ASIAN ARCHIVES OF PATHOLOGY

THE OFFICIAL JOURNAL OF THE ROYAL COLLEGE OF PATHOLOGISTS OF THAILAND

Volume 5
Number 1
January – March 2023

INDEX COPERNICUS INTERNATIONAL

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

EDITORIAL BOARD

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

CONTENTS

ORIGINAL ARTICLE

INJURY PATTERN AND DISTRIBUTION ACCORDING TO AUTOPSY FINDINGS AMONG PEDESTRIAN TRAFFIC FATALITIES

Gyan Gurung[1], Wisarn Worasuwannarak[2] and Smith Srisont[2*]

1 *Forensic Science Unit, Faculty of Science, Mahidol University, Bangkok, Thailand*

2 *Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*

* Correspondence to: Smith Srisont, MD, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. Telephone: +66 89 603 1578 Email: smithfa118@yahoo.com

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

Submitted: *26 November 2021*
Accepted: *30 November 2021*
Published: *1 March 2023*

Abstract

Background: Pedestrians are considered the most vulnerable group among all road users. In Thailand, the pedestrian has the highest fatality-to-injury ratio among other types of road users. The present study aims to evaluate the pattern and distribution of injuries among pedestrians involved in a fatal vehicle-pedestrian collision.

Methods: This is a retrospective study based on autopsy findings of 60 fatal pedestrian-vehicle collisions from two hospitals from 2013-2020. Autopsy records were reviewed to gather demographic characteristics, vehicle type, road type, and blood alcohol concentration (BAC).

Results: There were 37 males (61.7%) and 23 females (38.3%) included in the study. The largest group of fatalities was seen in the ≥60 years old age group (30%). Bonnet-front vehicle (43.3%) was the most common colliding vehicle, most of which occurred on the main-street (50%). The most frequently injured regions were the head (96.7%) and thorax (76.7%). The risk of sustaining injury was higher in all body regions when the collision occurred on the larger main street.

Conclusions: Although traffic crashes involving pedestrians constitute a relatively small proportion, they represent a much higher proportion of traffic fatalities when compared with

other types of road users. Countermeasures are important in place for reducing the mortality associated with pedestrians.

Keywords: Autopsy; Injury pattern; Pedestrian-Vehicle collision

Introduction

The victims of traffic accidents bear the brunt of the great conveniences today's motorized traffic offers. Deaths and injuries resulting from Road Traffic Accident remain a serious problem globally, causing more than 1.35 million deaths annually [(1)]. Pedestrians are considered the most vulnerable group among road users accounting for more than 26% of 1.35 million deaths annually [(1)]. A typical pedestrian accident involving a motorized vehicle and a pedestrian brings together quite unequal opponents. While the occupant of the vehicle is shielded by various safety technologies a modern vehicle can offer, the pedestrian's only protection is usually his or her clothing.

When reviewing the injury surveillance report of Thailand for the year 2017 and 2018, there were 4,753 and 4,327 total traffic fatalities, out of which 340 and 327 were pedestrians, which accounts for 7.1% and 7.7% of total traffic fatalities, respectively in the country [(2)]. As per the annual epidemiological surveillance report, ministry of public health, Thailand, though there has been a decrease in the number of fatalities in all road user types in the successive year, the so-called vulnerable road users remain to have higher percent fatality-to-injury ratios when involved in traffic crashes. Pedestrians had the highest percent fatality-to-injury ratio of 12 and 12.3 in 2017 and 2018, respectively. There is very few research done on vehicle-pedestrian collision in Thailand and in particular the injury pattern sustained by pedestrian during the collision. Therefore, this present study has been planned to determine the risk factors, distribution of injuries sustained and their association with risk factors involved in fatal pedestrian-vehicle collision.

Materials and Methods

The research design was a retrospective study consisting of data based on autopsy findings of fatal pedestrian traffic injury as obtained from two hospitals; Ramathibodhi for the duration of 2013-2020, and Chakri Naruebodindra Medical Institute for the duration of august 2018 to December 2020. The first hospital is centrally located in the heart of the capital city, Bangkok, and provide autopsy service within the jurisdiction of Bangkok metropolitan Police divisions 1 and 2, which also accommodates the 10 police stations in Bangkok. The second hospital provide autopsy service in Samut Prakan province.

The demographic variables of interest for this study included age, gender, date of the pedestrian traffic injury, type of vehicle involved in the collision, type of road where the collision occurred, blood alcohol concentration (BAC), and toxicology report of the decedents. The various injuries revealed in the autopsy report were broadly categorized as per the International classification of disease (ICD-10), which provides a common language for reporting and monitoring disease, and allows the world to compare and share data in a consistent and standard way.

Data analysis

Variables were checked and coded before entering data in SPSS. Data analysis was processed by using SPSS software version 22 for windows. Descriptive indices such as frequency, percentage, range, mean, and standard deviation were used to express the demographic factors and pattern of injuries sustained in pedestrian traffic collisions. For the relationship between the variables, the Chi-square test (Pearson χ^2 test) was used to test the association between injury pattern and other categorical variables, and Odds ratio (OR) was used to estimate the risk ratios between the injury pattern and the other variables (Gender, BAC, type of vehicle, and type of road).

Ethical Aspects

Human research ethics committee, faculty of medicine, Ramathibodhi hospital, Mahidol university authorized data collection and approved the study protocol.

Results

Sixty pedestrian traffic fatalities data were collected from autopsy reports. Ramathibodhi hospital registered 51 cases during 2013-2020 in Bangkok, and Chakri Naruebodindra medical institute registered 9 cases during 2018-2020 in Samut Prakan province. There were 37 males (61.7%) and 23 females (38.3%) involved in fatal pedestrian accidents. The mean age of all fatalities was 45 years with a standard deviation (SD) of 20.03 years (1 to 81 years). The age group ≥60 years represented the highest frequency of deaths with 30% of total deaths (Table 1).

Table 2 shows that vehicles with a bonnet front profile were most frequently involved with 26 fatal cases (43.3%) followed by motorcycle, 21 cases (35%). Half of the fatal pedestrian accidents occurred on the larger roads categorized as “main street” and there were only 9 cases of fatal accidents on the smaller “non-main street.” 35 out of 60 cases were subjected to blood alcohol concentration (BAC) assessment. The results were that 21 cases (35%) had no alcohol detection, 1 case had BAC less than 50mg/dl, and 13 cases (21.7%) had above 50

mg/dl. Mean BAC in all fatality was 65.21 mg/dl with SD of 97.66 mg/dl (range 0.0 to 324 mg/dl). For those who had BAC beyond 50 mg/dl, the ratio between males to females was 5.5:1.

Table 3 shows that Monday had the highest frequency of pedestrian fatalities with 13 cases (21.7%), followed by Tuesday and Thursday with 9 cases each. There was no association between the day of the week and time of day (*p*-value =0.751). There were 31 cases (51.7%) of pedestrian fatalities observed at nighttime and 29 cases (48.3%) at daytime. The number of fatalities on weekdays was 45 cases (75%, consisting of 48.9% in the daytime and 51.1% in nighttime) whilst the weekend was 15 cases (25%, consisted of 46.7 % in the daytime and 53.3% in nighttime). It was also observed that the average number of fatalities was slightly higher on weekdays than on weekends, with 9 and 7.5 cases per day, respectively.

Table 1 Distribution of fatal pedestrian traffic accident according to age and gender.

Age Group (years)	Male No of Deaths (%)	Female No of deaths (%)	Total (%)
<15	3 (5 %)	0 (0%)	3 (5 %)
15-29	8 (13.3 %)	5 (8.3%)	13 (21.7%)
30-44	10 (16.7 %)	3 (5 %)	13 (21.7 %)
45-59	7 (11.7 %)	6 (10 %)	13 (21.7 %)
≥60	9 (15%)	9 (15%)	18 (30%)
Total	37 (61.7 %)	23 (38.3 %)	60 (100%)

Table 2 Distribution of pedestrian fatalities by type of vehicle, road and BAC.

Characteristics		Number	Percentage
Type of vehicle	Bonnet front[a]	26	43.3
	Flat front[b]	11	18.3
	Motorcycle[c]	21	35.0
	Unknown	2	3.3
	Total	60	100
Type of road	Main street[d]	30	50.0
	Non Main street[e]	9	15.0
	Unknown	21	35.0
	Total	60	100
Blood alcohol concentration (mg/dl)	0.00	21 (M-10, F-11)	35
	1-50	1 (M-1, F-0)	1.7
	>50	13 (M-11, F-2)	21.7
	Unknown [f]	25 (M-15, F-10)	41.7
	Total	60	100

[a] Includes bonnet front vehicles: cars, SUVs, pickup truck;
[b] Includes larger vehicles with flat front: bus, truck, train, van;
[c] Includes motorized two wheelers and three wheelers;
[d] Includes expressway, national highway and rural highway;,
[e] Includes small lanes and foot path; f No evidence for BAC

Table 3 Distribution of pedestrian fatalities by time of accident

Time Classification		Time of day [a]		Total
		Daytime	Nighttime	
Day of week	Monday	6	7	13 (21.7)
	Tuesday	5	4	9 (15)
	Wednesday	4	3	7 (11.7)
	Thursday	5	4	9 (15)
	Friday	2	5	7 (11.7)
	Saturday	5	3	8 (11.7)
	Sunday	2	5	7 (11.7)
$\chi 2$ = 3.451 p-value =0.751				
Time of week	weekday	22	23	45 (75)
	Weekend [b]	7	8	15 (25)
Total		29(48.3)	31(51.7)	60 (100)
OR = 1.093 95% CI= 0.339, 3.525				
[a] 6:00 to 17:59 for daytime and 18:00 to 05:59 for nighttime [b] Saturday and Sunday				

The frequency of injury in each anatomic region is presented in table 4. Head was the most common body region to sustain an injury with an overwhelming 96.7 percent, followed by injuries to thorax region (76.7%), abdomen (58.3%), neck (41.7%), hip and thigh (26.7%), knee and lower leg (16.7%), shoulder and upper arm (13.3%), and elbow and forearm (11.7%). Subgaleal hemorrhage (90%) and subarachnoid hemorrhage (86.7%) were the most common type of injuries sustained in the head region. Skull fracture (63.3%), base of skull fracture (50%), brain laceration (48.3%) and subdural hemorrhage (SDH) (40%) were also not very uncommon. Multiple rib fractures (58.3%) and hemoperitoneum (48.3%) were noted most frequently in thorax and abdomen region, respectively.

Table 4 Distribution of injuries n=60

ICD codes	Injury descriptions	Number	Percent
S00-S09	HEAD	58	96.7
	Subgaleal Hemorrhage	54	90
	Sub-arachnoid Hemorrhage	52	86.7

	Skull Fracture	38	63.3
	Base skull Fracture	30	50
	Brain Laceration	29	48.3
	Subdural Hemorrhage	24	40
	Epidural Hemorrhage	3	5
S20-S29	THORAX	46	76.7
	Multiple rib fracture	35	58.3
	Hemo-thorax	34	56.7
	Lung laceration	24	40
	Hemo-pericardium	10	16.7
	Heart laceration	8	13.3
	Aortic laceration	7	11.7
S30-S39	ABDOMEN	35	58.3
	Hemo-peritoneum	29	48.3
	Liver laceration	22	36.7
	Kidney laceration	14	23.3
	Spleen laceration	10	16.7
	Pancreas laceration	10	16.7
S10-S19	NECK	25	41.7
	Cervical spine fracture	22	36.7
	Spinal Hemorrhage	18	30
	Atlanto occipital joint fracture	13	21.7
S70-S79	HIP AND THIGH	16	26.7
	Pelvic fracture	9	15
	Femur fracture	7	11.7
S80-S89	KNEE AND LOWER LEG	10	16.7
	Fracture of lower leg	10	16.7
S40-S49	SHOULDER AND UPPER ARM	8	13.3
	Fracture of shoulder and upper arm	8	13.3
S50-S59	ELBOW AND FOREARM	7	11.7
	Fracture of forearm	7	11.7

As shown in Table 5, compared to females, males were more likely of sustaining injuries in the neck (OR=2.16), thorax (OR=1.87), and shoulder region (OR=2.03). There were 37 pedestrians being hit by four-wheelers (included bonnet and flat front) vehicles and 21 pedestrians being hit by the motorcycle. The pedestrian being hit by four-wheelers were almost 5 times more likely to sustain injuries to the shoulder region (OR=4.66), and more than

2 times in the neck (OR=2.36) and abdomen (OR=2.03) than those who collided with the motorcycle. On the contrary, collisions with four-wheelers were found to have a lesser risk of sustaining injury to the head (OR=0.63) and forearm (OR=0.37) compared to collision with the motorcycle.

Table 5 Injuries sustained as classified by gender and type of vehicle involved.

Injuries sustained		Gender (n=60)		OR (95%CI)	Type of vehicle (n=58)		OR (95%CI)
		Male	Female		Four wheels	Motor cycle	
Head	yes	36	22	1.63 (0.10-27.51)	36	21	0.63 (0.517-0.77)
	no	1	1		1	0	
Neck	yes	18	7	2.16 (0.72-6.48)	18	6	2.36 (0.75-7.44)
	no	19	16		19	15	
Thorax	yes	30	16	1.87 (0.55-6.29)	29	16	1.13 (0.317-4.04)
	no	7	7		8	5	
Abdomen	yes	23	12	1.50 (0.52-4.32)	24	10	2.03 (0.68-6.04)
	no	14	11		13	11	
Pelvis and thigh	yes	10	6	1.04 (0.32-3.40)	11	4	1.79 (0.49-6.58)
	no	27	17		26	17	
Knee and lower leg	yes	7	3	1.56 (0.35-6.73)	6	3	1.16 (0.26-5.22)
	no	30	20		31	18	
Shoulder and upper arm	yes	6	2	2.03 (0.37-11.05)	7	1	4.66 (0.53-40.88)
	no	31	21		30	20	
Elbow and forearm	yes	4	3	0.80 (0.16-3.98)	3	4	0.37 (0.075-1.86)
	no	33	20		34	17	
Total		37	23		37	21	

Table 6 Injuries sustained as classified by BAC level and type of road.

Injuries sustained		BAC Level (n=34) >50 mg/dl	BAC Level (n=34) ≤50 mg/dl[a]	OR (95%CI)	Type of road (n=39) Main-street	Type of road (n=39) Non Main - street	OR (95%CI)
Head	yes	13	19	0.59 (0.44-0.79)	29	9	0.76 (0.63- 0.91)
	no	0	2		1	0	
Neck	yes	8	7	3.2 (0.75-13.49)	17	4	1.63 (0.36- 7.32)
	no	5	14		13	5	
Thorax	yes	10	17	0.78 (0.14-4.24)	24	6	2 (0.38-10.40)
	no	3	4		6	3	
Abdomen	yes	8	14	0.8 (0.19-3.37)	16	4	1.42 (0.31- 6.38)
	no	5	7		14	5	
Pelvis and thigh	yes	3	7	0.6 (0.12- 2.90)	10	2	1.75 (0.30- 10.02)
	no	10	14		20	7	
Knee and lower leg	yes	11	19	1.73 (0.21-14.05)	5	2	0.7 (0.11-4.42)
	no	2	2		25	7	
Shoulder and upper arm	yes	0	3	1.72 (1.27- 2.32)	5	1	1.6 (0.16- 15.79)
	no	13	18		25	8	
Elbow and forearm	yes	2	3	1.09 (0.15-7.59)	4	0	1.34 (1.10-1.63)
	no	11	18		26	9	
Total		13	21		30	9	

[a] included non BAC level

Table 6, Pedestrians who were found to have blood alcohol concentration (BAC) beyond the legal limit of 50mg/dl, were more than 3 times likely to sustain an injury in the neck region (OR=3.2) than those whose BAC was below 50mg/dl (included none BAC) but besides that, there were lesser odds in sustaining injuries in other regions, head (OR=0.59), pelvis (OR=0.594) and lower legs (OR=0.57). For those accidents that occurred in the larger main street, it was found more likely to sustain injury in almost all regions when compared with those who were hit on the smaller non-main street road. Compared to collisions on the non-main street, those who were hit on the main street were 2 times likely to sustain injury in the thorax (OR=2). No significant odds for other regions were observed.

Discussion

Pedestrians are the most vulnerable road users and carry a higher severity of injuries than other road users as they are rarely protected, unlike its counterpart. In Thailand, it continues to have a higher percent fatality to injury ratio than other road users [(2)]. This study attempts to shed light on the patterns of pedestrian injury relative to their personal characteristics and to a lesser extent, the association with the behavioral, vehicle, and environmental characteristics.

Males (61.7%) were much more likely to be involved in a fatal pedestrian-vehicle collision, (Table 1) and agrees with the findings from the National Highway Traffic Safety Administration (NHTSA), which shows that male pedestrians had a greater risk of being involved in fatal pedestrian crashes, with more than two-third (69%) of the total pedestrian fatalities [(3)]. Higher representation of males in our research also agrees with previous studies that found higher involvement of males over females in fatal pedestrian-vehicle collisions [(4–6)]. This higher frequency of males being involved in a fatal pedestrian collision may be because of various reasons, such as in previous studies they have attributed greater risk-taking behavior in men, walking after dark, alcohol or drug use, and non-compliance with traffic laws [(7)], or due to the fact that women tend to be more sensitive to traffic safety and appear to engage in fewer risk-taking behaviors [(8-9)]. The older pedestrian group showed a clear predominance in the number of pedestrian fatalities; the age group >60 years represented the highest frequency of deaths with 30% of total registered pedestrian deaths. As cited in previous studies, it may be due to the fact that older pedestrians were significantly more likely to be severely injured or killed than younger pedestrians [(7)] or that younger pedestrians are more likely to be in a better physical condition, are generally quicker to respond and tend to wear brighter clothes than the older group of pedestrians [(10)].

As shown in Table 2 collision with the motorcycle (35%) was also very common only second to bonnet front (43.3%) which was an anticipated finding considering the fact that motorcyclist still tops the type of road users involved in motor vehicle accidents in Thailand with an overwhelming 85.72% [(2)]. A closer look at the pedestrian-vehicle collision data, majority of pedestrian fatalities have occurred in bigger main-street (50%) where higher speed limits were set. The high ratio of males to females (5.5:1) in the BAC >50 mg/dl group, as revealed in our study, agrees with several previous studies [(11-12)]. It was observed that the average number of the fatal pedestrian-vehicle collision was higher on weekdays than on the weekends (9 and 7.5 cases per day, respectively) (Table 3). The number of crashes was more frequent during the nighttime, on both weekdays and weekends, which aligns with the findings of Holt et al which demonstrates 66% of the pedestrian fatalities occur between 6 p.m. and 6 a.m. or during the night or late evening [(13)].

Pedestrians being struck by a vehicle brings about a wide spectrum of injuries to the body ranging from primary injuries caused by the first impact of the vehicle on the victim to secondary injuries on other parts of the body caused by subsequent contact with the ground.

Thus, one victim often presents with injuries in multiple anatomic regions. Injuries to the head region were the highest, with an overwhelming 96.7 percent (Table 4). This finding is consistent with various studies, which also indicates that injury to the head is most common amongst fatal pedestrian traffic accidents [(14–16)].

Head and thorax region topped the frequency of injuries sustained for both the groups (97.3% and 78.38% for head and thorax respectively for four-wheelers and, 100% and 76.19% for head and thorax respectively for motorcycle) (Table 5). This distribution of injuries agrees with various past studies that demonstrate head, thorax, and lower extremities to be the most common anatomic region involved [(17-18)]. However, injury to the abdomen instead of lower extremities was the third most common finding in our study. This may be because the cases studied were of fatalities, and injuries of higher mortalities are located in the head, thorax, and abdomen rather than extremities. An analysis of the location of injuries shows that the risk of sustaining injuries in arm and forearm, and head is significantly higher (OR=2.7 and OR=1.58 respectively) in the case of impact with motorcycle than four-wheel vehicles. With the ever-increasing number of motorcycles on the road along with its overwhelming involvement in accident, further study involving pedestrian-motorcycle collision is highly required. On the contrary, collisions with four-wheel vehicles were almost 5 times more likely to sustain injuries to the shoulder region (OR=4.66) and more than 2 times in the neck (OR=2.36) and abdomen (OR=2.03) than those who collided with the motorcycle. Analysis of location of injuries showed that risk of sustaining injuries in shoulder and upper arm, and neck was higher (OR= 2.03 and OR =2.16 respectively) in case of male pedestrians, while no significant difference was observed in other body regions.

It is a well-known fact that alcohol is one of the important risk factors when it comes to motor vehicle accidents, and the strong positive association between blood alcohol concentration and road accidents involving pedestrian has been demonstrated in studies done by Irwin et al. [(19)]. In terms of total numbers of fatalities that were subjected to BAC analysis, it is clear that more than one-third of all fatally injured pedestrians had BAC of more than 50 mg/dl (13 out of 34) at the time of their accident (Table 6). Alcohol use by pedestrians impairs judgment and cognitive functions as they navigate their environment. As such, intoxicated pedestrians frequently lack the perceptual, cognitive, and physical skills which are necessary to navigate safely through complex traffic patterns [(19)]. And also, they are more likely to engage in risky street-crossing behavior such as not using the designated crosswalk or cross against the signal [(11)]. In our study, we found that the risk of sustaining injury to neck region was significantly higher (OR=3.2) in pedestrians who had BAC above 50 mg/dl.

In terms of the total number of fatalities according to the type of road (Table 6), it was found that accidents in the main street were more than three times that of Non-main Street (30 in Main Street and 9 in non-main street). The risk of sustaining injury was higher in all body regions when pedestrian-vehicle collisions occurred on the larger main street. They more

frequently had injuries in thorax (OR=2), pelvis (OR=1.75), neck (OR=1.63), shoulder and upper arm (OR=1.6), and elbow and forearm (OR=1.34). Impact speed has been found to be the most important factor in determining the severity of pedestrian injuries. A pedestrian may sustain only minor injuries at impacts below 20km/hr, whereas maybe fatal at speed in excess of 45km/hr. Larger main-streets have higher speed limits (up to 120 km/hr.) than the smaller non-main-street (less than 60 km/hr.), and as such, this trend of increasing risk of sustaining injury of this study is very consistent with the findings of Zhang et al. [(20)]. It is evident from this study that fatality is higher when pedestrian-vehicle collision happens on the main street, which suggests that the pedestrian-vehicle crash preventive measures can be focused on these larger highways.

Conclusion

This study shows that most of the fatal pedestrian-vehicle collision occurs in larger main street and older pedestrian (>60years) were the more frequently involved in fatal accidents. Our study also shows that bonnet front vehicle was the main vehicle involved in collision and head injuries remains the most common type of injuries sustained irrespective of colliding vehicle. Although traffic crashes involving pedestrians constitute a relatively small proportion, they also represent a much higher proportion of traffic fatalities when compared with other types of road user. It goes without saying the importance of having countermeasures in place for reducing the mortality associated with the pedestrian. Moreover, many pedestrian deaths are due to improper pedestrian behavior such as impairment by alcohol or drugs, or crossing against the signal, which can be easily avoided with proper awareness and sensitization amongst the pedestrians. Research done on injuries sustained in pedestrian-vehicle collision in Thailand is minimal and findings of this study can be used for similar studies in future.

Acknowledgments

I owe my immense gratitude to Thailand international cooperation agency (TICA) for funding this research.

References

(1). World Health Organisation. Global status report on Road safety 2018. 2018.

(2). Ministry of Public Health T. Annual Epidemiological Surveillance Report 2018. 2018: 266.

(3). National Highway Traffic Safety Administration. Traffic safety facts 2011 data--children. Ann Emerg Med. 2014; 63: 243.

(4). Onieva-garcía MÁ, Martínez-ruiz V, Lardelli-claret P, et al. Gender and age differences in components of traffic-related pedestrian death rates: exposure, risk of crash and

fatality rate. Injury Epidemiology [Internet]. 2016; Available from: http://dx.doi.org/10.1186/s40621-016-0079-2

(5). Zhu M, Zhao S, Coben JH, et al. Why more male pedestrians die in vehicle-pedestrian collisions than female pedestrians: a decompositional analysis. Inj Prev 2013; 19: 227 – 31.

(6). National Highway Traffic Safety Administration. National Pedestrian Crash Report. Springfield Virginia; June 2008.

(7). Clifton KJ, Burnier CV., Akar G. Severity of injury resulting from pedestrian-vehicle crashes: What can we learn from examining the built environment? Transportation Research Part D: Transport and Environment [Internet]. 2009; 14: 425 – 36. Available from: http://dx.doi.org/10.1016/j.trd.2009.01.001

(8). Transportation Research Board of the National Academies. Research on Women's Issues in Transportation. Washington DC; 2004.

(9). Sullman MJM, Gras ME, Font-mayolas S, et al. The pedestrian behaviour of Spanish adolescents. Journal of Adolescence [Internet]. 2011; 34: 531 – 9. Available from: http://dx.doi.org/10.1016/j.adolescence.2010.05.011

(10). Guo R, Xin C, Lin PS, et al. Mixed Effects Logistic Model to Address Demographics and Neighborhood Environment on Pedestrian Injury Severity. Transp Res Rec. 2017; 2659: 174 – 81.

(11). Dultz LA, Frangos S, Foltin G, et al. Alcohol use by pedestrians who are struck by motor vehicles: How drinking influences behaviors, medical management, and outcomes. J Trauma Inj Infect Crit Care. 2011; 71: 1252 – 7.

(12). Pedersen B, Oppedal K, Egund L, et al. Will emergency and surgical patients participate in and complete alcohol interventions? A systematic review. BMC Surg. 2011; 11: 26. Available from: http://www.biomedcentral.com/1471-2482/11/26.

(13). Holt DJ. Pedestrian Safety. Society of Automotive Engineers International. Warrendale, Philadelphia; 2004.

(14). Töro K, Hubay M, Sótonyi P, et al. Fatal traffic injuries among pedestrians, bicyclists and motor vehicle occupants. Forensic Science International. 2005; 151: 151 – 6.

(15). Huipeng C, Lianxue F, Heyue Z. A comparative study between China and IHRA for the vehicle-pedestrian impact. SAE Tech. 2009; 2: 1108 – 15.

(16). Yang J. Review of injury biomechanics in car-pedestrian collisions. Int J Veh Saf. 2005; 1: 100 – 17.

(17). Zhao H, Yin Z, Chen R, et al. Investigation of 184 passenger car-pedestrian accidents. Int J Crashworthiness. 2010; 15: 313 – 20.

(18). Rebollo-Soria MC, Arregui-Dalmases C, Sánchez-Molina D, et al. Injury pattern in lethal motorbikes-pedestrian collisions, in the area of Barcelona, Spain. J Forensic Leg Med. 2016; 43: 80 – 4.

(19). Živković V, Lukić V, Nikolić S. The influence of alcohol on pedestrians: A different approach to the effectiveness of the new traffic safety law. Traffic Inj Prev. 2016; 17: 233 – 7.

(20). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet [Internet]. 2015; 385: 117 – 71. Available from: http://dx.doi.org/10.1016/S0140-6736(14)61682-2

REVIEW ARTICLE

การแปรผันของจำนวนชุดในดีเอ็นเอ [Copy Number Variations (CNVs)]

พลภัทร์ เชื้อทอง, สุรศักดิ์ เนืองทอง และ ภัคจีรา ขันเงิน

นักศึกษาชั้นปีที่ 3 ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ
จังหวัดกรุงเทพมหานคร ประเทศไทย
โทรศัพท์: +66 (0) 95 675 8219 Email: Ponlapat.chueathong@g.swu.ac.th

ส่งต้นฉบับ: วันที่ 20 เดือนตุลาคม พ.ศ. 2564
รับลงตีพิมพ์: วันที่ 20 เดือนธันวาคม พ.ศ. 2564
ตีพิมพ์เผยแพร่: วันที่ 1 เดือนมีนาคม พ.ศ. 2566

บทคัดย่อ

การศึกษาทางอณูชีววิทยาเป็นกระบวนการที่ศึกษาหาความผิดปรกติของสารพันธุกรรมในผู้ป่วย และสามารถตรวจหาสารพันธุกรรมของเชื้อไวรัสได้ โดยเทคนิคที่ใช้กันอย่างแพร่หลายในปัจจุบัน ได้แก่ เทคนิคการเพิ่มปริมาณสารพันธุกรรม (polymerase chain reaction; PCR) ซึ่งสามารถตรวจหาการเพิ่มจำนวนและวัดปริมาณของ DNA บทความฟื้นวิชาการนี้ได้กล่าวถึงหลักการของ PCR ชนิดของ probe ที่ใช้ การคำนวณปริมาณของ DNA และความสำคัญของการแปรผันของจำนวนชุดใน DNA (copy number variations)

คำสำคัญ: Polymerase chain reaction, quantitative polymerase chain reaction, copy number variations

บทนำ

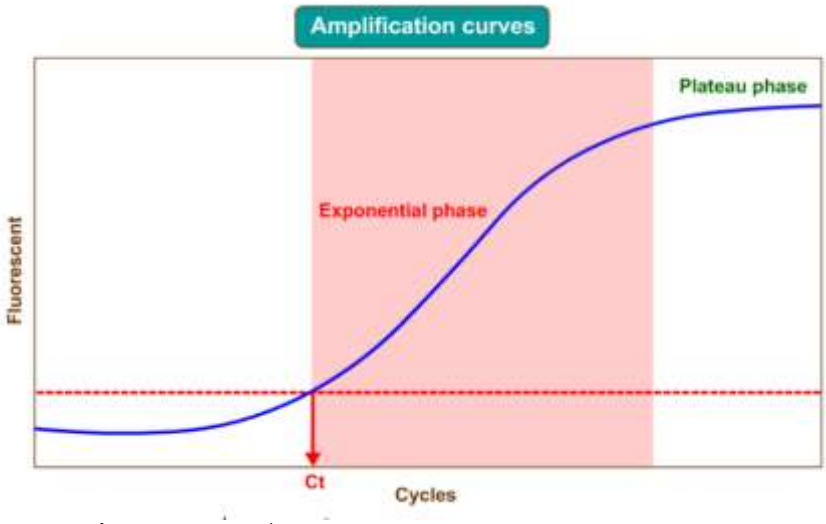
ในปัจจุบัน เทคนิคการเพิ่มปริมาณสารพันธุกรรม (polymerase chain reaction; PCR) ถือเป็นหนึ่งในกระบวนการตรวจที่ใช้กันอย่างแพร่หลายมากที่สุด ในงานวิจัยทางชีววิทยาโมเลกุล โดยเฉพาะอย่างยิ่งการตรวจหา การเพิ่มจำนวนดีเอ็นเอ (DNA) ที่ต้องการศึกษาอย่างจำเพาะและสามารถติดตามวัดปริมาณการเพิ่มจำนวนของดีเอ็นเอเป้าหมาย (DNA target) ได้ในทุกๆรอบของการเพิ่มจำนวน (quantitative analysis)

ในขณะที่ปฏิกิริยากำลังดำเนินอยู่ ตั้งแต่เริ่มต้นจนกระทั่งสิ้นสุดปฏิกิริยา (real-time detection) เรียกเทคนิคนี้ว่า real-time polymerase chain reaction (RT-PCR) ตัวอย่างที่ใช้ในการตรวจด้วย เทคนิค real-time PCR ได้แก่ การวัดระดับการแสดงออกของยีน (gene expression) การวัดปริมาณเชื้อไวรัสในผู้ป่วย (viral load) หรือใช้ในการตรวจหาเชื้อไวรัสสายพันธุ์ใหม่ เช่น coronavirus disease starting in 2019 (COVID-19) เป็นต้น เนื่องจากเป็นเทคนิคการตรวจที่ให้ผลถูกต้องและแม่นยำมากกว่าการตรวจวัดแบบ end-point detection

Real-time Polymerase Chain Reaction (RT-PCR) หรือ quantitative polymerase chain reaction (qPCR)

หลักการ

Real-time Polymerase Chain Reaction (RT-PCR) เป็นเทคนิคที่ใช้ในการเพิ่มจำนวนดีเอ็นเอที่ต้องการศึกษาอย่างจำเพาะและสามารถติดตามวัดปริมาณการเพิ่มจำนวนของดีเอ็นเอเป้าหมายได้ในทุก ๆ รอบของการเพิ่มจำนวนในขณะที่ปฏิกิริยากำลังดำเนินอยู่ ตั้งแต่เริ่มต้นจนกระทั่งสิ้นสุดปฏิกิริยา (real-time detection) เทคนิคนี้ทำได้โดยอาศัยการตรวจวัดสัญญาณสารเรืองแสง (fluorophore) ที่ถูกปล่อยออกมา ปริมาณแสงที่วัดได้จะเป็นสัดส่วนโดยตรงกับปริมาณดีเอ็นเอที่เพิ่มขึ้นจากปฏิกิริยาในแต่ละรอบ โดยทั่วไปดีเอ็นเอที่เพิ่มจากการทำปฏิกิริยา PCR จะเพิ่มเป็นลักษณะกราฟรูปตัว S (sigmoid หรือ exponential curve) โดยแกน X แสดงจำนวนรอบของการเพิ่มจำนวน (threshold cycles; Ct) และแกน Y แสดงสัญญาณการเรืองแสง (รูปที่ 1) ในการทำ real-time PCR เพื่อที่จะให้ได้ข้อมูลที่ถูกต้องแม่นยำ การตรวจวัดผลผลิต PCR ในเวลาที่เกิดขึ้นจะทำเฉพาะในช่วง exponential phase ซึ่งเป็นช่วงที่ระดับสัญญาณสารเรืองแสงสูงเหนือระดับ threshold และมีแนวโน้มการเพิ่มจำนวนแบบทวีคูณ (exponential) เท่านั้น[1]



รูปที่ 1 กราฟการเพิ่มจำนวนดีเอ็นเอด้วยเทคนิค real-time PCR
ที่มา : https://www.scispec.co.th/learning/index.php/blog/genomics/real-time-pcr

