined in footnotes. The tables and their legends and footnotes should be understandable without reference to the text. The authors should ensure that the data in the tables are consistent with those cited in the relevant places in the text, totals add up correctly, and percentages have been calculated correctly.

12. Figure Legends

The legends should be self-explanatory and typed on a separate page titled "Figure Legends". They should incorporate definitions of any symbols used and all abbreviations and units of measurement should be explained so that the figures and their legends are understandable without reference to the text.

If the tables or figures have been published before, the authors must obtain written permission to reproduce the materials in both print and electronic formats from the copyright owner and submit them with the manuscripts. These also follow for quotes, illustrations, and other materials taken from previously published works not in the public domain. The original resources should be cited in the figure captions or table footnotes.

13. Figures

All illustrations (line drawings and photographs) are classified as figures. The figures should be numbered consecutively in Arabic numerals (Figure 1, Figure 2, etc.). They are submitted electronically along with the manuscripts. These figures should be referred to specifically in the text of the papers but should not be embedded within the text. The following information must be stated to each microscopic image: staining method, magnification (especially for electron micrograph), and numerical aperture of the objective lens. The authors are encouraged to use digital images (at least 300 d.p.i.) in .jpg or .tif formats. The use of three-dimensional histograms is strongly discouraged when the addition of these histograms gives no extra information.

14. Components

14.1. Letters to the Editor

The Letter to the Editor manuscripts consist of the following order:

- *Title Page*
- *Main Text*
- *References*
- *Table (if needed)*
- *Figure Legend (if needed)*
- *Figure (if needed)*

14.2. Original Articles

The Original Article manuscripts consist of the following order:

- *Title Page*
- *Structured Abstract*
- *Introduction*
- *Materials and Methods*
- *Results*
- *Discussion*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

14.3. Review Articles

The Review Article manuscripts consist of the following order:

- *Title Page*
- *Unstructured Abstract*
- *Introduction*
- *Main Text*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

14.4. Case Reports

The Case Report manuscripts consist of the following order:

- *Title Page*
- *Unstructured Abstract*
- *Introduction*
- *Case Description*
- *Discussion*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

14.5. Case Illustrations

The Case Illustration manuscripts consist of the following order:

- *Title Page*
- *Clinical Presentation or Description*
- *Laboratory Investigations*
- *Discussion*
- *Final Diagnosis*
- *Multiple Choice Questions (MCQs)*
- *Take-Home Messages (Learning Points)*
- *Acknowledgements*
- *References*
- *Correct Answers to MCQs*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

14.6. Technical Notes

The Technical Note manuscripts consist of the following order:

- *Title Page*
- *Introduction*
- *Main text*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

Proofreading

The authors of the accepted manuscripts will receive proofs and are responsible for proofreading and checking the entire article, including tables, figures, and references. These authors should correct only typesetting errors at this stage and may be charged for extensive alterations. Page proofs must be returned within 48 hours to avoid delays in publication.

Revised Manuscripts

In many cases, the authors will be invited to make revisions to their manuscripts. The revised manuscripts must generally be received by the Editorial Board within 3 months of the date on the decision letter or they will be considered a new submission. An extension can sometimes be negotiated with the Editorial Board.

APPENDIX 2
BENEFITS OF PUBLISHING WITH ASIAN ARCHIVES OF PATHOLOGY

Asian Archives of Pathology (AAP) is an open access journal. Open Access makes your works freely available to everyone in the world. It provides a significant boost to the readership of your articles, and has been shown to have an increase in positive influence on citations and reuse. Hence, open-access leads to more recognition for our esteemed authors.

The journal has been sponsored by the Royal College of Pathologists of Thailand. We have the policy to disseminate the verified scientific knowledge to the public on a non-profit basis. Hence, we have not charged the authors whose manuscripts have been submitted or accepted for publication in our journal.

Since AAP is also a peer-reviewed journal, the submitted manuscripts will be reviewed by the members of the Editorial Board or the expert reviewers. The decision on these manuscripts is processed very fast without any delay and in shortest possible time. The processing period is 1 – 2 weeks. These decisions of the reviewers are unbiased and the decision (reject, invite revision, and accept) letter coming from the Editorial Board is always conveyed to the authors.

APPENDIX 3
SUBMISSION OF THE MANUSCRIPTS

Step 1: Access www.asianarchpath.com

Step 2: If you did not register before, please create an account first.

Step 3: Login with your username and password.

Step 4: Click the "+ New Submission" button on the upper right-hand side of the page.

Step 5: Proceed to fill up the Submission Form online and follow the directions given therein.

Step 6: Upload your manuscript file (s).

Step 7: Re-check the content of your manuscript (s) and the uploaded file (s) more carefully prior to the submission. If you have submitted your manuscript file (s) incorrectly, you must contact Editor-in-Chief of Asian Archives of Pathology immediately. The Editor-in-Chief can clear the incorrect attempt and allow you another submission.

Step 8: Click the "Submit Manuscript" button under Important Notice.

If you have any further enquiries, please do not hesitate to contact the Journal.

APPENDIX 4
CONTACT THE JOURNAL

The Editorial Office of Asian Archives of Pathology

Department of Pathology, Floor 6, Her Royal Highness Princess Bejaratana Building
Phramongkutklao College of Medicine
317 Rajavithi Road, Rajadevi, Bangkok 10400 Thailand

Telephone: +66 (0) 90 132 2047

Fax: +66 (0) 2 354 7791

Email: editor@asianarchpath.com

APPENDIX 5
SUPPORT THE JOURNAL

Asian Archives of Pathology (AAP) has a mission of disseminating the unbiased and reliable medical knowledge on a non-profit basis. If you consider that this journal is useful for the public, you can support us by submitting your advertisements via the contact information below.

Assistant Professor Dr Chetana Ruangpratheep
The Editorial Office of Asian Archives of Pathology
Department of Pathology, Floor 6, Her Royal Highness Princess Bejaratana Building
Phramongkutklao College of Medicine
317 Rajavithi Road, Rajadevi, Bangkok 10400 Thailand
Telephone: +66 (0) 90 132 2047
Fax: +66 (0) 2 354 7791

Email: editor@asianarchpath.com

Every support, small or big, can make a difference.

Thank you

Assistant Professor Dr Chetana Ruangpratheep
MD, FRCPath (Thailand), MSc, PhD
Editor-in-Chief of Asian Archives of Pathology

ACADEMIC MEETINGS AND CONFERENCES

Announcements of academic meetings and conferences that are of interest to the readers of Asian Archives of Pathology (AAP) should be sent to the Editor-in-Chief at least 3 months before the first day of the month of issue. The contact information is shown below.

Assistant Professor Dr Chetana Ruangpratheep
The Editorial Office of Asian Archives of Pathology
Department of Pathology, Floor 6, Her Royal Highness Princess Bejaratana Building
Phramongkutklao College of Medicine
317 Rajavithi Road, Rajadevi, Bangkok 10400 Thailand

Telephone: +66 (0) 90 132 2047

Fax: +66 (0) 2 354 7791

Email: editor@asianarchpath.com

WHAT IS INSIDE THIS ISSUE?

Original Article:

Original Article:

Review Article: