(TIJMS)

The International JOURNAL of MEDICINE and SCIENCES

VOL. 3 NO. 1 JUNE 2018

E-ISSN 2590-3713

Kulliyyah of Medicine & Health Sciences
Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah (UniSHAMS)

EDITORIAL BOARD

Kulliyyah of Medicine & Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah,
09300 Kuala Ketil, Kedah Darul Aman.
tijms.editor@gmail.com

www.tijms.unishams.edu.my

Original Article

EVALUATION OF MANNHEIM PERITONITIS INDEX IN SECONDARY PERITONITIS IN HUSM

Mehboob Alam Pasha*[1], Narendran Balasubbiah [2], Sangeta Vadiveloo [3], Nafeesa Banu Sathyamangalam Rahmathulla [1], Zaidi Zakaria [4]

[1] Faculty of Medicine, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah (UniSHAMS), Kuala Ketil, Kedah, Malaysia.
[2] Hospital Sultanah Bahiyah, Alor Setar, Kedah, Malaysia.
[3] Hospital Sultan Abdul Halim Shah, Sungai Petani, Kedah, Malaysia.
[4] School of Medicine, Universiti Sains Malaysia, Kubang Kerian, Malaysia.

ARTICLE INFO

Corresponding author:
AP Dr. Mehboob Alam Pasha

Email address:
mpasha49@hotmail.com

Received:
March 2017
Accepted for publication:
May 2017

Keywords:

Mannheim Peritonitis Index
Secondary peritonitis
Mortality
Prognosis

ABSTRACT

Management of peritonitis continues to be a challenge inspite of recent advances in surgical care and technology . Many scoring systems have been developed to study the associated risk factors in order to predict the outcome and develop strategies for improved care. The objective of this study was to evaluate the Mannheim Peritonitis Index (MPI) in determining the outcome of patients operated for secondary peritonitis in Hospital Universiti Sains Malaysia (HUSM). A total of 113 patients with peritonitis undergoing surgical treatment at HUSM between 1 Jan 2013 and 31Oct 2014 were included in the study. Demographic and clinical data, and findings at surgery were documented and analysed using SPSS software . Pearsons Chi-square was used as a statistical test for significance with p value ≤ 0.05. The mean MPI score was 25.22(±8.03) with the lowest score of 10 and highest of 43. The threshold MPI score was 26.5 and there was only 1 death which occurred below this score. The significant predictive factors for mortality were age >50 years, gender, organ failure and diffuse generalised peritonitis. Further, all parameters for MPI affected the scoring except source of sepsis from noncolonic origin. The ROC curve for mortality showed a sensitivity of 94.7% and specificity of 70.2% at a threshold MPI of 26.5. The MPI score is a simple and effective means to predict the outcome of patients with secondary peritonitis in HUSM.

INTRODUCTION

Peritonitis denotes inflammation of the serous membrane lining the peritoneal cavity and abdominal viscera from any cause [1]. It has been further classified into primary, secondary and tertiary. Secondary peritonitis or suppurative peritonitis is due to gastro-intestinal perforation, injury, haemoperitonitis, anastomotic dehiscence, or a gangrenous or infected hollow viscus. or organ. Until the end of the last century, peritonitis was treated medically, with a resultant mortality of over 90%. Since then many interventions have been made to reduce the incidence of mortality due to peritonitis, and is presently reported to be 13-43% [2]. With such high prevalence of mortality, management chiefly depends on early detection of peritonitis. In order to identify the high risk group in these patients, many simple scoring systems have been developed. One of them, which is very simple to apply, is Mannheim Peritonitis Index (MPI). This index is based on measuring very simple clinical parameters, which are routinely performed during admission to the hospital and findings during surgery available from the operation notes. MPI was developed by Wacha and Linder (1983) based on retrospective analysis of 1253 patients with peritonitis [3]. Twenty possible risk factors were taken into consideration, out of which eight were found to be of prognostic value (Table 1). The maximum possible value was 47 while the minimum was zero. The information is collected during the first laparotomy enabling immediate classification. The aim of this study was to evaluate the Mannheim Peritonitis Index in determining the outcome in patients operated for secondary peritonitis in Hospital Universiti Sains Malaysia, and to assess individual risk factors for their contribution towards mortality.

Table 1: MPI scoring with weightage for each of the eight risk factors

Number	Risk Factors	Weightage when present
1	Age >50 years	5
2	Female Sex	5
3	Organ Failure**	7
4	Malignancy	4
5	Preoperative Duration of Peritonitis >24 Hours	4
6	Origin of Sepsis Non-Colonic	4
7	Diffuse generalised Peritonitis	6
8	Exudate (Intra-operative)	
	• Clear	0
	• Cloudy/Purulent	6
	• Feculent	12
** Definition of Organ Failure		
Kidney	Creatinine level >177 umol/L, Urea level >16.7mmol/L, Oliguria <20ml/h	
Lung	PO_2 < 50 mmHg ,PCO_2 >50 mmHg	
Shock	Systolic Blood Pressure < 90 mmHg without inotropes	
Intestinal obstruction	Paralysis > 24 Hr or Complete Mechanical Obstruction	
Total MPI Score	=	

MATERIAL AND METHODS

This observational retrospective record review was conducted at the Surgical department of Hospital Universiti Sains Malaysia (HUSM), in the state of Kelantan, Malaysia. Ethical approval was obtained from the HUSM Ethics and Research Committee and permission to review the hospital records from the hospital director. The study included 113 patients aged above 12 years in whom secondary peritonitis was confirmed at laparotomy or laparoscopy. Exclusion criteria were patients below 12 years, those whose records were incomplete, those with primary and tertiary peritonitis, and those who underwent surgery for similar pathology elsewhere within the last six months. Proforma was designed to collect and enter demographic data and findings at surgery. Mannheim Peritonitis Index was then used to calculate prognostic score for each of the eight parameters according to the values set in Table 1. A cut-off score of 26 was set to predict mortality based on Billings (1994) study [4].

Data collected was analysed using SPSS version 21 (IBM, Chicago, IL,USA). Pearson Chi-square and Independent T-test was used to test validity of each of the eight MPI values. Results were summarised using Receiver Operating Characteristic (ROC) analysis and Area Under the Curve (AUC) was calculated. A p-value of <0.05 was considered statistically significant.

RESULTS

From January 1, 2013 to October 31, 2014, the records of 113 patients with secondary peritonitis who were operated were reviewed and included in the study. Of the 113 patients, 64 were males and 49 were females. There were more males than females (ratio 1.3:1). The ages ranged from 15 to 99 years, with a mean of 45 years. Among the causes of secondary peritonitis, appendicular perforation (49.6%) was the most common. Other causes were gastric perforation (14.2%), small bowel perforation (8.8%), colonic perforation of non-cancer origin (8%), perforated colon cancer (3.5%) etc (Table 2).

Out of the 113 patients in this study 19 patients died, with overall mortality of 16.8% (Figure1). In this study there were 66 patients aged 50 years and below, out of whom three patients died , giving a mortality of 4.5% (3/66). There were 47 patients aged ≥ 50 years old. Among this group the mortality rate is 34% (16/47). This suggests that age is a significant contributor to mortality. There were 64 male patients and 49 females. The mortality for males is 15.6% (10/64) and for females it is 18.4% (9/49). Females in this study had higher mortality. 41(36.3%) patients in this study had at least one type of organ failure most commonly shock followed by intestinal failure. The mortality rate of 46.3% is statistically significant (p=0.0001). Three of 10 patients who had malignancy of some organ system

Table 2: Causes of Secondary Peritonitis in HUSM

Causes	Frequency	Percentage
Small bowel perforation	10	8.8
Gastric perforation	16	14.2
Duodenal perforation	2	1.8
Appendicular perforation	56	49.6
Colon perforation other than cancer	9	8.0
Perforated colon carcinoma	4	3.5
Pelvic inflammatory disease	1	0.9
Tubo-ovarian abscess	2	1.8
Ruptured liver abscess	3	2.7
Perforated gall bladder	1	0.9
Post –bowel anastomotic leak	4	3.5
Other causes	5	4.4
Total	113	100

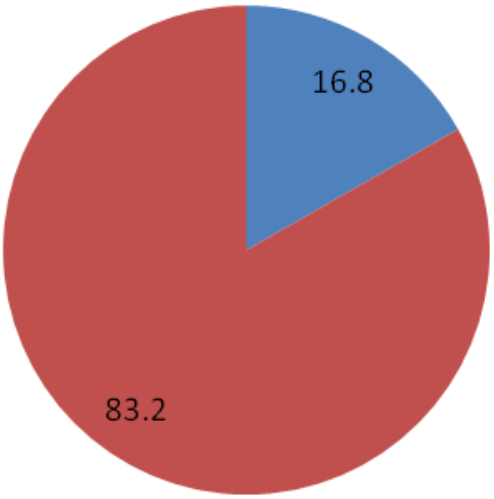


Figure 1:
Percentage of patients who died or survived (N=113)

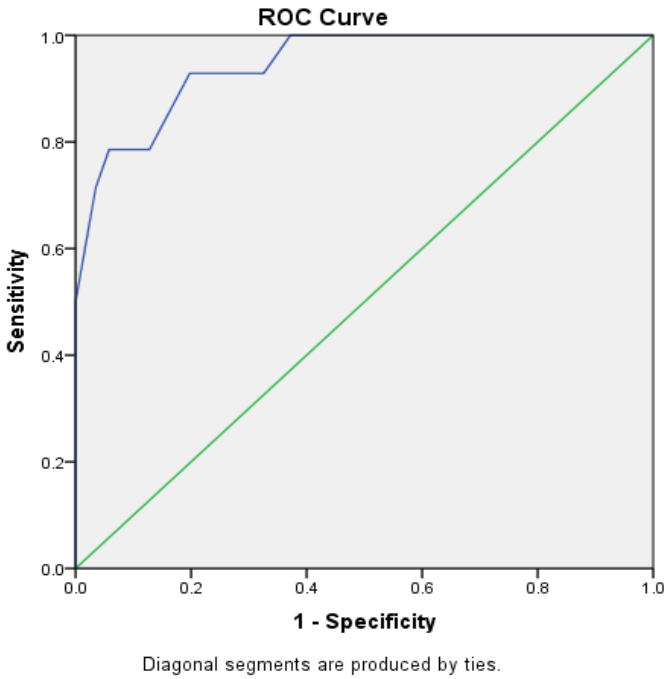


Figure 2: ROC Curve For Sensitivity and Specificity

died {Mortality30% (3/10)}. On MPI analysis this was found to be a significant risk for mortality. In our study, in patients having duration of peritonitis < 24 hours, the mortality rate is 4.3% (1/23). Meanwhile, in the other group when duration of preoperative peritonitis was >24 hours, the mortality rate is 20% (18/90). Late presentation is a significant risk for mortality. The mortality for diffuse peritonitis in this study is 21% (18/65), compared to localised peritonitis where mortality was only 3.6% (1/28). This is significant. Similarly, the mortality when exudate was purulent or cloudy was 17% (15/88) and when the exudate was feculent the mortality was 16%(4/25).

Mannheim Peritonitis Index was used to evaluate each of the eight parameters mentioned in Table 1, and assess the statistical significance of each factor to predict mortality (Table 3). When Pearson Chi-square test was applied to test each of the eight MPI parameters, only 4 factors had a significant outcome to the survival of patients (Table 3). The four factors are age more than 50 years, female sex, presence of

organ failure, and diffuse generalised peritonitis (p=0.0001).

The other four factors namely; the presence of malignancy, source of sepsis, preoperative duration of peritonitis more than 24 hours, and nature of peritoneal exudates did not contribute to survival outcome as an independent variable. But when all the eight parameters were studied independently for relationship to mean MPI scores using the Independent T-test, all showed significance except for non-colonic origin of sepsis (p=0.079).

In this study the mean MPI score is 25.22 (SD ±8.03) with a score of 10 as the lowest and 43 as the highest. The minimum possible score is 0 and the maximum is 47. The ROC curve for mortality showed the best sensitivity at 94.7% and specificity of 70.2%, corresponding to the MPI score of 26.5.

The MPI threshold score of 26.5 was analysed to the survival outcome. In this study only 1 death was recorded among the patients with secondary peritonitis having MPI score of 26.5 and below . The remaining 18 deaths were recorded in patients whose MPI score was greater than 26.5. (Figure 3). Higher MPI scores are predictors of mortality (p<0.0001).

DISCUSSIONS

Inspite of recent advances in surgical techniques and postoperative care, the mortality in secondary peritonitis remains unacceptably high. Billings (1994) in a multicenter study reported a mean mortality of 19.5% [4]. Others have reported mortalities reaching up to 60% in their studies[5,6]. In this study the mortality rate was 16.8%, comparing favourably with most other studies [1,4,7]. Surgical and medical management may be favourably influenced by early prediction of mortality. Out of the several scores available, the Mannheim Peritonitis Index and the Apache II score can independently predict the outcome of sepsis in peritonitis [8]. The MPI however is easier to apply and uses details readily available from the patient records, with accuracy matching the APACHE II scores [9,10].

In previous studies patients with MPI score of less than 21 had mortality rate ranging from 0% - 2.3%, and with MPI more than 29, highest mortality rates even up to 100% was observed [4,11]. However, in a study by Qureshi (2005) at a threshold MPI score of 26 , the mortality was 4.3 % in patients having MPI below 26 , and 28.1% when MPI was above 26 [12].

In the present study, mortality was 1.5% when MPI is less than 26 whereas it was 39.0% with MPI score of >26 comparing favourably with other studies [1,7] (sensitivity 94.7%, specificity 70.2%). An optimal cut off point for MPI is one at which the maximum values of sensitivity and specificity of the score can be obtained and it is identified from the ROC curve. In this study the AUC of 0.947 indicates that MPI is a good indicator of mortality.

The age range of patients in this study is 15-99 years , similar to a study in Srinagar, India where the age range was 15-90 years [13]. The mean age was 45 years similar to the Iranian study by Notash [14] where the mean age was 44 years . However studies from Western populations show relatively higher age ranges from 46-64.8 [1-3,15]. This is probably due to higher life expectancy and higher prevalence of colon related pathology [15]. Most studies reported age >50 years to be a significant risk factor for mortality. This can be explained on the basis of poorer physiological and immunological responses to the stress caused by sepsis in older patients[16]. In our study, the mortality rate for patients over 50 years was 34%, which is strongly significant (p=0.0001). Boey (1982) did not find age to be a significant risk factor for mortality [17]. Majority of the patients included in his study had

Table 3:Distribution of MPI variables and outcome of patients

Variable	Outcome		Chi-square	Mean MPI	T-test
	Survived n(%)	Dead n(%)			
Age>50yrs	31 (66)	16 (34)	0.0001	31	0.0001
Female Sex	40 (82)	9 (18)	0.0001	28	0.0001
Organ failure	22 (53.7)	19 (46.3)	0.0001	32	0.0001
Duration>24 hrs	72 (80)	18 (20)	0.115	27	0.0001
Malignancy	7 (70)	3 (30)	0.368	35	0.0001
Diffuse peritonitis	67 (78.9)	18 (21.1)	0.039	28	0.0001
Peritoneal Exudate					
- Cloudy	73 (83)	15 (17)	1.000	24	0.0001
- Fecal	21 (84)	4 (16)	1.000	31	0.0001
- Noncolonic origin	78 (84.8)	14 (15.2)	0.343	25	0.0079

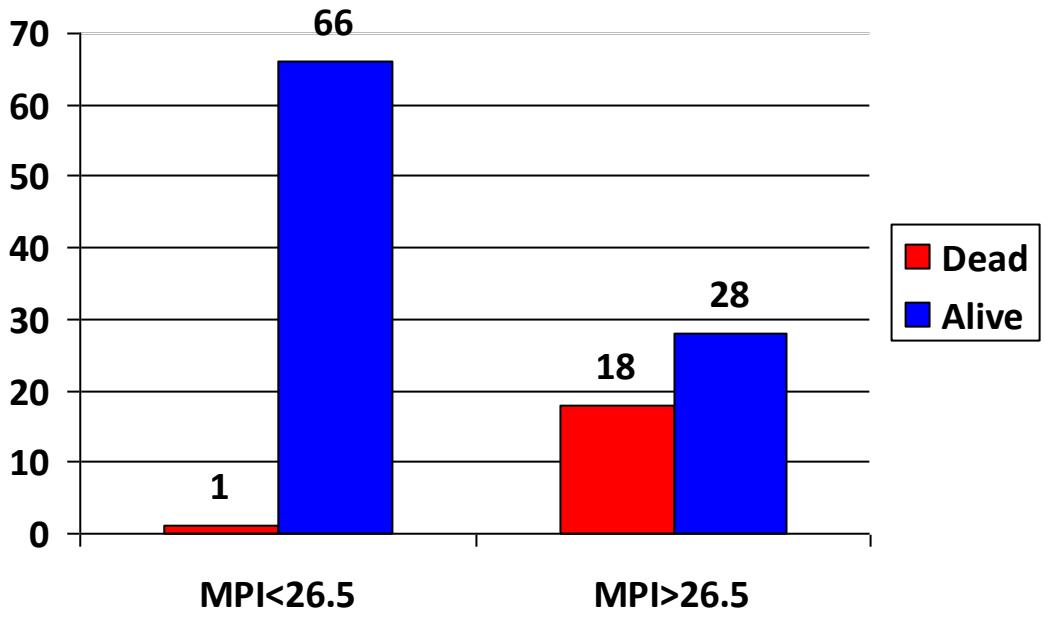


Figure 3: MPI threshold score related to the survival outcome

duodenal perforation, where risk for sepsis is less than other types of peritonitis.

In the Linder scoring scale for MPI 5 points have been added to the MPI if the patient is female [3]. Most studies have reported a higher mortality among females with peritonitis [18-22]. The mortality rate for females in this study was 18.4% (8/49). The mean MPI score of 28 was strongly significant (P=0.0001). It is possible that in females with their smaller peritoneal cavity, infections tend to spread faster leading to higher mortality. Nevertheless, other studies have not found female gender to be a significant risk factor for mortality [12,23-25]. This may be due to possible differences in demographic pattern and cut-off values for MPI in those studies.

Almost all studies done worldwide quote organ failure as a major risk factor for death in peritonitis [12,14,16,18,24-25]. The systemic inflammatory response (SIRS) induced by the peritoneal infection usually leads to septic shock and multiorgan failure. In our study 19 out of 51 patients had at least one failed organ at the time of death , The high MPI score of 33 with a mortality rate of 46.3% is strongly significant (p=0.0001), conforming to most other studies. Probable reasons could be late presentation of the patient and time taken to stabilise the patient before laparotomy.

Presence of malignancy in any system produces destruction of anatomical barriers and probable alterations of immune systems by decreased phagocytic activity, humoral and cellular responses. Hence peritonitis in oncologic patients presents with higher mortality as reported by various studies [12,15,24,26]. However other studies were inconclusive probably due to the small number of patients in their studies [21,25]. In this study, three of 10 peritonitis patients who had malignancy died (30% mortality). The MPI scores for those with and without malignancy were 35 and 21 respectively. This was strongly significant (p=0.0001). Hence malignancy in our study is a useful prognostic indicator.

The majority of patients in this study presented to the hospital after 24 hours from the onset of symptoms . 20% of them died as a result of late presentation. With a MPI score of 27 on Independent T-test, this was significant (p=0.0001). Probable causes are tendency of the local population to neglect their symptoms, belief in traditional medicinal systems, or lack of proper referral systems. Some authors have noted zero mortality when duration of peritonitis is less than 24 hours[16]. On the other hand Notash (2005) found mortality to be 11.4% when patients presented within 24 hours[14] . Differences in demographics and types of pathology could be the reason.

14 (15.2%) of our patients in this study died when their perforation was not from the colon. With a MPI score of 25 this was not found to be statistically significant (0.079). This correlates with findings in other studies [12,15]. Our local population unlike their Western counterparts are

probably less prone for colonic pathology and hence less risk for mortality.

Diffuse generalised peritonitis denotes the spread of the inflammatory process within the peritoneal cavity. This is identified at laparotomy by the finding of cloudy, purulent or faecal exudates in two or more quadrants. In this study, 75% of patients had diffuse generalised peritonitis, with a MPI score of 28 and a mortality of 21.1%. This was a significant MPI predicator (p=0.0001), comparing favourably with previous studies [1,10,21,25].

Peritoneal exudates can be clear, and considered to be probably sterile in the early stages, purulent or cloudy, and frankly feculent. Faecal exudates are generally of colonic origin with a high microbial content mainly due to gram negative organisms. In this study there were no clear exudates. 15 out of 88 patients with purulent exudates died (mortality 17% and MPI of 24). Of the 25 who had faecal exudates, four patients died (mortality 16% and MPI of 31). Pearsons Chi-square showed no significance (p=1.000) but between groups analysis showed presence of faecal exudates to be a significant risk factor (p=0.0001) .Our finding compares favourably with previous studies [9,12,18,25].

This retrospective study is limited by the small population in Kelantan, which may not be representative . There are limited or scarce reports available to compare our results within the Malaysian population. Sepsis of noncolonic origin was not a significant risk factor in this study. Hence, the MPI can be widened to include colonic perforation as a risk factor for sepsis to increase its validity.

CONCLUSION

The results of our study conform favourably with previous studies done elsewhere. All the MPI adverse factors except origin of sepsis being noncolonic, behaved as expected, with age ,gender, organ failure and diffuse peritonitis showing strong significance. High MPI scores were found to be associated with higher mortality. We can conclude that MPI score is a safe and reliable predictor for mortality in patients with secondary peritonitis.

ACKNOWLEDGEMENT

We thank the HUSM Director for permission to use the records and the Head of Surgical Department, HUSM, for supporting this study.

CONFLICTS OF INTEREST : Nil

REFERENCES

1. Seiler CA, Brügger L, Forssmann U, Baer HU, Büchler MW. Conservative surgical treatment of diffuse peritonitis. Surgery 2000;127:178-84.
2. Wittman DH. Intra-abdominal Infections: Pathophysiology and Treatment. New York: Marcel Dekker; 1991. p. 48-51.
3. Wacha H, Linder MM, Feldman U, WeschG, Gundlach E, Steifensand RA. Mannheim peritonitis index – prediction of risk of death from peritonitis: construction of a statistical and validation of an empirically based index. Theoretical Surg. 1987;1:169-77.
4. Billing A, Fröhlich D, Schildberg FW. Prediction of outcome using the Mannheim peritonitis index in 2003 patients. Peritonitis Study Group. Br J Surg. 1994 Feb;81(2):209-13.
5. Bohnen J, Boulanger M, Meakins JL, Mclean APH. Prognosis in generalized peritonitis: relation to cause and risk factors. Arch Surg. 1983;118:285-90.
6. Schein M, Saadia R, Freinkel Z and Decker GAG (1983). Aggressive treatment of severe diffuse peritonitis from intestinal origin. *World Journal of Surgery* **7** 762-6.
7. Bielecki K, Kaminski P, Klukowski M. Large bowel perforation: morbidity and mortality. Tech Coloproctol 2002; **6**: 177-82.
8. Malik AA, Wani KA, Dar LA, Wani MA, Wani RA, Parray FQ, et al. Mannheim Peritonitis Index and APACHE II - prediction of outcome in patients with peritonitis. *Ulus Travma Acil Cerrahi Derg.* 2010;16(1):27-32.
9. Ohmann C, Wittmann DH, Wacha H. Prospective evaluation of prognostic scoring systems in peritonitis. Peritonitis Study Group. *The European journal of surgery = Actachirurgica*. 1993;159(5):267-74.
10. Pacelli F, Doglietto GB, Alfieri S, Piccioni E, Sgadari A, Gui D, et al. Prognosis in intra-abdominal infections. Multivariate analysis on 604 patients. Arch Surg 1996; 131(6): 641-5.
11. Fugger R, Rogy M et al Validation study of the Mannheim peritonitis index. Chirurg. 1988; 59:598-601.
12. Qureshi AM, Zafar A, Saeed K, Quddus A. Predictive power of Mannheim peritonitis index. J Coll Physicians Surg Pak. 2005;15(11):693-6.
13. Khan SP**,** Dar LA, Hayat H. Predictors of mortality and morbidity in peritonitis in a developing country. *Turkish Journal of Surgery*. 2013;29(3):124-130.
14. Notash AY, Salimi J, Rahimian H, Fesharaki Ms, Abbasi A. Evaluation of Mannheim peritonitis index and multiple organ failure score in patients with peritonitis. Indian J Gastroenterol. 2005 Sep-Oct;24(5):197-200.
15. Correia MM, Thuler LCS, Velasco E and Vidal EMSA (2001). Prediction of Death using the Mannheim Peritonitis Index in Oncologic Patients. *Revista Brasileira de Cancerologia* .2001:47(1) 63-68

16. Tushar D, Ramachandra L , Rajesh N, Digvijoy S. Evaluation of prognosis in patients with perforation peritonitis using Mannheim's peritonitis index International Journal of Scientific and Research Publications.2015:5(5):1 -35
17. Boey J, Wong J, Ong GB. A prospective study of operative risk factors in perforated duodenal ulcers. *Ann Surg* 1982; 195(3): 265-269.
18. Chaudhari ND, Nakum A, Mahida H. Mannheim's peritonitis index validation study in the Indian set-up. Int J Sci Res 2014;3:1808-11.
19. Sharma S, Singh S, Makkar N, Kumar A, Sandhu MS. Assessment of severity of peritonitis using Mannheim peritonitis index. Niger J Surg 2016;22:118-22.
20. Wabwire B, Saidi H. Stratified outcome evaluation in peritonitis. Ann Afr Surg. 2014;11 (2):29-34.
21. Jain S, Jain M, Jain R. Validation of Mannheim peritonitis index in a tertiary care center in Rajasthan. Int J Med Sci Public Health 2015;4:664-668.
22. Kusumoto Yoshiko, Neyagawa Masayuki, et al. Study of Mannheim Peritonitis Index to Predict Outcome of Patients with Peritonitis. *Japanese Journal of Gastroenterological Surgery.* 2004;37(1):7-13.
23. Yilmazlar T, Toker S, Zorluoglu A. Non-traumatic colorectal perforations. Int Surg 1999; 84: 155- 8.
24. Ntirenganya F, Ntakiyiruta G, Kakande I. Prediction of outcome using the Mannheim peritonitis index in patients with peritonitis at Kigali University Teaching Hospital. East Cent Afr J Surg. 2012;17(2):52-64.
25. Rudofo L, Bracho-Riquelme, Armando Melero-Vela M C , Aidee Torres-Ramirez M C . Mannheim Peritonitis Index validation study at the Hospital General de Durango (Mexico) Cirugia y Cirujanos.2002: 70(4) , 217-225.
26. Koppad SN, Vandakudri AB, Desai M, Kodliwadmath H. Analysis of Mannheim peritonitis index scoring in predicting outcome in patients with peritonitis secondary to hollow viscous perforation. Int Surg J 2016;3:1116-20

Original Article

DISTRIBUTION OF FINGERPRINT PATTERNS AMONG YOUNG ADULTS AND SIBLINGS IN MALAYSIA

Gan Shyang Heng[1], Normaizatul Afizah Ismail*[2], Zury Azreen Azizul Rahman[3] and Amidon Anan[1]

[1] Forensic Science Program, Universiti Kebangsaan Malaysia, Malaysia.
[2] Kulliyyah of Medicine & Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah (UniSHAMS), Kuala Ketil, Kedah, Malaysia.
[3] Faculty of Medicine, Lincoln University, Kelana Jaya, Selangor, Malaysia.

ARTICLE INFO

Corresponding author:
Normaizatul Afizah Ismail

Email address:
normaizatul@unishams.edu.my

Received:
April 2018
Accepted for publication:
May 2018

Keywords:

Fingerprints
Forensic
Siblings
Malaysia

ABSTRACT

A study was conducted to study the distribution of fingerprint patterns among Malaysian population, specifically on the right and left hands, gender, major ethnic groups and siblings. A total of 192 subjects in the age of 14 and above were involved. For public citizen, 96 subjects were selected from University Kebangsaan Malaysia Kampus Kuala Lumpur (UKMKKL) while 96 siblings were recruited from families around Kuala Lumpur. Ten fingerprints were collected from each subject and the pattern was classified into whorls, loops, arches and composites patterns. The study revealed the most likely fingerprint patterns to occur on a specific finger as well as in a specific ethnicity. Fingerprint patterns were dependent upon the finger on which they occur. Statistical analysis indicated that right and left hands could be distinguished by whorl pattern. However, fingerprint patterns did not show any differences between males and females. Loops and whorls were the most predominant pattern in all studied ethnic groups. Malays and Chinese had similar distributional patterns which was different with Indians. Fingerprint patterns showed a significant difference among three major ethnic groups ($p < 0.01$) especially on the left and right thumb, right index as well as right middle finger. Siblings demonstrated greater similarity of all fingerprint patterns than non-siblings except for the arch pattern. The present study suggested that fingerprint pattern could be inherited genetically but not linked to sex chromosome.

INTRODUCTION

Among all the methods of identification, fingerprint has proved to be both infallible and feasible. Its superiority over the older methods, such as branding, tattooing, distinctive clothing, photography, and body measurements, has been demonstrated time after time [1]. So far, fingerprints have been used for more than 100 years as the most popular biometric signs of identity in both civil and criminal cases because of their unique properties of absolute identity [2]. It has been estimated that chances of two persons having identical finger impression is about one in sixty four thousand million population of the world. Identical twins share the same DNA profile, yet their fingerprints are as distinctive as any unrelated persons. Therefore, no two fingers are found to have identical prints [3].

The ridge patterns are formed in the human fetus before birth and remain the same throughout a person's life except in the case of accidents, such as bruises and cuts on the finger tips [4, 5]. Anyway, fingerprints remain the same even after small cuts or abrasions affecting the skin surface because the skin's regeneration was based on the original dermis pattern. Only deep cuts that damaged the dermis will result in a permanent scar [6]. The patterns of fingerprints become fixed when a person is about 14 years or older [7].

Fingerprint classification refers to the problem of assigning a fingerprint to a class in a consistent and reliable way. Fingerprints are made up of a number of easily recognizable features that permit them to be classified and filed for later reference [8]. It is an important indexing scheme to narrow down the search of fingerprint database for efficient large-scale identification. Therefore, the identification process can be speeded up by reducing the number of comparisons that are required to be performed. However, it is still a challenging problem due to the intrinsic class ambiguity and the difficulty for poor quality fingerprints [9]. Most of the classification schemes currently used worldwide is variants of Henry's classification scheme which include four most common classes of fingerprint, i.e. arch, loop, whorl and composite [10].

Even though every fingerprint pattern occurred in every ethnic, some fingerprint patterns noticeably dominant in some ethnic group than the others did. For instance, whorl is the most dominant pattern in fingerprint of Asians. On the other hand, there is some correlation between both the class and minutiae-based similarity between the fingerprints of parents and their children, and the same pattern was also observed for identical twins. The similarity between the fingerprints of siblings was found to be higher than that between those of parents and their children [4].

The purpose of this research is to study the distribution of fingerprint patterns among Malaysians, specifically on public citizen and siblings. There are no studies available on the distributional pattern of fingerprint for siblings in Malaysian population. In Malaysia, there are three major ethnic groups which are Malay, Chinese and Indian as well as other minorities such as Iban and Kadazan. The three major ethnic groups in Malaysia were selected to be participated in this study. The population in Malaysia now is constituted of 65.1% of Malay, 26% of Chinese, 7.7% of Indian and the balance 1.2% containing others minority groups [11]. Thus, the ratio of each ethnic selected is representing Malaysian population.

MATERIALS AND METHODS

The study was conducted on 192 subjects aged from 14 years old and above [7]. For public citizens, 96 subjects were chosen randomly from Universiti Kebangsaan Malaysia Kampus Kuala Lumpur (UKMKKL) with 64 Malays, 25 Chinese and 7 Indians. To be confirmed as Malaysian, subject was asked to present their Malaysian Identification Card (Mykad). Another 96 siblings were chosen randomly around Kuala Lumpur. Only those siblings with blood relationship in a family were allowed to take part in this study. A total of ten fingerprints were taken from each subject by using *Perfect Ink PI-10* (Identicator Inc, California). Individuals that have at least three generations of same ethnic group marriage were included and those who are not were classified as “unpure” and omitted. On the other hands, subjects with any evidence of disease and injury of the fingertips that are likely to alter the fingerprint pattern such as leprosy, scars of the fingertips and lacerations were excluded. Only those with ten fingers were included in this study.

This study was approved by UKM ethics committee prior to commence of the study. Consent was obtained in writing prior to the collection of the samples using black ink and white paper method. Subjects were asked to wash and dry their hands to remove dirt and grease. They were then asked to roll the finger pads very gently on the ink pad and then let the researcher to roll it slowly and very gently on the form with labels. The subject was asked to keep his/her arm relaxed and not to try to help in rolling the fingers as this may cause smudging.

The fingers were always rolled away from the body of the subjects [3]. Besides, the fingers were rolled from side to side in order to obtain all available ridge detail [1]. Hence, the ten prints were taken individually – thumb, index, middle, ring and little fingers of each hand in the order named. The pattern of each fingerprint was then determined by researcher with the aid of a hand magnifier *Armor Forensics* 5-1000 (Lightning Powder Co., Germany). The patterns used in this study included arch, loop, whorl and composite [6].

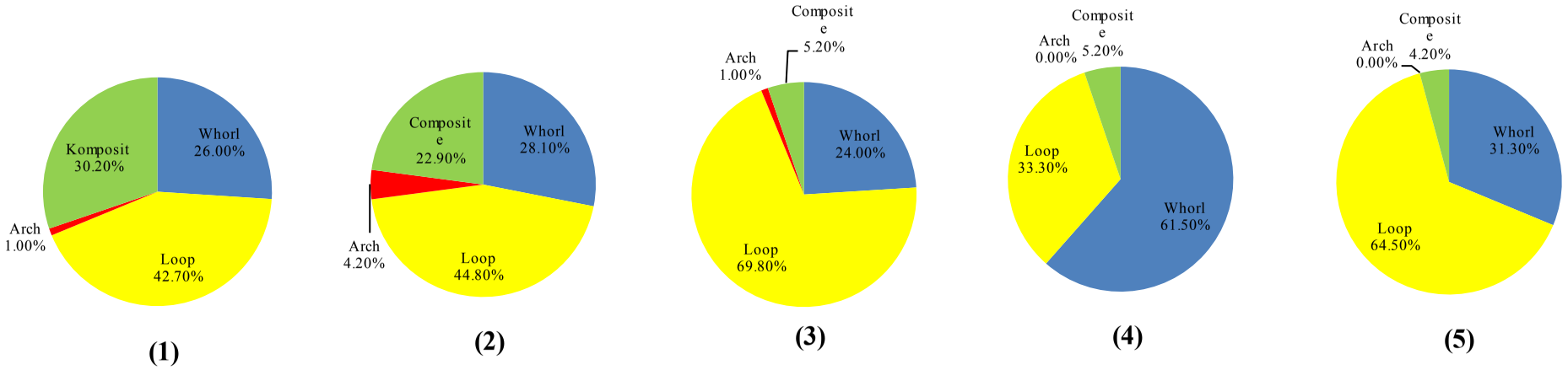
The data were then subjected to statistical analysis by using Statistical Package for Social Science (SPSS) 23.0. Mann-Whitney U test was used to determine the presence of significant difference of fingerprint pattern between right and left hands. In addition, the differences of fingerprint pattern between gender as well as its similarity among siblings and non-siblings were determined by the same test. On the other hand, Chi-Square test for independence was used to study the distribution of fingerprint pattern among races in Malaysia.

RESULTS

Digits were numbered according to Henry’s classification system in which 1 to 5 were designated for fingers on right hand while 6 to 10 for the left hand arranged accordingly from thumb to little finger [3]. Figure 1 shows the distribution of fingerprint patterns across all the fingers among Malaysian. On the whole, loop was the most frequent fingerprint pattern. Notwithstanding this, frequency of loop pattern was the highest on little fingers and followed by middle fingers from both hands. Whorl pattern was most noticeably observed only on ring fingers from both hands. In contrast, arch pattern was rarely observed across all the fingers with the highest percentage of merely 5.2% on index finger from left hand. In addition, ring fingers and little fingers did not show any presence of arch pattern. As regard to composite pattern, thumb and index fingers from both hands indicated the most evident frequency compared to other fingers.

The distribution of fingerprint patterns for left and right hands was showed in Table 1. Overall, both hands demonstrated the same distribution pattern arranging from the highest to the lowest frequencies accordingly i.e., loop, whorl, composite and arch pattern. The frequency of loop, arch and composite pattern was greater on left hand while the right hand showed higher frequency of whorl pattern. The output of statistical analysis showed that frequency of whorl pattern was statistically significant different ($p < 0.05$) between left and right hands. In contrast, both hands showed no statistically different ($p > 0.05$) for loop, arch as well as composite pattern.

Right hand



Left hand

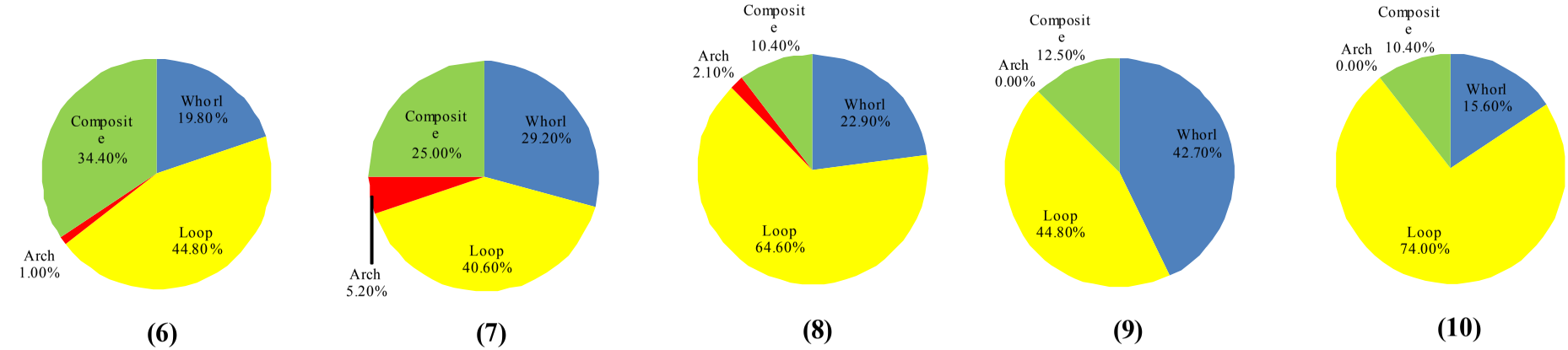


Figure 1: Distribution of fingerprint patterns across all fingers

Table 1: Percentage of fingerprint patterns for public citizens

Fingerprint Patterns	Hands (%)		Genders (%)		Races (%)		
	Left	Right	Males	Females	Malays	Chinese	Indians
Loop	53.75	51.04	48.14	55.85	55.30	43.20	58.60
Whorl	25.83	33.96	32.80	27.74	28.60	36.00	22.90
Composite	18.75	13.75	17.91	14.72	15.30	20.00	8.60
Arch	1.67	1.25	1.16	1.70	0.80	0.80	10.00

With regard to gender, the distribution of fingerprint pattern was showed in Table 1. Male and female possessed the same distribution pattern. In spite of this, male displayed the greater occurrence of whorl and composite pattern whereas female showed the higher frequency of loop and arch pattern. Regardless of this, the frequency differences of fingerprint patterns was vague among the gender. With statistical analysis, the relationship of the fingeprint patterns and the gender failed to be significant ($p > 0.05$).

In respect of the ethnic groups, the frequency distribution of fingerprint patterns was showed in Table 1. Similar distribution pattern was observed in Malays and Chinese. In other words, Indians showed different distribution pattern if compared with Malays and Chinese. Apparently, percentage of loop pattern was extensively greater than the other patterns especially for Malay and Indian. Whorl pattern was the most frequent to be observed after loop pattern. All races demonstrated high frequency of whorl pattern with the highest being observed in Chinese. Percentage of arch pattern was markedly high in Indian (10%) by comparing with Malay (0.8%) and Chinese (0.8%). Moreover, composite pattern was present in every ethnic groups with the greatest frequency in Chinese. As a whole, Malay showed a moderate frequency of fingerprint pattern in comparison with the other studied races.

In accordance with statistical analysis using the chi-square test, there was significant difference ($p< 0.01$) in distribution of fingerprint patterns in all ten fingers among three major ethnic groups in Malaysia. Statistically, the alpha level of less than 0.01 resulted in a high dependency between finger-print patterns and ethnic groups. Furthermore, pat-tern frequency on four out of ten fingers had been proved to be significantly different ($p < 0.05$) among the ethnic groups. The mentioned fingers including left and right thumb, right index as well as right middle finger. On the other hand, Malaysian population demonstrated 30.10% whorl, 52.40% loop, 1.50% arch and 16.00% composite pattern. Loop and whorl patterns were the most dominant pattern followed by composite and arch patterns in Malaysian population.

The average of similarity for fingerprint pattern had been compared between siblings and non-siblings group. The distribution of similarity was showed in Figure 2. Generally, similarity of all studied patterns was greater and manifest in siblings in comparison with non-siblings. The output of statistical analysis showed that there was a significant different ($p< 0.05$) of similarity for all the studied patterns between siblings and non-siblings with the exception of arch pattern ($p > 0.05$).

DISCUSSIONS

The findings for distribution of ten fingerprints were in agreement with the studies by Swofford (2005) and Nithin et al. (2009) [3, 12]. This study revealed that fingerprint pattern was dependent upon the finger on which they occur. Despite the fact that every fingerprint pattern occurred on every finger, some fingerprint patterns noticeably dominated specific fingers more than others did. With the outcome of present study, the fingers on which the fingerprints occur can be predicted in order to expedite the comparison of latent prints found at crime scene to known ten prints database. This can be done by narrowing the search parameters as to the most likely finger before comparison.

The findings of same distribution pattern for both hands were in agreement with Endom et al. (2009), Narahari & Padmaja (2006) and Nithin et al. (2009) [3, 13, 14]. In addition, the findings of pattern frequencies on right and left hands were in agreement with Narahari et al. (2008) [15]. However, Segura-Wang & Barrantes (2009) reported a different findings [16]. The difference could be due to different target population which may showed dissimilar distribution of fingerprint patterns.

This study revealed that both right and left hands may be differentiated through the pattern of whorl. This findings partially supported previous studies saying that fingerprint patterns and the hands on which they occur were not dependent upon each other [12, 17]. In fact, along with bilateral symmetry of both hands, there could be a relatively equal number of the same fingerprint patterns occuring on the two hands [12]. Moreover, fingerprint patterns on an individual's left and right hands were often

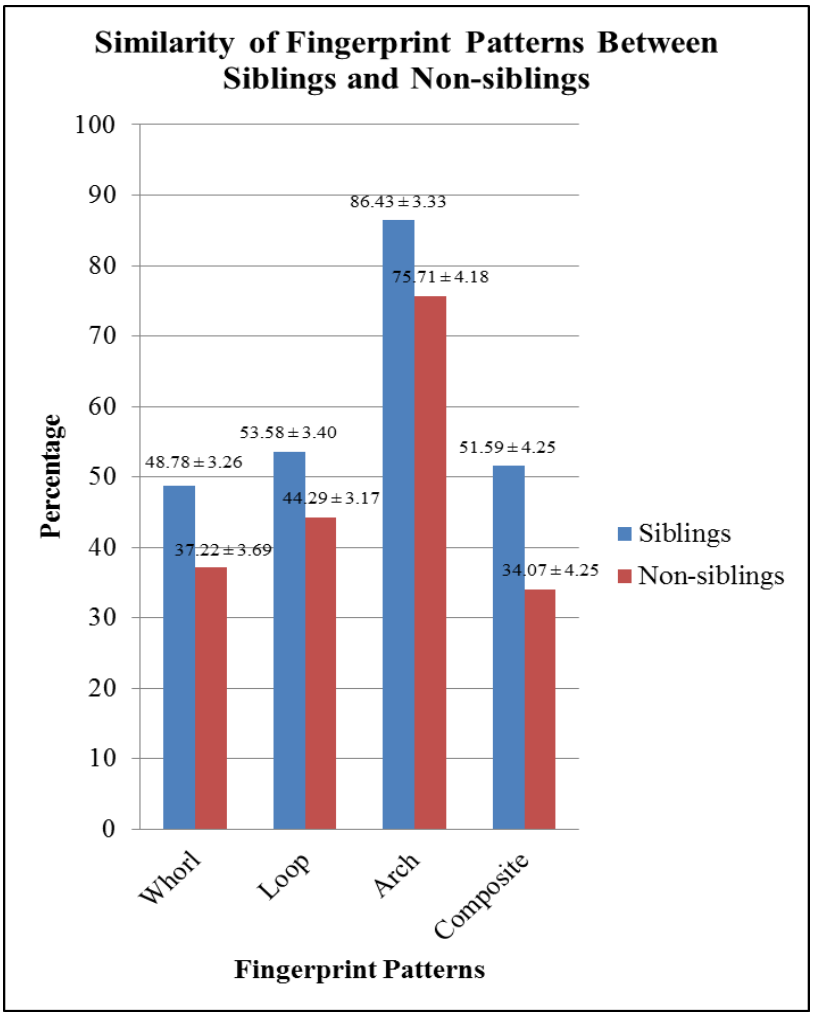


Figure 2: Similarity of fingerprint patterns between siblings and non-siblings

similar to mirror image of each others. This was due to the genetic basis for volar pad formation [18]. Nevertheless, the percentage differences of every single patterns was indistinct as a whole.

The same distribution pattern between males and females was in agreement with Gutiérrez-Redomero et al. (2010) [19]. Regarding the pattern frequencies among genders, a number of previous studies reported the similar findings with the present study [3, 15, 20-23]. This tendency was probably indicative of the modulatory influence of genes on the segments of the sex chromosomes have on the several ontogenic processes regulating ridge pattern morphogenesis in utero [20].

Fingerprint patterns and the gender on which they occur were not dependent upon each other. Hence, the present study revealed that gender could not be differentiated by using fingerprint patterns. The findings were in agreement with [13, 14, 19, 22, 24]. With respect to the findings, it suggested that fingerprint pattern did not inherited genetically via chromosome Y. If the gene determination for fingerprint pattern was located on chromosome Y, it was very likely that higher frequency of certain fingerprint patterns can be observed on male. Furthermore, with no significant different among gender, it could also proposed that fingerprint pattern did not inherited genetically via sex chromosome. Nonetheless, further study on this is necessarily to confirm the reliability of the fact.

With regard to ethnic groups, the findings of similar distribution pattern among Malays and Chinese were in agreement with previous similar study on Malaysian population by Endom et al. (2009) [13]. The present study revealed that even though every fingerprint pattern occurred in every race, some fingerprint patterns markedly being observed in some races more than the others. Sharma et al. (2008) [25] and Endom et al. (2009) [13] reported the similar findings with the present study in which races could be differentiated by fingerprint patterns. It is concluded that races could be differentiated by fingerprint patterns in regard to genetic variation among different races in Malaysia [13]. Sharma et al. (2008) [25] also suggested that differences of fingerprint features might be according to the genetic differences among the studied populations, characterized by different geographical conditions, ethnicity and linguistic backgrounds.

With respect to the significant different among ethnics, it is proposed the usage of these four specific fingers to discriminate major ethnics in Malaysia. Despite the fact that fingerprint pattern could not be used to identify individual, it may reduce the database search scope and further decrease the necessary comparison to be proceed [9]. For instance, timesaving database searching can be achieved by identifying or narrow down the race for fingerprint pattern which was found at crime scene. In this manner, this could possibly increase the effectiveness in solving the criminal cases in the country.

The similar distribution pattern with Malaysian population had been reported in a number of previous studies [13, 26, 27]. The fingerprint patterns were predominantly affected by two combined timing events i.e., the onset of epidermal cellular proliferation and the timing of the regression of the volar pads [12]. Early ridge formation was associated with whorl, later formation with arch, and intermediate formation with loops [28]. Since loop was the most dominant pattern, it can be concluded that fetus from Malaysian population has the onset of ridge proliferation during the middle stages of volar pad regression while the volar pad is most likely asymmetrical.

Because of the genetic basis for the formation of volar pad, overall ridge flow or pattern classification is often similar between siblings, especially identical twins [18]. On the other hand, siblings shared 50% of their genetic information [29, 30] while non-siblings did not share any of their genetic material. For this reason, the present study revealed that fingerprint pattern may be inherited genetically with the greater similarity of patterns among siblings. Rastogi and Pillai (2010) [23] reported that there was a strong assocsiation between human blood group with fingerprint patterns. In actual fact, people inherit two genes for blood type or more accurately two alleles, one from each parents, which determine the blood type [31]. Hence, it suggested that fingerprint pattern was indirectly inherited genetically from parents.

The statistical outcome may indicates that siblings and non-siblings could be distinguished by similarity of whorl, loop and composite patterns on their fingers. Although the similarity of arch pattern was higher among siblings, it was not statistically different with similarity among non-siblings. Notwithstanding this, its significant level ($p = 0.054$) was very close with the border line of significance ($p = 0.05$). This could probably due to insufficient sample size to show its significance.

CONCLUSION

The most predominant pattern among Malaysian population was loop, followed by whorl, composite and arch. Fingerprint patterns were dependent upon the finger on which they occur. Right and left hands could be distinguished by whorl pattern. Irrespective of the gender, fingerprint patterns did not show any difference. It is suggested that fingerprint pattern can be used to narrow down the races in Malaysia especially left and right thumb, right index as well as right middle finger. Siblings showed greater similarity of all fingerprint patterns than non-siblings except for the arch pattern. Fingerprint pattern could be inherited genetically but not linked to sex chromosome.

ACKNOWLEDGEMENT

My upmost appreciation goes to scientific officers, friends and family members for their guidance, assistance, advice and enthusiastic support without which this study would not have been possible.

REFERENCES

1. Hoover, J.E., *The science of fingerprints: Classification and uses.* 2006, United States: Federal Bureau of Investigation.
2. Sudesh Gungadin, M., *Sex Determination from Fingerprint Ridge Density.* Internet Journal of Medical Update, 2007. **2**(2).
3. Nithin, M.D., et al., *Study of fingerprint classification and their gender distribution among South Indian population.* J Forensic Leg Med, 2009. **16**(8): p. 460-463.
4. Han, Y., et al., *A Study on Evaluating the Uniqueness of Fingerprints Using Statistical Analysis.* Information Security and Cryptology–ICISC 2004, 2005: p. 467–477.
5. Nayak, V.C., et al., *Sex differences from fingerprint ridge density in Chinese and Malaysian population.* Forensic Sci Int, 2010.
6. Galloway, V. and D. Charlton, *Fingerprints*, in *Forensic Human Identification: An Introduction*, T. Thompson and S. Black, Editors. 2007, CRC Press: United States of America. p. 57-72.
7. Hsieh, C.T., S.R. Shyu, and C.S. Hu, *An Effective Method of Fingerprint Classification Combined with AFIS.* Embedded and Ubiquitous Computing, 2005: p. 1107–1122.
8. Maltoni, D., et al., *Handbook of fingerprint recognition*. 2 ed. 2009, London: Springer-Verlag New York Inc.
9. Liu, M., *Fingerprint classification based on Adaboost learning from singularity features.* Pattern Recognit, 2010. **43**(3): p. 1062–1070.
10. Mihăilescu, P., K. Mieloch, and A. Munk, *Fingerprint Classification using Entropy Sensitive Tracing.* Progress in Industrial Mathematics at ECMI 2006, 2008: p. 928–932.
11. JPM. *Penduduk mengikut jantina, kumpulan etnik dan umur, Malaysia, 2010: Jabatan Perangkaan Malaysia*. 2010 [11 Jun 2010]; Available from: http://www.statistics.gov.my

12. Swofford, H.J., *Fingerprint patterns: a study on the finger and ethnicity prioritized order of occurrence.* Journal of Forensic Identification, 2005. **55**(4): p. 480.
13. Ismail, E., et al., *Dermatoglyphics: Comparison between Negritos Orang Asli and the Malays, Chinese and Indian.* Sains Malaysiana, 2009. **38** (6): p. 947–952.
14. Narahari, S. and J.S. Padmaja, *Finger and Palmar Dermatoglyphic Study Among the Bondos of Orissa.* Anthropologist, 2006. **8**(4): p. 237–240.
15. Narahari, S., K.S.K. Malati, and K. Dev, *The Khond: A Dermatoglyphic Study.* Anthropologist, 2008. **10**(3): p. 207–210.
16. Segura-Wang, M. and R. Barrantes, *Dermatoglyphic traits of six Chibcha-speaking Amerindians of Costa Rica, and an assessment of the genetic affinities among populations.* Rev Biol Trop, 2009. **57**(1): p. 357–369.
17. Pour-Jafari, H., M.H. Chaleshtori, and D.D. Farhud, *Dermatoglyphics in Patients with Oligo/ Azospermia.* Iranian J Publ Health, 2005. **34**(3): p. 56–61.
18. Li, S.Z. and A.K. Jain, *Encyclopedia of biometrics*. 2009: Springer Verlag.
19. Gutiérrez-Redomero, E., et al., *Distribution of the minutiae in the fingerprints of a sample of the Spanish population.* Forensic Sci Int, 2010.
20. Jaja, B.N.R., et al., *Asymmetry and pattern polarization of digital dermal ridges among the Ogoni people of Nigeria.* Scientific Research and Essays, 2008. **3**(2): p. 051–056.
21. Ekanem, E.P., et al., *Digital Dermatoglyphic Patterns Of Annang Ethnic Group In Akwa Ibom State Of Nigeria.* The Internet J Biol Anthropol, 2009. **3**(1).
22. Banik, S.D., P. Pal, and D.P. Mukherjee, *Finger Dermatoglyphic Variations in Rengma Nagas of Nagaland India.* Collegium antropologicum, 2009. **33**(1).
23. Rastogi, P. and M.K.R. Pillai, *A study of fingerprints in relation to gender and blood group.* J Indian Acad Forensic Med, 2010. **32**: p. 1
24. Gutiérrez-Redomero, E., et al., *Variability of fingerprint ridge density in a sample of Spanish Caucasians and its application to sex determination.* Forensic Sci Int, 2008. **180**(1): p. 17–22.
25. Sharma, P.R., A.K. Gautam, and P.K. Tiwari, *Dermatoglyphic variations in five ethno-geographical cohorts of Indian populations: A Pilot Study.* The Internet J Biol Anthropol, 2008. **2**(1): p. 57–66.
26. Bhasin, M.K., *Genetics of Castes and Tribes of India: Dermatoglyphics.* Int J Hum Genet, 2007. **7**(2): p. 175.
27. Yao-Fong, C., et al., *A dermatoglyphic study of the Amis aboriginal popula-tion of Taiwan.* Sci China Ser C-Life Sci, 2008. **51**(1): p. 80–85.
28. Elsaadany, H.M., et al., *Can Dermatoglyphics be used as an Anatomical Marker in Egyptian Rheumatoid Patients?* J Am Sci, 2010. **6**(11).
29. Harden, K.P., et al., *Gene-environment correlation and interaction in peer effects on adolescent alcohol and tobacco use.* Behav Genet, 2008. **38**(4): p. 339–347.
30. Brock, J.A.K., et al., *Family History Screening: Use of the Three Generation Pedigree in Clinical Practice.* J Obstet Gynaecol Can, 2010. **32**(7): p. 663–672.
31. Criswell, D., *ABO Blood and Human Origins.* Acts & Facts, 2008. **37**.

Original Article

REPEATED INTRAVENOUS MERCURY CHLORIDE ADMINISTRATION INDUCES REGENERATION AND BIPHASIC RENAL DAMAGE IN RATS

Suhaidah Ibrahim*[1], Noordin Mohamed Mustapha [2], Rasedee Abdullah [2], Noor Azhar Mohamed Shazili [3]

[1]Kulliyyah of Medicine & Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah, 09300 Kuala Ketil, Kedah, Malaysia.
[2]Faculty of Veterinary Medicine, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[3]School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.

ARTICLE INFO

Corresponding author:
Dr. Suhaidah Ibrahim

Email address:
suhaidahibrahim2013@gmail.com

Received:
January 2018
Accepted for publication:
March 2018

Keywords:

Mercury chloride
Toxicity
Renal
Epithelisation

ABSTRACT

Mercury and its compounds are well known to be very toxic to kidneys. Forty Sprague-Dawley rats treated with intravenous injection of 0.5 mg/kg of body weight mercuric chloride ($HgCl_2$) in 1.0 mL 0.85% NaCl through tail vein. Another group served as a control and received 1.0 mL 0.85% NaCl. The treatments were repeated every other day for ten days. Renal tissue Hg concentration of the treated group increased significantly on day 14 with a value of 2064.5 ppb/g compared to control value of 19.16 ppb/g. The renal Hg content reached 2116.89 ppb/g on day 22 and kept decreasing to its lowest value on day 38 post-treatment; 310.47 ppb/g. The necrotic cells increased significantly with time reaching peak on day 42; 6007.67 damaged cells, compared to the control count of 50.75 damaged cells. The necrosis process was accompanied by regeneration of young cells which appeared bluish in colour and could be seen as early as on day 14 with a cell count of 58 cells/10 fields. The number decreased significantly on day 22 and 26. By day 30, these young cells were no longer seen. No evidence of tissue regeneration was observed in all control samples. Repeated intravenous mercury chloride administration was observed to cause biphasic renal damage. The early damaging phase was accompanied by a high reparative epithelisation process and the severe tubular necrosis began on day 18 as soon as the reparative phase has getting waned off.

INTRODUCTION

Mercury and its compounds have been used in human history for at least 3000 years. Mad hatter's and Minamata disease are the classical examples of mercurial poisoning. It's commonly found in various sources e.g. fish, poultry, insecticides, fungicides, pesticides, disinfectants, dental amalgam, thimerosal-containing vaccines and petroleum and its derivatives [1,2]. Urban discharges, agricultural materials, mining, hydrocarbon combustion and industrial discharges are major anthropogenic sources of Hg emissions into the environment [1,3,4]. It was reported in 2007 that 20,000 tons of mercury are annually released to the environment due to many human activities [5]. In many cases, the contamination chain of Hg follows closely the cyclic order: industry, atmosphere, soil, water, phytoplankton, zooplankton, fish and human [6].

Mercury and its compounds are highly toxic to kidneys causing necrosis [7,8]. It is suggested to bind to sulfhydryl group of enzymes inhibiting mitochondrial respiration [9]. Mercury intoxication has been observed to cause gross tubular lesion and thickening of glomerular basement membrane [10]. This study was designed to determine the pattern damaging effect of renal tissue following repeated intravenous mercury administration on rats.

MATERIALS AND METHOD

Animals**:** A total of 80 Sprague-Dawley rats aged between eight and ten weeks were divided into two groups. Treated group was given intravenous injection of 0.5 mg/kg of body weight mercuric chloride ($HgCl_2$) in 1.0 mL 0.85% NaCl through tail vein. Control group received 1.0 mL 0.85% NaCl. The treatments were repeated every other day for ten days. All rats were housed in cages fitted with urine collection trays, fed and given water *ad libitum*. Five rats from each group were sacrificed every four days commencing from the last day of the treatment (Day 14).

***Mercury Standard solutions*:** The content of 1.0 g Mercury titrisol ampoule (Merck, Germany) was transferred to chemically sterile Erlenmeyer containing 0.5 g potassium dichromate ($K_2Cr_2O_2$) and 50.0 mL concentrated HNO_2. The solution was later on made up to 1.0 L with distilled water. This solution was used as a stock mercury solution (1.0 mg/mL). Standard solutions of 5.0, 10.0, 15.0, and 20.0 µg Hg/ mL were prepared from the stock solution.

***Trace Element Analysis*:** Kidneys and livers of sacrificed rats were removed and kept in chemically sterile sealed plastic bags at -20°C until used. Approximately 0.5 – 1.0 g (wet weight) of kidney tissue samples were placed in 50.0 mL borosilicate test tubes. 4.0 mL of concentrated H_2SO_4 and 1.0 mL HNO_2 were added slowly to sample while in ice. The tubes containing sample were placed in a water bath and maintained at 50.0 – 60.0°C until all tissue samples were digested and solutions became clear. While in ice, 20.0 mL of 6.0% (w/v) potassium permanganate ($KmnO_4$) were added slowly to the solution. The samples were then left overnight at room temperature for further digestion. A blank solution was prepared in the same way except no sample was added to the preparation. After being left overnight, 5.0% (w/v) of hydroxylamine hydroxide was added slowly to all sample tubes to remove excess permanganate ions. All samples were then diluted to 20 times and made up to a final volume of 100.0 mL. The mercury content of the solutions were analysed using a cold-vapour atomic absorption spectrophotometer (Perkin Elmer 3100).

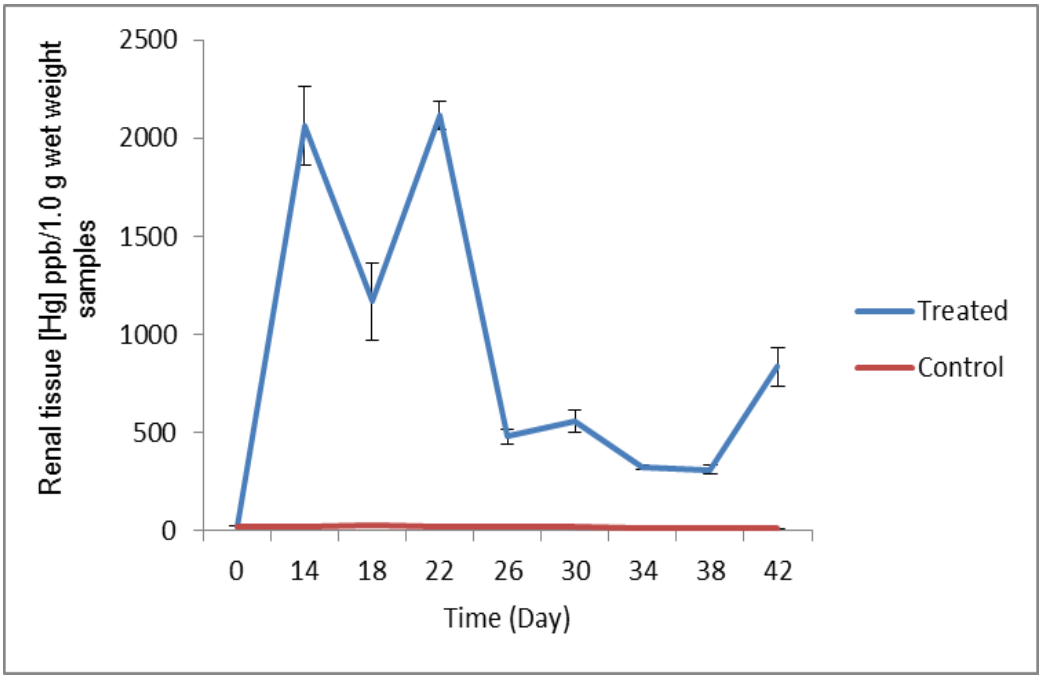
***Histopathology*:** Kidney samples from both treated and control rats were fixed in 10.0% formalin, embedded in paraffin wax and sectioned at 5.0 µm thickness. The sections were then stained with haematoxylin and eosin (H&E) and observed under light microscope (200x) for abnormal cells in 10 randomly selected fields per slide.

***Statistical analysis*:** All the data collected were analysed using analysis of variance (ANOVA) and Duncan test. Value of $p \leq 0.05$ were considered to be statistically significant and all results were expressed as mean ± standard error.

RESULTS

***Renal tissue mercury*:** The renal tissue Hg concentration of treated group increased significantly on day 14 with a value of 2064.5 ppb/ g compared to control value of 19.16 ppb/g (Figure 1). The renal Hg content of treated group significantly decreased on day 18 to 1167.8 ppb/g. The Hg content remained high on day 22; 2116.89 ppb/g, before kept decreasing to its lowest value on day 38 post-treatment; 310.47 ppb/g.

***Gross and histopathology*:** The kidney samples of treated group were grossly enlarged and pale in colour (Figure 2a). Gross observations noted pin-point lesions; resulted from necrosis and healing process, were noted on the surface of the



*Error bars represent standard error calculated from a triplicate samples

Figure 1: Analysis of results showed a renal tissue mercury concentration in rats sacrificed every 4 days following repeated exposure to $HgCl_2$.

treated kidney samples from day 26 onward. The lesions were not clearly seen on day 14, 18, and 22 on organ samples.

Light microscopic observation revealed evidence of tubular epithelial cell damage (Figure 2b). For purpose of quantification, cells which were described as vacuolated cytoplasm (hydropic changes), pyknotic, karyorrhectic, kayolytic and necrotic sloughed cells in the lumen of tubules were considered as damaged cells. Renal tissue samples of the treated group showed a significant early damage evidence by increased number of damaged cells on day 14 post-treatment; 367.2 damaged cell count/10 fields, compared to samples of control group; 22.33 damaged cell count/10 fields. In fact, the number increased significantly with time (Figure 3) reaching peak on day 42; 6007.67 damaged cells, compared to the control count of 50.75 damaged cells. Tissue samples of treated group showed increase of karyolysis and vacuolation. Also by day 26 sloughed off cells began to appear in the lumen of the tubules and the number increased by day 38 post-treatment.

Light microscopic observations also revealed evidence of tubular tissue regeneration through epithelisation in samples of treated group (Figure 4). These regenerative cells appeared bluish in colour in H&E tissue sections. The cells could be seen as early as on day 14 with a cell count of 58 cells/10 fields and the number significantly decreased to 15.67 and 20.67/ 10 fields on day 22 and 26 respectively. However, the cells were totally absent on day 18 in all tissue samples (Figure 5). By day 30 they were no longer seen. No evidence of tissue regeneration was observed in all control samples.

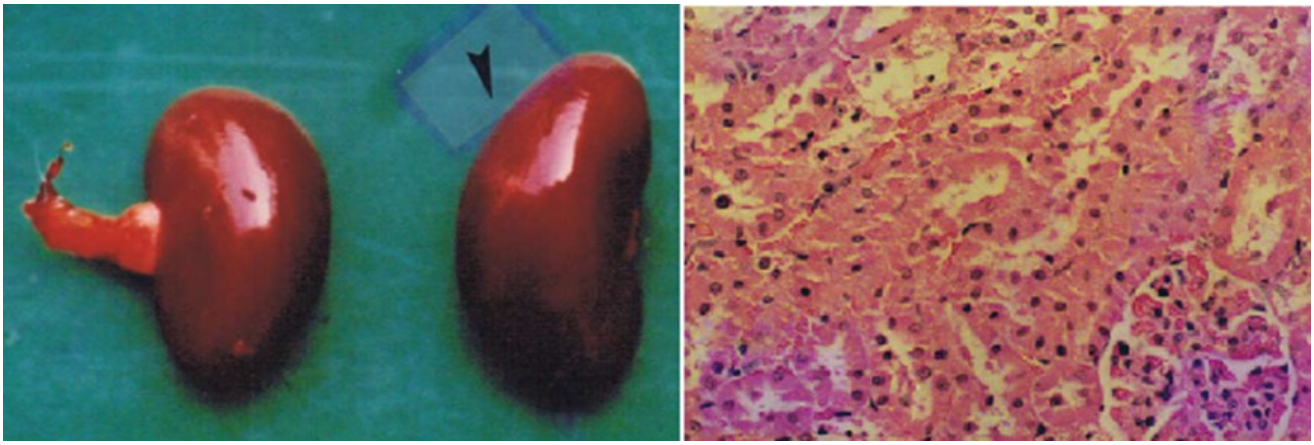
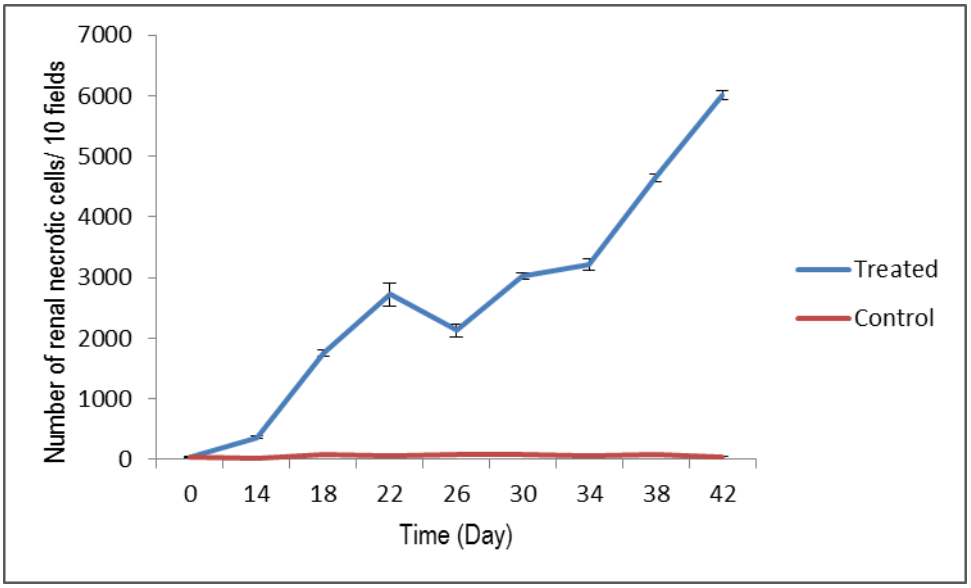


Figure 2: Kidney sample of treated group was enlarged (arrowhead; a) and photomicrograph showed an acute tubular necrosis (b); 200x magnification.



*Error bars represent standard error calculated from a triplicate samples

Figure 3: Light microscopic observation revealed numbers of damaged renal tubular cells in rats sacrificed every 4 days following repeated exposure to $HgCl_2$.

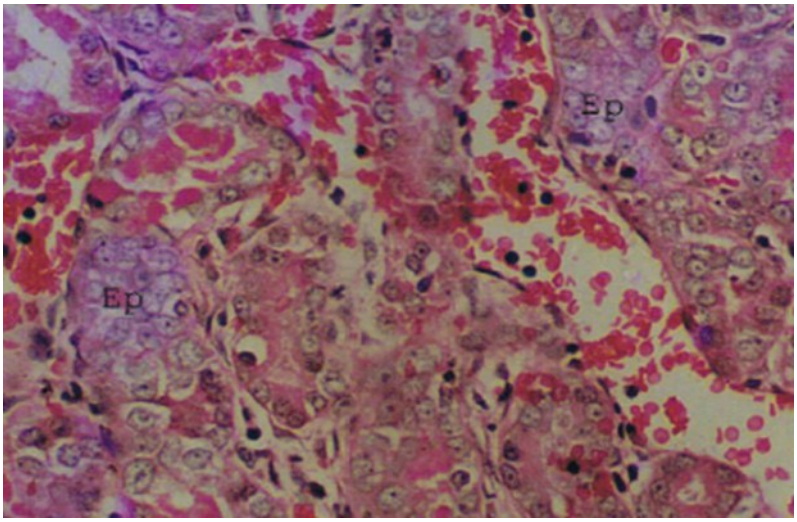
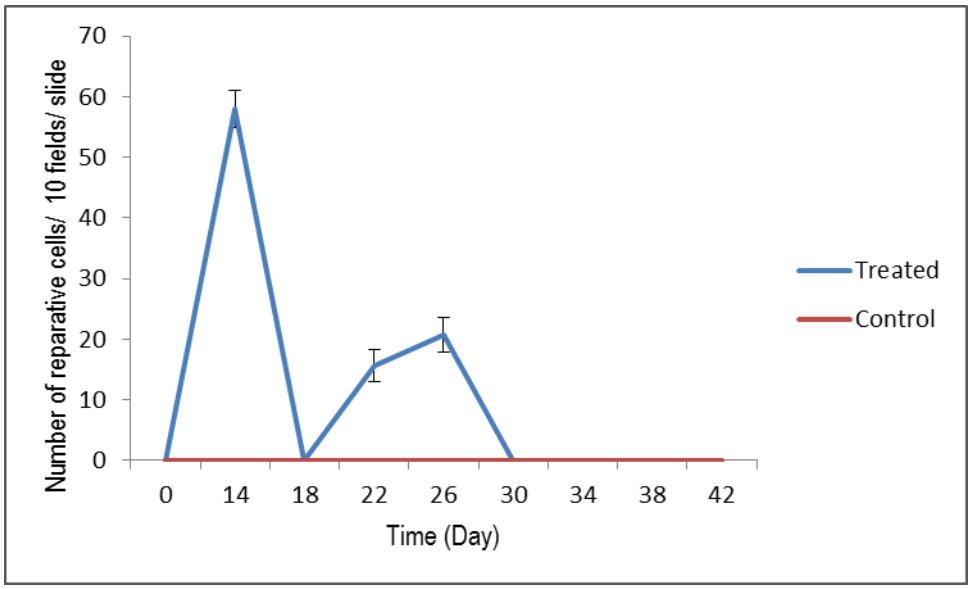


Figure 4: Light microscopic observation revealed epithelisation of renal tubular cells in rats sacrificed every 4 days following repeated exposure to $HgCl_2$. The young cells were bluish in colour (Ep); 200x magnification



*Error bars represent standard error calculated from a triplicate samples

Figure 5: Light microscopic observation revealed numbers of regenerative renal tubular cells in rats sacrificed every 4 days following repeated exposure to $HgCl_2$.

DISCUSSIONS

Mercury chloride has been recognised to be nephrotoxic reagent causing renal tubular vacuolation, interstitial inflammation and cell degeneration [11]. In this study, Sprague-Dawley rats were injected with $HgCl_2$ to induce acute nephrotic syndrome. The data showed a significant steady increase in tubular epithelial cell damage beginning on day 14 till the end of study period. The damage seemed to be more severe in the outer cortex region. This phenomenon could be due to higher accumulation of Hg in the region as suggested by Clarkson, 1972. It is believed that tubular secretion is the main process of Hg elimination from the body [5,13] and lysosome was observed to be the major site of Hg deposition in the renal tubules [14,15]. This may well be true since the characteristics associated with the damaged cells observed in this study; karyolysis, vacuolated cytoplasm, sloughing-off cells into the tubular lumen and swollen cells, are of the tubular cells.

Mercuric cations; Hg^{+} and Hg^{2+}, have high electro-negativity of 2.0 on the Pauling scale and large ionic radius of 0.111 - 0.116 nm. Owing to those characteristics, Hg-cations belong to “soft” acids, and thus having a strong binding affinity to “soft” bases like thiols and selenols.

Many proteins; enzymes and hormones, and cofactors feature thiols or so called sulfhydryl groups, -SH. Thiols appear to be not only of importance for oxidation-reduction mechanisms of mitochondrial respiration but also for the proper functioning of all thiol-featured proteins and cofactors [16]. Therefore, binding of Hg to thiol residues would result in activation of sulphur and functional attenuation or blockage of related enzymes, cofactors and hormones [2,17]. The injury of renal tubules in this study was suggested to be due to ischaemia and also direct nephrotoxicity of Hg. Mercury, as in previous studies, was observed to inhibit the mitochondrial electron transport chain (ETC) leading to cell death [9,18]. Production of reactive oxygen species (ROS) was also reported to be increased [19,20] and activity level of endogenous antioxidants; GSH, SOD, GPx, GR, was decreased [21,22] following administration of organic Hg; thus, putting the cells in oxidative stress.

In this study, there was a drastic regeneration of new cells observed following repeated exposure to $HgCl_2$. Those newly formed cells of tubular epithelium observable as early as on day 14 post-treatment but disappeared completely by day 30 post-treatment. However, those cells were absent in all samples on day 18; giving rise to a biphasic pattern. A previous study showed that regenerative response to Hg-induced injury was as early as 3 to 5 days following $HgCl_2$ administration [23]. Chen *et. al.* (2016) suggested $HgCl_2$ exposure stimulates the cell division of stem cells. The dying cells sent signal to the surrounding stem cells to initiate tissue regeneration by accelerating the proliferation and differentiation of the stem cells, which led to the generation of new-born cells.

The second bout of cell regeneration on day 22 and 26, even the number of newborn cells was significantly much lower, could be due to high compensatory effort of the kidney to go for repair against the injury. Mercury was observed to induce metallothionein and has a strong binding to it [12,25,26]. This ability contributed to longer retention of Hg in tissue and longer exposure of tissue to its toxicity. The excess exposure was suggested giving rise to severe injuries that beyond the cure by tissue regeneration.

CONCLUSION

In conclusion, this study showed that repeated intra-venous mercury chloride administration caused biphasic renal damage. The first phase of the damage was accompanied by a high reparative epithelisation phase. The second phase started as severe tubular necrosis began on day 18, which was as soon as the repair phase has getting waned off. This study suggested that it was possible for the kidney to repair the damage if the injury was well controlled and properly treated.

REFERENCES

1. Wilhelma, S.M and Bloomb, N. (2000). Mercury in petroleum. *Fuel Processing Technol*. **63 (1):** 1 -27.
2. Geir Bjørklund, Dadar, M., Mutter, J., and Aaseth, J. (2017). The toxicology of mercury: Current research and emerging trends. Environm. Res. **159**: 545 -554.
3. Jackson, T.A. (1997). Long-range atmospheric transport of mercury to ecosystems, and the importance of anthropogenic emissions—a critical review and evaluation of the published evidence. *Environ. Rev*. **5:** 99–120.
4. Zhang, I., Wong, M.H. (2007). Environmental mercury contamination in China: Sources and impacts. *Environ. Int*. **33:** 108–121.
5. Dias, D., Bessa, J., Guimar˜aes, S., Soares, M.E., Bastos, M.L., and Teixeira, H.M. (2015). Inorganic mercury intoxication: a case report. *Forensic Science International.* **256**: 20 – 24.
6. Kadar, I., Koncz, J., Fekete, S. (2000). Experimental study of Cd, Hg, Mo, Pb and Se movement in soil-plant-animal systems. In: Kniva, International Conference Proceedings, Patija, Croatia, pp. 72–76.
7. Ganote, C.E., Reimer, K.A., and Jennings, R.B. (1974). Acute mercury chloride nephrotoxicity: An electron microscopic and metabolic study. *Lab. Invest*. **31 (6)**: 633 – 647.
8. Sapin, C., Druet, E., and Druet, P. (1977). Induction of anti-glomerular basement membrane antibodies in the Brown-Norway rats by mercury chloride. *Clin. Exp. Immunol.* **28**: 173 – 179.
9. Taylor, N.S. (1965). Histochemical studies of nephrotoxicity with sublethal doses of mercury in rats. *Am. J. Path*. **46**:1 – 21.
10. Mandema, E., Arends, van Zeijst, J., Vermeer, G., van der Hem, G.K. and van der Slikke, L.B. (1963). Mercury and the kidney. *Lancet* **1**: 1266.
11. Liu, J., Lu, Y.F., Li, W.K., Zhou, Z.P., *et al*. (2016). Mercury sulfides are much less nephrotoxic than mercury chloride and methyl-mercury in mice. *Toxicol. Letters* **262**: 153 – 160.
12. Clarkson, T.W. (1972). The Pharmacology of mercury compounds. *Ann. Rev. Pharmacol*. **12**: 372 – 406.
13. Berlin, M., Zalups, K. R., Fowler, B. A. (2005). Mercury. In Nordberg, G. F. *et al.* (3rd Ed.) Handbook on the Toxicology of Metals. Academic Press.

14. Fowler, B.A. (1972). Ultrastructural evidence for nephropathy induced by long-term exposure to small amounts of methylmercury. *Science* **175**: 780.
15. Ammerman, C.B., Miller, S.M., Fick, K.R., and Hansard II, S.L. (1977). Contaminating elements in mineral supplements and their potential toxicity: A review. *J. Anim. Sci*. **44(3)**: 485 – 508.
16. Greenstein, J.P. (1938). Sulfhydryl groups in proteins. *J. Biol. Chem*. 501-513.
17. Mathieson, P.W. (1995). Mercury: god of TH2 cells. *Clin. Exp. Immunol*. **102**: 229–230.
18. Robinson, M. and Hesketh, A. (1976). Effect of mercury chloride on the structure and function of the kidney of sheep. *J. Comp. Path*. **86**: 307 – 318.
19. Moss, D.W., Bates, T.E. (2001). Activation of murine microglial cell lines by lipopolysaccharide and interferon-gamma causes NO-mediated decreases in mitochondrial and cellular function. *Eur. J. Neurosci*. **13**: 529–538.
20. Ali, S.F., LeBel, C.P., Bondy, S.C. (1992). Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. *Neurotoxicology* **13**: 637–648.
21. Franco, J.L., Braga, H.C., Stringari, J., Missau, F.C., Posser, T., Mendes, B.G., *et al*. (2007). Mercurial induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. *Chem. Res. Toxicol*. **20**: 1919–1926.
22. Mori N, Yasutake A, Hirayama K. (2007). Comparative study of activities in reactive oxygen species production/defense system in mitochondria of rat brain and liver, and their susceptibility to methylmercury toxicity. *Arch. Toxicol*. **81**:769–776.
23. Cuppage, F.E. and Tate, A. (1967). Repair of nephron following injury with mercury chloride. *Am. J. Path*. **51**: 405 – 417.
24. Chen, Z., Wu, X.Z., Lou, H.J., Zhao, L.L, *et al*. (2016). Acute exposure of mercury chloride stimulates the tissueregeneration program and reactive oxygen species production in the *Drosophila* sp. midgut. *Environ. Toxicol. Pharmacol*. **41**: 32 – 38.
25. Piotrowski, J.K., Trojanowska, B., Winiewska-Knypl, J.M., and Bolanowska, W. (1974). Mercury binding in the kidney and liver of rats with repeated exposure to mercury chloride: Induction metallothionein by mercury and cadmium. *Toxicol. Appl. Pharmacol*. **27**: 11 – 19.
26. Rana, S.V.S. (2008). Metals and apoptosis: Recent developments. *J. of Trace Elements in Med. and Biol.* **22**: 262–284.

Original Article

VERNAL KERATOCONJUNCTIVITIS IN THE TROPICAL CLIMATE: EPIDEMIOLOGY AT A TERTIARY CARE CENTER.

Rupini Yogesvaran*[1], Fiona Chew Lee Min [2]

[1] Department of Ophthalmology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Malaysia.
[2] Department of Ophthalmology, Hospital Selayang, Selangor, Malaysia

ARTICLE INFO

Corresponding author:
Dr. Rupini Yogesvaran

Email address:
rupini.y@gmail.com

Received:
November 2017
Accepted for publication:
December 2017

Keywords:

Vernal keratoconjunctivitis
Tropical climate
Ocular allergy

ABSTRACT

Vernal keratoconjunctivitis (VKC) is a chronic persistent disease in our country with a tropical climate. It mainly affects young school going children and if not treated in time may lead to irreversible blinding complications. A total of 41 patients under Selayang Hospital follow up were studied in a retrospective manner to provide epidemiological data, precipitating factors, common presenting symptoms, treatment options and outcomes. Our aim is to create awareness among all practitioners so that early Ophthalmology referral is commenced in aid to achieve clinical remission in the fastest time with the least complications.

INTRODUCTION

Vernal keratoconjunctivitis (VKC) is a severe component of the allergic conjunctivitis spectrum. It is a bilateral, inflammatory ocular surface disease which targets the paediatric age group. The most striking difference between the milder forms of allergic conjunctivitis and VKC is that, the later can compromise the cornea, leading to ulceration and later scarring. This disease is an important public health issue, as it is responsible for visual impairment in children, especially school going children, and have severe blinding complications if not treated appropriately and in time. Vernal keratoconjunctivitis has a variety of precipitating factors ranging from environmental causes, food elements and also familial preponderance. These patients' more often than not, present initially to general practitioners, paediatricians or allergists prior to being seen by an ophthalmologist. Hence, this study was done to evaluate the epidemiology, precipitating factors, clinical features and also treatment outcomes of VKC patient, in aim to increase awareness of this debilitating disease.

MATERIALS AND METHODS

A total of 41 patients diagnosed with Vernal Kerato-conjunctivitis (VKC) under Hospital Selayang Ophthalmology clinic care between January to December 2016 were studied. This was done by a retrospective, noncomparative case series using our patient's clinical records. Patient's data were extracted from the computer database using key-words such as vernal keratoconjunctivitis, VKC and shield ulcer. All epidemiological data, clinical symptoms and slit lamp examination findings during each appointment were obtained from the clinical records. These data were subsequently used to classify the severity of VKC. The types of drugs and other treatment modalities were obtained from the case sheets as well as pharmacy orders database for each patient. Precipitating factors were obtained based on history taking, skin prick test and allergy blood testing where available.

RESULTS

Out of the 41 patients, 87.5% (35 patients) were male and the remaining 12.5% (6 patients) were female. 78.0% (32 patients) were Malay, 14.6% (6 patients) were Chinese and the least were among the Indians which amounted to 7.3% (3 patients). The age range of patients were from 6 to 24 years. The mean age of presentation to our clinic was 10.5 years of age and the mean age of onset of symptoms was 8.3 years of age (Figure 1).

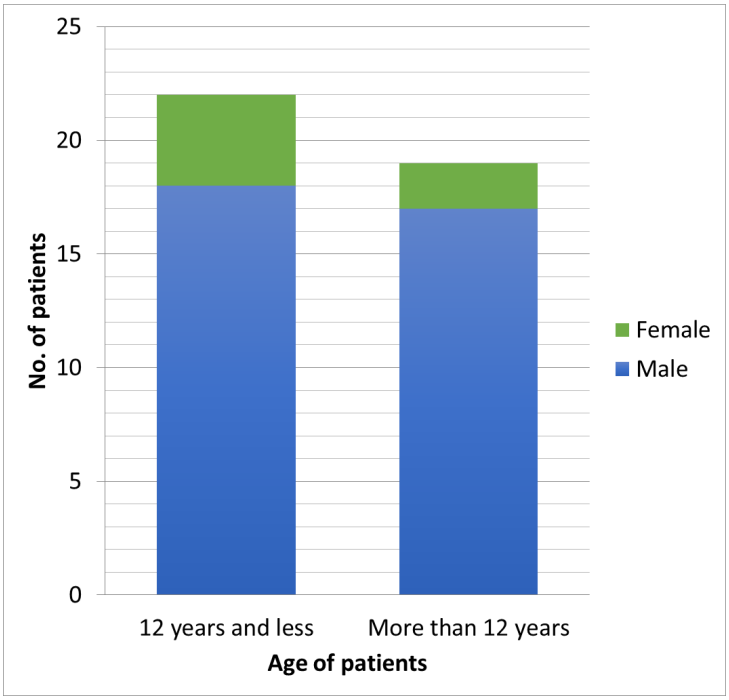


Figure 1: Bar chart of demographic distribution of age and sex of patients with Vernal keratoconjunctivitis VKC under Hospital Selayang follow up for the year 2016.

Most of our patients were referred to us by nearby general practitioners. A total of 24 patients (58.5%) were first seen at either government or private polyclinics before being referred to the Ophthalmology clinic. The remaining patients were referred to us by other specialist departments of the same hospital namely the Peadiatrics and Otorhinolaryngology department. This amounted to about 12 patients (29.2%). 2 patients (4.9%) were referred from private ophthalmologists for continuation of care due to financial constraints and 3 patients (7.3%) source of referrals were not documented.

When reviewing patient's risk factors, we noticed 51.2% (21 patients) had concurrent allergic rhinitis, 41.5% (17 patients) had underlying Bronchial Asthma and 2.4% (1 patient) had eczema. 43.9% (18 patients) informed us regarding family history of atopy. Out of all of our patients, only 12 patients had either a skin prick test or a blood allergy test done to identify their precipitating factors. 41.6% (5 patients) had dust mite allergies, 33.3% (4 patients) were allergic to egg and seafood, 4.9% (2 patients) were allergic to milk, 2.4% (1 patient) had allergy to dog and cat dander and 2.4% (1 patient) was allergic to benzoyl peroxide.

The common presenting symptoms were itchiness (70%), redness of eyes (51%) and tearing of eyes (29%). All 41 patients gave history of symptoms being persistent throughout the year. Objective examination showed all patients having tarsal component of VKC with 47% having cobblestone papillae, 29% having mild papillae and the remaining 24% having giant papillae. A total of 34% of our patients had a combination of limbal component. 29.2% (12 patients) had limbitis, 12.1% (5 patients) had tranta's dot sign and 4.9% (2 patients) had pseudogerontoxon. A total of 18 patients (44%) developed shield ulcer during the course of treatment. Out of these, eight of them had recurrent episodes of shield ulcer and 14 patients developed ulcer during the first year of presentation. Based on our hospital records, patient's presenting with acute exacerbations did not show any seasonal pattern and were distributed through all 12 months.

All patients were treated with mast cell stabilisers and lubricants. 34 patients received a short duration of topical steroids (dexamethasone 0.1% or fluoromethalone 0.1%). Out of these 34 patients, one patient developed steroid induced glaucoma with a highest documented intraocular pressure (IOP) of 28mmHg. It was well controlled with two topical antiglaucoma drops and IOP returned to a normal range after topical corticosteroids were removed. Twelve patients in this series required topical cyclosporine 0.5% during the course of treatment. Out of all the case series only five patients received supratarsal injection with triamcinolone and all of them had documented reduction in papillae post procedure (Figure 2).

The visual acuity (VA) on presentation shows 36 patients with visual acuity of 6/18 or better, one with visual acuity less than 6/18 to 6/60, three with visual acuity of less than 6/60 to 3/60 and one patient with a vision less than 3/60. The final visual outcome showed improvement with 39 of them having a visual acuity of 6/18 or better and two of

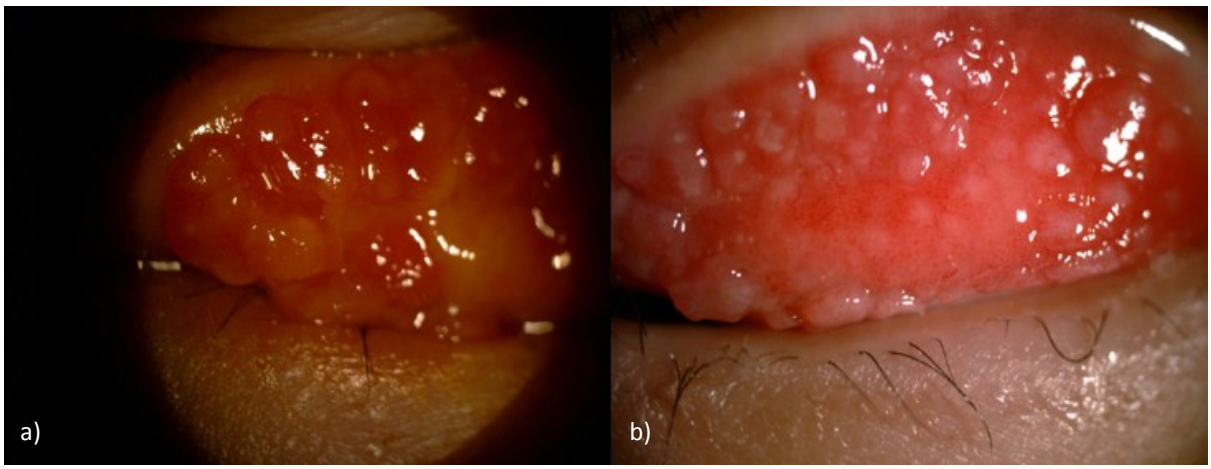


Figure 2a shows the severity of a patient's papillae prior to commencement of supratarsal triamcinolone and Figure 2b shows the same patient showing improvement after treatment.

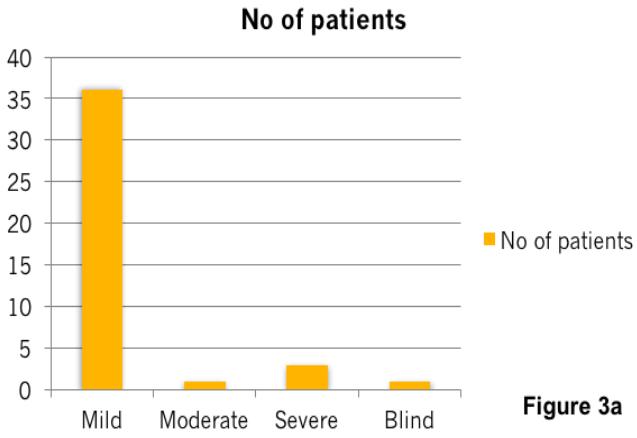


Figure 3a

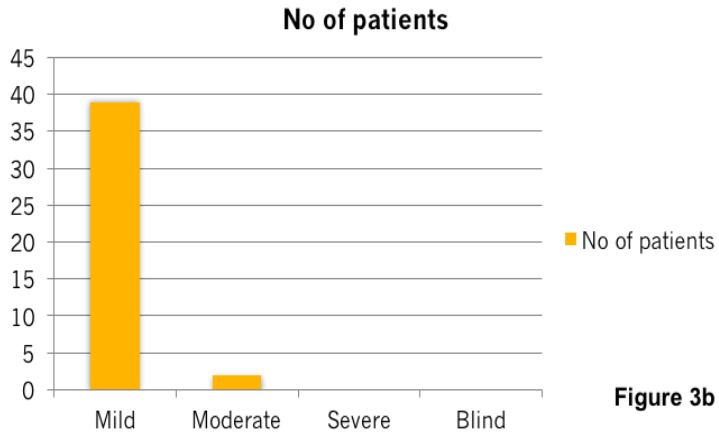


Figure 3b

*Patients were categorised based on the WHO classification of visual impairment

Figure 3: The bar chart in Figure 3a shows the distribution of patients based on their presenting vision and Figure 3b shows their vision at the latest follow up.

them with visual acuity of worse than 6/18 to 6/60 (Figure 3).

DISCUSSION

A greater part of the patients suffering from VKC in this study were young boys who have been under our sub-specialty follow up for at least 2 years. Although the mean age of presentation to our clinic was 10.5 years, the age of onset of symptoms was 8.3 years. This correlates with epidemiological data from numerous studies stating the age of onset is before the age of 10 years and majority of them outgrow their VKC at puberty [1-3]. The distribution of cases based on ethnicity in our series, represented the local population around the hospital.

All of our patients had a perennial pattern of symptoms. This is most likely due to the fact that Malaysia has an equatorial climate of being hot and humid, which leads to the persistent symptoms throughout the year. These constant symptoms are comparable to that documented by our neighbors with very similar weather [1,4]. More than half of the patients exhibit some atopy sensitization suggesting a similar pathological pathway in these clusters of diseases. Dust mites seem to be a common allergen, but it is not possible to draw conclusions as not many of our subjects were tested for it and among those who were, different tests were done.

VKC can be classified into three forms and commonly, the limbal form and mixed form has

been reported in Asia [4], while the tarsal form seems to be more prevalent in Europe [5]. However, studies from India [6] and Singapore [1] showed the tarsal form more predominant in certain areas. Most of our patients had predominantly tarsal form followed by a mixed form of VKC and none demonstrated the limbal form alone. Corneal involvement is something all Ophthalmologists are concerned about as it has a possibility to lead to severe vision- threatening sequela [7]. 18 (44%) of our patients developed shield ulcer which is much higher than the reported 3-11% [8]. This is because all of our patient had changes in the tarsal conjunctiva predisposing them to cornea compromise. 14 out of these 18 patients had corneal ulcers in the first year in our clinic suggesting, often they are referred for an eye assessment in severe ends of the disease spectrum.

The treatment of VKC is mainly medical treatment and it depends on the stage of the disease. Milder cases can be treated with avoidance of triggering factor and use of antihistamic eyedrops and mast cell stabilisers. Severe varieties are treated with either topical corticosteroids to control the disease especially in exacerbations or topical immunosuppressants to reduce cellular level inflammation which in turn shows symptomatic and structural improvements [9,10]. 83% of our patients used a course of topical corticosteroids during the course of treatment. This is similar to other studies in the east [1] and west [5] where topical corticosteroids were used 85% and 75% of patients respectively. Twelve of our patients were clinically stable while on topical cyclosporine as demonstrated in other studies [11,12]. In severe cases refractive to medical therapy, supratarsal corticosteroid injections has been shown to be successful [13]. Five of our patients had supratarsal triamcinolone injection and all showed a positive change following it.

CONCLUSION

Our series clearly demonstrates most of these patients do not present to Ophthalmologist firsts, but in fact are seen by general practitioners or other specialists before being referred for an eye assessment. The purpose of this article is to give an overview of VKC to all, so that practitioners are aware of the risk factors and presenting symptoms, thus referral to the Ophthalmologist will be early which is imperative for prompt treatment for these patients.

REFERENCES

1. Studies from Tan Tock Seng Hospital Add New Findings in the Area of Allergic Conjunctivitis (Allergic conjunctivitis in Asia). Obesity, Fitness & Wellness Week. 2017:5085.
2. Leonardi A, Busca F, Motterle L, et al. Case series of 406 vernal keratoconjunctivitis patients: a demographic and epidemiological study. Acta Ophthalmol Scand 2006: 84: 406–10.
3. Katelaris CH. Ocular allergy in the Asia Pacific region. Asia Pacific Allergy. 2011;1(3):108-114. doi:10.5415/apallergy.2011.1.3.108.
4. Kosrirukvongs P, Vichyanond P, Wongsawad W. Vernal keratoconjunctivitis in Thailand. Asian Pac J Allergy Immunol 2003: 21: 25–30.
5. Bonini S, Lambiase A, Marchi S, et al. Vernal keratoconjunctivitis revisited: a case series of 195 patients with long-term followup. Ophthalmology 2000: 107: 1157–63.
6. Sofi RA, Mufti A. Vernal Keratoconjunctivitis in Kashmir: A temperate zone. International ophthalmology. 2016 Mar 10.
7. Cameron JA. Shield ulcers and plaques of the cornea in vernal keratoconjunctivitis. Ophthalmology. 1995;102(6):985-93.
8. Vichyanond P, Pacharn P, Pleyer U, Leonardi A. Vernal keratoconjunctivitis: A severe allergic eye disease with remodeling changes. Pediatr Allergy Immunol 2014: 00.
9. Mantelli F, Lambiase A, Bonini S, Bonini S. Clinical trials in allergic conjunctivits: a systematic review. Allergy. 2011;66(7):919-24.
10. De Smedt S, Wildner G, Kestelyn P. Vernal keratoconjunctivitis: an update. Br J Ophthalmol 2013: 97: 9–14.
11. Secchi AG, Tognon MS, Leonardi A. Topical use of cyclosporine in the treatment of vernal keratoconjunctivitis. Am J Ophthalmol 1990: 110: 641–5.
12. Power WJ, Tugal-tutkun I, Foster CS. Long-term follow-up of patients with atopic keratoconjunctivitis. Ophthalmology. 1998;105:637–642.
13. Zaouali S, Kahloun R, Attia S, Jelliti B, Trigui M, Yahia SB, et al. Supratarsal injection of triamcinolone acetonide and childhood allergic keratoconjunctivitis. International Ophthalmology. 2012;32(2):99-106

Original Article

EMPIRICAL FIXED RADIOIODINE THERAPY FOR HYPERTHYROIDISM: A SENSIBLE OPTION AND ITS OUTCOME AT A LOCAL NUCLEAR MEDICINE REFERRAL CENTRE.

Ahmad Zaid Zanial*[1], Mahayuddin Abdul Manap [2], Fadzilah Hamzah [3]

[1] Nuclear Medicine Department, Hospital Kuala Lumpur
[2] Advanced Medical and Dental Institute, Universiti Sains Malaysia
[3] Nuclear Medicine Department, Hospital Pulau Pinang

ARTICLE INFO

Corresponding author:
Dr. Ahmad Zaid Zanial

Email address:
ahmadzaidx@gmail.com

Received:
April 2018
Accepted for publication:
June 2018

Keywords:

Empirical fixed
Radioiodine theraphy
Hyperthyroidism

ABSTRACT

Fixed empirical radioiodine therapy is one of the treatment options for hyperthyroidism. Although several methods to calculate administered radioiodine activity have been proposed previously, no clear advantages could be proven in using adjusted dosage over fixed dosage. Hence, our aim was to determine the outcome following fixed 15 millicurie (mCi) therapy among local hyperthyroidism patients and factors associated with euthyroid status post-treatment. Patients undergoing first-time radioiodine therapy and achieved pre-treatment urinary iodine level <50 µg/L following minimum of 1 week dietary restriction preparation were recruited (n=49). Majority were middle aged females with small to moderate goitre. Anti-thyroid drugs consumption was stopped for at least a week prior to empirical 15 mCi radioiodine therapy. Favourable treatment outcome includes euthyroid and hypothyroidism determined at 9th month follow-up post-therapy. Collected clinical data were analysed. Majority (88%) achieved favourable thyroid status (euthyroid, n=26 and hypothyroidism, n=17). None developed any major therapy complication. No significant association between age, gender, goitre classification and duration of illness with euthyroid post-treatment status. However, patients with optimised baseline free thyroxine, fT4 values within normal range were associated with euthyroid status (p<0.05). Multiple logistic regression analysis revealed that optimised baseline fT4 was the only factor associated with developing euthyroid status (OR 19.48, 95% CI 1.253-302.692, p<0.05). Majority of our hyperthyroidism patients achieved favourable outcome following empirical fixed 15 mCi radioiodine therapy. Optimised baseline fT4 was significantly associated with euthyroid status post-treatment.

INTRODUCTION

Hyperthyroidism can be defined as a state of hyper-functioning thyroid causing excessive circulating thyroid hormones. Various aetiologies such as Graves' disease, toxic thyroid adenoma and multinodular goitre lead to hyperthyroidism. Nuclear medicine therapy utilising radioactive Iodine-131 or radioiodine is an established treatment method for thyroid diseases. Oral administration of radioiodine and its role in the management of hyperthyroidism related benign disorders and thyroid malignancy have been well documented [1]. After being taken up by thyroid gland and processed similarly as dietary iodine, radioactive Iodine-131 will emit beta-radiation destroying thyroidal follicular cells and gradually leading to disease control [2].

At present, outpatient radioiodine therapy for hyper-thyroidism has become one of the feasible treatment options in Malaysia mainly available at several tertiary hospitals and referral institutions with nuclear medicine facilities [3]. Although majority of our hyper-thyroidism patients are medically treated with thionamide anti-thyroid drugs, some of them might be referred for radioiodine therapy. Reason for Iodine-131 administration among this subset of patients is essentially due to failure of medical treatment [4]. The aim of radioiodine therapy in hyperthyroidism is to achieve euthyroid state or iatrogenic hypothyroidism which require thyroxine supplement [1].

Most local nuclear medicine centres prescribe empirical fixed radioiodine dosage of generally <30 millicurie (mCi) for hyperthyroidism with consideration given to thyroid size based on physical examination [5]. Although several methods in calculating appropriate and almost accurate Iodine-131 dosage were previously proposed, no clear advantages could be proven in using adjusted over fixed dose regimen [6, 7]. Hence, our aim was to determine the outcome following common fixed 15 millicurie (mCi) therapy among local hyperthyroidism patients particularly euthyroid state post-therapy and factors associated with it.

METHODOLOGY

A prospective study was conducted, involving hyperthyroidism patients regardless of underlying of aetiologies referred for first-time radioiodine therapy between January and September 2014, at Nuclear Medicine Department, Hospital Pulau Pinang. The patients received briefing and written instruction on relevant preparation measures and pre-therapy dietary restriction. Anti-thyroid drugs consumption except for lithium was stopped for at least a week prior to and after radioiodine therapy. Subsequently, pre-treatment urinary iodine test was performed to ensure compliance towards the dietary preparation. Altogether, 49 patients who achieved urinary iodine level <50 µg/L following minimum of 1 week dieting were recruited.

All patients received with empirical fixed 15 mCi radioiodine. They were then monitored and attended clinic sessions scheduled every 3 months. Favourable treatment outcome includes euthyroid and hypothyroidism determined at 9th month follow-up post-radioiodine therapy. Relevant clinical data including age, gender, duration of illness, pre-therapy free thyroxine (fT4) level, thyroid size based on the WHO/UNICEF/ICCIDD goitre grading as well as treatment adverse effect and outcome were collected. Statistical analysis was performed using SPSS version 19.0 for Windows. Written consents were obtained from all subjects prior to their enrolment in this study. This study has received ethical approvals from Medical Research Ethics Committee, Kementerian Kesihatan Malaysia and Human Research Ethics Committee, Universiti Sains Malaysia.

RESULTS

Out of 49 patients included in this study, 73% of them were females. Average age of patients was 45.5 ± 12.5 years. Mean duration of hyperthyroidism was 40.2 ± 29.8 months with 55% of patients having the illness for more than 24 months. Nearly 61% of patients presented with small to moderate sized goitre. Mean fT4 level pre-radioiodine therapy was 21.0 ± 14.2 pmol/L. Approximately 71% of patients had optimised pre-therapy fT4 values (within normal range of 12–22 pmol/L). At 9th month follow-up, majority of patients (88%) achieved favourable treatment outcome with overall no reported major adverse effects. Clinical characteristics and treatment outcomes are summarised in Table 1.

Among patients with favourable outcome, about 60% attained euthyroid state without thyroxine supplement post-radioiodine therapy. Patients with optimised pre-therapy fT4 level showed significant correlation with euthyroid status (p=0.037). However, there was no significant association found between euthyroid status and gender (p=0.625), age (p=0.551), duration of illness (p=0.204) as well as goitre classification (p=0.299). Furthermore, multiple logistic regression analysis revealed that optimised fT4 was the only significant factor associated with developing euthyroid (OR 19.478, 95%CI 1.253–302.692, p<0.05) as shown in Table 2.

Table 1: Clinical characteristics and treatment outcomes of the patients (n=49)

Variables		n (%)
Gender	Females	36 (73%)
	Males	13 (27%)
Mean Age		45.5 ± 12.5 years
Mean Duration of Illness		40.2 ± 29.8 months
Illness Duration	≤ 24 months	22 (45%)
	> 24 months	27 (55%)
Goitre Grading	Small to Moderate	30 (61%)
	Large	19 (39%)
Mean Baseline fT4		21.0 ± 14.2 pmol/L
Category of fT4	Deranged	14 (29%)
	Optimised	35 (71%)
Outcome	Euthyroid	26 (53%)
	Hypothyroidism	17 (35%)
	Hyperthyroidism	6 (12%)

DISCUSSION

Generally, radioiodine therapy for hyperthyroidism is relatively safe and has minimal adverse effects. However, the protocol for radioiodine therapy varied between centres and there were differences in the doses of Iodine-131 activity being used to cure hyperthyroidism [8]. Empirical fixed dose regimen for radioiodine therapy is being described as simple, more convenient to use and effective in achieving therapeutic goals compared to calculated dose method [2, 9]. Calculated dose regimen is associated with prolongation of treatment duration and has not been demonstrated to have significant benefits in terms of improving cure rates [10]. Fixed doses of radioiodine activity are generally in the range of 5–15 mCi which enable further incremental dose of Iodine-131 when the thyroid gland is large [11]. A Summary of Consensus for Management of Thyroid Disorders in Malaysia has highlighted the role of radioiodine therapy and fixed dose approach using 10–15 mCi Iodine-131 for hyperthyroidism [12].

An empirical fixed dose regimen of 15 mCi was selected and administered for all patients in this current study. Overall success rate at 9th month follow -up following fixed empirical 15 mCi radioiodine therapy among our cohort of patients was 88% with 26 patients achieving euthyroid status and 17 patients noted to be hypothyroid requiring thyroxine supplement. An early analysis of 478 hyperthyroidism patients showed 44% of them developed euthyroid and 35% became hypothyroid at four months after 15 mCi of Iodine-131 therapy [10]. In another study, Lewis *et al*. (2013) reported favourable outcome with 74% of subjects became hypothyroid and 19% developed euthyroid at one year follow-up post radioiodine therapy with approximately 15 mCi for 449 hyperthyroidism patients with Graves' disease, toxic MNG and indeterminate aetiology [8].

Majority of patients in this study were middle aged females having hyperthyroidism with small to moderate sized goitre for more than 2 years prior to radioiodine therapy. Apart from radioiodine dosage, there are several other parameters such as age and gender of the patients, goitre size as well as disease severity that may influence Iodine-131 treatment outcome in hyperthyroidism [13]. Lower cure rates were associated with males, young patients, large goitre and higher disease severity [14, 15]. The statistical analysis revealed that patients with optimised pre-therapy fT4 level were significantly associated with post-treatment euthyroid status while age, gender, duration of illness and goitre classification showed no significant correlation. Optimised pre-therapy fT4 level was the only significant factor associated with developing euthyroid based on the multiple logistic regression analysis.

A retrospective study by Khalid *et al.* (2011) involving 584 hyperthyroidism patients receiving approximately 15 mCi radioiodine therapy revealed that 93% of subjects achieved favourable outcome and their pre-treatment fT4 level was the only factor which independently influenced therapy outcome. High fT4 level of >45 pmol/L predicted a lower cure rate [16]. Although Lewis *et al.* (2013) reported that patients with medium to large sized goitre were found to have persistent hyperthyroidism (14.7%, p=0.001) than those with small or no goitre, the influence of goitre size was not significant in the logistic regression model after inclusion of baseline fT4. Moreover, patients with markedly high baseline fT4 level (>80 pmol/L) in that study were

Table 2: Association between clinical parameters and euthyroid state post-radioiodine therapy by Multiple Logistic Regression model (n=43)

Variables	Regression coefficient (b)	Adjusted Odd Ratio (95% CI)	Wald statistic	Significance value
Age (years)	-0.019	0.98 (0.87, 1.10)	0.10	0.753
Illness duration (months)	0.002	1.00 (0.96, 1.05)	0.01	0.920
Gender				
Female	0	1		
Male	0.501	1.65 (0.25, 10.86)	0.27	0.602
Goitre				
Small to medium	0	1		
Large	0.248	1.28 (0.19, 8.49)	0.07	0.797
Baseline fT4 category				
Deranged	0	1		
Optimised	2.969	19.48 (1.25, 302.69)	4.50	0.034

demonstrated to have increased failure rate (16.7%, p=0.003) compared to those with lower ranges of fT4 [8].

Limitations of this study included the small sample size and short duration of follow-up compared to other previous studies. Long term effect especially hypothyroidism might manifest later with permanent hypothyroidism seemed to be unavoidable in Graves' disease [13, 17]. Additionally, information on the exact aetiologies of hyperthyroidism was not evaluated due to limited data available, while test for thyroid autoantibodies, ultrasonography and radioiodine uptake study were not routinely done for all patients. Another study limitation was determinant of goitre grading done by physical evaluation which could be subjected to observer variation and less accurate compared to ultrasonography. However, estimation of goitre by similar clinical method and grading have been utilised in other previous studies [9, 10].

CONCLUSION

Empirical fixed common dosage of 15 mCi radioiodine therapy could well be utilised for hyperthyroidism treatment. Majority of patients from our cohort of predominantly females with small to moderate goitre achieved favourable outcome at 9th month follow-up. No major therapy complications observed. Optimised pre-therapy fT4 level was significantly associated with developing euthyroid status post Iodine-131 treatment. Future study with larger cohort and longer monitoring period is recommended.

REFERENCES

1. Silberstein EB, et al. (2012). The SNM Practice Guideline for Therapy of Thyroid Disease with 131 -I. Journal of Nuclear Medicine 53(10): 1-19
2. Mumtaz M, et al. (2009). Radioiodine I-131 for the Therapy of Grave's Disease. Malaysian Journal of Medical Sciences 16(1): 25-32
3. Zanial AZ, et al. (2016). Management of Hyperthyroidism in Malaysia and the Contemporary Role of Radioiodine Therapy – A Review. The International Journal of Medicine and Sciences 1 (1): 23-25
4. Sztal-Mazer S, et al. (2012). Evidence for higher success rates and successful treatment earlier in Grave's disease with higher radioactive iodine doses. Thyroid 22: 991-995
5. Zanial AZ and Hamzah F (2015). Understanding Issues in Preparing Patients for Radioactive Iodine Treatment of Thyroid disorders. Malaysian Journal of Nursing 7(1): 3-6
6. Leslie WD, et al. (2003). A randomized comparison of radioiodine doses in Graves' hyperthyroidism. J Clin Endocrinol Metabolism 88(3): 978-83
7. Jaiswal AK, et al. (2014). Comparison of clinical outcome after a fixed dose versus dosimetry-based radioiodine treatment of Graves' disease: Results of a randomized controlled trial in Indian population. Indian J Endocrinol Metabolism 18 (5): 648–654
8. Lewis A, et al. (2013). Outcome of 131I therapy in hyperthyroidism using a 550MBq fixed dose regimen. Ulster Med J 82(2): 85-88
9. Yau JSY, et al. (2009). Usage of a fixed dose of radioactive iodine for treatment of hyperthyroidism: one-year outcome in a regional hospital in Hong Kong. Hong Kong Medical Journal 15: 267 -273
10. Gupta SK, et al. (2010). Fixed dose (555 MBq; 15 mCi) radioiodine for treatment of hyperthyroidism: Outcome and its predictors. Internal Medicine Journal 40(12): 854-857
11. Iagary A and McDougall IR (2007). Treatment of Thyrotoxicosis. Journal of Nuclear Medicine 48: 379-389
12. Zainudin S, et al. (2012). A Summary of the Consensus for the Management of Thyroid Disorders in Malaysia. Journal of ASEAN Federation of Endocrine Society 27(1): 40-43
13. Bonnema SJ and Hegedus L (2012). Radioiodine therapy in benign thyroid disease: effects, side effects, and factors affecting therapeutic outcome. Endocrine Reviews 33(6): 920-980
14. Allahabadia A, et al. (2001). Radioiodine Treatment of Hyperthyroidism–Prognostic factors for Outcome. J Clin Endocrinol Metabolism 86(8): 3611-3617
15. Alexender EK and Larsen PR (2002). High Dose 131I Therapy for the Treatment of Hyperthyroidism Caused by Grave's Disease. J Clin Endocrinol Metabolism 87(3): 1073-1077
16. Khalid Y, et al. (2011). Efficacy of Fixed High Dose Radioiodine Therapy for Hyperthyroidism – a 14 year Experience: A focus on Influence of Pre-treatment Factors on Outcomes. British Journal of Medical Practitioners 4(3):a435
17. Collier A (2012). Thyrotoxicosis and radioiodine therapy: Dose the dose matter? Indian J Endocrinol Metab 16(Suppl 2): S147-S149

Review Article

A REVIEW ON CARDIOPROTECTIVE EFFECT OF AN ANTIOXIDANT, VITAMIN-E AGAINST MYOCARDIAL INFARCTION.

Nuruddin Mohammed Nur*[1], Abdelbaset Taher Abdelhalim[2]

[1]Biochemistry Department, Kulliyyah of Medicine & Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah, 09300 Kuala Ketil, Kedah, Malaysia.

[2]Pharmacology Department, Kulliyyah of Medicine & Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah, 09300 Kuala Ketil, Kedah, Malaysia.

ARTICLE INFO

Corresponding author:
Dr. Nuruddin Mohammed Nur

Email address:
nuruddinnur@yahoo.com

Received:
April 2018
Accepted for publication:
May 2018

Keywords:

Vitamin E
Antioxidants
Myocardial infarction,
Cardioprotective

ABSTRACT

Vitamin E (Vit E), an antioxidant, is a compound that has an important role in maintaining health because it can capture free radical molecules that inhibit oxidative reactions in the body, is considered to prolong survival in patients and animals. Myocardial infarction (MI), known as a heart attack, is the formation of a necrosis in heart muscle cells following inadequate blood supply. This study tested the hypothesis that early treatment with Vit E reduces mortality because of its protective effects against myocardial infarction by arresting free radical molecules and reactive oxygen species that cause degenerative diseases. Miocardial Infarction is one of the clinical manifestations of coronary heart disease, which is a major cause of morbidity and mortality worldwide. Early mortality rate of 30 days in acute MI patients is 30% with more than half deaths before the patient reaches the hospital.

INTRODUCTION

Myocardial infarction is the damage or death of an area of the heart muscle (myocardium) resulting from a blocked blood supply to the area, causing the death of heart tissue [1]. Myocardial infarction (MI) usually results from an imbalance in oxygen supply and demand, which is most often caused by plaque rupture with thrombus formation in a coronary artery [2]. This is a complicated event which not only affects mechanical and electrical properties of myocardium but also its structural and biochemical properties [3].

The rates of death from MI have diminished in most high income countries, despite 1 in 3 of all deaths in the USA in 2008 was due to cardiovascular disease [4].

Inspite it is accepted that cardiovascular disease is a common cause of death in the developing world. For example, ischemic heart disease had become the leading cause of death by 2004 accounting for 1.46 million deaths in India (14% of total deaths) and the same were expected to double during next decade [5].

The general assumption is that adequate intake of nutrients like fruits, grains and vegetables and moderate degree of exercise can help prevent coronary heart disease of the populations in advanced and 'near – advanced' countries [6].

Some antioxidants like ascorbate, tocopherols, tocotrienols, flavonoids, other phenols and carotenoids (found in plants) are taken up by humans. The important food antioxidant can significantly reduces the side effects of reactive species, which are involved in chronic diseases and can protect myocardium [7 & 8]. There are documentation about the oxidative damage contributes to the pathology of atherosclerosis and Reactive oxygen or nitrogen species play an integral role in myocardial injury [9].

MYOCARDIAL INFARCTION

Myocardial infarction, known as a heart attack, is the formation of a necrosis in myocardial muscle cells due to inadequate blood supply to an area initiated with ischemic [2]. Transmural and Subendocardial

are the two basic types of acute myocardial infarction [10].

Clinically, myocardial infarction can be subclassified into a ST elevation MI (STEMI) versus a non-ST elevation MI (non-STEMI) based on ECG changes [11]. A coronary occlusion occurs when there a thrombus covers on a uncerated or unstable plaque, which results in break down in the supply of myocardial oxygen and nutrients [12]. Job stress show a minor role computing for only about 3% of cases of myocardial disease, while Smoking appears to be 36% of cardivascular disease, obesity 20% and lack of exercise 7-12% [13].

Myocardial ischemia is a consequence of reduced blood flow in coronary arteries, due to a combination of fixed vessel narrowing following gradual buildup of cholesterol and fibrous tissue in plaques in the wall of arteries and abnormal vascular tone as a result of atherosclerosis and endothelial dysfunction. This blood column irregularities can be visible on angiography for long period of time [14, 15].

Several factors affect the hemodynamic significance of a stenotic myocardial lesion such as length of the lesion and more importantly the degree of vessel narrowing, amount of compensatory vasodilatation that smaller, distal resistance vessels are able to achieve and Myocardial oxygen demand. Distal vessels are affected more when there is long term occlusion of vessels [16]. The report from different studies say that tissue damage in myocardial infarction is due to apoptosis which is also called cell death [17].

Oxidative stress in cardiovascular diseases

Normal mediators in cell signaling are very important for regulating the functions of vessels, which is done by Reactive oxygen species (ROS) [18]. ROS are produced in endothelium, smooth muscle and adventitia of the vessel wall [19]. Under pathophysiological conditions, these free radicals play important role in various conditions, including atherosclerosis, ischemic heart diseases, arrhythmias, cardiomyopathy and congestive heart failure [20].

Oxidative stress and atherosclerosis

Free radical induced oxidative stress that influences the occurrence of degenerative diseases such as heart disease causes atherogenic process and its steps of pathogenic consequences [21]. The collective confirmation that oxidative modification of low density lipoprotein (LDL) plays an important role in the pathogenesis of atherosclerosis [22].

Management of myocardial infarction

Myocardial infarction can be diagnosed by assessing the chief complaints of the patients and physical examination. Some investigations like canhges in ECG and coronary angiogram and increase in levels of the cardio-markers guide us to establish the diagnosis. The main aspect of ECG is to let us know the site of infarction (heart muscle damage), while coronary angiogram locates the exact site of narrowing or obstructions in coronary vessels [23].

In myocardial infarction, the cardiac enzymes those raised are aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) and one cardiac marker is very important is Troponin T [24]. Creatine kinase (CK) is an enzyme that has been measured for the detection of MI, is used confidently since old age. CK is increased not only in myocardial injury and but also in other tissues like muscle injury. After a heart attack, rise in CK occurs 4 to 9 hours after the onset of chest pain, peaks at 24 hours, and returns to baseline at 48 to 72 hours [25].

Cardiac troponins I and troponin T is now been popular for their speficity and accuracy for myocardial damage and has beed used for the diagnosis. These parameters are routinely used in hospitals for diagnostic purposes. [26]. After a myocardial infarction, both troponins starts increasing in serum within 4 to 9 hours, goes to peak at 12 to 24 hours, and remain elevated for up to 14 days [27].

Nowadays the occurance of myocardial infarction can decreased by restricting and managing some of the risk factors like control of blood pressure, lifestyle modificaton, cessation of smoking, regular exercise, a balanced healthy diet for cardiact problems, and limitation of alcohol intake [28]. Among the drug therapy Beta blocker such as metoprolol or carvedilol is used to reduce the risk of MI [29]. Some high-risk patients particularly MI with left ventricular dysfunction or continuing cardiac ischaemia shows great benefit from risk factors modification [30].

ANTIOXIDANTS

Free radical is an atom or molecule having an unpaired electron. Free radicals are considered to be harmful because they become highly reactive in the effort to get their electron pairs, as well as new free radicals from the atoms or molecules whose electrons are donated to pair with previous free radicals. The free radicals usually come from oxygen, nitrogen and sulfur molecules [31]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are described free radicals and other non-radical reactive derivatives. For example, ROS includes free radicals such as superoxide anion ($O2^{-\bullet}$), perhydroxyl radical ($HO2\bullet$), hydroxyl radical ($\cdot OH$), nitric oxide (NO), and other species such as hydrogen peroxide ($H2O2$), singlet oxygen ($1O2$), hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$) [31].

The development of natural antioxidants has received great attention over the last few years. Natural antioxidants in addition to protecting the

body from free radicals can also slow the occurrence of chronic diseases caused by reduced reactive oxygen species (ROS), especially hydroxyl radicals and radical superoxide. [32].

Antioxidant is a compound inhibit that have an important role in protecting health due to it can absorb free radical molecules and inhibit oxidative reaction which cause any kinds of diseases [33]. Anti-oxidants delay or inhibit oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons, ending the carbon-stealing reaction [34].

Fruits and vegetables are loaded with key antioxidants such as vitamin A, C, E, betacarotene that can play important roles as cellular antioxidants [35]. The sources of the natural antioxidants are also spices, grains, and herbs such as ginseng, curcuma, ginkgo, rosemary, green tea, grape, ginger and garlic. The main antioxidant compounds that these foods contain are phenol, polyphenols, flavonoids, carotenoids, steroids and thiols [36].

Overproduction of the free radicals can responsible for tissue injury. Cell membranes are made of unsaturated lipids and these unsaturated lipid molecules of cell membranes are particularly susceptible to free radicals. Oxidative damage can direct to a breakdown or even hardening of lipids, the main component of all cell walls. Breakdown or hardening is due to lipid peroxidation leads to death of cell or it becomes unfeasible for the cell to properly get its nutrients or get signals to achieve another. [37]. These antioxidant enzymes such as xanthine oxidase (XO), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) provide a crucial fence against free radicals [38].

VITAMIN E

The natural forms of vitamin E include α-tocopherol, β -tocopherol, γ-tocopherol, and δ-tocopherol as well as the tocotrienol forms of each of these. The α-tocopherol and γ-tocopherol isoforms. The tocotrienols from vitamin are alpha-, beta-, gamma-, and delta-tocotrienols. The human body maintains effectively alpha-tocopherol, the only form of vitamin E; therefore, this form of vitamin E found in the largest quantities in blood and tissues [39].

Vitamin E may exert their activity by several mechanisms, like by suppressing the production of active species by reducing hydroperoxides and H2O2, by sequestering metal ions, termination of chain reaction by scavenging active free radicals and also caused repairing and/or clearing damage of cell, thus stop cancer and cardiovascular disease. The effect of protection of vitamin E supplements against oxidative stress in humans protects other fat-soluble vitamins from destruction[40].

Human body enriched with vitamin E as antioxidants can prevent the onset as well as treat diseases caused and/or fostered due to free-radical mediated oxidative stress and anti-inflammatory processes. This can also inhibit platelet aggregation and can cause immune enhancement [41].

Atherosclerosis and Vitamin E

Of all the antioxidants, vitamin E can easily be assimilate into low density lipoprotein (LDL) molecule and ultimately can protect against LDL oxidation which is the initial stage of atherosclerosis [42]. There is an evident relationship between dosage and effectiveness of vitamin E. The higher the dose taken for vitamin E the higher is the chance of safeguard against oxidative damage to LDL cholesterol. From the previous explanation we can see Vitamin E can reduce may LDL cholesterol peroxidation and increase plasma LDL breakdown. This signifies the prevention activity of cardiovascular disease by inhibiting excessive platelet aggregation and increase in fibrinolytic process [42].

FREE RADICALS AND CARDIOVASCULAR DISEASES

A free radical may be defined as a molecule or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbital [43]. This radical is likely to have a chain reaction which occurs in the body can cause continuous damage. The number of free radicals can increase due to stress, radiation, cigarette smoke and environmental pollution causing an inadequate body defense system thus result in several chronic disease such as cardiovascular diseases, neurological diseases, cancer [44].

Recent researches have shown that the antioxidants of plant origin with free-radical scavenging properties could have great importance as therapeutic agents in several diseases caused due to oxidative stress which is a common mechanism of molecular and cellular damage. Plant extracts and phytoconstituents found effective as radical scavengers and inhibitors of lipid peroxidation [45].

Free radicals are always produced in our body system by various mechanisms and physiological processes. These include mitochondrial respiration, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidoreductase and uncoupled nitric oxide (NO) synthases [46].

There are several theories about the mitochondrial production of ROS. One of popular theory says about the electron transport where about five percent electron is escaped and reacts with oxygen, which results in formation of ROS. Another theory about the generation of ROS is Mitochondrial superoxide production, which occurs during normal cellular activity [47].

Redox potential maintains the electron balance between oxidants and antioxidants which ultimately guard the mitochondrial permeability to electrons which is also described in chemiosmotic theory. Diseases occur due to any imbalance between coupling and coupling of electrons and generation of ROS [48]. The vascular process of atherosclerosis and LDL cholesterol disease is closely related to ROS/NO abnormal production [49].

ROS and cardiovascular diseases

Among the long list of heart disease risk factors endothelial dysfunction is important, as it is the source of atherosclerosis. The theory around how the cardiovascular disease starts is mainly through the endothelial dysfunction where coronary walls become more vulnerable to atherosclerosis and coronary heart disease [50].

Early atherosclerosis is due mainly to mitochondrial overproduction of ROS and uneven distribution of antioxidants and oxidants. When there is endothelial damage of the coronary vascular wall, there is multiple cell populations resulted from abnormal ROS signaling [51]. A number of process that occurs during over production of free radical that leads to bizarre oxidation of LDL which ultimately causes vascular endothelial damage. Then the pathogenesis of atherosclerosis starts and myocardial infarction occurs [52].

REFERENCES:

1. Rajadurai M. and Stanely Mainzen Prince P. (2007): Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in wistar rats. Toxicology 2007;230:178-88.
2. Bono DP and Boon NA. (1992): Diseases of the cardiovascular system. In: Edwards CRW, Boucheir IAD, editors. Davidson's Principles and Practice of Medicine. Hong Kong: Churchill Livingstone; 1992. pp. 249–340.
3. Petrich ER, Schanne OF. and Zumino AP. (1996): Electrophysiological responses to ischemia and reperfusion. In: Karmazyn M, editor. Myocardial ischemia: Mechanism, reperfusion, protection. Basel: Birkhauser Verlag; 1996. p. 115-33.
4. Roger VL, Go AS, Lloyd-Jones DM, et al. (January 2012). "Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association". Circulation 125 (1): 188–97.
5. Gupta R, Joshi P, Mohan V, Reddy KS and Yusuf S (January 2008). "Epidemiology and causation of coronary heart disease and stroke in India". *Heart* 94 (1): 16–26.
6. La Vecchia C, De Carli A and Pagano R. (1998): Vegetable consumption and risk of chronic disease. Epidemiology 1998;9:208–210.
7. Halliwell B and Gutteridge JMC. (1999): Free radicals in biology and medicine,: ed. 3, Oxford: Oxford University Press, Oxford,1999.
8. Wiseman SA, Balentine DA. and Frei B. (1997): Antioxidants in tea. Crit Rev Food Sci Nutr 1997;37:705–718.
9. Rosenfeld ME. (1998): Inflammation, lipids and free radicals: lessons learned from the atherogenic process. Semin Reprod Endocrinol 1998;16:249–261.
10. Reznik, AG. (2010): "[Morphology of acute myocardial infarction at prenecrotic stage]" (in Russian). Kardiologiia 50 (1): 4–8. PMID 20144151
11. Moe KT. and Wong P. (March 2010): "Current trends in diagnostic biomarkers of acute coronary syndrome". Ann. Acad. Med. Singap. 39 (3): 210–5. PMID 20372757.
12. Cotran RS, Kumar V. and Robbins SL. (1994): Robbins Pathologic Basis of Disease. 5th ed. Philadelphia: WB Saunders, 1994.
13. Kivimäki, Mika; Nyberg, Solja T; Batty, G David et al (2012). "Job strain Hung J, Lam JYT, Lacoste L and Letchacovski G. Cigarette smoking acutely increases platelet thrombus formation in patients with coronary artery disease taking aspirin. Circulation. 1995, 92: 2432-2436.
14. Woollard KJ. and Geissmann F. (February 2010). "Monocytes in atherosclerosis: subsets and functions". Nat Rev Cardiol 7 (2): 77–86.
15. Spaan J, Kolyva C, van den Wijngaard J, et al. (September 2008). "Coronary structure and perfusion in health and disease". Philos Transact a Math Phys Eng Sci 366: 3137–53.
16. Tsujita K, Kaikita K, Soejima H, Sugiyama S and Ogawa H (April 2010): "[Acute coronary syndrome-initiating factors]" (in Japanese). Nippon Rinsho 68 (4): 607–14.
17. Krijnen PA, Nijmeijer R, Meijer CJ, Visser CA, Hack CE and Niessen HW. (2002): "Apoptosis in myocardial ischaemia and infarction".JClin Pathol 55 (11): 801–11.
18. Griendling K K, sorescu D, Lassigue B and Ushio-Fukai M. (2000): Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. Arteriosclero Thromb Vasc Biol 20, 2175-2183.
19. Lassegue B and Clem Pus R E (2003): Vascular NAD(P)H oxidase: specific features, expression and regulation. Am J pysiol Regul Integr comp physiol 285,R277-297.
20. Zalba G, San Jose G, Moreno M U, Fortuno M A, Fortuno A, Beaumont F J and Diaz J. (2001): Oxidative stress in arterial hypertension: role of NAD(P)H oxides. Hypertension 38, 1395-1399.
21. S V Vijaya Lakshmi, G Padmaja, Periannan Kuppusamy and Vijay Kumar Kutala. (2009): Oxidative stress in cardiovascular disease Indian journal of Biochemistry & Biophysics Vol.46, December 2009, pp421-440.