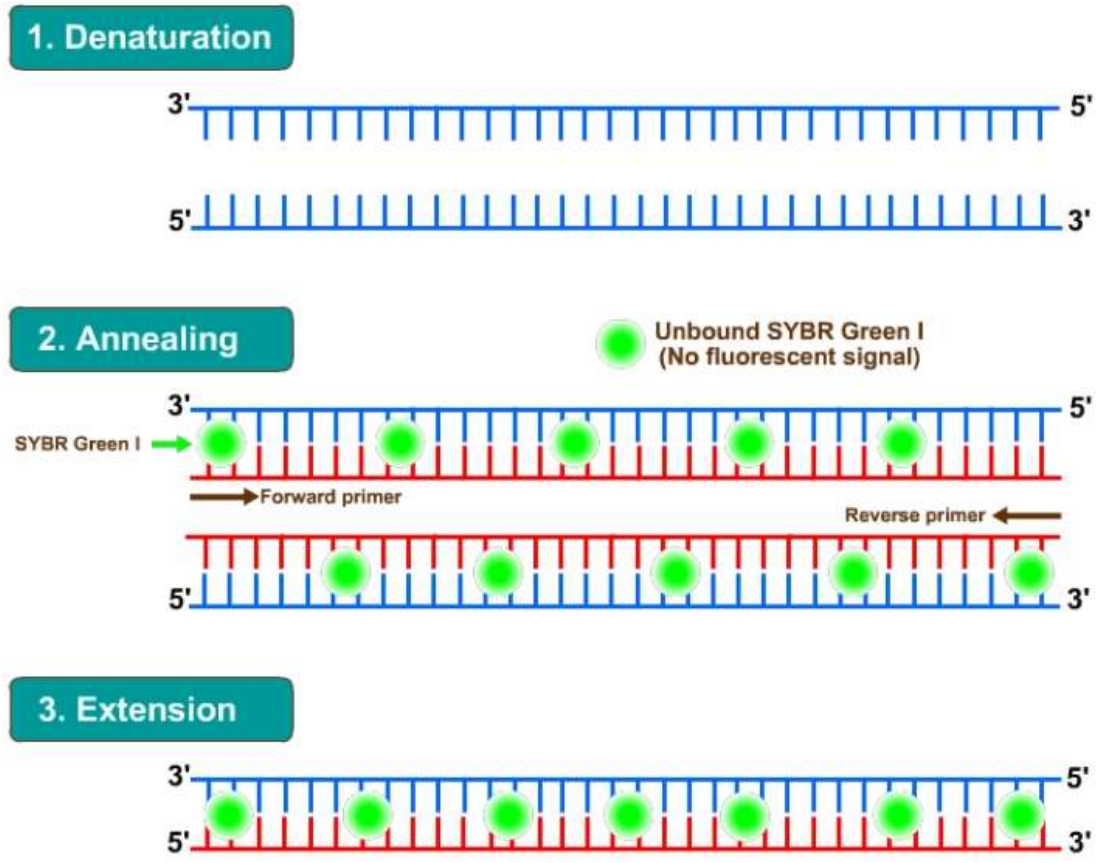
Real-time PCR detection

วิธีการตรวจวัดปริมาณสารพันธุกรรมที่เพิ่มขึ้นด้วยสารเรืองแสงแบบ real time มีอยู่ 2 วิธีด้วยกัน คือ การใช้ สารฟลูออเรสเซนซ์ (fluorescence) จับกับดีเอ็นเอสาย (DNA binding fluorescent dyes) ได้แก่

SYRB Green และ การใช้ probe ไปทำให้เกิดการไฮบริไดเซชัน (hybridization) กับสายดีเอ็นเอที่สร้างขึ้นใหม่ ได้แก่ Taqman probe

DNA binding fluorescent dyes

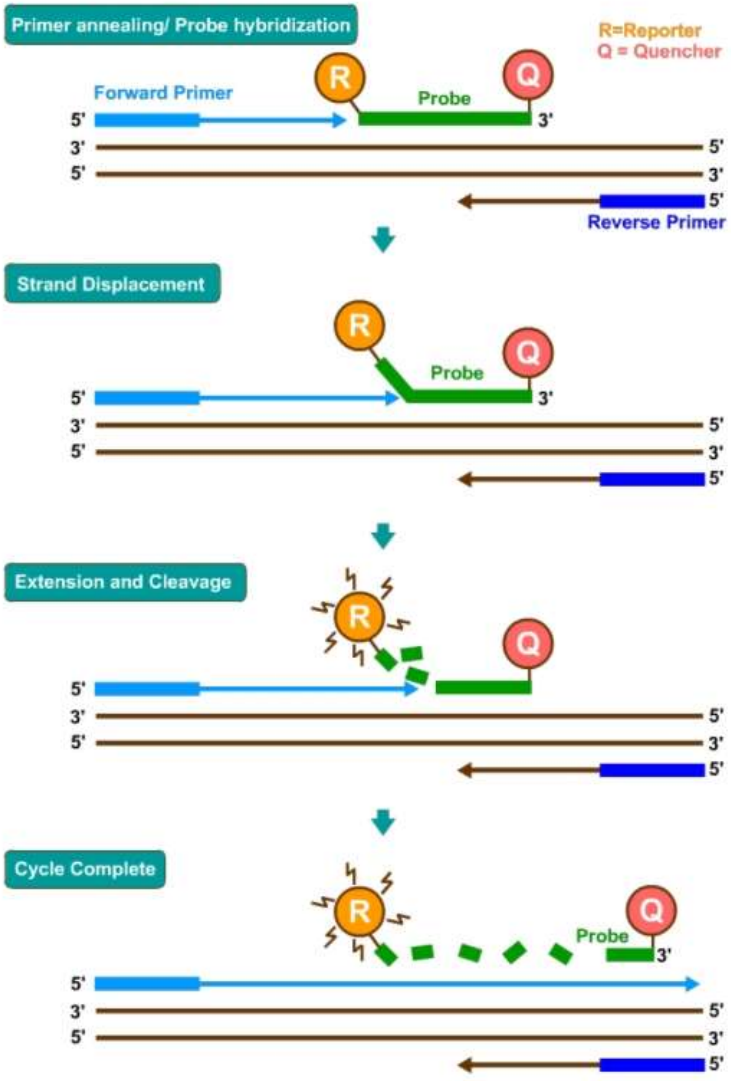
เป็นเทคนิคที่ใช้สีย้อมเรืองแสงย้อมกรดนิวคลีอิก โดยเฉพาะดีเอ็นเอแบบเกลียวคู่ (double strand DNA) ซึ่งวิธีนี้ ใช้เพื่อหาปริมาณของ PCR product เมื่อจับกับดีเอ็นเอคอมเพล็กซ์ (DNA Complex) สีย้อมดีเอ็นเอที่ได้จะดูดซับแสงสีน้ำเงินและเปล่งแสงสีเขียว เกิดขึ้นเนื่องจากการเปลี่ยนแปลงโครงสร้างที่เกิดขึ้นในโมเลกุลของสีย้อมเมื่อจับกับดีเอ็นเอแบบเกลียวคู่ เมื่อ PCR สร้างดีเอ็นเอมากขึ้นเรื่อยๆ โมเลกุลของสีย้อมจะจับกับดีเอ็นเอมากขึ้น ทำให้เกิดการเรืองแสงมากขึ้น ดังนั้นการเรืองแสงจึงเพิ่มขึ้นตามการสะสมของ PCR product ที่เพิ่มขึ้น ทำให้สามารถวัดได้ในเชิงปริมาณ โดยการตรวจจับการเรืองแสงของ SYBR Green (รูปที่ 2) ในปัจจุบัน การใช้เทคนิค DNA binding fluorescent dyes ถือเป็นหนึ่งในวิธีการตรวจวัดปริมาณ DNA ที่ใช้กันอย่างแพร่หลายมากที่สุด ในงานวิจัยทางชีววิทยาโมเลกุล เนื่องจากมีราคาถูกกว่าการใช้ Taqman probe [(1)]



รูปที่ 2 หลักการทำงานของ SYBR Green I fluorescent dye ซึ่งจะปล่อยสัญญาณฟลูออเรสเซนซ์ออกมาเฉพาะกรณีที่จับกับดีเอ็นเอสายคู่เท่านั้น

Probe-based assay

การตรวจวัดปริมาณสายดีเอ็นเอที่สร้างขึ้นใหม่โดยใช้ probe ไปทำให้เกิดการไฮบริไดเซชัน (hybridization) กับสายดีเอ็นเอที่สร้างขึ้นใหม่ โดย probe ที่ใช้จะเป็นนิวคลีโอไทด์สายเดี่ยว ขนาดสั้น ๆ (oligonucleotide) และมีลำดับนิวคลีโอไทด์ที่เข้าคู่ได้กับบริเวณที่ต้องการศึกษา probe ที่นิยมใช้ในปัจจุบัน ได้แก่ TaqMan probe ซึ่งเป็น oligonucleotide สายเดี่ยวที่มีการติดฉลากสารเรืองแสง (fluorophore) ที่ปลาย 5′ และติดตัวบดบัง (quencher) ที่ปลาย 3′ ซึ่งในสภาวะปกติจะไม่มีการเรืองแสงเนื่องจาก quencher จะยับยั้งโมเลกุลของสารเรืองแสงที่ติดไว้ที่ probe ไม่ให้ปล่อยพลังงานออกมา แต่ในขณะที่เกิดปฏิกิริยา PCR ในขั้นตอน annealing probe จะเข้าไปจับดีเอ็นเอต้นแบบในบริเวณที่มีลำดับนิวคลีโอไทด์ที่เข้าคู่กันได้ และในขั้นตอน extension เมื่อเอนไซม์ DNA polymerase ทำการสร้างสายดีเอ็นเอสายใหม่ โดยเอนไซม์ชนิดนี้มีคุณสมบัติ 5′->3′ exonuclease activity ซึ่งสามารถย่อย TaqMan probe ได้ ทำให้โมเลกุลของสารเรืองแสงและ quencher หลุดออกจากกัน (รูปที่ 3) ดังนั้นจึงเกิดการเรืองแสงขึ้น โดยปริมาณสารเรืองแสงจะแปรผันกับปริมาณของสายดีเอ็นเอที่เพิ่มขึ้น[1]