22. Torzewsky M and Lackner K J (2006): Initiation & progression of atherosclerosis-enzymatic or oxidative modification of low- density lpoprotein? Clin Chem Lab Med 44, 1389-1394.
23. Sudheer, MD. (2011): "Vessel localisation and Prognostication". Life Hugger. Retrieved 21 January 2011.
24. J. Mair. (1997): Progress in myocardial damage detection: new biochemical markers for clinicians, Crit. Rev. Clin. Lab. Sci. 34 (1997) 1-66.
25. Apple FS, Quist HE, Doyle PJ, et al., (2003): Plasma 99th percentile reference limits for cardiac troponin and creatine kinase MB mass for use with European Society of cardiology/American college of cardiology consensus recommendations. Clin Chem 2003; 49:1331.
26. Tobias Reichlin, Willibald Hochholzer, Stefano Bassetti et al. (2009): Early Diagnosis of Myocardial Infarction with Sensitive Cardiac Troponin Assays. N Engl J Med 2009;361:858-67.
27. Wu AH, Apple FS, Gibler WB, et al. (1999): National Academy of clinical Biochemistry Standards of Laboratory Practice C recommendations for the use of cardiac markers in coronaryartery diseases. Clin Chem. 1999;45:1104-1121.
28. Ross R. (1999): Atherosclerosis: an inflammatory disease. N Engl J Med. 1999; 340: 115–126.
29. Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, Lang CC, Rumboldt Z, Onen CL, Lisheng L, Tanomsup S, Wangai P Jr, Razak F, Sharma AM. and Anand SS. (2005): INTERHEART Study Investigators. "Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study". Lancet 366 (9497): 1640–9.
30. Dargie H J. (2001). Effect of carvedilol on outcome after myocardial infarction in patients with left-ventricular dysfunction: the CAPRICORN randomised trial. Lancet. 2001 May 5;357 (9266):1385-90
31. Vajragupta O, Boonchoong P and Berliner LJ. (2004): Manganese complexes of curcumin analogues: evaluation of hydroxyl radical scavenging ability, superoxide dismutase activity and stability towards hydrolysis. Free Radic Res. 2004;38:303 –14.
32. Geier DA, Kern JK, Garver CR, et al. (2009): A prospective study of transsulfuration biomarkers in autistic disorders. Neurochem Res. 2009;34:386–93.
33. Jian-Ming Lü,[a] Peter H. Lin, Qizhi Yao, and Changyi Chen. (2010): Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. J Cell Mol Med. 2010 April; 14(4): 840–860.
34. DeFeudis FV, Papadopoulos V. and Drieu K. (2003): Ginkgo biloba extracts and cancer: a research area in its infancy. Fundam Clin Pharmacol. 2003;17:405–17.
35. Rababah TM, Hettiarachchy NS. and Horax R. (2004): Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-butylhydroquinone. J Agric Food Chem. 2004;52:51836.
36. Lotito SB. and Frei B. (2006): Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Biol Med. 2006;41:1727–46.
37. Panchatcharam M, Miriyala S, Gayathri VS. and Suguna L. (2006): Curcumin improves wound healing by modulating collagen and decreasing reactive oxygen species. Mol Cell Biochem. 2006;290:87–96.
38. Shih PH, Yeh CT. and Yen GC. (2007): Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. J Agric Food Chem. 2007;55:9427–35.
39. Bella DL, Schock BC, Lim Y, Leonard SW, Berry C, Cross CE, Traber MG (2006).Regulation of the alpha-tocopherol transfer protein in mice: lack of response to dietary vitamin E or oxidative stress. Lancet. 2001 May 5;357(9266):1385-90.
40. Taş U, Verhagen AP, Bierma-Zeinstra SM, Hofman A, Odding E, Pols HA, Koes BW. Incidence and risk factors of disability in the elderly: the Rotterdam Study. Prev Med. 2007 Mar;44(3):272-8. Epub 2006 Dec 20.
41. Gore Thornton J, Kowdley KV. To band or to block for primary prophylaxis against variceal bleeding? Gastroenterology. 2006 Jan;130 (1):275-7; discussion 277.
42. Pizzorno Jr, Joseph E, Michael T (2000). Textbook of Natural Medicine, 2nd. 2. Churchill Livingstone, 1118-9.
43. Gutteridge JM and Halliwell B.(2000): Free radicals and antioxidants in the year. A historical look to the future. Ann N Y Acad Sci. 2000;899: 136-47.
44. Pacher P. and Szabo C. (2008): Role of the peroxynitritepoly (ADP-ribose) polymerase pathway in human disease. Am J Pathol. 2008;173:2-13.
45. Vassalle C, Pratali L, Boni C, Mercuri A. and Ndreu R. (2008): An oxidative stress score as a combined measure of the pro-oxidant and antioxidant counterparts in patients with coronary artery disease. Clin Biochem. 2008;41:1162-7.
46. Sanjib Bhattacharya, KK Mueen Ahmed. and Subhankar Chakraborty. (2011): Free Radicals and Cardiovascular Diseases: An Update Free Radicals and Antioxidants 21 Vol 1, Issue 1, Jan-Mar, 2011.
47. Kovacic P, Pozos RS, Somanathan R, Shangari N and O'Brien PJ. (2005): Mechanism of mitochondrial uncouplers, inhibitors and toxins: focus on electron transfer, free radicals and structure-activity relationships. Curr Med Chem. 2005;12:2601-23.

48. Bodrova ME, Brailovskaya IV, Efron GI, Starkov AA and Mokhova EN. (2003): Cyclosporin A-sensitive decrease in the transmembrane potential across the inner membrane of liver mitochondria induced by low concentrations of fatty acids and Ca2+. Biochemistry. 2003;68:391-8.
49. Liu Y, Zhao H, Li H, Kalyanaraman B, Nicolosi AC. and Gutterman DD. (2003): Mitochondrial sources of H2O2 generation play a key role in flow-mediated dilation in human coronary resistance arteries. Circ Res. 2003;93:573-80.
50. Munzel T, Sinning C, Post F, Warnholtz A. and Schulz E. (2008): Pathophysiology, Diagnosis and prognostic implications of endothelial dysfunction. Ann Med. 2008;40:180–196.
51. Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO. And Kobeissi ZA. (2001): Allopurinol Improves Myocardial Efficiency in Patients With Idiopathic Dilated Cardiomyopathy. Circulation. 2001;104:2407-11.
52. Heymes C, Bendall JK, Ratajczak P, Cave AC, Samuel JL, Hasenfuss G and Shah AM. (2003): Increased myocardial NADPH oxidase activity in human heart failure. J Am Coll Cardiol. 2003;41:2164-71.

Original Article

STUDY ON THE EFFECT OF AGEING TO GEL PEN INK ON PAPERS USING ATTENUATED REFLECTANT MODE FOURIER TRANSFORM INFRARED (ATR-FTIR) SPECTROSCOPY .

Hafizah Mohamed Said*[1], Dzulkiflee Ismail[1]

[1]School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Malaysia.

ARTICLE INFO

Corresponding author:
Hafizah Mohamed Said

Email address:
hafizah299@gmail.com

Received:
May 2018
Accepted for publication:
June 2018

Keywords:

Ink ageing analysis
IR spectroscopy
ATR-FTIR
forensic questioned document

ABSTRACT

Forensic analysis of writing ink ageing on document is one of the most challenging parts in forensic investigation. It can significantly prove to be useful in forensic document examination as it helps to build a timeline of an event or provide link to investigation. The objective of this study was to study the effect of ageing to gel pen ink on different papers i.e. A4, envelop, newspaper and manila card using Attenuated Reflectant Mode Fourier Transform Infrared (ATR-FTIR) spectroscopy. Inks were deposited on four different types of papers and subsequently subjected to ageing at different interval (7, 14, 30, 60 and 90 days). The aged and fresh inks were compared through visual examination and statistical evaluation using paired t-test was then carried out based on their mean absorbance. The results showed that the gel pen ink deposited on different types of papers subjected to ageing could not be differentiated from their fresh counterpart using ATR-FTIR and statistical analysis. As a conclusion, under the experimental parameters used in this study, the writing inks deposited on different types of papers subjected to ageing could not be differentiated from their fresh counterpart using ATR-FTIR and statistical analysis.

INTRODUCTION

In forensic science, document associated with criminal cases in which its authenticity and author-ship are questionable or dubious are termed as questioned documents (QD). They can be prepared either manually using writing instruments such as ballpoint pen, gel pen, marker pen, fountain pen and pencil or automatically using computer, commonly on paper although other materials are also possible. In forensic science, careful analysis of QD could reveal useful information regarding the authorship, authenticity and type of writing instruments being used to prepare the documents. Writing ink on paper is particularly important because of the significance of signatures and handwritten records. The paper document is the most popular writing medium in daily life until today.

In 2007, a study [1] had shown that Solid Phase Micro-extraction (SPME) technique can be employed to determine the volatile components of ball point inks quantitatively by direct analysis on paper prior to Gas Chromatography-Mass Spectrometry (GC–MS) [1]. The aim of SPME technique used was to monitor the evaporation of ink's volatile components as the ink ages on document [1]. A previous study [2] also reported that SPME extraction was capable to monitor the ink ageing analysis. SPME have high sensitivity and limit of detection. However, this technique requires sample preparation and time consuming.

Gel pen inks

Gel ink pen is a popular writing material due to its smooth and interesting writing appearances [3]. They are used in daily activities to sign contracts, checks, loans and other documents. Gel pen inks are water-based inks which contain dyes or pigments as colorants, water as vehicles, resins, nonionic surfactants and other additives [4]. Acid dyes and related organic compounds containing several sulphonic groups generally used as the colouring agents in gel pen inks [4][5]. Nowadays, the growths of modern techniques allow easy and efficient way to analyse and identify gel pen inks.

Previous study [6] showed that Ion Pairing High Performance Liquid Chromatography (IP-HPLC) was capable to detect the composition changes in blue gel pen ink deposited on paper stored at different lighting conditions and also in natural environment. Chromatograms were developed to interprete the ageing mechanism of ink to establish the differences between natural and artificial ink aging [6]. Besides, it also provided an explanation on the changes the relative component of the dyes in the blue gel pen ink [6].

The aim of this study were to analyse fresh and aged writing inks on the different papers using ATR-FTIR, to manually evaluate the spectra of fresh and aged gel pen ink on the different papers and to determine the most optimum IR spectra region for statistical comparison of aged inks with their fresh counterparts.

MATERIALS & METHODS

Sample collection

Gel pen, fountain pen, marker pen, white A4 paper (70 gsm), envelope, newspaper and manila paper where purchased from a book store located in Kubang Kerian area. Table 1 shows the list of samples used in this study and their respective reference code.

Sample preparations

Each of the paper was cut into small rectangular square with dimension of 1 cm x 1 cm using a pair of scissors. The inks from the ballpoint pen and gel pen were transferred onto the paper by scribbling ensuring that the whole area of the papers were covered with inks. The paper cuts were secured onto a board using hair pins and then left to age at room condition for 90 days. For the purpose of sampling identification of the paper cuts, they were given reference codes according to the list in Table 2.

FTIR Spectral acquisitions

The spectra of the inks were acquired using a Bruker Tensor 27 (Bruker Optics, UK) FTIR spectrometer equipped with a diamond ATR sampling attachment. Prior to acquiring the spectra of the inks, a standard polystyrene film was scanned to ensure that the ATR-FTIR was working correctly. Spectral measurements were taken over the entire spectral range of 4000 cm^{-1} to 600 cm^{-1} with resolution of 4 cm^{-1}. Within this ageing period, spectra of the inks were acquired at 0 day (i.e. immediately after preparation), 7 days, 14 days, 30 days, 60 days and 90 days interval.

Spectral evaluations by direct visual comparisons

The effect of ageing to gel pen ink on the different papers was evaluated by directly comparing the spectra of aged inks to the spectra of their fresh counterparts which were acquired immediately after preparation (i.e. 0 day). This method was necessary in order to narrow down the selection of infrared region to perform statistical analysis.

Statistical analyses

Paired t-test was performed to statistically evaluate the effect of ageing using IBM SPSS statistical software (SPSS 2.0, IBM, USA). Data pre-processing (i.e. standardisation) was performed prior to statistical test in order to overcome 'one-to-one' variation within a large data set. To assess the effect of selection of spectral region to ageing evaluation, the paired t-test was first performed to the frequency region and then to the fingerprint region (1500 cm^{-1} – 600 cm^{-1}). The

Table 1: List of samples used in this study and its respective code

Sample	Reference code
Gel pen	B
A4 paper	a
Envelope	b
Manila paper	c
Newspaper	d

Table 2: List of sample cuts with their respective reference code

Sample description	Reference code
Gel pen on white A4	Ba
Gel pen on white envelope	Bb
Gel pen on white manila paper	Bc
Gel pen on newspaper	Bd

parameters set-up for the paired t-test is shown in Table 3. Null hypothesis will be accepted when the computed p-value exceeded the α value, and vice versa.

RESULTS

Repeatability and reproducibility studies

Repeatability and reproducibility studies were performed prior to performing the ageing study of the writing inks on the different papers to evaluate the intra and inter precision or robustness of the techniques employed. The repeatability and reproducibility were measured by calculating the percentage (%) Relative Standard Deviation (RSD) of the absorbancies of six prominent bands or peaks was selected from the resultant spectra of the writing inks on the papers. The selected bands are as shown in Figure 1.The %RSD calculated for both repeatability and reproducibility studies were less than 5% and less than 20% respectively which indicating good intra and inter precision of the techniques.

Visual examinations of the infrared spectra

Figure 2 to 5 displays the spectra of the gel pen ink on the different papers after being aged in the environment for 7, 14, 30, 60 and 90 days. The 0 day spectrum was obtained from the writing ink immediately after its deposition on the paper. It acted as reference spectrum where the spectra of aged inks were compared.

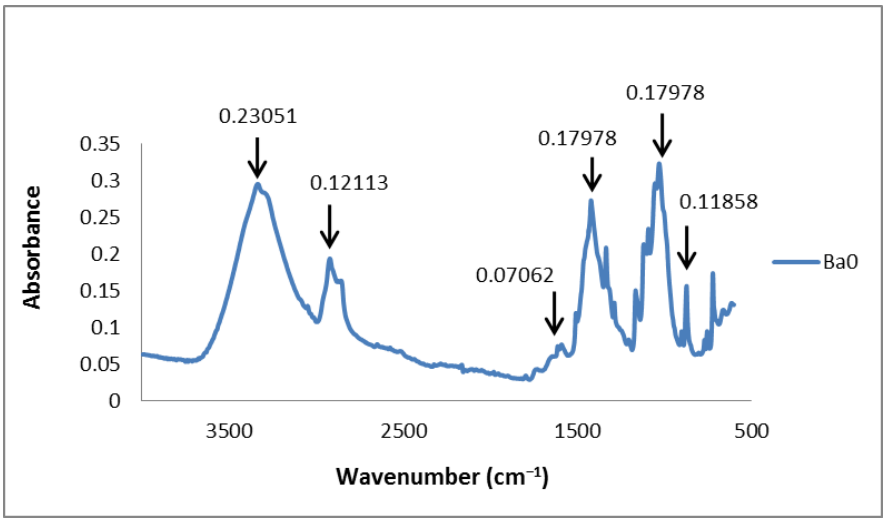


Figure 1: The infrared spectra of gel pen ink on A4 paper. The arrows show the six prominent bands used to calculate the %RSD for repeatability and reproducibility studies.

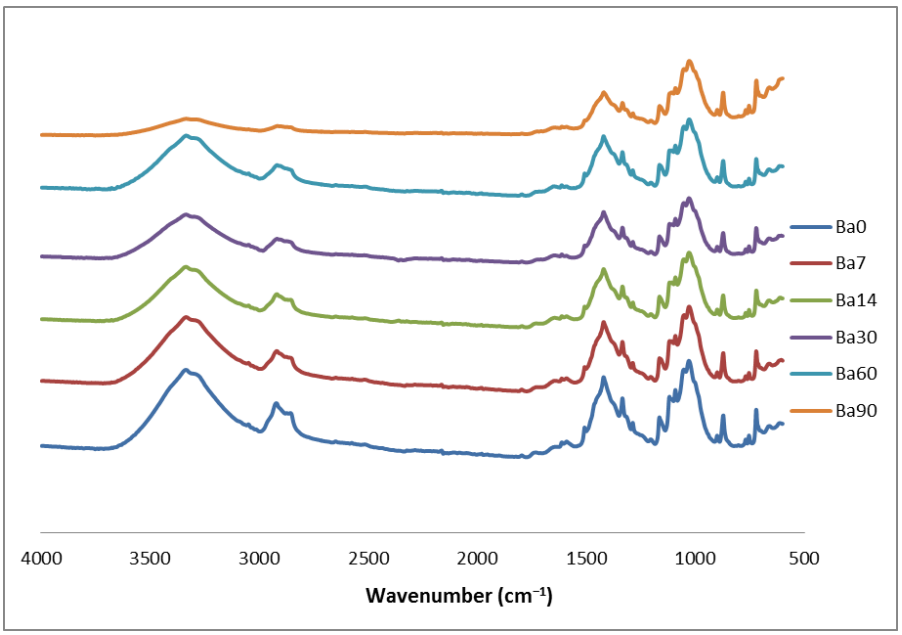


Figure 2: Infrared spectrum of gel pen ink on white A4 paper

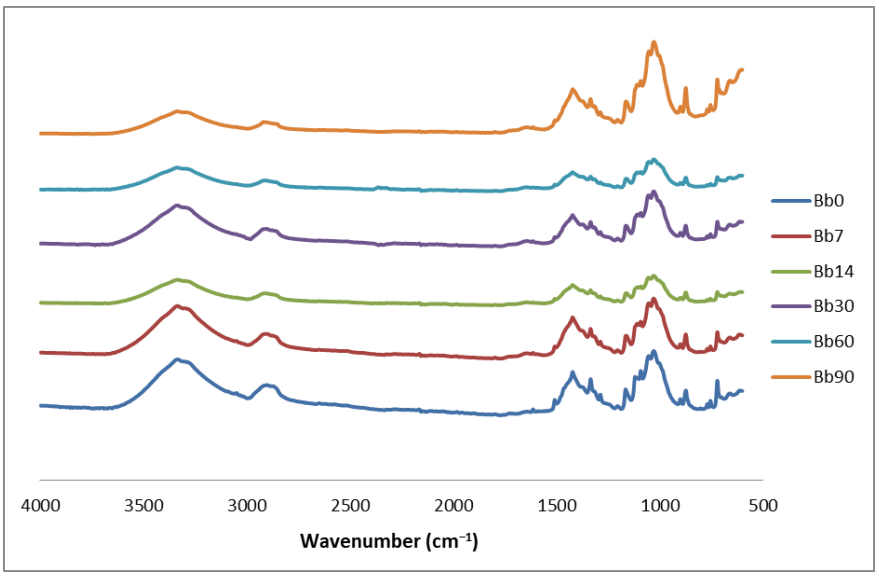


Figure 3: Infrared spectrum of gel pen ink on white envelope

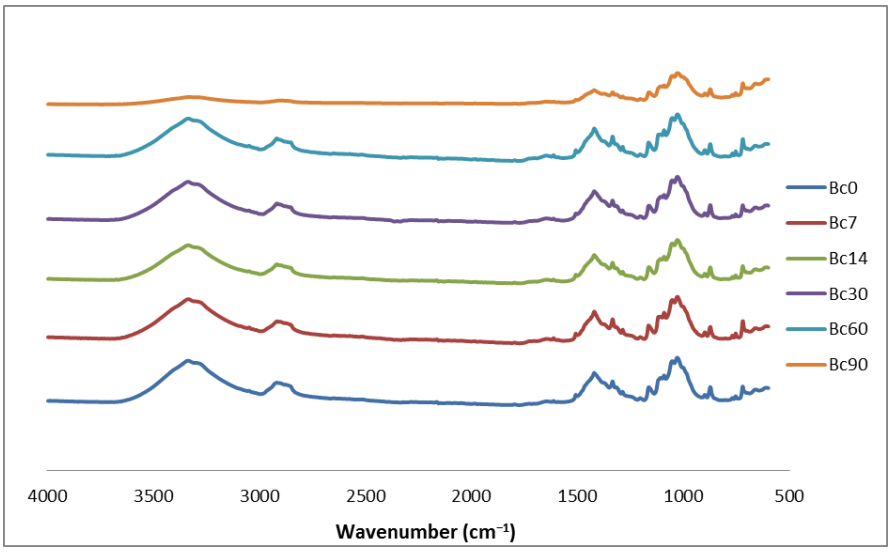


Figure 4: Infrared spectra of gel pen ink on manila paper

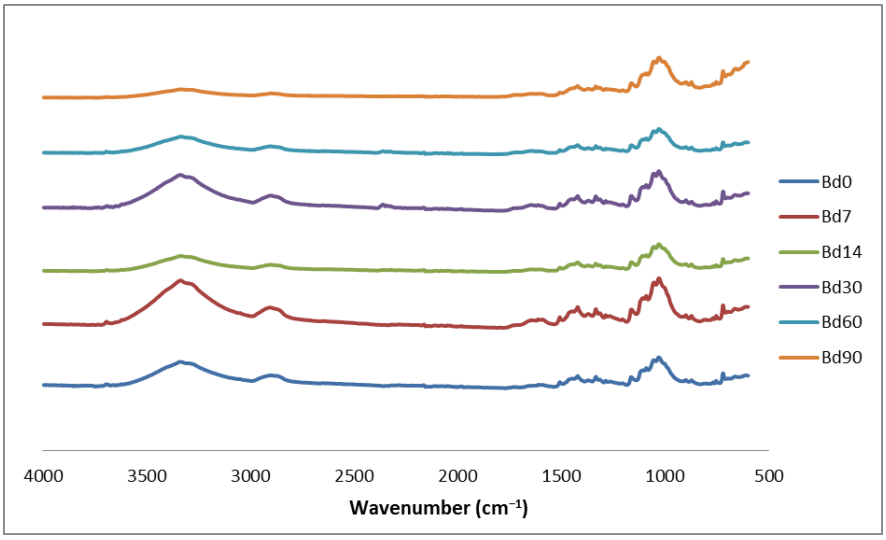


Figure 5: Infrared spectra of gel pen ink on newspaper

Statistical analyses
Paired t-test was used to evaluate the effect of ageing of writing inks on different papers to the environment. This particular statistical test was chosen to assess pre and post treatment effect, in this case, before and after ageing in the environment. The frequency and fingerprint regions were tested separately. The main reason was to see which regions can successfully capture the ageing effect. The frequency region at 3700 cm^{-1} – 3000 cm^{-1} was selected as this corresponds to the O-H group region that had shown remarkable changes over 90 days of exposure from visual examinations. Table 4 shows the outcomes of paired t-test for gel pen inks on white A4 paper.

DISCUSSION

Repeatability and reproducibility studies
The %RSD calculated for both repeatability and reproducibility studies were less than 5% and less than 20% respectively which indicating good intra and inter precision of the techniques.

Visual examinations of the infrared spectra
In general, the spectra of the gel pen inks on the different papers did not show any remarkable changes especially at the fingerprint region, in other words were quite consistent except for O-H group within the group frequency region which showed marked decrement particularly after 90 days of ageing. This is because ink components could oxidise, cross-link, polymerise, and evaporate when exposed to the environment [7].

Based on visual examinations alone, it was extremely difficult to conclusively judge or said that the writing inks had experienced significant changes after 90 days of ageing. These situations hence justify the use statistical technique to evaluate the effect of ageing. In this study, paired t-test was used and its outcomes are further discussed in the following section.

Statistical analyses
Table 4 showed that the p values of the paired t-test for all conditions at fingerprint region were greater than 0.05. This means that there was no significant difference before and after ageing. In other words, no changes in terms of organic components of ballpoint pen inks. Samples deposited on the different papers after 7, 14, 30, 60 and 90 days of exposure p value was equal to 1.000 (p = 1.000).

However, it showed different outcomes of p value of the paired t-test for all conditions at frequency region (3700 cm^{-1} – 3000 cm^{-1}) where p <0.05 for all conditions. This means that there was no significant difference before and after ageing. Similar results can be observed for gel pen ink deposited on envelope, manila and newspaper.

The significant difference occurred at O-H absorption band was due to moisture of environment and from the solvent within the ink component. Besides that, it was also influenced by behaviour of solvent that contain O-H group was easily evaporated when the ink affixed on paper. This change was not necessary that the ink component changed over 90 days because at fingerprint region, it composed of other ink components. Thus, it was conclusively judge the profile of ink does not change over 90 days.

CONCLUSION

From the result obtained, visual examinations could not objectively evaluate the effect of ageing to gel pen inks. On the other hand, statistical analysis i.e. using paired t-test can objectively evaluate the effect of ageing to writing inks as demonstrated in the present study. The statistical results suggested that there were no significance

Table 4: Results of paired t-test of gel pen on A4 paper.

Sample	IR region			
	3700 cm^{-1} – 3000 cm^{-1}		1500 cm^{-1} – 600 cm^{-1}	
	t-stats (df)	p value*	t-stats (df)	p value*
Ba0-Ba7	-35.575 (364)	0.000	0.000 (467)	1.000
Ba0-Ba14	-50.750 (364)	0.000	0.000 (467)	1.000
Ba0-Ba30	45.961 (364)	0.000	0.000 (467)	1.000
Ba0-Ba60	-49.240 (364)	0.000	0.000 (467)	1.000
Ba0-Ba90	45.983 (364)	0.000	0.000 (467)	1.000

difference between aged inks with their fresh counterparts on after 90 days of exposure to the environment. Meanwhile, the paired t test calculated using the frequency region (3700 cm^{-1} – 3000 cm^{-1}) showed that there were significant changes between aged inks with their fresh counterparts. However this could not conclusively reflect on the overall change to inks compositions since this region corresponds to O-H which might due to solvent used in the manufacturing of the inks for example phenoxyethanol or perhaps moisture absorbed from the environment.

Needless to say, evaporation of these substances is inevitable process as such could not be used to judge changes experienced by the inks. Fingerprint region is suggested to be the region to study the effect of ageing because it composes of more complex ink's components. These findings also infer that organic components in writing ink might be stable and did not change significantly up to three month of exposure to the environment.

ACKNOWLEDGEMENT

We would like to thank to the School of Health Sciences for furnishing us with the essential skills to excel in both theoretical and practical works especially for ATR-FTIR instrument.

REFERENCES

1. Brazeau, L., & Gaudreau, M. (2007). Ballpoint pen inks: The quantitative analysis of ink solvents on paper by solid-phase microextraction. *Journal of Forensic Sciences*, *52*(1), 209–215.
2. Andrasko, J. (2003). A simple method for distinguishing between fresh and old ballpoint pen ink entries. *Forensic Science International*, (136), 80–81.
3. Liu, Y. Z., Yu, J., Xie, M. X., Liu, Y., Han, J., & Jing, T. T. (2006). Classification and dating of black gel pen ink by ion-pairing high-performance liquid chromatography. *Journal of Chromatography A*, *1135*(1), 57–64.
4. Brunelle, R. L., & Crawford, K. R. (2002). *Advances in the Forensic Analysis and Dating of Writing Ink, IL, 2002.*
5. Gernandt, M. N., & Urlaub, J. J. (1996). An Introduction to the Gel Pen. *Journal of Forensic Science*, *3*(41), 503.
6. Liu, Y. Z., Yu, J., Xie, M. X., Chen, Y., Jiang, G. Y., & Gao, Y. (2006). Studies on the degradation of blue gel pen dyes by ion-pairing high performance liquid chromatography and electrospray tandem mass spectrometry. *Journal of Chromatography A*, *1125*(1), 95–103.
7. Jian Wang, Guoan Luo, Suqin Sun, Zhiqiang Wang, and Y. W. (2001). Systematic Analysis of Bulk Blue Ballpoint Pen Ink by FTIR Spectrometry. *Journal of Forensic Science*, *46*(5).

Review Article

DIAGNOSTIC DELAY AND REDUCING THE DIAGNOSTIC INTERVAL IN CHILDREN WITH BRAIN TUMOR

Mossad Abdelhak Shaban*[1], Abdelbaset Taher Abdelhalim[2,3]

[1]Paediatric Department, International Islamic University of Malaysia (IIUM), Kuantan, Pahang, Malaysia.
[2]Pharmacology Department, Faculty of Medicine, Alazhar University, Cairo, Egypt.
[3]Pharmacology Department, Kulliyyah of Medicine & Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah, 09300 Kuala Ketil, Kedah, Malaysia.

ARTICLE INFO

Corresponding author:
Dr. Mossad Abdelhak Shaban

Email address:
mossadshaban@iium.edu.my

Received:
May 2018
Accepted for publication:
June 2018

Keywords:

Diagnostic delay
Brain tumor in children
Post symptomatic diagnostic interval

ABSTRACT

Brain tumor account for a quarter of all childhood cancers, affecting about 1 in 2400 United Kingdom (UK) children under the age of 16 annually, while brain and other nervous system cancer incidence per 100,000 population in Peninsular Malaysia for age 0-19 years was 84/100000. Death occurs either as a result of catastrophic presentations with raised intracranial pressure or as a result of tumor recurrence and resistance to further treatment. Five-year survival rates became over 70%, and the majority of these patients go on to be long-term survivors. Despite this, 60% of long-term survivors of childhood brain tumors are moderately or severely neurologically disabled. Delayed diagnosis of brain tumor among children is a concern around the globe and has been reported by parents, in the media, and in the courts. These reports can disturb public confidence in healthcare systems. This literature searches studied diagnostic delay and reducing the diagnostic interval in children with brain tumor, using online databases and a manual search. Main keywords used were diagnostic delay, brain tumor in children and post symptomatic diagnostic interval. In some studies post symptomatic diagnostic interval (PDI) was 28 weeks with a parental delay of 11.1 weeks and a doctor's delay of 16.9 weeks. We concluded many recommendations as programmes to raise public and professional awareness of the symptomatology of brain tumor, early referral, CT scan fast track. Along with many other recommendations were discussed in this article.

INTRODUCTION

Mortality and morbidity from cancer is worse in many countries, the reasons for this are multi-factorial, but diagnostic delays and consequent later stage diagnoses are likely to be major contributory factors [1]. Brain tumor account for a quarter of all childhood cancers, affecting about 1 in 2400 UK children under the age of 16 annually [2]. The incidence of brain and other nervous system cancer in Malaysia per 100,000 population in Peninsular Malaysia for age 0-19 years was 84/100000 in 2006 [3]. Primary malignant central nervous system tumors (CNS) are the second most common childhood malignancies, after leukaemia [4]. They are the most common paediatric solid organ tumor [5]. It is the leading cause of death from child-hood cancer, surpassing the mortality rate of acute lymphoblastic leukaemia [6]. CT is used for initial workup, but MRI is superior and essential if CT finds abnormalities or inconclusive, MRI spectroscopy can be useful as elevated choline [7]. Although advances in surgical intervention, radiation therapy, and chemotherapy have improved the survival rates in children with central nervous system tumors, mortality and morbidity associated with these disorders persists [8].

Survival

Five- and 10-year survival rates for children with central nervous system tumors are 73% and 70%, respectively .The likelihood of survival depends upon the type of tumor. Survival has improved, in part due to advances in diagnostic techniques and histological classification of tumours, improvement in neurosurgical and radiation oncology techniques, and the utilization of new single and combination chemotherapeutic agents. Despite advances in the care of children with CNS tumors, improvement in survival and durable remissions has been slower in patients with CNS tumors compared with other cancers, particularly leukemias and lymphomas [9].

Long term morbidity

Paediatric survivors with CNS tumors often have neurologic, cognitive, psychological, and endocrine complications that are due to damage from the tumor itself, its treatment (surgery, radiation, and/or chemotherapy), or subsequent secondary

malignancy. In the Childhood Cancer Survivor Study, 82 present of the 2821 five-year survivors reported having at least one chronic medical condition. Compared with their siblings, survivors had an increased risk of developing a new endocrine condition, sensory deficit as hearing loss, and neurologic problem. Cranial radiation therapy was associated with an increased risk of subsequent malignancy and neurocognitive impairment [10].

Diagnostic delay and, diagnostic interval

Diagnosis of brain tumors in children is often delayed in relation to the presenting symptoms. In some studies, Post symptomatic diagnostic interval was 28 weeks with a parental delay of 11.1 weeks and a doctor's delay of 16.9 weeks. Main clinical symptoms were headache (66.7%), vomiting (57.7%), vision (46.2%) and gait (41.6) disorders and fatigue (41.0%) followed by other neurological signs [11] (Figure 1). Another cohort study and meta-analysis of symptomatology and referral practice for childhood brain tumors, total diagnostic interval (TDI, time between first symptom onset and diagnosis) ranged widely from a day to 6.9 years, with a median of 3.3 months (14 wk) [12] (Figure 2).

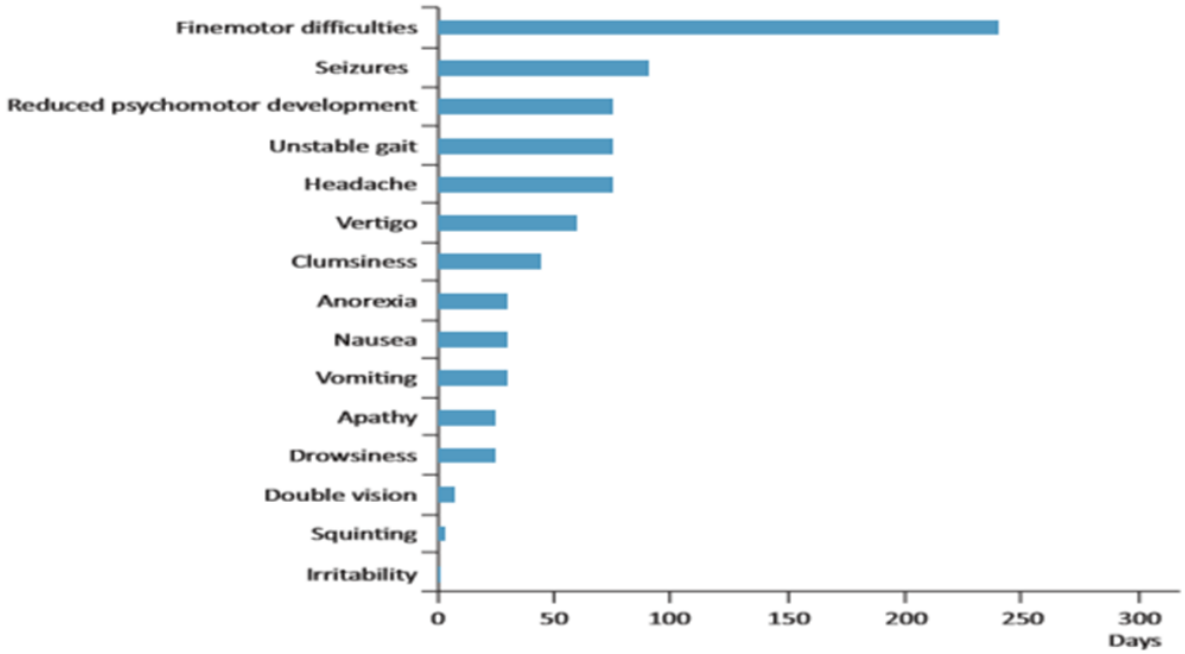


Figure 1: Key milestones and time intervals in the pathways from first symptom until start of treatment.