รูปที่ 3 หลักการทำงานของ Taqman probe โดยโมเลกุลของ quencher (Q) จะยับยั้งโมเลกุลของสารเรืองแสง (Reporter; R) โดยปฏิกิริยา PCR ในขั้นตอน annealing probe จะเข้าไปจับดีเอ็นเอต้นแบบ และในขั้นตอน extension เมื่อเอนไซม์ DNA polymerase สร้างสายดีเอ็นเอสายใหม่ TaqMan

probe จะถูกทำลายโดยคุณสมบัติ 5′->3′ exonuclease activity ของเอนไซม์ ทำให้โมเลกุลของ reporter และ quencher หลุดออกจากกัน จึงเกิดการเรืองแสงขึ้น

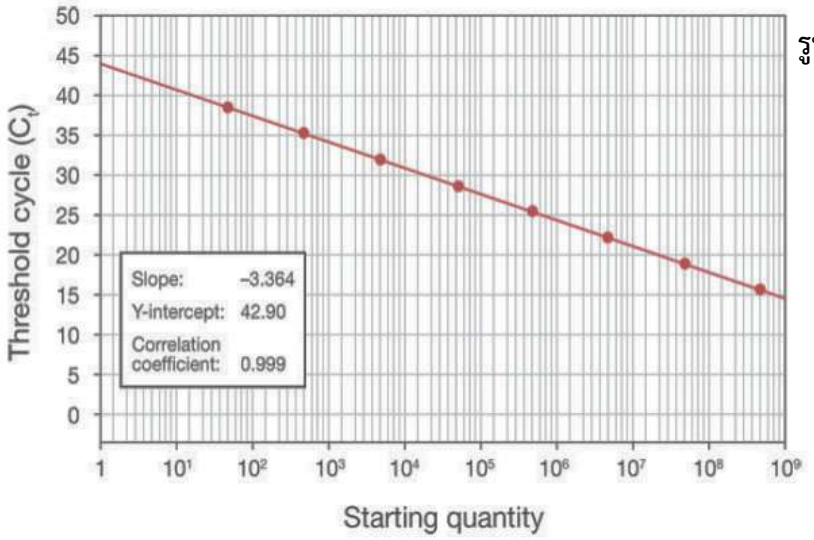
ทั้งนี้ได้สรุปความแตกต่างระหว่างการตรวจทั้ง 2 วิธี ไว้ในตารางที่ 1[(2)]

ตารางที่ 1 แสดงข้อแตกต่างระหว่างการตรวจโดยใช้ SYBR Green กับ Taqman probe

	SYBR Green	Taqman probe
	ขึ้นอยู่กับสีย้อมดีเอ็นเอ (fluorescent dye)	ขึ้นอยู่กับ hybridization probe และกิจกรรม
Probe ที่ติดฉลากเรืองแสง	ไม่จำเป็นต้องใช้ probe ที่มีฉลากเรืองแสง	จำเป็นต้องมี probe ที่มีฉลากคู่
การวิเคราะห์ยีนมัลติเพล็กซ์	ไม่สามารถใช้สำหรับเป้าหมายยีนมัลติเพล็กซ์	สามารถใช้สำหรับเป้าหมายยีนมัลติเพล็กซ์
ค่าใช้จ่าย	ราคาไม่แพง	ราคาแพง
ความจำเพาะ	มีความจำเพาะน้อยกว่า	มีความจำเพาะสูงกว่า
ประสิทธิภาพ	มีประสิทธิภาพน้อยกว่า	มีประสิทธิภาพสูงกว่า

การสร้างและวิเคราะห์ผลด้วย Standard curve

กราฟมาตรฐาน (standard curve) หรือกราฟมาตรฐานความเข้มข้น (concentration standard curve) จัดเป็นการเทียบสารมาตรฐานทางอ้อมซึ่งกราฟที่สร้างขึ้นเป็นความสัมพันธ์ระหว่างสัญญาณตอบสนองที่วัดได้ (แกน y) กับความเข้มข้นของสารที่สนใจวิเคราะห์ (analyte) ที่เตรียมเป็นสารมาตรฐาน (แกน x) ดังนั้น การหาค่า copy number จาก standard curve สามารถสร้างได้จากการเจือจางความเข้มข้นของ DNA แม่แบบที่รู้ความเข้มข้น (แกน x) จะถูกพล็อตกับค่า threshold cycle (Ct) (แกน y) (รูปที่ 4)[(3)]
ค่า threshold cycle (Ct) คือ cycle number ที่สามารถตรวจพบสัญญานของสารเรืองแสงได้ โดยค่า Ct ที่ได้จะนำมาใช้ในการคำนวณหาค่า DNA copy number เนื่องจากค่า Ct จะมีความสัมพันธ์แบบผกผัน (inverse) กับปริมาณที่เพิ่มขึ้นของ DNA เป้าหมาย



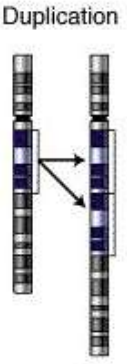
รูปที่ 4 ตัวอย่างของ standard curve ที่ได้จากข้อมูลการทำ real-time PCR โดยแกน Y แสดงค่า threshold cycle (Ct) แกน X แสดงปริมาณของ DNA เป้าหมาย โดยค่าที่ใช้ในการวิเคราะห์ได้แก่ ค่า slope y-intercept และ correlation coefficient [6]

สาเหตุของ Copy number variations มาจากการที่มนุษย์มีการสัมผัสกับสิ่งต่างๆทั้งที่เป็นสารเคมี รังสี อาหาร รวมไปถึงสิ่งแวดล้อม ซึ่งการสัมผัสสิ่งต่างๆเมื่อเข้าสู่ร่างการอาจก่อให้เกิดการเปลี่ยนแปลงลักษณะของสิ่งมีชีวิตได้ โดยหากการเปลี่ยนแปลงนั้นเกิดขึ้นกับยีน หรือเกิดขึ้นกับสารพันธุกรรมจะทำให้สารพันธุกรรมมีลักษณะเปลี่ยนแปลงไปจากเดิม เช่นการเกิด Gene mutation และลักษณะดังกล่าวก็จะสามารถถ่ายทอดไปสู่ลูกหลานได้

Copy number variations (CNVs)

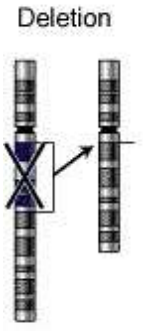
ค่า Copy number variations (CNVs) คือ ค่าการแปรผันของจำนวนชุดใน DNA เป็นส่วนหนึ่งของ DNA ที่มีความแตกต่างของจำนวนชุด (copy number) ในจีโนมสองชุดหรือมากกว่า ส่วนที่ซ้ำกันนี้อาจมีขนาดได้ตั้งแต่หนึ่งร้อยคู่เบสจนถึงหลายล้านคู่เบส อาจได้รับการถ่ายทอดมาจากรุ่นสู่รุ่นหรือเกิดขึ้นใหม่ก็ได้[5] CNVs มีรูปแบบการแปรผันทางโครงสร้างคือมีการเปลี่ยนแปลงของ DNA ในจีโนมที่ส่งผลก่อให้เกิดความผิดปกติ ตั้งแต่หนึ่งส่วนโดยอาจถูกลบจำนวนชุด DNA หรือมีการเพิ่มในโครโมโซมบางตัว ซึ่งส่งผลให้ค่า CNVs มีมากกว่าหรือน้อยกว่าปกติ[10]

การเพิ่มขึ้นของ copy number (copy number gain) เกิดจากการ duplication ของโครโมโซม การ duplication ของโครโมโซม หมายถึง โครโมโซมที่ผิดปกติ อันเกิดจากการที่มีส่วนหนึ่งของโครโมโซมเพิ่มเติมขึ้นมาจากสภาพปกติ (รูปที่ 5)[9]



รูปที่ 5 การ Duplication ของโครโมโซม

การลดลงของ copy number (copy number loss) เกิดจากการ deletion ของโครโมโซม[8] การ deletion ของโครโมโซม หมายถึง โครโมโซมที่ผิดปกติ ซึ่งเกิดขึ้นจากการที่ส่วนหนึ่งของโครโมโซมขาดหายไป (รูปที่ 6)[9]



รูปที่ 6 การ deletion ของโครโมโซม

ดังนั้น การเพิ่มขึ้นหรือลดลงของ CNVs ทำให้เกิดความแปรผันของจำนวนชุด DNA

ความสำคัญของ CNVs สามารถเป็นตัวบ่งชี้แนวโน้มของการเกิดโรคมะเร็งได้หลายชนิดและยังมีส่วนร่วมในการเป็นตัวบ่งชี้ของความโรคทางจิตเภท ออทิสติก และโรคความบกพร่องทางการเรียนรู้ ตัวอย่างเช่น โดยค่าจำนวนชุดของยีน EGFR จะสูงกว่าปกติ และยีน CCL3L1 สามารถตรวจพบได้กับคนที่เป็นโรคภูมิคุ้มกันอ่อนแอ หรือกลุ่มคนที่ติดเชื้อ HIV[10]