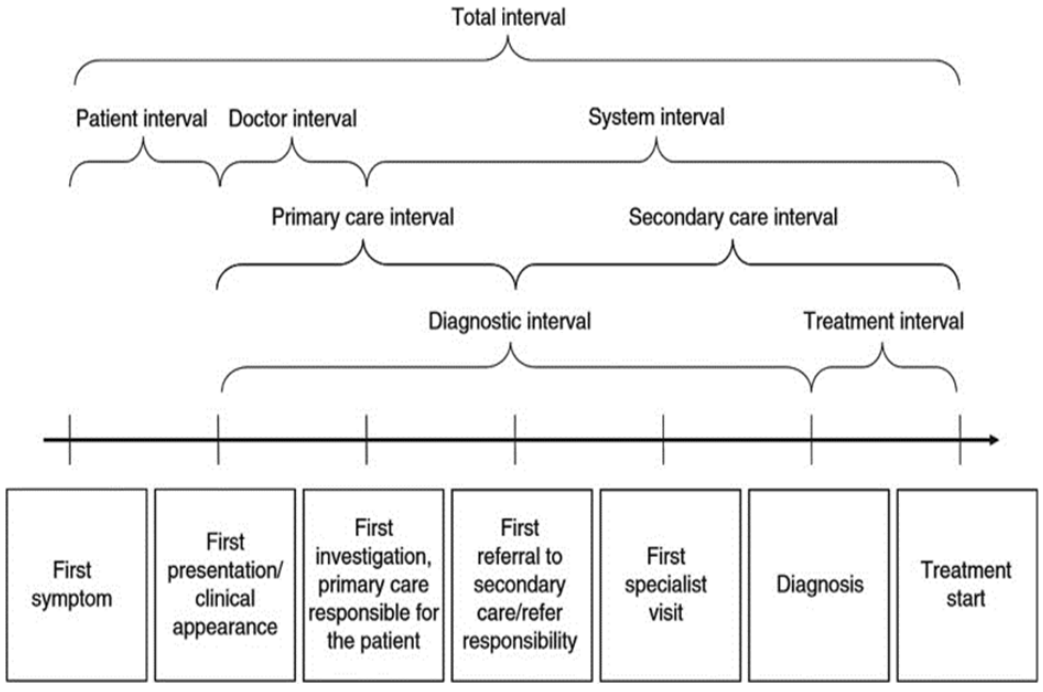


Figure 2: Relationship in median days between symptoms and pre-diagnostic symptomatic interval

Recommendations [7, 11-15]

- Community education (without making them panic).
- Identify that the red flag symptoms have been explored. However, they do not yield significant positive predictive values for individual symptoms that can be used for stratifying childhood patients in primary care.
- Be Cancer Alert Campaign. One of the example is a collaborative project between the University of Malaya, Queen's University Belfast and the National Cancer Society Malaysia.
- Using an awareness intervention (Designed awareness age-stratified symptom) as this offered the opportunity to change practice. The symptoms of brain tumor include persistent or recurrent headache, persistent or recurrent vomiting, balance or coordination problem, abnormal eye movement, or blurred or double vision, behavioural problems, fits or seizures, abnormal head position as wringing head or stiff neck. If a child has one of these symptoms consultation from a doctor is a must, but if two or more symptoms occurred, urgent referral is needed. The risk of excessive public alarm and the potential for swamping imaging facilities at the top of our risk assessment and designed our materials to prioritize reassurance. Materials to be distributed to health care professionals via conferences and seminars and to general practitioner surgeries, health organizations, and professional bodies by direct mail. Materials also distributed to the public through community champions (directly to local schools, nurseries, hospital waiting rooms, etc.), as well as via local authorities and other charities and commercial networks.
- Design an open access decision support website as Head Smart Website.
- Conferences and Education Outreach Events.
- Professional education and system track by reinforcing an on-going need for population-based surveillance and further etiologic studies. Guideline to Examinations and procedure to follow in children with suspected brain tumor and, linking the revised guideline to policies of child-hood and cancer practice raised awareness about features of childhood cancers among paediatricians and were associated with reduction in total diagnostic interval. Conferences and specialised continuous medical education Outreach Events.
- Change in referral practice with early referral was most pronounced in the time from first medical contact to CNS imaging. Computed tomographic fast track. MRI protocols for imaging paediatric brain tumours: Further awareness of the revised protocol, improved access to the guidelines, and strict adherence to the protocols. Computed tomographic study of epilepsy in children as the primary diagnosis of a brain tumor, There were also formulated the practical recommendations concerning carrying out of CT investigations in children with prolonged resistant epileptic syndromes.
- EEG, brain magnetic resonance imaging and tumor markers (CSF/EEG) for early detection of an evolving occult hypothalamic-stalk lesion in idiopathic central diabetes insipidus as Determination of CSF hCG at the first presentation may be useful, because an increased CSF level of hCG precedes MRI abnormalities.
- The development of paediatric neurosurgery subspecialty, How to create as Correlation of neurosurgical subspecialisations with good out-comes in children with malignant brain tumors.

CONCLUSION

Diagnosis of paediatric brain tumor is often delayed in relation to the presenting symptoms. The duration between first symptom and a cancer diagnosis is important because, if shortened, may lead to earlier stage diagnosis and improved cancer mortality and morbidity. Recent programs have sought to improve survival while decreasing neurologic sequels, design community awareness without making them panic as age-stratified symptom checklist, with instructions that one symptom required medical assessment and two required an urgent referral. So if parents report a combination of headache with other neurological abnormalities, a brain tumor should always be considered. Another pathway for professional and system track with revise of guidelines and policies, continuous medical education, CT fast track, MRI updated protocols, and other special recommendation as EEG and CSF analysis and their role in early diagnosis.

REFERENCES

1. McPhail S, Johnson S, Greenberg D, et al. Stage at diagnosis and early mortality from cancer in England. Br J Cancer.2015 Mar 31;112 Suppl 1:S108-15.
2. Cancer Research UK. Childhood cancer incidence statistics; 2012. cancerre-searchuk.org/cancerinfo/cancerstats/ childhoodcancer/incidence/. Accessed Septem-ber2015.
3. Omar ZA, Ali ZM, Taming NS, .Malaysian cancer association , Brain and Other Nervous System Age specific Cancer Incidence per 100,000 population (CR) by sex, Peninsular Malaysia 2006.p 92.National Cancer , Ministry of Health Malaysia.
4. Kraft P. National integrated cancer pathways for children. National Board of Health, Denmark: Publ 2009/SUPL (1 May 2011)

5. Linabery AM, Ross JA. Trends in childhood cancer incidence in the U.S. (1992-2004). Cancer. 2008; 112(2):416.
6. Ostrom QT, de Blank PM, Kruchko C, et al. Alex's Lemonade Stand Foundation Infant and Childhood Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2007-2011. Neuro Oncol 2015; 16 Suppl 10:x1
7. Craig E[1], Connolly DJ, Griffiths PD, et al. MRI protocols for imaging paediatric brain tumours. Clin Radiol. 2012 Sep;67(9):829-32.
8. Ostrom QT, Gittleman H, Liao P, Rouse C, et al. Overview of the management of central nervous system tumors in children'statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007-2011.Neuro Oncol. 2014;16 Suppl 4.
9. Alikhanov AA, Petrukhin AS, Mukhin KIu, et al. A computed tomographic study of epilepsy. Russian. PMID.1997;97(8):13-7.
10. Pollack L, Stewart S, Thompson T. Trends in childhood cancer mortality--United States. Centers for Disease Control and Prevention (CDC 1990-2004. MMWR Morb Mortal Wkly Rep 2007; 56:1257.
11. Klitbo D, Nielsen R, Illum NO, et al. Symptoms and time to diagnosis in children with brain tumours Dan Med Bul 2011 Jul;58(7):A4285.
12. Trusler J, Evans A, J, A new clinical guideline from the Royal College of Paediatrics and Child Health with a national awareness campaign accelerates brain tumor diagnosis in UK children. Neuro Oncol. 2016 Mar 18(3):445-54.
13. Tao Y, Lian D, Hui-juan Z, et al. Value of brain magnetic resonance imaging and tumor markers (CSF/EEG) in the diagnosis and treatment of intracranial germinoma in children. 2011 Apr;33(2):111-5. doi: 10.3881.
14. Hirsch JF.The development of pediatric neurosurgery in Paris. How to create something out of nothing: reflexions on the methodology. Childs Nerv Syst. 1997 Aug-Sep;13(8-9):465-7
15. Albright AL, Sposto R, Holmes E, et al. Correlation of neurosurgical subspecialisations with outcomes in children with malignant brain tumors. Neurosurgery 2000; 47:879.

Original Article

FORENSIC DISCRIMINATION OF LIPSTICK SMEARS USING ATTENUATED TOTAL REFLECTANCE–FOURIER TRANSFORM INFRARED (ATR-FTIR) SPECTROSCOPY WITH CHEMOMETRICS TECHNIQUES

Farhana Mohamed Ghazali*, Dzulkiflee Ismail

School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Malaysia.

ARTICLE INFO

Corresponding author:
Farhana Mohamed Ghazali

Email address:
farhana.mohamedghazali@gmail.com

Received:
May 2018
Accepted for publication:
June 2018

Keywords:

Lipstick analysis
trace evidence
FTIR spectroscopy
chemometrics techniques

ABSTRACT

Lipstick smear is one of the trace evidence that may transfer due to contact between suspect, victim and crime scene. The main objective of this study was to discriminate and classify 12 red lipstick samples of local and international brands using ATR-FTIR spectroscopy coupled with chemometrics techniques of principle component analysis and hierarchical cluster analysis. The result obtained from hierarchical cluster analysis is in conformance with principle component analysis. The application of chemometrics techniques had successfully separated and discriminated the red lipstick samples into six distinctive clusters according to their brands.

INTRODUCTION

Cosmetic products are used by individuals with intention to improve their appearances as well as to "express" their beauty to the public. Lipstick is an emblem of feminism, allure and elegance. Individuals apply lipstick to make their appearances much more attractive. The main ingredients of lipstick are wax, oil and colourants [1, 2]. The vivid colour of lipstick is due to the presence of one or combination of organic dyes and inorganic pigments [3]. Some examples of dyes commonly used in lipstick are eosin, phloxine and erythroxine.

In forensic caseworks, lipstick is commonly found in the form of smear and present in minute or trace amount [4-8]. The smears can be encountered on various types of substrates such as paper, fabric, tissue paper, drinking glass, paper cup, cigarette butt and skin. Lipstick smear is the result of physical contact between a person wearing lipstick with another person and/or with object(s) [9-12]. Hence lipstick smear is very helpful and useful in providing link between victims, suspects and also crime scene [13].

Two techniques are generally approached in forensic lipstick analysis, namely, destructive and non-destructive techniques. Most chemical analyses involve in lipstick smears are destructive in nature [14-19], which mean that the analyses result in loss due to extraction and dissolution in organic solvents. In order to maintain sample integrity, non-destructive techniques which have known to be straight forward analysis is highly recommended as it does not require any form of sample preparation, allows *in-situ* analysis and most importantly does not result in sample loss.

FTIR spectroscopy is one of the examples of non-destructive method. In this study, FTIR spectroscopy together with ATR accessory was chosen for the lipstick analysis. Previous study using FTIR spectroscopy in this area is very limited. A study conducted by Pasieczna-Patkowskaa and Olejnik (2013) [20] to red lipstick samples using different IR spectroscopy techniques including ATR-FTIR obtained similar spectra pattern regardless of lipstick formulations. Anthocyanin is one of the natural pigment contain in the lipstick formulation. Its absorption towards skin had successfully been studied by Westfall (2015) [21]. Most of the research study had focused and discovered only at spectroscopy spectral pattern. However, the interpretation of spectral pattern using conventional direct manual examination is very tedious, challenging as well as very time consuming. In this case, chemometrics techniques of principle component analysis (PCA) and hierarchical cluster analysis (HCA) were applied and give more

objectives and definite outcomes.

PCA and HCA are described as unsupervised pattern recognition methods which are commonly used in forensic caseworks due to their ability to reduce a large number of datasets and presenting the data in the form of graphical presentation for an easy interpretation [22]. The result of PCA is presented in the score plot which reveals the relative position of the samples where the samples having similar scores are located closely together. Meanwhile in HCA, the data analysis will be signified in a dendrogram showing the successive stages of grouping. Both techniques enable a classification and characterisation of sample in more objectives and reproducible manner [23].

The aim of this study is to characterise and discriminate a set of red lipstick smears of local and international brands of similar shades using ATR-FTIR spectroscopy and analyse the spectroscopic data using chemometrics techniques of PCA and HCA.

METHODOLOGY

Sample Collection

A total of 12 red lipstick samples, comprising of local and international brands by six different manufacturers (SilkyGirl, Sendayu Tinggi, Simplysiti, Avon, NYX, Revlon) were used in this study. The selection of the brands was based on their commonality in the market. Each of the lipstick samples having similar hue and shade indistinguishable by eyes were selected. Table 1 lists the lipstick samples, their shades, manufacture and reference codes. The reference codes were given for ease of identification of the lipstick samples. Another 6 lipstick samples of local brand named Peinifen13 (code P13) with same red shade was purchased specifically for repeatability and reproducibility examination.

ATR-FTIR Spectroscopy

The FTIR analysis in this study was performed using a Bruker Tensor 27 FTIR spectrometer (Bruker Tensor, UK) equipped with a crystal diamond ATR sampling interface. The spectrometer was calibrated using a polystyrene film standard prior to performing any analysis.

The ATR sampling interface was thoroughly clean by wiping it using a tissue paper soaked with methanol before and after scanning was done to the sample to ensure that the interface was free from any contaminants or sample carry-over effect. Background scanning was done to confirm that the sampling interface was thoroughly clean.

The lipstick sample was smeared directly from its container onto the sampling interface. This was to ensure that the spectra were obtained without the interference from substrate or background matrix. The smear was scanned using the parameter mentioned in Table 2.

The repeatability and reproducibility of the analysis were assessed by calculating the percent relative standard deviation (%RSD) of peak absorbancies. All the samples were analysed in six replicates.

Chemometrics Analyses

The chemometrics analyses of principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using Minitab Version 16.2.3 statistical software (Minitab Inc., State College, PA, USA). Prior to importing the dataset into the Minitab environment, the raw data were first pre-processed

Table 1: List of the lipstick samples used in this study.

Categories	Brands	Lipstick shade	Manufacture code	Reference code
Local	SilkyGirl	Foxy red	08	SK3
		Spicy marsala	10	SK5
	Sendayu Tinggi	Red desire	NA	ST2
		Red ruby	NA	ST3
	Simplysiti	Deep plum	LC15	SS2
		Chilli red	LC25	SS4
International	Avon	Berry-berry nice	1402	AV2
		Poppy love	1400	AV4
	NYX	Russian roulette	SR01	NYX2
		Seduction	SR05	NYX3
	Revlon	Really red	006	REV1
		Retro red	004	REV3

to compensate for run-to-run variations and to minimise the masking effect.

RESULTS

Repeatability and Reproducibility Studies

Peinifen 13 (reference code: P13) lipstick samples were used for repeatability and reproducibility studies. These studies aimed to evaluate the precision and robustness of the techniques used in this study. Figures 1 and 2 display the spectra obtained for the repeatability and reproducibility study, respectively. The figures have presented that the spectra of the P13 lipstick was overlapping one another and the calculated %RSD for both repeatability and reproducibility studies were less than 5%, indicating that the technique was robust.

Hierarchical Cluster Analysis (HCA)

HCA was performed to the spectra lipstick samples using the Euclidean distance and single linkage clustering strategy mechanism. The wavenumber region 1730 cm^{-1} – 1701 cm^{-1} was chosen and the outcome of HCA is presented in a dendrogram as shown in a Figure 3.

Principle Component Analysis (PCA)

Similar to HCA, the wavelength region of 1730 cm^{-1} – 1710 cm^{-1} was used for PCA analysis. The outcome is presented in a score plot and the final interpretation was made using the first two PCs; i.e. PC1 and PC2. In order to elucidate the outcomes, the clusters were manually circled and labelled as shown in Figure 4.

DISCUSSIONS

Hierarchical Cluster Analysis (HCA)

Wavenumber region 1730 cm^{-1} – 1701 cm^{-1} was chosen because it describe most of the variability concerning on the characteristics of samples hence allowing the samples to be discriminated from one another. As can be seen from the Figure 3, all the samples formed distinctive clusters. At similarity index approximately 87%,

Table 2: Parameter of ATR-FTIR Tensor 27 System

Item	Specifications
Software	OPUS 7.0 (20110823)
Resolution	4 cm^{-1}
Sample scan time	16
Background scan time	16
Range of wavenumber	4000 cm^{-1} – 600 cm^{-1}
Accessory	MIRacle, Diamond #141ADDF0

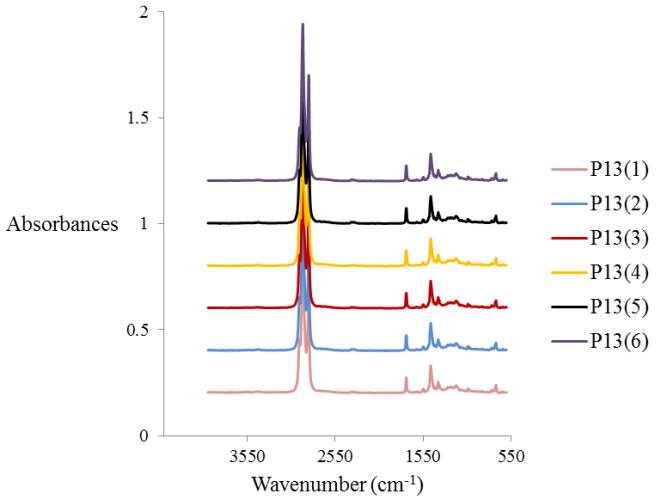


Figure 1: ATR - FTIR spectra for repeatability study

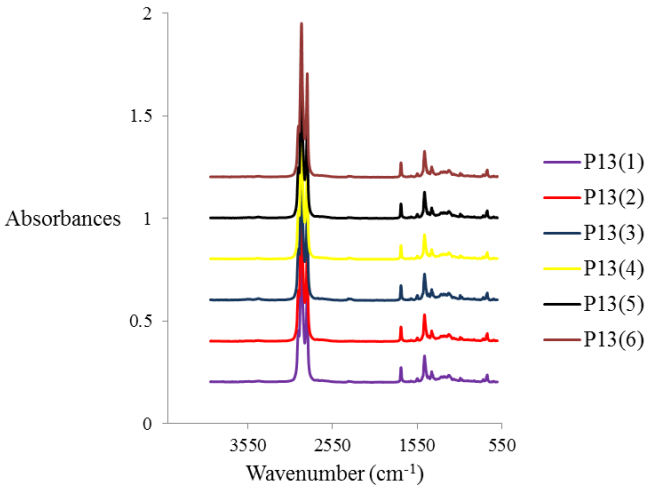


Figure 2: ATR – FTIR spectra for reproducibility study

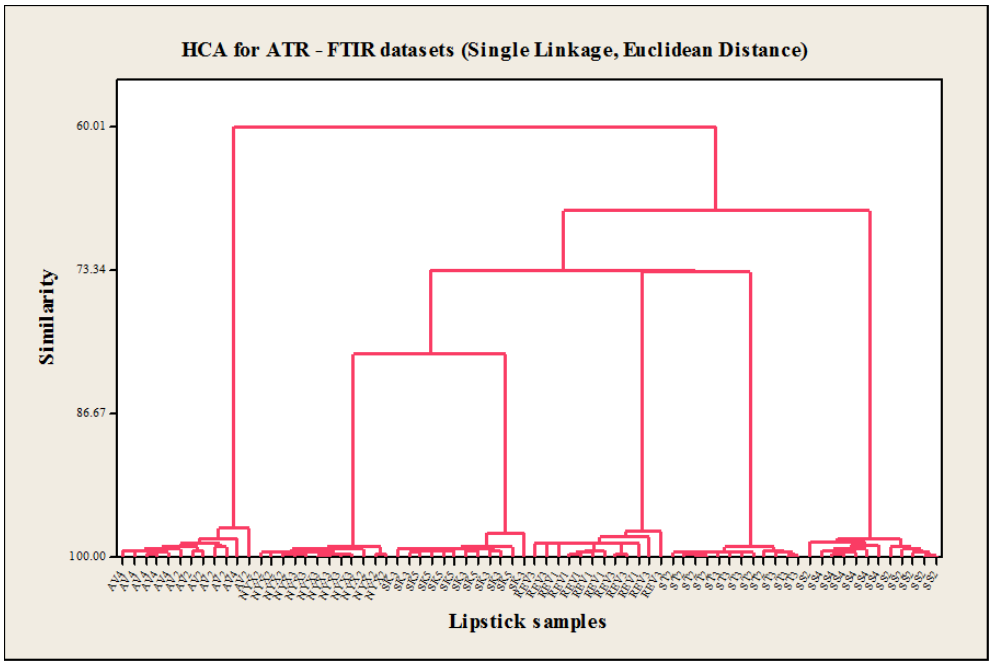


Figure 3: A dendrogram generated from the red lipsticks smeared dataset using Single Linkage and Euclidean Distance

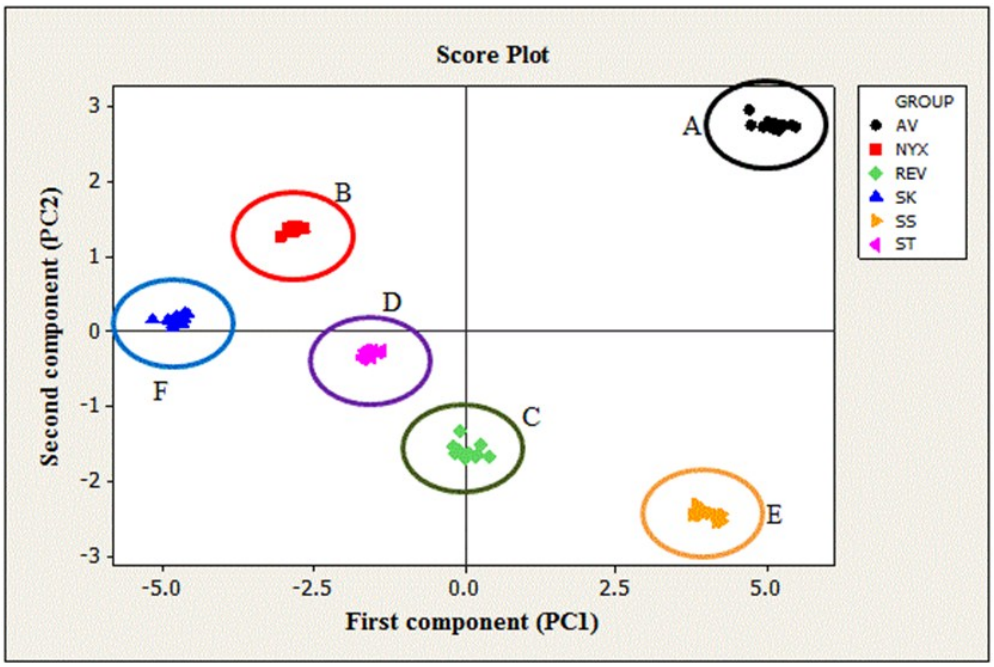


Figure 4: The principle component score plot for the lipstick samples

six clusters which were designated as cluster 1, 2, 3, 4, 5 and 6 (cluster 1: Avon, cluster 2: NYX, cluster 3: Silkygirl, cluster 4: Revlon, cluster 5: Sendayu Tinggi, cluster 6: Simplysiti) are evident in the dendrogram.

Principle Component Analysis (PCA)

The first principle component (PC1) accounted for 80% of the variation in the dataset. The second principle component (PC2) accounted for 19% of the variation in the dataset. Hence, the combination of these two PCs accounted for 99% variation in the dataset. As shown in the score plot, the lipstick smears are successfully classified into six distinctive clusters equivalent to the six different lipstick brands used in this study.

The six different clusters are labelled as cluster A (Avon), B (NYX), C (Revlon), D (Sendayu Tinggi), E (Simplysiti) and F (Silkygirl). These six distinctive

clusters have been separated very well. The separation could have been due to the different dyes formulation and composition in the lipstick samples used in this study.

CONCLUSION

The variations in lipstick colours arise from different combinations of dyes and pigments available in the market. Generally, lipsticks of different colours for examples red and brown can be readily discriminated between one another however it is not the case with lipsticks of similar colours and shades. The latter when discovered at crime scene poses a very challenging task to forensic scientists.

In this study, red lipsticks from local and international brands were studied using ATR - FTIR coupled with chemometrics techniques with the aim to differentiate them. The combination of ATR – FTIR coupled with PCA and HCA had successfully discriminated the lipstick samples into six distinctive clusters according to their brands. These chemometrics techniques give more objective outcomes (delivered by the score plot) compared to direct visual examinations of FTIR spectra alone. Hence, it shows that these approaches are powerful procedures in characterising and discriminating the lipsticks with different brands.

ACKNOWLEDGMENT

The author would like to thank to Dr. Wan Nur Syuhaila bt. Mat Desa and Dr. Ahmad Fahmi Lim bin Abdullah for all the supports and motivation given also thank you to everyone who participated in this research study.

REFERENCES

1. M. Shibata, K. Shimizu, K. Nojima, K. Yoshino, et al., Application of self-organizing silicone polymers for long-wearing lipstick, Stud. Surf. Sci. Catal. 132 (2001) 1071-1074.
2. F. Salahioglu, M.J. Went, Differentiation of lipsticks by Raman spectroscopy. Forensic Sci. Int. 223 (2012) 148-52.
3. L.W. Russell, A.E. Welch, Analysis of Lipsticks. Forensic Sci. Int. 25 (1984) 105-116.
4. S. Singh, V. Saran, M. Mishra, A. Gupta, Forensic examination of lipstick by the various physio-chemical and instrumental method, Int. J Soc. Relev. Concern 3 (2005) 1-7.
5. D. Ismail, N.N. Daeid, Comparison of smears of wax-based products using thin-layer chromatography and microspectrophotometry, J. Forensic Identification 16 (2011) 136-146.
6. B. Joshim, A Comparison of Red Pigments in Different Lipsticks Using Thin Layer Chromatography (TLC), J. Anal. Bioanal. Tech. 4 (2013) 1-4.
7. J. Andrasko, Some examples of applications of a microthermal desorption device in the forensic laboratory, J. Forensic Sci. 54 (2009) 1055-8.
8. J. Andrasko, Forensic analysis of lipsticks, Forensic Sci. Int. 17 (1981) 235-251.
9. P. Gardner, M.F. Bertino, R. Weimer, E. Hazelrigg, Analysis of lipsticks using Raman spectroscopy, Forensic Sci. Int. 232 (2013) 67-72.
10. A.F.L. Abdullah, Y. Marimuthu, C.K. Haw, N.F.M. Said, et al., Forensic discrimination of lipstick by thin layer chromatography and gas chromatography-mass spectrometry, Malaysian J. Forensic Sci. 2 (2011) 22-28.
11. M. Choudhry, Comparison of minute smears of lipstick by microspectrophotometry and scanning electron microscopy/energy-dispersive spectroscopy, J. Forensic Sci. 36 (1991) 366-375.
12. A.M.L. Barker, P.D.B. Clarke, Examination of small quantities of lipsticks, J. Forensic Sci. Soc. 12 (1972) 449-451.
13. Y. Ehara, Y. Marumo, Identification of lipstick smears by fluorescence observation and purge-and-trap gas chromatography, Forensic Sci. Int. 96 (1998) 1-10.
14. H. Wang, Z. Li, Q. Niu, J. Ma, et al., Preparation of a zeolite-modified polymer monolith for identification of synthetic colorants in lipsticks. Appl. Surf. Sci. 353 (2015) 1326-1333.
15. E. Guerra, M. Celeiro, J.P. Lamas, M. Llompart, et al., Determination of dyes in cosmetic products by micro-matrix solid phase dispersion and liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. A 1415 (2015) 27-37.
16. M. Haunschmidt, W. Buchberger, C.W. Klampfl, Identification and semi-quantitative analysis of parabens and UV filters in cosmetic products by direct-analysis-in-real-time mass spectrometry and gas chromatography with mass spectrometric detection, Anal. Methods 3 (2011) 99-104.
17. O.P. Jasuja, R. Singh, Thin-layer chromatographic analysis of liquid lipsticks, J. Forensic Identification 55 (2005) 28-35.
18. L. Gagliardi, G. Cavazzutti, A. Amato, A. Basili, et al., Identification of cosmetic dyes by ion-pair reversed-phase high-performance liquid chromatography, J. Chromatogr A 394 (1987) 345-352.
19. J.J. Giles, The analysis of waxes and greases using high resolution gas chromatography, J. Forensic Sci. Soc. 27 (1987) 231-239.
20. S. Pasieczna-Patkowskaa, T. Olejnik, Analysis of cosmetic products using different IR spectroscopy techniques, Ann. UMCS, Chemia 68 (2013) 95-106.
21. Westfall, A., Evaluation of the efficacy of anthocyanins as biologically active ingredients in lipstick formulations, in Food Science and Technology. 2015, Ohio State University. p. 1-102.
22. M. Kamil, M.N.M. Asri, W.N.S.M. Desa, and D. Ismail, Fourier transform infrared (FTIR) spectroscopy and principal component analysis (PCA) of unbranded black ballpoint pen inks, Malaysian J. Forensic Sci. 6 (2015) 48-53.
23. M.N.M. Asri, W.N.S.M. Desa, and D. Ismail, Fourier transform infrared (FTIR) spectroscopy with chemometric techniques for the classification of ballpoint pen inks, Arab J. Forensic Sci. Forensic Med. 1 (2015) 194-200.

Original Article

CASE SERIES OF INFECTIOUS RETINITIS : A 2 YEAR REVIEW IN HOSPITAL SELAYANG

Mahani Mastor*[1,2], Azian Adnan[1], Amelia Lim Lay Suan[1], Hanizasurana Hashim[1]

[1]Department of Ophthalmology, Hospital Selayang
[2]Department of Ophthalmology Hospital Universiti Kebangsaan Malaysia

ARTICLE INFO

Corresponding author:
Dr. Mahani Mastor

Email address:
mahanibintimastor@yahoo.com

Received:
May 2018
Accepted for publication:
June 2018

Keywords:

Infectious retinitis
Immunocompromised
Immunocompetent

ABSTRACT

In Malaysia and globally, infectious diseases remain a complicated and never- ending series of problems affecting multiorgan and system including the eyes. Retinitis as a consequence of infection is potentially sight threatening and occurs in both immunocompetent and immunocompromised individuals. Retinitis may affect human in eye per se or often as a manifestation of systemic illness. A total of 24 patients and 31 eyes were included in this retrospective electronic observational case series review. Only cases with positive history and clinical findings together with positive laboratory findings in Hospital Selayang from 2015 until 2017 were analysed. We excluded Retroviral cases as they may have multifactorial cause of immunodeficiency as a result of opportunistic infections. Our target is to identify common cause of infectious retinitis in immunocompetent and immunocompromised patients hence can offer good treatment and prevent blindness. In the future we plan to extend period of our study to include more cases of infectious retinitis besides to compare differences between those groups.

INTRODUCTION

Retinitis as a consequence of infection is potentially sight threatening and can occur in both immunocompetent and immunocompromised individuals. Infectious retinitis is caused by different types of pathogens including viruses, bacteria, fungi or parasites. The symptoms include floaters, blurring of vision and eye discomfort. The clinical signs are anterior uveitis, vitritis, yellow plaque-like lesion, with some may have vasculitis and choroiditis. Retinal detachment and retinal atrophy may complicate infectious retinitis if not treated accordingly.

MATERIALS AND METHODS

A total of 24 patients and 31 cases from January 2015 until December 2017 were studied. All these data were extracted electronically using the keywords ' Retinitis, Infectious, immunocompetent, and immunocompromised'. The cases were reviewed individually from the first presentation, symptoms, clinical findings, diagnosis and treatment. Only those with positive serology findings were chosen and included in this study. Subsequently those data were classified into immunocompetent and immunocompromised groups and the number of the causative agents were calculated. Vision of each patient was also analysed.

RESULTS

The patients involved in this study aged between 8 and 77 years old. The mean age group is 28years old. The causes of infectious retinitis varies in causative agents (Figure 1) with Bartonella (Figure 2) being the highest, 54%, viral causes namely Cytomegalovirus (Figure 3) accounts for 12.5% and Varicella Zoster Virus 8.5% while Leptospira, Burkholderia Pseudomallei (Figure 4), Tuberculosis and Toxosplasma (Figure 5) accounts for 4.1% respectively.

Immunocompetent patients who developed retinitis occurred in 21patients (87.5%) and in immunosuppressed, 3 patients (12.5%) (Figure 6). Patients in immunosuppressed group are patients with malignancy who underwent chemotherapy and those with autoimmune disease on immunosuppressant drugs. Bartonella is the most common cause of retinitis in immunocompetent patients (54%). In contrast Cytomegalovirus retinitis

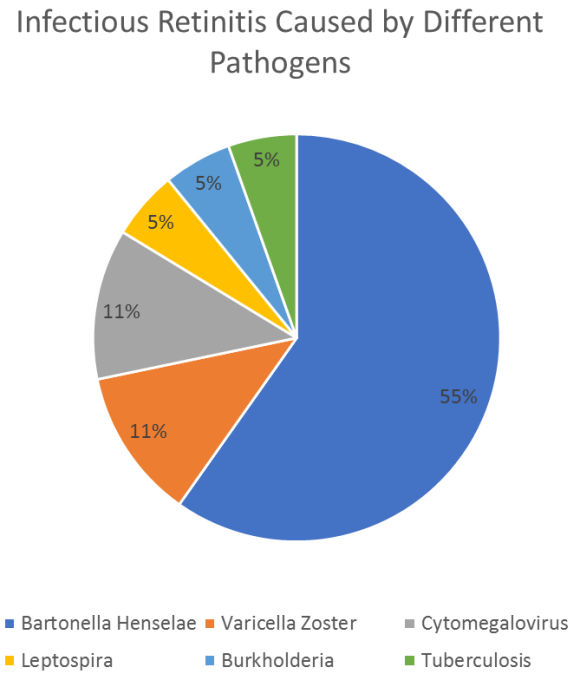


Figure 1: Pie chart of different causative agents of Infectious retinitis

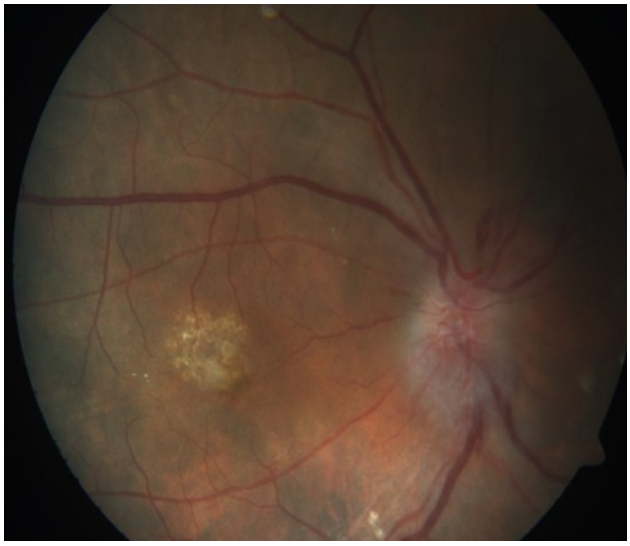


Figure 2: Fundus photograph of patient with Neuroretinitis secondary Bartonella infection.

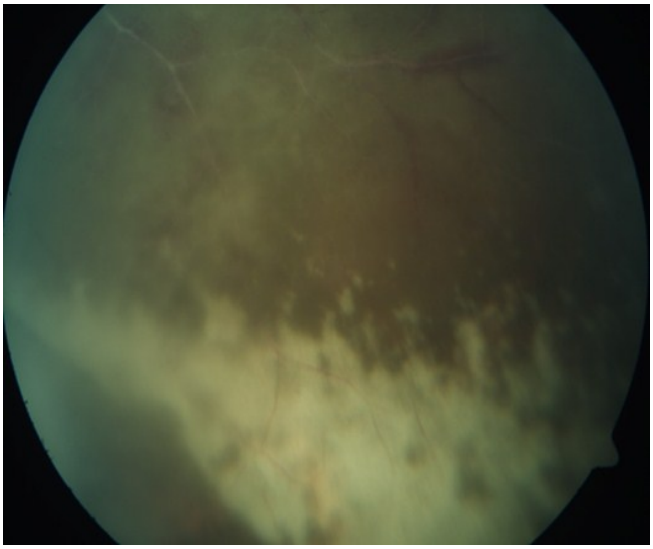


Figure 3: Fundus photograph of patient with Cytomegalovirus retinitis.

(11.11%) is the cause seen in 3 immunosuppressed patients.

All patients received treatment accordingly with complete resolution of the lesion. However final visual outcome varies among patients. In immuno-competent group 38% of the patients gain vision of ≥6/9 and another 62% has vision which ranges from 6/18 to Counting Fingers at a distance of 3feet. All the 3 patients in the immunosuppressed group had vision < Counting Fingers. Macular scar, epiretinal membrane and lamella hole are the main causes of poor vision in both group.

DISCUSSION

Bartonellosis is the commonest cause of infectious retinitis seen in these case series which probably due to geographical factor as a tropical country [1,2]. Bartonella Henselae which is a gram negative rod infects human via traumatic contact or by vectors

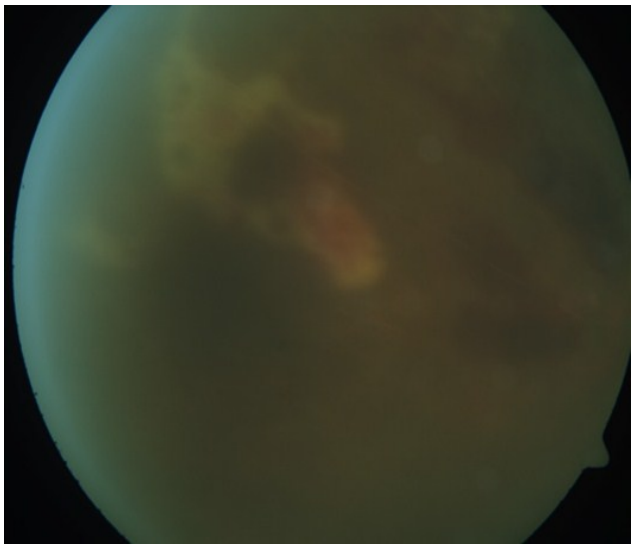


Figure 4: Fundus photograph of patient with Melioidosis.

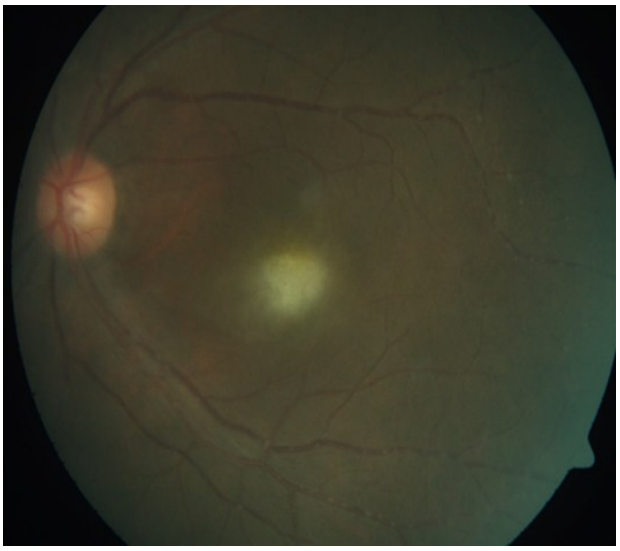


Figure 5: Fundus photograph of patient with Toxoplasma Retinitis

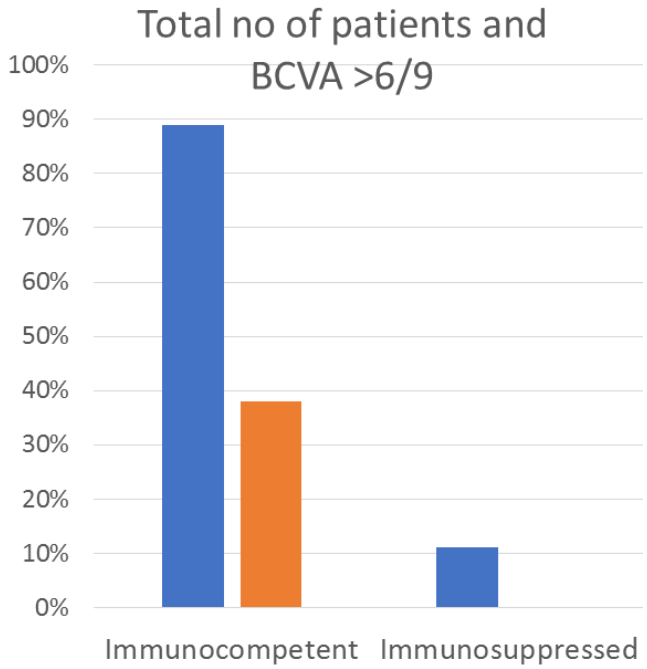


Figure 6 : Total no of patients in immunocompetent group is 90% and Immunosuppressed group 10%. 38% of immunocompetent group gained vision ≥ 6/9 and none in immunosuppressed group has vision > 6/9

like cat flea, mosquitoes or sand fly. It makes a protein binder, adheres to red blood cells, penetrate into endothelial cell and colonise there. Therefore immunocompetent individual is also vulnerable to the disease. Besides retinitis or neuroretinitis, it causes various ocular manifestation such as conjunctivitis, focal chorioretinitis, subretinal fluid and vasculitis. Tuberculosis is still endemic in Malaysia. It is 1 of the most infectious disease with rates of incidence of 79.8 per 100000 [3]. It may present with large variations of clinical presentation including vitritis, vasculitis, and retinitis. Thus, it is a great mimicker and must be ruled out in all suspected infectious case. Cytomegalovirus in immunocompetent patients are not well documented [4]. It frequently reported in immunocompromised patients such as transplant recipient and HIV positive patient. However a study by Dowling et al [5] revealed that 8 of 14 patients receiving immunosuppressive drugs infected with Cytomegalovirus systemically. Cytomegalovirus causes full-thickness retinal necrosis with pathognomonic cytomegalic cells with intranuclear

inclusions. *Leptospira and Burkhoderia sp*, are not to be missed out during investigating cause of retinitis. Clinical examinations and laboratory diagnostics are mandatory for diagnosis and successful treatment. Likewise knowledge of the immune status of the host is essential since immune modifying strategies may be needed to complement the anti infective treatment in those patients who have been on immunosuppressant drugs.

CONCLUSION

Infectious retinitis can occur regardless immune status of a host. Bartonella is the commonest cause but mainly among immunocompetent individuals whereby Cytomegalovirus in immunocompromised group. Early diagnosis and aggressive treatment are crucial to prevent debilitating visual complications.

REFERENCES

1. Cunningham ET, Koehler JE.Am J Ophthalmol. 2000 Sep;130(3):340-9
2. Bartonella species in small mammals and their potential vectors in Asia Oct 2014.Asian Pacific Journal of Tropical Biomedicine. Volume 4, Issue 10, October 2014, Pages 757-767
3. http://www.moh.gov.sg/content/moh_web/home/publications/Reports/2016/communicable-diseases-surveiilance-in-Singapore-2015.html
4. 4.T-G wreghitt E.L Teen Osule_Clinical infectious disease, Volume 37, Issue 12, 15Dec2003 page 1603-1606.
5. 5.Dowling et al J infect Dis 1976 Cytomegalovirus infection in patients receiving immunosuppressive drugs.

Case Report

SEVERE OCULAR NEONATAL HERPES SIMPLEX VIRUS TYPE 2 INFECTON: A CASE REPORT

Muhammad Najmi Khairudin[1], Aida Zairani Mohd Zahidan[1], Norshamsiah Md Din[1], Ropilah Abd. Rahman*[2]

[1] Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre, Cheras, Kuala Lumpur, Malaysia.
[2] Kulliyyah of Medicine and Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah (UniSHAMS), Kuala Ketil, Kedah, Malaysia.

ARTICLE INFO

Corresponding author:
Prof. Dr. Ropilah Abd. Rahman

Email address:
ropilah_ar@yahoo.com

Received:
May 2018
Accepted for publication:
June 2018

Keywords:

Herpes simplex virus
Acute retinal necrosis
Acyclovir
tractional retinal detachment,
neonate

ABSTRACT

We reported a case of a 42 days old baby girl who was diagnosed with bilateral acute retinal necrosis with tractional retinal detachment. She was earlier diagnosed to have disseminated Herpes Simplex Virus (HSV) type 2 infection with encephalitis and treated by the paediatrics team with intravenous aciclovir. She was referred for routine eye assessment and dilated fundus examination revealed extensive peripheral retinal necrosis with some areas of resolving retinitis involving the right macular but sparing the left macular. The ocular disease progressed and tractional retinal detachment developed bilaterally needing vitreoretinal intervention. However, her systemic diseases worsened and she passed away at another tertiary hospital while waiting for the vitreoretinal surgery. This case demonstrates that a patient with such severe retinal necrosis might have normal anterior segment findings. Therefore, it is recommended that all neonates with systemic herpetic infection should be referred ophthalmological assessment early even in the absence of external eye signs as early antiviral treatment can minimise complications of acute retinal necrosis.

INTRODUCTION

Acute retinal necrosis (ARN) is an uncommon condition in immunocompetent patients. The age of presentation is usually between 20 to 60 years old (1). The most common causative organisms are Herpes Simplex Virus (HSV) type 1, HSV type 2, Varicella Zoster virus and rarely cytomegalovirus and Epstein Barr virus (1). In paediatric group, the most common organism is the HSV-2 as it is commonly seen as a feto-maternal transmission (2). This case highlights the ocular involvement in HSV type 2 infection which can be devastating despite treatment.

A 42 days old baby girl who was diagnosed of disseminated Herpes Simplex Virus (HSV) type 2 infection with encephalitis was referred for routine eye assessment. She was born via spontaneous vaginal delivery at full term with no antenatal, intrapartum or postnatal complications but her mother was tested positive for HSV-2 infection. She was started on intravenous acyclovir by the paediatrics team seven days before the referral to the ophthalmology team. Ocular examination done on the first review revealed normal anterior segment. There was no relative afferent pupillary defect. Conjunctiva in both eyes were white and there was no eye discharge, lid swelling or eyelid skin changes. Except for bilateral mild cataract, the anterior segments findings were unremarkable in both eyes. The right and left intra-ocular pressure were 8 mmHg and 9 mmHg consecutively. Dilated fundus examination of both eyes revealed extensive peripheral retinal necrosis with some areas of resolving retinitis involving the right macular (Figure 1) but sparing the left macular (Figure 2). There were multiple areas of retinal haemorrhages and mild traction band seen but there was no retinal detachment noted. Both optic discs were pale.

She was diagnosed with bilateral acute retinal necrosis secondary to disseminated HSV-2 infection. The ocular management at this point was to continue with intravenous treatment of acyclovir 500mg/m2 three times daily as prescribed by the paediatric team for a total duration of six weeks. Dilated fundus examinations were done by the ophthalmology team twice weekly to monitor the progress of the retinal necrosis. However, fundus review after two weeks revealed an inferior tractional retinal detachment in the right eye which then progress to involve the macular after 4 days.

(Figure 3). The left eye also started to develop tractional retinal detachment one week later. Vitreoretinal team at another tertiary hospital was consulted regarding the case and she was transferred there for further management and assessment pending surgical intervention for the retinal detachment.

There, her systemic condition deteriorated despite continuation of treatment. She developed multiple episodes of seizure secondary to the HSV encephalitis. Thus surgery to correct the retinal detachment had to be postponed. Unfortunately, baby Tan XL passed away after 2 weeks

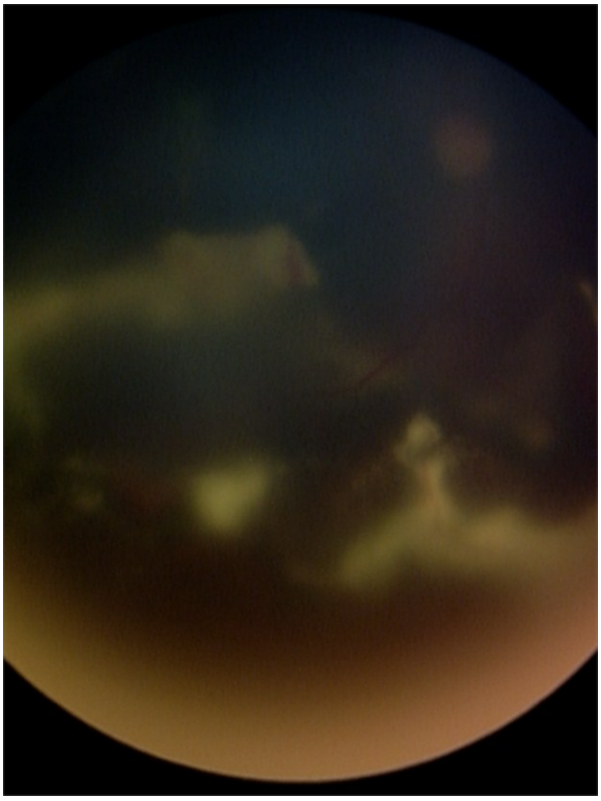


Figure 1: Retcam photograph of the right fundus showing extensive retinal necrosis involving the macular with hazy media due to vitritis

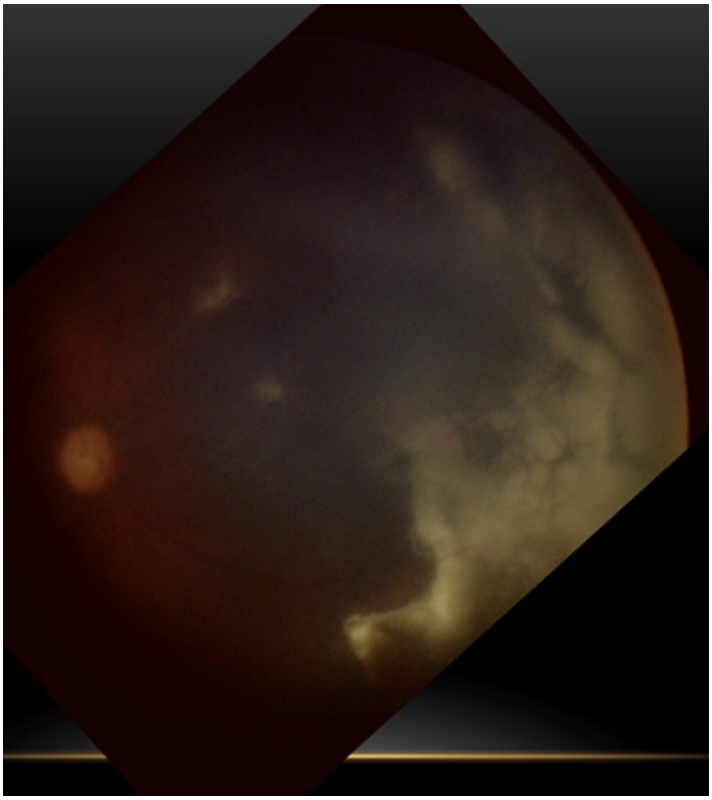


Figure 2: Retcam photograph of left fundus showing extensive necrotic retina mainly involving the temporal retina

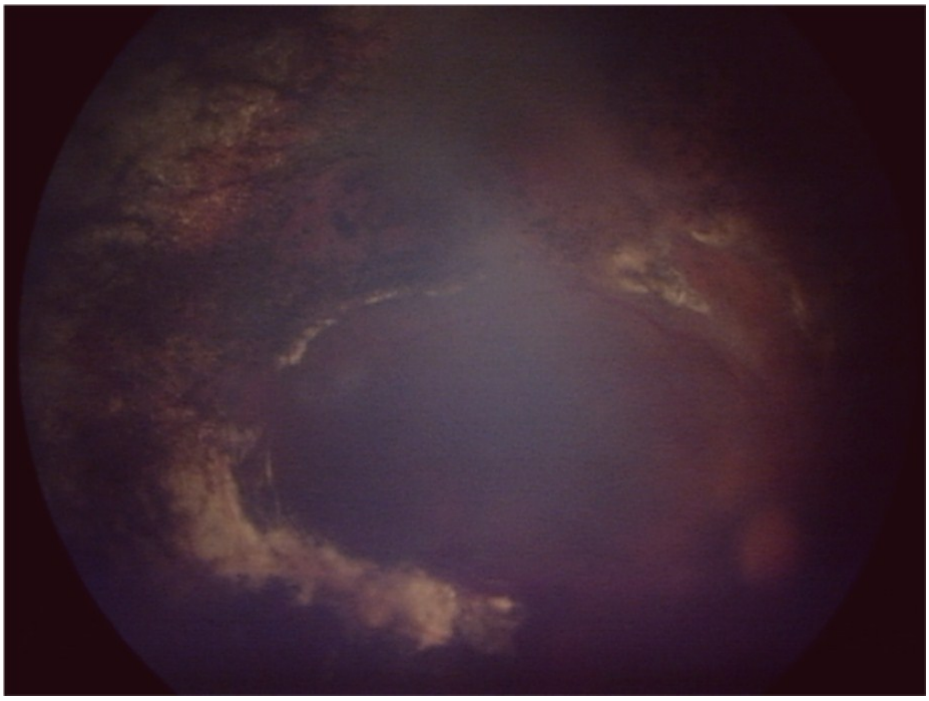


Figure 3 : Retcam photograph of the right fundus at the time of referral to vitreoretinal unit showing extensive retinal scarring with tractional retinal detachment.

of intensive therapy at the paediatric intensive care unit.

DISCUSSION

Acute retinal necrosis is a triad of peripheral necrotising retinitis, retinal arteritis and anterior uveitis or vitritis (1). The condition is relatively rare in paediatric age group. There are only several case reports published with the largest case series reported by Silva et al (2) in which they reported total number of 14 patients, aged from new-born to 21 years old who were diagnosed between 1995 until 2009 in a large tertiary centre.

There are several ways in how ARN can happen in a paediatric group. These patients might be having neonatal herpetic infection such as a disseminated infection, isolated encephalitis or mucosal herpetic infection of the skin, eye and mouth. These infections then spread hematogenously into the ocular structure causing ARN. It is also postulated that the virus can spread from the brain via axonal transmission from suprachiasmatic or periventricular nuclei towards the retina.(2) Another possible way is that ARN can also be a result of a reactivation of a latent neonatal infection which had previously taken place (3).

The most severe clinical features of ARN usually confined to the retina but anterior segment may show some periorbital skin vesicles, conjunctivitis or anterior uveitis. Retinal changes are divided into acute and late stage. Peripheral necrotising retinitis which is rapidly progressing is the most stand-out features. Other posterior segment findings in the acute stage would be vitritis, retinal arteritis and retinal edema. Late stage disease would reveal chorioretinal atrophy with scarring and tractional band. Some of the tractional band would progress into tractional retinal detachment.

Most cases of ARN can be diagnosed clinically based on dilated fundus examination due to the obvious necrotising retinitis present in all patients. It is sometime possible to identify the causative organism by obtaining sample from the ocular fluids and send it for polymerase chain reaction (PCR), culture or immunohistochemical investigations (4). In our case, the HSV-2 was detected in cerebrospinal fluid by PCR.

The mainstay of treatment in cases of ARN would be a systemic antiviral therapy. The recommended antiviral for HSV-1 and HSV-2 is intravenous acyclovir.(3) The duration of treatment depends on the clinical course. During active inflammation, it is important to administer parenteral treatment for 10 to 15 days and this might be continued with oral antiviral for another 3 months up to a year (5). Valaciclovir is reported to have better absorption compared to acyclovir thus having better ocular penetration (5).

Recent studies have suggest that there is additional benefit of combination of systemic and intravitreal foscarnet in reducing the rate of retinal detachment and visual loss (6). However, it is also reported that intravitreal injection itself carries some risk for complications such as endophthalmitis, retinal detachment, vitreous haemorrhage and lens trauma. In addition, the availability of the medication is limited to certain countries. Our patient was only seen most likely at the late stage of the disease with retinal detachment occurring not long after the first ophthalmological review.

The role of systemic corticosteroid is controversial. However in cases with very dense vitritis, corticosteroid might be helpful. Other potential benefit of systemic corticosteroid includes minimisation of fibrosis and tractional bands formation. Some ophthalmologist also advocates barricade retinal laser photocoagulation surrounding the traction and necrotic areas as a prophylaxis to prevent retinal detachment. In cases which progressed into retinal detachment, patient needs to undergo pars plana vitrectomy and endolaser with silicone oil as tamponade to treat the retinal detachment.

This case demonstrates that a patient with such severe retinal necrosis might have normal anterior segment findings. Therefore, it is recommended that all neonates with systemic herpetic infection should be referred to ophthalmology for eye assessment even in the absence of external eye signs because early antiviral treatment can minimise complications of ARN.

REFERENCES

1. Holland GN. Standard diagnostic criteria for acute retinal necrosis syndrome. Am J Ophthalmol 1994;117:663-667
2. Silva RA, Berrocal AM, Moshfeghi DM et al. Herpes simplex virus type 2 mediated acute retinal necrosis in paediatric population: Case series and review. Graefes Arch Clin Exp Ophthalmol 2013;251:559-566
3. Corey L, Wald A. Maternal and neonatal herpes simplex virus infections. N Engl J Med 2009;361:1376-85
4. Pepose JS, Keadle TL, Morrison LA. Ocular herpes simplex: Changing epidemiology, emerging disease patterns and potential of vaccine prevention and therapy. Am J Ophthalmol 2006;141:547-57
5. Grose C. Acute retinal necrosis caused by herpes simplex virus type 2 in children: Reactivation of an undiagnosed latent neonatal herpes infection. Semin Paediatr Neurol 2012;19:115-118.
6. Flaxel CJ, Yeh S, Lauer AK. Combination systemic and intravitreal antiviral therapy in the management of acute retinal necrosis (An American Ophthalmological Soc Thesis). Trans Am Ophthalmol Soc 2013;111:133-14.

Original Article

COMPARISON OF CENTRAL CORNEAL THICKNESS BETWEEN DIABETIC AND NON-DIABETIC POPULATION

KS Wan[1], HF Oh[1], NA Abdul Latiff[1], A Farrah Qistina[1], SL Chieng[1], R Mohd Akmal[1], Aida Zairani MZ[2], Then KY [2]

[1] Medical Undergraduates, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Cheras, Kuala Lumpur, Malaysia.
[2] Department of Ophthalmology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Cheras, Kuala Lumpur, Malaysia.

ARTICLE INFO

Corresponding author:
Dr. Aida Zairani Mohd Zahidin

Email address:
aidazahidin@gmail.com

Received:
May 2018
Accepted for publication:
June 2018

Keywords:

Diabetes
Central Corneal Thickness
Glaucoma

ABSTRACT

A case control study was conducted to compare the central corneal thickness between diabetic and non-diabetic population. The subjects were 185 Malaysian adults who came to the Universiti Kebangsaan Malaysia Medical Centre (UKMMC) Eye Clinic. The sample was divided into two groups in which there were 90 diabetic and 95 non-diabetic participants. All subjects who fulfilled the inclusion and exclusion criteria were recruited after obtaining informed consents. Central cornea thickness (CCT) measurement was carried out on right eye with a Topcon SP-2000P non-contact specular microscope. Readings would only be taken from the left eye if the right eye did not meet the inclusion criteria. Three measurements were taken and the mean was used as the final result. Over half were Malays (n=103, 55.7%) followed by Chinese (n=69, 37.3%) and Indians (n=13, 7.0%). The mean age for diabetic participants was 59.23±10.02 years, ranging from 38-74 years. Among the non-diabetic participants, the mean age was 57.07±13.68 years, ranging from 23-78 years. The difference between age of diabetics and non-diabetics was not statistically significant (p=0.22). CCT of all participants was normally distributed, with the mean of 526.55± 31.82 µm. The mean CCT in diabetic participants was 531.48± 32.88 µm whereas it was 521.88± 30.22 µm in non-diabetic participants. The increase in CCT found in diabetic participants was statistically significant (p=0.04). This study showed that diabetes is associated with thicker CCT which might contribute to overestimation of intraocular pressure in the management of suspected glaucoma patients.

INTRODUCTION

Diabetes mellitus is a common worldwide disease. It is a cause of major and growing concern due to its high prevalence, chronic complications and high mortality rate, affecting approximately 180 million people around the world [1]. The prevalence of diabetes in Malaysia has been increasing steadily over the years with an estimate of 0.65% in 1960, to 2% in 1982. In 1986, the prevalence was estimated to be 6.3% and is further increased to 8.3% in 1996 [2].

The measurement of central cornea thickness (CCT) helps in the clinical assessment of glaucoma [3]. Because diabetes mellitus is a common condition in most countries, the association between diabetes and CCT is important as it affects the measurement of true intraocular pressure (IOP). Ocular Hypertension Treatment Study has demonstrated that CCT was a predictor for the development of primary open angle glaucoma (POAG) [4]. The thickness of cornea has a significant effect on the measurement of intraocular pressure. A thicker cornea leads to an overestimation of IOP while a thinner cornea causes an underestimation of IOP.

There have been reports of increased central corneal thickness in patients in whom diagnosis of ocular hypertension was made. It had been found that CCT of eyes of patients with ocular hypertension was significantly greater (606±41µm) than that of eyes of patients with glaucoma (554±22µm) or of eyes of normal controls (561±26µm) [5]. These variations of CCT in eyes of different groups have also been shown in many other studies such as study by Patwardhan A et al in United Kingdom who found that the mean ± standard deviation of CCT was 561.5 ± 35.7 µm, 538.9 ± 41.4 µm, 538.3 ± 40.3 µm for ocular hypertension (OHT), primary open angle glaucoma (POAG) and normal pressure glaucoma (NPG) subjects respectively [6].

This overestimation or underestimation of IOP has important implications in diagnosis and management of patients. In term of diagnosis, a population may have higher prevalence of ocular hypertension if the population has a thicker CCT and vice versa. This would influence epidemiological study of the disease of that particular population.

On top of that, CCT must be considered when developing a treatment approach for a patient with ocular hypertension since the patient's ocular hypertension can be due thick CCT rather than other pathological changes. The same study by Patwardhan A et al demonstrated that CCT and adjusted IOP measurement can influence glaucoma management in a clinical context [6]. It helps attribute risk and hence aids patient management decisions. Their study revealed that the IOP adjustment was greater than ±2 mmHg in 33.9% (n=304) of eyes. This CCT and adjusted IOP information had led to different treatment option in 37% (n=152) of cases. Of the most important changes, 20.4% cases would have been commenced on additional IOP-lowering medication, 2.0% would have been counseled for trabeculectomy surgery and 3.3% of the cohort would have been observed rather than treated [6]. This clearly shows the importance of central corneal thickness in clinical decision making.

As diabetes is common in Malaysia, it is justified to study the effect of the disease on the CCT. To our best knowledge, there is little known about the CCT among diabetic and non-diabetic persons in Malaysia. Therefore, this study is designed to compare the CCT between those with and without diabetes, since CCT has been shown to influence diagnosis and management of the patients.

MATERIALS AND METHODS

This is a case-control study which was carried out in April 2009 among 90 diabetic and 95 non-diabetic participants who came to Ophthalmology Clinic, Universiti Kebangsaan Malaysia Medical Centre (UKMMC). The sampling frame consisted of all main races in Malaysia which are Malay (n=103, 55.7%), Chinese (n=69, 37.3%) and Indian (n=13, 7.0%); ages from 23 to 78 years old. Approval for the study protocol was granted by UKMMC Research Secretariat. Written informed consent was obtained from all the participants before enrollment.

All participants had gone through a standardized interview to determine if they were eligible to participate in the research based on the inclusion and exclusion criteria. Diabetes mellitus was defined in this study as those who have been diagnosed by a physician to have Diabetes Mellitus previously, and on diabetic medication or having non-fasting blood glucose of ≥11.1mmol/l. Non-diabetic participants were those who denied of having diabetes mellitus and having non-fasting blood glucose of <11.1mmol/l. Those with history of corneal disease, glaucoma, previous eye surgery including laser refractive eye surgery and usage of contact lens at the time of participation were excluded from the study. The subjects' non-fasting blood glucose was tested using OneTouch R Horizon glucometer with OneTouch R Horizon test strips. Three measurements of the CCT were obtained from the right eye with a Topcon SP-2000P non contact specular microscope (Topcon Corp., Tokyo, Japan). If the participant's right eye did not meet the inclusion criteria, measurements from the left eye were taken provided the left eye fulfilled the criteria. The mean CCT based on 3 measurements was used as the final result.

Statistical analysis was performed using SPSS for Window (version 13.0, SPSS Inc. Chicago. IL). The correlation of central corneal thickness between diabetic and non diabetic subjects was analyzed using the Student t-test.

RESULTS

A total of 185 subjects were recruited in this study and CCT measurements were taken. Over half were Malays (n=103, 55.7%) followed by Chinese (n=69, 37.3%) and Indians (n=13, 7.0%). The sample was divided into two groups in which there were 90 diabetic and 95 non-diabetic subjects. The mean age for diabetic subjects was 59.23 ± 10.02 years, ranging from 38-74 years. Among the non-diabetic subjects, we found that the mean age was 57.07 ± 13.68 years, ranging from 23-78 years old. The age difference between diabetic and non-diabetic subjects was statistically not significant. (p=0.22, Student t-test) (Table 1). The number of left eye measured is 9 (4.9%), while the remaining CCT were measured using right eye, 176 (95.1%).

CCT was normally distributed with a mean of 526.55± 31.82 µm. The mean CCT in diabetic subjects was 531.48± 32.88 µm whereas it was 521.88± 30.22 µm in non-diabetic subjects. The increase in CCT found in diabetic subjects was statistically significant (p=0.04, Student t-test). (Table 2 and Figure 1).

DISCUSSIONS

Since diabetes mellitus is a very common disease in Malaysia with increasing prevalence over the years, it is potentially important to associate diabetes and CCT. CCT must be considered when developing a treatment approach for a patient with glaucoma or ocular hypertension, since a thicker central cornea causes overestimation of IOP rather than presence of pathological changes. In addition, persons with diabetes are thought to be at higher risk of glaucoma, possibly due to the influence of chronic hyperglycaemia on corneal thickness and IOP measurement. The Blue Mountains Eye Study

Table 1: Demographic Data

Characteristic	Diabetic	Non-diabetic	Total	P value
Age				
Mean ±SD	59.23±10.02	57.07±13.68		0.22
Range	38-74	23-78		
Gender				
Male, n = (%)	44(51.8%)	41(48.2%)	85	0.09
Female, n = (%)	46(46.0%)	54(54.0%)	100	
Race				
Malay, n = (%)	48(46.6%)	55(53.4%)	103	0.26
Chinese, n = (%)	31(44.9%)	38(55.1%)	69	
Indian, n = (%)	11(84.6%)	2(15.4%)	13	

Table 2: Mean of Central Corneal Thickness

Characteristic	N	Mean ± SD (µm)	Student t-test P value
Diabetic	90	531.48±32.88	0.04
Non-diabetic	95	521.88±30.22	
Total	185	526.55± 31.82	

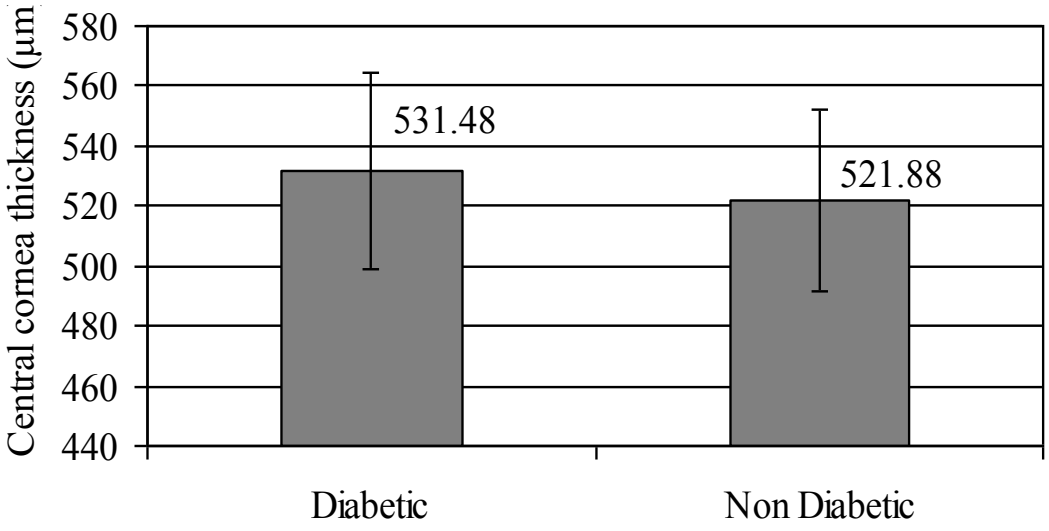


Figure 1: Mean (Standard Deviation) of Central Corneal Thickness (CCT)

and other studies showed that persons with diabetes are at increased risk of developing glaucoma [7]. Thus, the relation between diabetes, CCT, IOP and glaucoma plays an important aspect in our study.

This study demonstrates a relationship between CCT and diabetes. In our study, on average, the central cornea of a diabetic person is 9.6μm thicker than a person without diabetes mellitus (531.48μm vs 521.88μm). The difference between age of diabetics and non-diabetics was statistically insignificant (p=0.22), indicating that age is not a confounding factor in this study. The effect of diabetes on CCT is also seen in other races as shown by several studies done by other researches. A study by Brandt JD et al amongst Caucasians showed that mean CCT of subjects reporting a history of diabetes at the baseline was statistically significantly greater compared with subjects not reporting a history of diabetes (diabetics, n=128; 580.1±42.0 μm; non-diabetics, n=1101; 572.2±38.6 μm, p=0.02) [8]. The Singapore Malay Eye Study showed that the central corneas were significantly thicker in diabetic subjects as compared to non-diabetic subjects (diabetic, n=748, 547.2 μm vs non-diabetic, n=2491, 539.3 μm, p<0.001). The difference is comparable to our study. However, this study also demonstrated that thicker CCT was also associated with higher serum glucose (p=0.023) and higher HbA1c levels (p<0.001) [9]. A study by Claramonte PJ et al also showed that persons with diabetes had a thicker CCT than persons without diabetes (571.96±26.81μm vs 544.89±35.36μm) [10]. Data from a randomized clinical trial, the European Glaucoma Prevention Study, showed that participants with diabetes had thicker central corneas than persons without diabetes (588μm vs 571μm) [11]. As a result, our study implies that thicker central corneas have been associated with diabetes which may further influence the readings of IOP in the diagnosis of glaucoma in suspected patients.

Apart from that, our study also shows that the mean CCT in both diabetic and non-diabetic subjects are lower than the measurements found in the studies mentioned. This is possibly due to the usage of Topcon SP-2000P non-contact specular microscope as a tool to measure CCT in our study, as compared to the other studies which used ultrasonic pachymeter. According to a study by Shigenobu Suzuki et al, central corneal thickness measurements using noncontact specular microscope (Topcon SP-2000P) (525.3±31.4μm) gave significantly lower readings than scanning-slit topography (546.9±35.5μm) and ultrasonic pachymeter (548.1±33.0μm) [12]. Another study by Sallet G also showed that the mean CCT measured using non-contact specular microscope (Topcon SP-2000P) was significantly lower than mean CCT measured using ultrasonic pachymeter (535μm vs 540μm) [13]. K Kawana et al also demonstrated that the value obtained with SP-2000P non-contact specular microscope was significantly smaller than that taken with ultrasonic pachymetry (467.9±40.2μm vs 478.8±41.9μm, p<0.001) [14]. On top of that, the reason we use non-contact specular microscope to measure CCT is that we could eliminate the human error when using ultrasonic pachymetry. Because the latter is handheld, we may not be able to center the probe centrally. Even though ultrasonic pachymetry is a common approach and an efficient way to measure CCT, this probe must touch the corneal surface and topical anaesthesia is thus required. More over, its accuracy is dependent on the perpendicularity of the probe's application to the cornea and reproducibility relies on the precise probe placement on the corneal center [12]. Thus, due to the bias induced by ultrasonic pachymeter placement, the measurement of CCT is not consistent from one operator to another. Therefore, measurement of CCT using Topcon SP-2000P specular microscope is more consistent [15]. This is also the reason why we use the non-contact specular microscope to conduct this study as the CCT measurements could be taken by different operators in our study. Besides, the other advantages of using non-contact specular microscope are that it is easily mastered by a technician, there is no risk of transmitting infectious disease, the central alignment is easily achieved and there is no topical anaesthesia needed [13].

Several mechanisms can be speculated about the basis of association between diabetes and CCT. Hyperglycaemia may contribute to cornea endothelial dysfunction resulting in stromal hydration and cornea swelling. This was supported by McNamara NA et al who pointed that corneal structures are altered in diabetic patients, suggesting that hyperglycaemia affects control over corneal hydration, thus varying corneal thickness in diabetic patients [16]. In fact, abnormalities of cornea endothelial morphology such as polymorphism, polymegatheism, decrease in percentage of hexagonal cells, higher coefficient of variation, and increased CCT have been detected on specular microscope in persons with diabetes [17, 18].

On the other hand, we would like to make some recommendations after completing this study. We recommend that a study is done to show the relationship between duration of diabetes and CCT. A separate study to demonstrate relationship between CCT and adjusted IOP measurement amongst glaucoma patients could also be conducted. It is necessary to complement the findings in our study with the results of those recommended studies mentioned in an attempt to provide an appropriate management to suspected glaucoma patients.

CONCLUSION

In conclusion, our study showed that persons with diabetes have thicker central corneas than those without diabetes. These findings suggested that

CCT measurements were affected by the presence of diabetes. Thus, IOP must be adjusted when treating diabetic patients with suspected glaucoma or ocular hypertension.

ACKNOWLEDGMENT

We would like to thank Prof. Dr. Ropilah Abdul Rahman for her guidance and help during the research.

REFERENCES

1. William R, Airey M, Baxter H, Forrester J, Kennedy-Martin T, Girach A. Epidemiology of diabetic retinopathy and macular edema: a systemic review. Eye 2004; 18: 963-983.
2. Shafie O, Fatanah I, Noor Azah D. Diabetes program in Malaysia- current and future. NCD Malaysia 2004; vol 3, no 2: 6-12.
3. Shih CY, Graff Zivin JS, Trokel SL, Tsai JC. Clinical significance of central cornea thickness in the management of glaucoma. Arch Ophthalmol 2004; 122: 1270-75.
4. European Glaucoma Prevention Study (EGPS) Group. Predictive factors for open-angle glaucoma among patients with ocular hypertension in the European Glaucoma Prevention Study. Ophthalmology 2007; 114: 3-9.
5. Herndon LW, Choudhri SA, Damji KF, et al. Central cornea thickness in normal, glaucomatous, and ocular hypertensive eyes. Arch Ophthalmol 1997; 115: 1137-41.
6. Patwardhan A, Khan M, Mollan S, et al. The importance of central cornea thickness and decision making in general ophthalmology clinic. J Cataract Refract Surgery 2006; 326: 1018-21.
7. Mitchell P, Smith W, Chey T, Healey PR. Open-angle glaucoma and diabetes: Blue Mountains Eye Study, Australia. Ophthalmolgy 1997; 104: 712-8.
8. Brandt JD, Beiser JA, Kass MA, et al. Central corneal thickness in the Ocular Hypertension Treatment Study (OHTS). Ophthalmology 2001; 108: 1779-88.
9. Daniel HW Su, Tien Y Wong, Wan-Ling Wong, Seang-Mei Saw, Donald TH Tan, Sunny Y Shen, Seng-Chee Loon, Paul J Foster, Tin Aung. Diabetes, hyperglycaemia, and central cornea thickness, The Singapore Malay Eye Study. Ophthalmology 2008; 115: 964-8.
10. Claramonte PJ, Ruiz-Moreno JM, Sanchez-Perez SP, Leon M, Grino C, Cervino VD, Alio JL. Variation of central cornea thickness in diabetic patients as detected by ultrasonic pachymetry. Arch Soc Esp Oftalmol 2006;81: 523-6.
11. European Glaucoma Prevention Study Group. Central cornea thickness in the European Glaucoma Prevention Study. Ophthalmology 2007; 114: 454-9.
12. Shigenobu Suzuki, Tetsuro Oshika, Kotaro Oki et al. Corneal thickness measurements: Scanning-slit corneal topography and noncontact specular microscopy versus ultrasonic pachymetry. J Cataract Refract 2003; 29:1313-8.
13. Sallet G. Comparison of optical and ultrasound central cornea pachymetry. Bull. Soc. Belge Ophthalmol 2001; 281: 35-38.
14. K Kawana, T Tokunaga, K Miyata, F Okamoto, T Kiuchi, T Oshika. Comparison of cornea thickness measurements using Orbscan II, non-contact specular microscopy and ultrasonic pachymetry in eyes after laser in situ keratomileusis. Br J Ophthalmol 2004; 88(4): 466-8.
15. Rence Bovelle, Stephen C. Kaufman, Hilary W. Thompson, Hikaru Hamano. Corneal thickness measurements with the Topcon SP-2000P specular microscope and an ultrasound pachymeter. Arch Ophthalmol 1999;117:868-870.
16. McNamara NA, Brand RJ, Polse KA, Bourne WM. Corneal function during normal and high serum glucose levels in diabetes. Invest Ophthalmol Vis Sci 1998; 39: 3-17.
17. Larsson LI, Bourne WM, Pach JM, Brubaker RF. Structure and function of the corneal endothelium in diabetes mellitus type I and II. Arch Ophthalmol 1996; 114: 9-14.
18. Schultz RO, Matsuda M, Yee RW, et al. Corneal endothelial changes in type I and type II diabetes mellitus. Am J Ophthalmol 1984; 98: 401-10.

Original Article

THE USE OF MULTIFOCAL ELECTRORETINOGRAM TO PREDICT PROGRESSION OF DIABETIC RETINOPATHY

Haizul Ikhwan Murat[1], Faridah Hanum Annuar[1], Norshamsiah Md Din[1], Ropilah Abd Rahman[2], Sabrizan Osman[3], Hazlita Isa*[1]

[1] Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia.
[2] Kulliyyah of Medicine & Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah (UniSHAMS), Kuala Ketil, Kedah, Malaysia.
[3] Department of Ophthalmology, Hospital Tengku Ampuan Afzan,Kuantan, Pahang Darul Makmur, Malaysia.

ARTICLE INFO

Corresponding author:
Dr. Hazlita Isa

Email address:
drhdmi@yahoo.co.uk

Received:
May 2018
Accepted for publication:
June 2018

Keywords:

Diabetic Retinopathy;
Macular edema;
Multifocal Electroretinogram;
Predictive Diabetic retinopathy progression

ABSTRACT

The primary cause of visual loss in diabetic retinopathy (DR) is macular edema. Predicting the occurrence of diabetic macular edema may allow institution of early treatment in diabetic patients. A prospective observational study was conducted to determine whether abnormal implicit time in multifocal ERG or mfERG (mfERG IT) within the macular region can predict progression of DR after one year. A total of fifty patients with type 2 diabetes and mild to moderate non-proliferative diabetic retinopathy (NPDR) was utilized. At baseline, patients' mfERG from 61 retinal points within 35 degrees from the center of fovea were recorded and fundus photographs were taken at baseline and 12 month. mfERG IT at baseline were measured and fundus photograph were used to monitor progression of DR within 1-year. The result revealed that 1552 retinal points with abnormal mfERG IT showed DR progression after 1 year. Relative risk of DR progression among retinal points with abnormal mfERG IT at baseline were 6 times greater than retinal points with normal mfERG IT (RR 6.21; p < 0.001). mfERG IT at baseline has 89.9% sensitivity and 81.7% specificity to predict progression of DR. In conclusion, abnormal mfERG IT provides an objective assessment of local retinal health in diabetes and may be useful to predict DR progression.

INTRODUCTION

The prevalence of Diabetes Mellitus (DM) is expected to increase two- fold from 2.8% or 171 million population in the year 2000 to 4.4% or 366 million population in 2030 [1]. In10.2 million adults aged 40 years and older with DM, the estimated crude prevalence rates for retinopathy and vision threatening retinopathy were 40.3% (4.1 million person) and 8.2% (836,400 person) respectively [2]. The primary cause of visual loss in diabetic retinopathy (DR) is macular edema, caused by leakage from micro aneurysms and dilated capillary segments. Diabetic macular edema (DME) can occur at any stage of DR and may result in devastating visual complication. Therefore, predicting and preventing macular edema in "at risk" individuals would be of great benefit for diabetic patients.

Recently, multifocal ERG (mfERG) has emerged as a new technique for exploring human retinal function including DR [3]. Several studies have shown that mfERG implicit times (IT) were significantly increased and the mfERG amplitude (Amp) were significantly reduced in diabetic patients with or without retinopathy [3,4]. Other relevant study reported that the relative risk (RR) of developing new DR over one year in the areas with abnormal mfERG IT was approximately 21 times greater than in eyes with normal baseline mfERG IT [4]. Nevertheless the study only involved a small number of subjects and used larger retinal zones rather than small unit of retinal points.

The purpose of this study is to assess whether abnormal mfERG IT within the macular region at baseline are predictive of new development or worsening of DR after 12 months and to study the correlation between the number of retinal points with abnormal mfERG IT that has developed new or worsening DR after 12 months with glycosylated hemoglobin (HbA1c) level, duration of diabetes and diabetic treatment.

METHODOLOGY

Subjects

This was a prospective observational study among type 2 diabetic patients with mild to moderate

NPDR. This study was conducted at the Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Kuala Lumpur, Malaysia. The study followed the tenets of the Declaration of Helsinki and was approved by the UKMMC Ethics Committee for protection of Human Subjects. Patients with only mild to moderate NPDR were included in the study. All subjects were free from other ocular diseases apart from DR and had the best corrected visual acuity (BCVA) of 20/20 or better.

Baseline

At baseline, all patients had their visual acuities measured using the Snellen chart, a complete dilated ocular examination using the slit lamp and blood taken for HbA1c level. Fundus photographs using Topcon TRC 50DX Type 1A Retinal Camera were obtained. The macular area was determined by an imaginary circle of which the radius is a distance between the center of the macular and including the temporal 1/3 of the optic disc. Only one eye with the clearest fundus image was included in the study. The images were cropped and printed out using A4 size glossy paper.

MfERG procedure

After 15 minutes exposure to ordinary room light (recovery period), mfERG examination were performed using the Espion mfERG by Diagnosys, LLC. The selected eye was anesthetized with topical instillation of Proparacaine Hydrochloride 0.5% and the patient's temporal part of the scalp, midline of the forehead and both sides of the nasal bridge were cleaned with alcohol swab and scrubbed using the skin prep paper. The Dawson-Trick-Litzkow (DTL) fibers were then placed on the inferior fornices of the tested eye. The fibers were held by two skin electrodes, one placed on the temporal scalp and the other on the sides of the nasal bridge. A ground electrode was placed on the midline of the forehead and an eye occluder covered the untested eye

The patient was then seated comfortably 30 cm from the LCD screen with their head placed in the built-in headrest. The 61 hexagons 35 degree protocol was used for this study which was an array of 61 hexagonal elements delivered within a field of 35 degrees. During stimulation, the displays appeared as a flicker of each hexagon that will go through a binary m- sequence of black (Lmin = 0cd/m^2) and white (Lmax = 1000cd/m^2). In order to increase the reliability of the test, blink rejection was set up to 20μV and noise rejection was set up to 50μV to filter the unnecessary waveforms produced by noise and blinking reflex. A reliability index of 98 -100% was aimed for each test. To increase patient concentration, the test was paused and re- run repeatedly to allow the tested eye to rest for a few seconds. The overall test took about 8- 10 minutes to complete. In this study, a standard algorithm of m- sequence which was recommended by the International Society of Clinical Electrophysiology of Vision (ISCEV)standard for clinical mfERG (2011 edition) was used [5]. The m-sequence controls the sequences of change between light and dark stages of each hexagon. This caused each hexagon to change with every different frame. The mfERG test was repeated twice and the mean from all three readings were taken.

At the end of the test, the trace array, topographic 3-D density plots and concentric rings averages were obtained. In trace array, the smoothed (filtered) rather than raw data was used. This smoothed data eliminated surrounding noise and thus further smoothed the raw data.

Trace array MfERG result were printed on an A4 size overhead projector transparent paper and superimposed on the printed A4 size color fundus photo on high quality glossy paper. This was used as the reference point during the patient's follow up at 12 months.

Follow up

At 12 months follow up, fundus photographsof the study eye was repeated. The images were cropped around the area of interest for analysis and printed out. The trace array mfERG at baseline on the OHP transparent papers were then superimposed on the 12 months printed color fundus photo.

Outcome measures

The mfERG IT was defined as the time from the beginning of the wave until the first prominent response peak (P1). Abnormal mfERG IT was defined as mfERG IT longer than the normal IT of the local population normative database.

Each of the retinal points were classified into different outcomes which included true positive (TP), false positive (FP), true negative (TN), and false negative (FN). True positive was defined as retinal points with abnormal mfERG IT at baseline and the DR progressed. False positive was defined as retinal points with abnormal mfERG IT without DR progression. True negative was defined as retinal points with normal mfERG IT and no DR progression and false negative was defined as retinal points with normal mfERG IT that progressed. The definitions of DR progression and no DR progression are illustrated in Figure 1 and Figure 2. In order to reduce bias in the results, mfERG at baseline were analyzed by the primary investigator, and fundus photo at baseline and month 12 were analyzed by 2 different medical retina experts.

Data analysis

Retinal points with TP, FP, TN, and FN results were summed up. The mean of mfERG intrinsic time between TP and FP, TN and FN were analyzed using independent T- test. The area under curve (AUC) and relative risk (RR) for the development of new or worsening DR in the macular region were calculated.

RESULTS

Patients' characteristics

A total of 50 eyes from 50 participants were included

in this study. The mean age was 59.5 ± 8.3 years (range 42- 77). There was a slight female preponderance with the male to female ratio of 1: 3. There was no racial predilection (p= 0.737). The over-all mean duration of diabetes was 12.1 ± 7.0 years (range 2- 32 years). The mean HbA1c of all 3 months was 8.4 ± 1.76 mmol/ml.

Implicit times and development of DR

The mean number of retinal points with abnormal mfERG IT at baseline that had DR progression after 12 months i.e TP were found to be significantly more (31.06 retinal points, p< 0.001, 95% CI 23.20- 29.32) compared to mean number of retinal points that did not showed progression DR despite an abnormal mfERG IT at baseline i.e FP. In addition, the mean number of retinal points with normal mfERG IT at baseline that did not showe DR progression i.e TN were significantly more (21.40 retinal points, p< 0.001, 95% CI 15.52-20.68) compared to mean number of retinal points which showed DR progression despite a normal mfERG IT at baseline i.e FN (3.48 retinal points). Implicit times provide good accuracy (p< 0.001, AUC = 0.89, 95% CI 0.87-0.90) in predicting DR in 12 months. (Figure 3) Based on the calculation, mfERG IT at 38.40ms is a cut off point to predict DR progression. Our study also found that retinal points with abnormal mfERG IT at baseline were found to be 6 times more likely to develop DR in 12 months compared to retinal points with normal mfERG IT(RR= 6.23, 95% CI 5.43 – 7.17). The sensitivity and specificity of mfERG IT to predict DR progression was 89.9% and 81.7% respectively (Table 1).

DISCUSSIONS

This study was aimed to see whether abnormal mfERG IT within the macular region at baseline are predictive of Diabetic Retinopathy progression after 1 year. This is a large scale study involving 3050 retinal points from 50 subjects. This study demonstrated that abnormal mfERG IT may be useful in determining DR progression.

Patient characteristics

The mean duration of diabetes among patients within the study is 12.1 ± 7.0 years with the shortest duration of 2 years and the longest duration of 32 years old. This was consistent with other studies that showed the estimated onset of detectable retinopathy is between 4 to 7 years before the diagnosis type 2 DM. Duration of DM type 2 has been proven to be a strong risk factor for DR progression [6,7,8,9].

Abnormal mfERG IT and the progression of DR

This study highlighted that mfERG IT has a strong predictive value in predicting the development and progression of DR. mfERG IT also has a high sensitivity (89.9%) and specificity (81.7%) in predicting DR progression. We also found that retinal points with abnormal mfERG IT is 6 times more at risk of developing new or worsening of DR within 1 year. The mfERG IT measurements can be used to monitor the progression of DR during the early stages of disease and can also evaluate the effectiveness of preventative drug therapies that are currently being developed [4].

Interestingly, these results also match those observed in an earlier study that demonstrated that implicit times rather than the amplitude of mfERG was more sensitive in assessing retinal function in diabetics than amplitude mfERG [10,11]. Over a 1 year period, mfERG IT increased in most of the retinal areas of eyes with NPDR but remained constant in normal subjects and in diabetic patients without retinopathy [4]. In contrast, amplitude mfERG were not associated with DR and did not predict retinopathy.The reason for prolonged implicit times prior to the occurrence of diabetic lesions has been described by Cao et al.[12]. Prior to visible fundus lesion, pericyte apoptosis and basement membrane thickening results in the occurrence of acellular capillaries indicating early vasculopathy in diabetic eyes. Four major theories have been proposed to explain how chronic hypoglycemia and subsequent retinal hypoxia might lead to these anatomic changes: 1) increased formation of advanced glycosylation end products; 2) abnormal by pass of glucose metabolism through the sorbitol pathway; 3) activation of growth factors such as the vascular endothelial growth factor; and 4) oxidative stress and free radical generation which promotes the development of diabetic lesions. These compromised local metabolisms affect the functions

Table 1: Analysis between implicit times mfERG and progression of diabetic retinopathy

		p	95% CI
RELATIVE RISK	6.32		5.41- 7.19
SENSITIVISITY (%)	89.9		
SPECIFICITY (%)	84.2		
AREA UNDER CURVE (AUC) (%) OF ROC CURVE	0.89	<0.001	0.86- 0.90
BEST CUT OFF POINT (ms)	38.40		

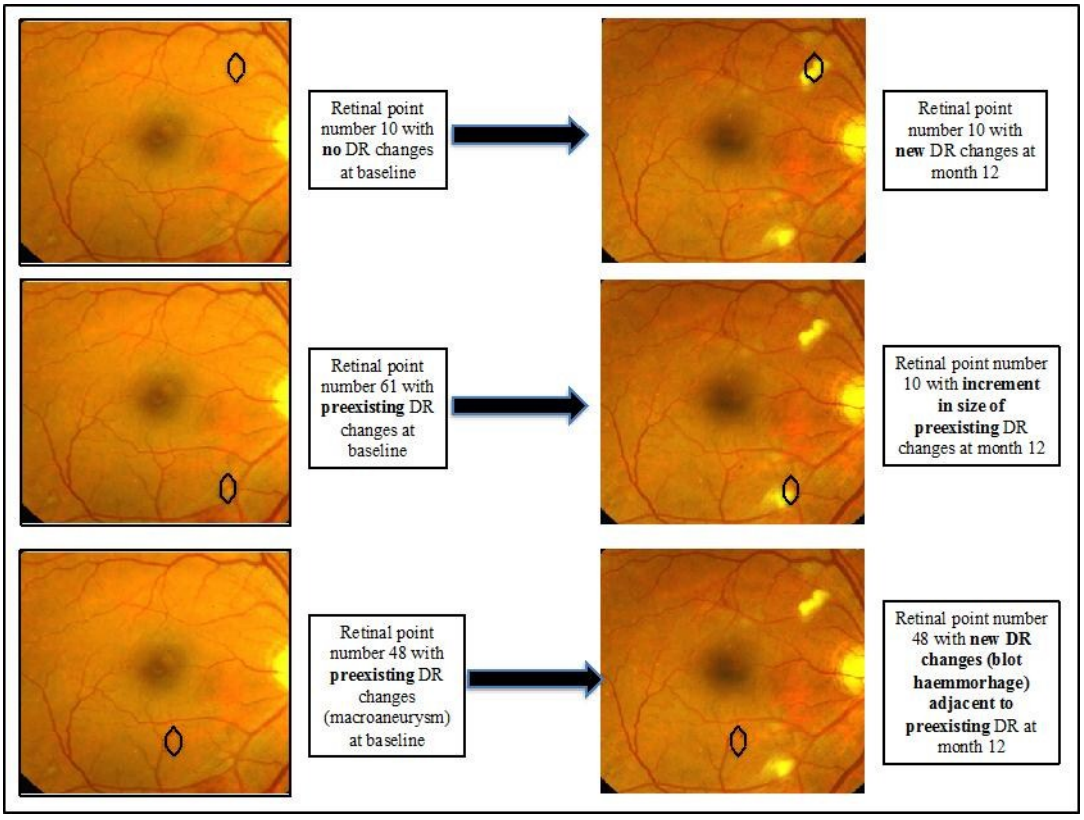


Figure 1: 35 degree fundus photograph at baseline (left) and at month 12 (right) showing DR progression

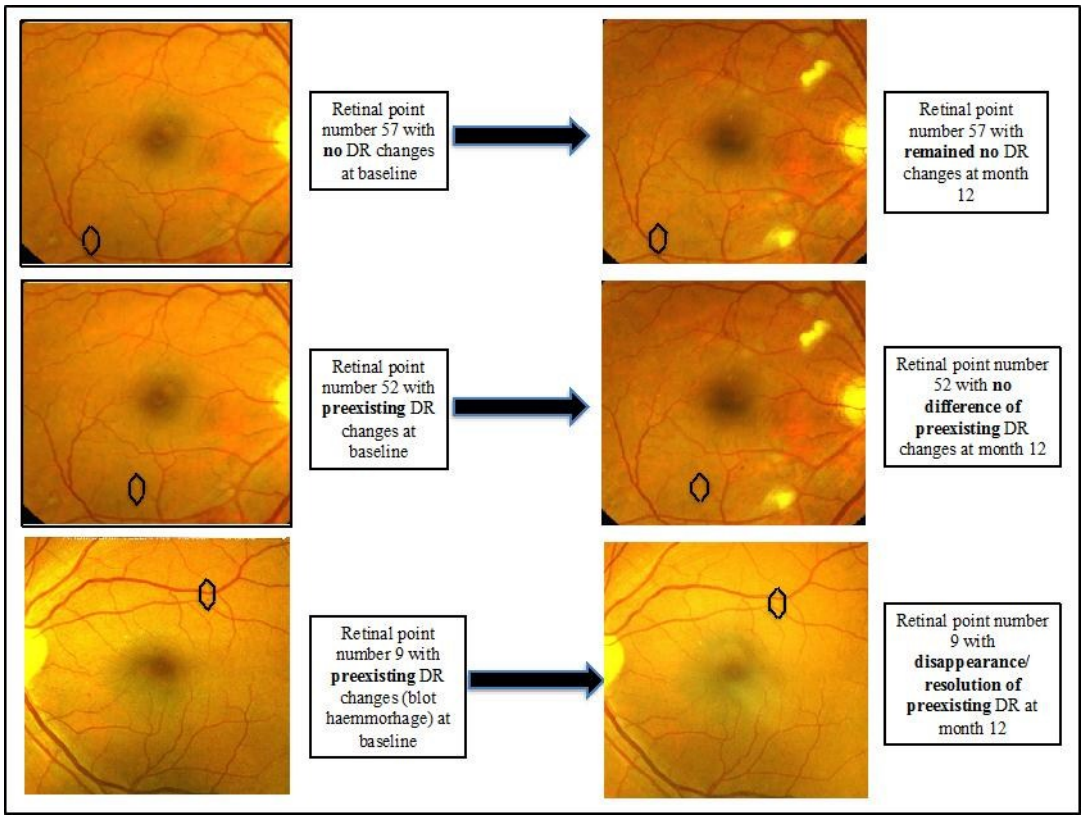


Figure 2: 35 degree fundus photograph at baseline (left) and at month 12 (right) showing no DR progression

of mfERG generators (photoreceptors and bipolar cells), leading to delayed neural conduction and prolonged mfERG IT, subsequently resulting in an abnormal mfERG reading despite normal retinal appearance.

Fortune et al has demonstrated that the presence of significant delayed local response in mfERG among retinal points without retinopathy is a very early indicator of local retinal dysfunction in diabetes [13]. It is possible that early local mfERG delays, found in the absence of retinal vascular findings are caused by early diabetic choroidal lesion. Retinal hypoxia is thought to be a major stimulus leading to increased expression of vascular endothelial growth factor (VEGF) [14]. In turn, increased expression of VEGF is likely to be a critical factor in the development of even the earliest retinal vascular lesion in diabetic retinopathy.

This study also found that mfERG is well suited in the study of DR. This is based on the fact that, DR is a retinal disorder with localized lesion typically confined to the posterior pole, the same site where standard mfERG tests local retinal function (across 35 to 45 degree). Secondly, DR is largely caused by defects of retinal capillaries in the inner nuclear layer of the retina, where bipolar cells' cell bodies, the primary generators of the mfERG are located [15]. Taken together, these results suggest that the mfERG IT could play an important role for monitoring local metabolic condition in diabetes.

In conclusion, this study demonstrated that abnormal mfERG IT has a good predictive value for DR progression. The mfERG provides a very sensitive and objective assessment tool for local retinal health in diabetes and would benefit in the assessment and management of DR in the future. Further future work may be performed to show whether mfERG IT could also predict the development of DR in eyes without retinopathy. Concurrent use of other modalities such as the OCT and FFA in the assessment of macular area would indeed give a more accurate anatomical macular assessment in relation to mfERG.

Financial Support
This study was funded by the Universiti Kebangsaan Malaysia.

Conflict of Interest
No conflicting relationship exists for any author.

REFERENCES

1. Sarah W, Gojka R, Anders G, Richard S, et al. Global Prevalence of Diabetes, Estimates for the year 2000 and projection for 2030. *Diabetes Care* 2004;27 (1): 1047- 1051.
2. John HK, Benita J, O'Colmain.The Prevalence of Diabetic Retinopathy Among Adults In The United States. *Arch Ophthalmology*. 2004; 122(9): 552- 562.
3. Mariani E, Moreo G, Colucci GB. Study of visual evoked potentials in diabetics without retinopathy: correlations with clinical findings and polyneuropathy. *ActaNeurolScand.* 1990; 81(3):337–340.
4. Ying Han. Multifocal ERG Delays predict sites of subsequent diabetic retinopathy. *IOVS.* 2004; 45 (8): 948- 954.
5. Hood DC, Bach M, Britgell M, Keati, et al. ISVEC standard for clinical mfERG (2011 edition). *Ophthalmol.* 2011;124(8): 1- 13.
6. Matthews DR, Stratton IM, Aldington SJ, et al. UK Prospective Diabetes Study Group Risks of progression of retinopathy and vision loss related to tight blood pressure control in type 2 diabetes.*Arch Ophthalmology.2004;*122(2):1631-1640.
7. Paul Mitchell, Wayne Smith, Jie Jin Wang, et al. Prevalence of Diabetic Retinopathy In An Older Community. The Blue Mountain Eye Study. *Ophthalmology.*1998;105(7): 406-411.
8. Harris MI, Klein R, Welborn TA, et al. Onset of NIDDM occurs at least 4-7 years before clinical diagnosis. *Diabetes Care.*1992;14(1): 914-918.
9. Moss SE, Klein R, Klein BE. Association of cigarette smoking with Diabetic Retinopathy. *Diabetes care*.1991;14(7): 119 - 126.
10. Diabetes control and complication trial study group. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complication trial. *Diabetes.*1995;44(5):968- 983.
11. Palmowski AM, Sutter EE, Bearse MA. Mapping of retinal function in diabetic retinopathy using the multifocal electroretinogram. *Invest Ophthalmol Vis Sci.*1997;38(2):2586–2596.
12. Cao J, McLeod S, Merges CA, et al. Choriocapillaries degeneration and related pathologic changes in human diabetic eyes. *Arch Ophthalmol.*1998;116(6):189-222.
13. Fortune et al. Multifocal ERG Delays Reveal Local Retinal Dysfunction in Early Diabetic Retinopathy. *IOVS.*1999*;* 40(2):2638-2651.
14. Linsenmeter RA, Braun RD, McRipley MA.Retinal hypoxia in long-term diabeticeyes. *Invest Ophthalmol Vis Sci.*1998; 37 (6):1647- 57.
15. Hood DC, Frishman IJ, Saszik S. Retinal origins of the primate mfERG: implications for the human response. *Invest Ophthalmol Vis Sci.* 2002;105(4):189.

Kulliyyah of Medicine & Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah,
Kuala Ketil Campus, 09300 Kuala Ketil, Kedah Darul Aman.
tijms.editor@gmail.com