แม้ว่ากลไกการก่อโรคจะนำไปสู่โรคมะเร็งหลายชนิดแต่ยังไม่ชัดเจนอย่างไรก็ตามมะเร็งเป็นผลมาจากความผิดปกติจากกิจกรรมหรือการแสดงออกของยีนที่ความคุมการเจริญของเซลล์[10]

การหาค่า copy number สามารถคำนวณได้จากสูตร ดังแสดง[5]

$$Copies\ number = 10^{\left(\frac{n-b}{m}\right)}$$

m = slope

b = intercept

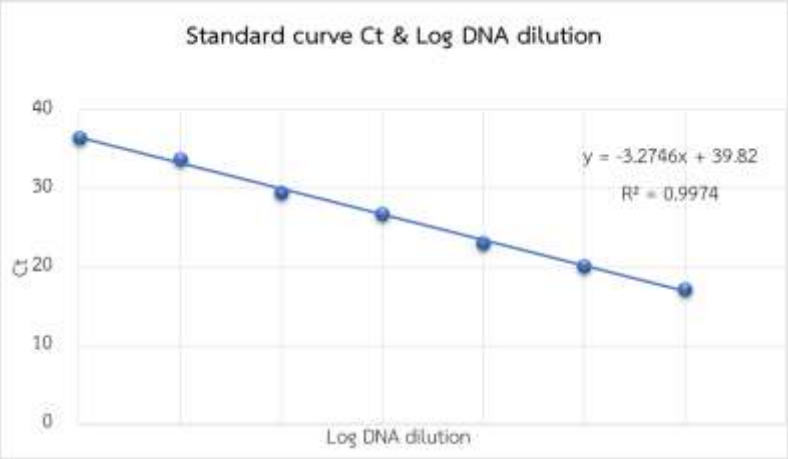
ตัวอย่างการหาค่า copy number ของยีน TFRC จาก standard curve

Number of DNA copies of DNA template								
Number	sample	Conc. (ng)	Primer	length (bp)	Avogadro number	Conversion factor	Mass of 1 bp	Copy number
1	dsDNA	200	TFRC	78	6.022E+23	1.00E+09	660	2.34E+12

ทำการเจือจาง DNA แม่แบบ ที่อัตราส่วน 1/10, 1/100, 1/1,000, 1/10,000, 1/100,000, และ 1/1,000,000, ก่อนตรวจวัดปริมาณยีน TFRC ด้วย RT-PCR ได้ค่า copies/reaction ดังแสดงในตารางที่ 2 การ plot กราฟผลการทดลองพบว่ามีช่วงความสามารถในการตรวจวัดที่เป็นเส้นตรง (linearity detection range) ที่ R2 = 0.9974 ซึ่งใช้ตัวอย่าง DNA ปริมาตร 1 ไมโครลิตร (µl) (รูปที่ 5)

ตารางที่ 2 แสดงค่า Ct กับ DNA แม่แบบที่ทำการเจือจาง

Ct	Log DNA dilution
17.16	10^{-0}
20.14	10^{-1}
23.11	10^{-2}
26.85	10^{-3}
29.49	10^{-4}
33.83	10^{-5}
36.47	10^{-6}



รูปที่ 5 แสดง Standard curve ของยีน TFRC ที่ได้จากการทำ RT-PCR

การหาค่า copy number DNA จากตัวอย่างผู้ป่วยจำนวน 2 ราย (การทำซ้ำ 3 ครั้ง)

จากสมการ y = (mx + b); m = slope, b = intercept

จะได้ y = ((-3.2746)x + 39.82)

หาค่า copy number DNA จากสูตร $Copies\ number = 10^{\left(\frac{n-b}{m}\right)}$

ถ้าตัวอย่างมีค่า CT= 21.25 (example)

ดังนั้น $Copies\ number = 10^{\left(\frac{21.25-39.82}{-3.2746}\right)}$

copies number = 468728.8007

ตารางที่ 3 แสดงตัวอย่างการคำนวณ ค่า absolute copies number ของยีน ในตัวอย่างผู้ป่วยจำนวน 2 ราย

ตัวอย่าง	ทำครั้งที่	Ct	Copies
C01	1	23.25	114857.67
C01	2	23.51	95666.75

C01	3	23.00	136932.09
ค่าเฉลี่ย			115818.84

ตัวอย่าง	**ทำครั้งที่**	**Ct**	**Copies**
C02	1	17.10	8674650.78
C02	2	17.06	8922103.00
C02	3	17.58	6189694.46
ค่าเฉลี่ย			7928816.08

จากตารางที่ 3 พบค่าเฉลี่ยของ Copies number ของผู้ป่วย C01 เท่ากับ $1.15x10^5$ พบค่าเฉลี่ยของ Copies number ของผู้ป่วย C02 เท่ากับ $7.67x10^6$ แสดงว่า ปริมาณการแสดงของยีนTFRC ในผู้ป่วย C02 มีมากกว่า ผู้ป่วย C01

การตรวจหาค่า copies number ของโครโมโซมหรือยีน รวมถึงการตรวจหาปริมาณเชื้อไวรัส โดยใช้เทคนิค RT-PCR มีประโยชน์ต่อการศึกษาทางอณูชีววิทยา ทั้งนี้การออกแบบการทดสอบโดยเฉพาะการออกแบบ standard curve พบว่ามีความสำคัญต่อการหาค่า absolute copies number ในการทำ RT-PCR

<u>เอกสารอ้างอิง</u>

(1). ดร. อารีย์รัตน์ หนูนวล.Molecular genetic testing techniques-Types of polymerase chain reaction. ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์[อินเทอร์เน็ต].[เข้าถึงเมื่อ 8 ก.ค. 2564], เข้าถึงได้จาก https://meded.psu.ac.th/binlaApp/class02/B2_364_221/Molecular_genetic_part2/index7.html

(2). ความแตกต่างระหว่าง SYBR GREEN และ TAQMAN. [อินเทอร์เน็ต]. 13-14 มีนาคม 2560 [เข้าถึงเมื่อ 8 ก.ค. 2564], เข้าถึงได้จาก: https://th.strephonsays.com/sybr-green-and-vs-taqman-463#menu-2

(3). ผู้ช่วยศาสตราจารย์ ดร.วรวิทย์ จันทร์สุวรรณ. หลักการวิเคราะห์ด้วยเครื่องมือโดยอาศัยกราฟมาตรฐาน. [อินเทอร์เน็ต]. 10 มีนาคม 2564 [เข้าถึงเมื่อ 8 ก.ค. 2564], เข้าถึงได้จาก: https://web.rmutp.ac.th/woravith/?page_id=4559

(4). ธีรวุฒิ วงศ์วรัตน์ และ ศุจิรัตน์ สงวนรังศิริกุล. การประเมินปริมาณเชื้อไฟโตพลาสมาโรคใบขาวอ้อยด้วยวิธีAbsolute และ Relative quantification real-time PCR [อินเทอร์เน็ต]. 11 มิถุนายน 2556 [เข้าถึงเมื่อ 8 ก.ค. 2564], เข้าถึงได้จาก: http://annualconference.ku.ac.th/cd53/01_022_O77.pdf

(5). การแปรผันของจำนวนชุดดีเอ็นเอ[อินเทอร์เน็ต]. 8 มีนาคม 2556 [เข้าถึงเมื่อ 8 ก.ค. 2564], เข้าถึงได้จาก: https://th.wikipedia.org/wiki/การแปรผันของจำนวนชุดดีเอ็นเอ

(6). Bio-Rad Laboratories, Inc. Real-Time PCR Applications Guide [Internet]. California: Bio-Rad Laboratories, Inc; 2006 [cited 2021 July 1]. Available from: https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_5279.pdf

(7). Life technologies. Real-time PCR handbook. [Internet]. California: Thermo fisher scientific Inc.; 2014 [cited 2021 July 1]. Available from: https://www.thermofisher.com/content/dam/LifeTech/global/Forms/PDF/real-time-pcr-handbook.pdf

(8). ฉริยาวรรณ จรัลสวัสดิ์. การประยุกต์ใช้ไมโครอาร์เรย์ทางการแพทย์ [อินเทอร์เน็ต]. สงขลา: มหาวิทยาลัยสงขลานครินทร์, คณะแพทยศาสตร์, ภาควิชาพยาธิวิทยา; 2556 [เข้าถึงเมื่อ 19 ก.ค. 2564]. เข้าถึงได้จาก: https://li01.tci-thaijo.org/index.php/gst/article/view/12368/11126

(9). Narjes Khatoon Shab ani Sadr. Copy number variations and cancer [Internet]. Ahvaz: university of Ahvas; 2015 [cited 2021 July 19]. Available from https://www.slideshare.net/NarjesSadr/cancer-and-cnv

(10). เตือนใจ โก้สกุล. sc.chula.ac.th [อินเทอร์เน็ต]. กรุงเทพฯ: จุฬาลงกรณ์มหาวิทยาลัย; 2021 [เข้าถึงเมื่อ 19 ก.ค. 2021]. เข้าถึงได้จาก http://www.sc.chula.ac.th/courseware/2305262/context/text10/subtext10/body_subtext10.html

CASE REPORT

AN UNUSUAL CASE OF HEMOSIDEROTIC SYNOVITIS OF THE KNEE IN A NON-HAEMOPHILIC ELDERLY FEMALE

Smita Singh and Neha Suman

Department of Pathology, Lady Hardinge Medical College, New Delhi

* Correspondence to: Dr Neha Suman, Department of Pathology, Lady Hardinge Medical College, Shaheed Bhagat Singh Marg, New Delhi. Email: drnehasuman@gmail.com

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

Submitted: *11 December 2021*
Accepted: *26 December 2021*
Published: *1 March 2023*

Abstract

Hemosiderotic synovitis develops due to recurrent hemorrhages in the joint. The most common affected joint is knee. Repeated hemarthrosis may lead to chronic osteomyelitis in the long run. The most common cause is haemophilia. However, causes other than haemophilia has also been reported but the incidence is low. Here we report a rare case of non-haemophilic hemosiderotic synovitis of the knee joint in which the patient lacked history of any bleeding diathesis. The definitive diagnosis was made by histopathological examination. Early diagnosis leads to appropriate therapy and hence less joint destruction.

Keywords: Hemosiderotic, knee, synovitis

Introduction

Synovium is a specialized connective tissue that lines the inner surface of capsules of synovial joints and tendon sheaths. Many characteristic pathologic processes may occur at this site [(1)]. Synovial proliferative disorders include pigmented villonodular synovitis (PVNS) and hemosiderotic synovitis. Hemosiderotic synovitis is caused by clotting factor deficiency [(2)]. Correct diagnosis requires detailed clinical history and examination. However, the definitive diagnosis is possible by histopathological examination [(3-4)]. The patient complains of pain and stiffness of the involved joint. Chronic intraarticular haemorrhages may result in chronic osteoarthritis. The knowledge and recognition of this entity leads to appropriate treatment and better outcome. Here, we report a rare case of non-haemophilic hemosiderotic synovitis of knee in an elderly female.

Case Report

A 55 year old female patient presented with complaints of pain, stiffness and swelling in right knee joint. Fixed flexion deformity was observed in the patient. History and clinical examination excluded any clotting factor deficiency and collagen vascular diseases. There was no history of any anti-coagulant drug intake. No history of trauma was present. The prothrombin time (PT), activated thromboplastin time (APTT) and platelet count were within normal range. Sickling test was negative on high performance liquid chromatography (HPLC). Total knee replacement was done and synovium was sent for histopathological examination. Grossly, we received a specimen of multiple greyish brown tissue bits varying in size from 0.5x0.5x0.3 cm to 2.5x2x2 cm with few rust brown coloured areas. On microscopic examination, the sections showed synovial proliferation. The synovial cells contained deep brown pigment [Figure1] which showed positive staining for hemosiderin on Prussian blue stain [Figure2]. These brown hemosiderin pigment were also present in the macrophages. No proliferation of lipid laden cells or multinucleated osteoclast like giant cells were seen hence possibility of PVNS was excluded. Hence the final diagnosis of hemosiderotic synovitis was made.

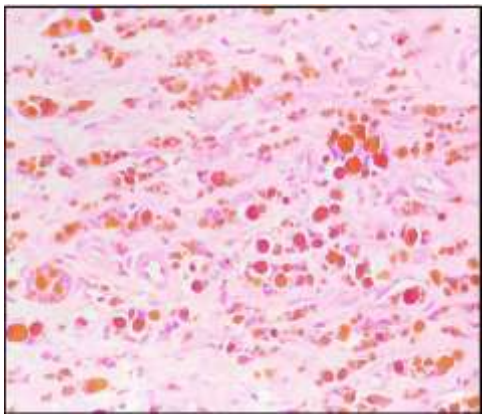


Figure1: Synovial cells with brown hemosiderin pigment (Hematoxylin and eosin stain; x400)

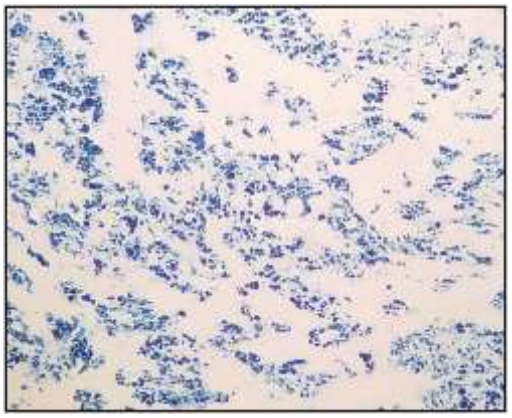


Figure2: Iron present in the synoviocytes on Prussian blue staining confirming deposition of hemosiderin (Prussian blue stain; x100).

Discussions

Hemosiderotic synovitis develops with recurrent intraarticular haemorrhages. The most common cause is clotting factor deficiency such as haemophilia. The most commonly affected joint is knee joint. However, causes other than haemophilia are oral anticoagulant use, trauma, rheumatoid or psoriatic arthritis, osteoarthritis, collagen vascular disease, pigmented villonodular synovitis, hemochromatosis, scurvy, sickle cell anaemia, synovial hemangioma and myeloproliferative disorders [(4)]. In both PVNS and hemosiderotic synovitis, the synovium appears brown or rust coloured with loss of normal glistening appearance of the synovial

tissue. With repeated haemorrhages in the joint, the synovium thickens and becomes opaque leading to fibrous scarring [5]. Clinical and radiological findings may not give a definitive diagnosis. In these cases histopathological examination plays an important role in reaching at the correct diagnosis as was possible in the present case. In hemosiderotic synovitis, hemosiderin is deposited in the synovial lining cells and macrophages. Multinucleated osteoclast like giant cell with hemosiderin deposits is not seen. Similar findings were seen in the present case. There is presence of osteoclast-like multinucleated giant cells in PVNS [6], hence ruled out.

The recurrent hemorrhages lead to formation of a hyperplastic vascular tissue. The breakdown of haemoglobin leads to release of hemosiderin. The accumulation of these hemosiderin results in rusty brown discoloration of synovium with loss of normal glistening appearance. As bleeding inside the joint increases, there is more deposition of hemosiderin and the synovium becomes darker and later opaque [4]. It has been studied that iron deposition is associated with release of pro-inflammatory cytokines and also it inhibits the formation of the cartilage matrix. Thus, iron may play a role in the resultant synovial changes and catabolic mediators that affects the articular cartilage. However, whether the hemosiderin is the direct stimulator for production of the cytokines is not clear. There are possibilities that phagocytosis by synovial cells and macrophages into the affected joints result in cytokine production [7-8]. However, the exact mechanism of pathogenesis of non-hemophilic hemosiderotic synovitis is still not clear.

Conclusion

Non-haemophilic hemosiderotic synovitis occurs in patients without history of bleeding diathesis. Definitive diagnosis is not possible by clinical and radiological examination alone. Hence proper histopathological examination required to reach at a definitive diagnosis. Correct diagnosis leads to appropriate treatment, less destruction of the joint and better patient outcome.

References

(1). O'Connell JX. Pathology of the synovium. Am J Clin Path. 2000; 114: 773 - 84.

(2). Humphrey PA. Joints and synovium. In: Humphrey PA, Dehner LP, Pfeifer JD, editors. The Washington Manual of Surgical Pathology. 2nd Edition. PA: Lippincott Williams and Wilkins; 2012: 822 - 5.

(3). Yalçin N, Bektaþer B, Ciçekli O, et al. An unusual cause of recurrent joint effusions: Nonhemophilic hemosiderotic synovitis of the knee. Acta Orthop Traumatol Turc. 2010; 44: 162 - 5.
(4). France MP, Gupta SK. Nonhemophilic hemosiderotic synovitis of the shoulder. A case report. Clin Orthop Relat Res. 1991; 262: 132 - 6.
(5). Roosendaal G, Lafeber FP. Joint damage as a result of hemarthrosis. In: Caviglia HA, Solimeno LP. Orthopedic Surgery in Patients with Hemophilia. First Edition. Italy: Springer; 2008; 5 - 14.
(6). Bullough PG. Joint diseases. In: Mills SE, editor. Sternberg's Diagnostic Surgical Pathology. Fifth Edition. PA: Lippincott Williams and Wilkins; 2010: 211 - 2.
(7). G. Roosendaal, Vianen ME, M. J. G. Wenting, et al. Iron deposits and catabolic properties of synovial tissue from patients with haemophilia. J Bone Jt Surg. 1998; 80: 540 - 5.
(8). Stein H, Duthie R. The pathogenesis of chronic haemophilic arthropathy. J BoneJt Surg. 1981; 63: 601 - 9.

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*
- *Conclusions: Brief summary and potential implications*
- *Keywords: 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:

Review Article:

Case Report: