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Case Report

SUPPLEMENTARY ROLE OF FDG PET-CT IMAGING IN THE MANAGEMENT OF A RARE CASE OF MALIGNANT PERITONEAL MESOTHELIOMA.

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ABSTRACT

A case report to highlight the clinical presentation of malignant peritoneal mesothelioma (MPM) and illustrate the role of fluorodeoxyglucose (FDG) positron emission tomography-CT (PET-CT) imaging in the management of this rare carcinoma. A middle-age male with chronic ascites and acute intestinal obstruction was initially diagnosed with metastatic adenocarcinoma to the porta hepatic nodes and omentum. However, CT scan done after completion of chemotherapy still demonstrated gross ascites, omental caking, peritoneal nodules and enlarged porta hepatic nodes. Thus, a review of the earlier histology slides was requested. Evaluation and consensus interpretation by pathologists concluded that the overall histological features and immunostaining were in favour of mesothelioma than metastatic adenocarcinoma. Subsequent FDG PET-CT to further assess the patient and exclude other possible primary malignancy has revealed a metabolically active porta hepatic lesion with multiple peritoneal and nodal deposits in the absence of other abnormal lesion in the thorax or solid organs, in keeping with the clinical diagnosis of peritoneal mesothelioma.

Keywords*: peritoneal, malignant mesothelioma, FDG PET-CT*

INTRODUCTION

Malignant mesothelioma is a rare neoplasm of the mesothelial lining within the human body. Peritoneal mesothelioma constitutes the major form of the disease after pleural mesothelioma. Mesothelioma may arise from both visceral and parietal peritoneum [1]. The incidence of MPM is approximately 6-10% and the disease is characterised by its difficulty to diagnose, poor response to treatment and high mortality [2,3,4]. It is generally more common in men with the incidence rate in industrialised countries ranges between 0.5 and 3 cases per million among men and between 0.2 and 3 cases per million among women [5]. In the United States, its overall prevalence is reported to be approximately 1-2 cases per million people with an estimated incidence of 200-400 new cases annually [1].

Exposure to asbestos is the main known cause of MPM [5]. As a comparison, the lifetime risk of developing mesothelioma of the thorax in heavily exposed individuals is as high as 10% [6]. Latency period between exposure and onset of malignant mesothelioma may be delayed and ranging from 15-60 years. However, several reported MPM cases showed no prior exposure to asbestos [2,3,7]. Symptoms of peritoneal mesothelioma include abdominal pain, ascites, abdominal mass, weight loss and fever [1,3]. In Malaysia, a middle-aged male was previously reported to be diagnosed with MPM after he presented with ascites of unknown origin [8]. Nevertheless, patients' clinical features and history can be elusive [1,2]. Although most often non-confirmatory, imaging techniques such as conventional CT scan as well as functional imaging like PET-CT scan may offer some supportive analytical assistance. Thus, the aim of this case report is to illustrate the role of FDG PET-CT in the evaluation and management of a rare case of MPM that was associated with diagnostic predicament.

CASE REPORT

A 40-year old male with underlying chronic ascites and post emergency surgery for acute intestinal obstruction was initially diagnosed with metastatic adenocarcinoma to the porta hepatic nodes and omentum. Preliminary imaging with CT scan showed only enlarged porta hepatic nodes while the peritoneal fluid cytology was previously inconclusive. However, histopathological and immuno-

histochemistry examination of samples from porta hepatic nodes and omental lesion demonstrated features of metastatic adenocarcinoma with the lung as the most probable occult primary site. Hence, chemotherapy with combined cisplatin and gemcitabine was initiated and later completed in October 2014 after 12 cycles.

Post chemotherapy CT scan in December 2014 showed omental caking and multiple peritoneal nodules with the largest measuring 0.6 x 0.9 cm. No significant change was seen in the enlarged porta hepatic nodes. There was also no CT scan evidence of pleural based lesion, bowel related mass or even other structural lesion seen elsewhere. Thus, a review of previous histology samples was then requested following a multi-disciplinary discussion. Samples were reassessed and microscopic findings revealed cohesive epithelioid cells with tubular and reticular patterns in the background of myxoid stroma. Apart from previously positive for cytokeratin AE1&3 and cytokeratin 7, repeat immunostaining was positive for calretinin. Therefore, consensus interpretation by pathologists in May 2015 concluded that overall these histopathological examination features were in favour of mesothelioma.

On further questioning, he denied any prior respiratory symptoms, exposure to asbestos and family history of malignancy. A PET-CT scan was requested by the attending oncologist for further evaluation to determine the extent of disease and exclude other possible primary malignancy. FDG PET-CT in August 2015 revealed a metabolically active lesion exhibiting the highest standardised uptake value (SUV) at the porta region with SUV 8.9 as well as an enlarged paracaval lymph node with SUV 5.9 and several other peritoneal and omental nodules with SUV 2.0 to 2.4 as shown in Figure 1, 2 and 3. Subsequently he continued to be under oncology management for further chemo-radiation therapy and follow-up visits.

DISCUSSION

The histopathological findings of mesothelioma generally can be divided into 3 pathological types; (a) epitheliod (55-66%) which can appear pathologically similar to adenocarcinoma, (b) sarcomatoid (10-15%) and (c) bi-phasic (20-35%) which has both epithelioid and sarcoma-toid features [9]. In addition, a histological subtype car-ries an important prognostic factor with epitheloid sub-type showing the longest survival while the sarcomatoid subtype has the worst prognosis [10]. At present, surgical resection by cytoreductive surgery combined with either heated intraperitoneal chemotherapy or systemic chemotherapy is among the treatment strategies that have been described [1,2]. Despite the current multimodality approach, small improvement in survival has underscored the obvious need for less morbid and more effective interventions for peritoneal mesothelioma patients.

In this case report, establishing a diagnosis was a clinical dilemma as the initial reported histopathological findings did not match the subsequent overall condition and progress of the patient. Accurate diagnosis of peritoneal mesothelioma depends on histologic and immunohisto-chemical examination. Several immunohistochemical

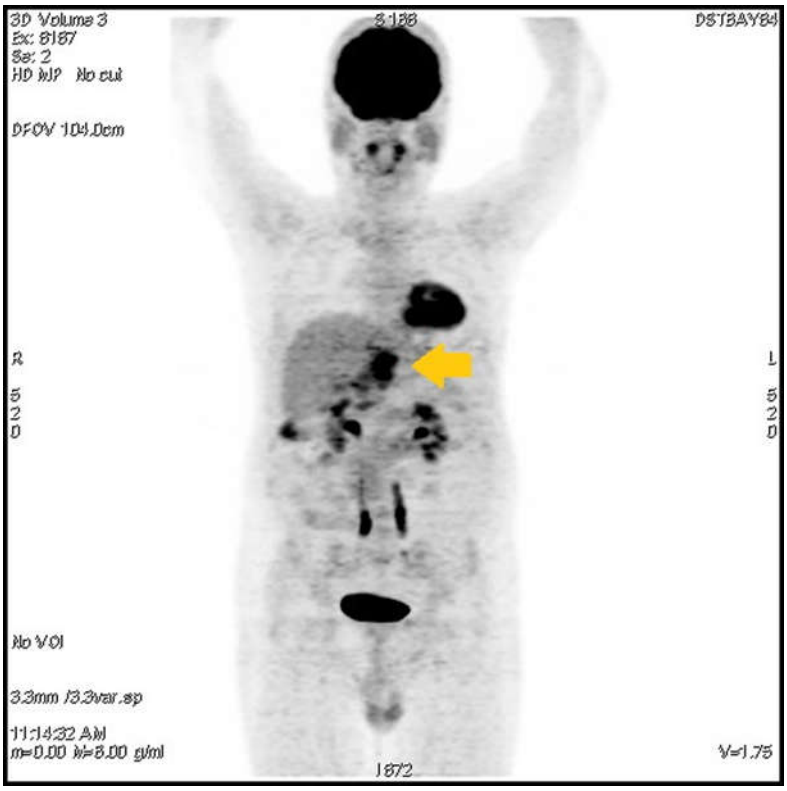

Figure 1: Maximum intensity projection (MIP) image of FDG PET-CT showing abnormal increased radiotracer uptake in the midline abdomen adjacent to the liver (arrow).

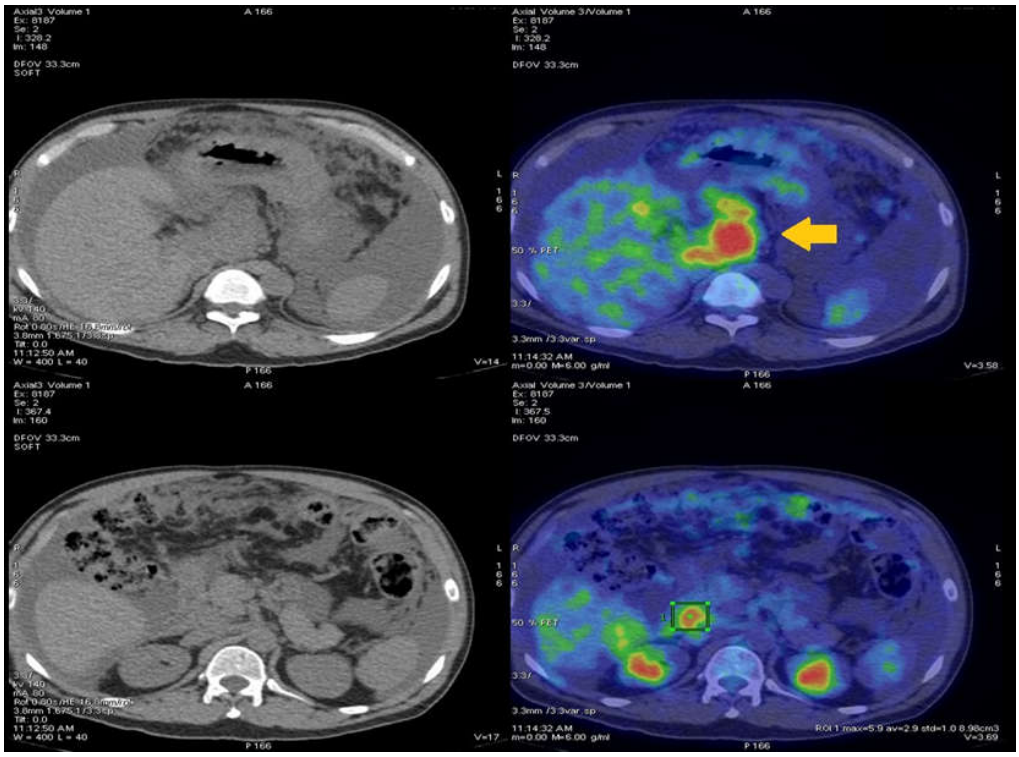

Figure 2: Fused axial images of FDG PET-CT showing metabolically active porta hepatic lesion (arrow) and enlarged paracaval lymph node.

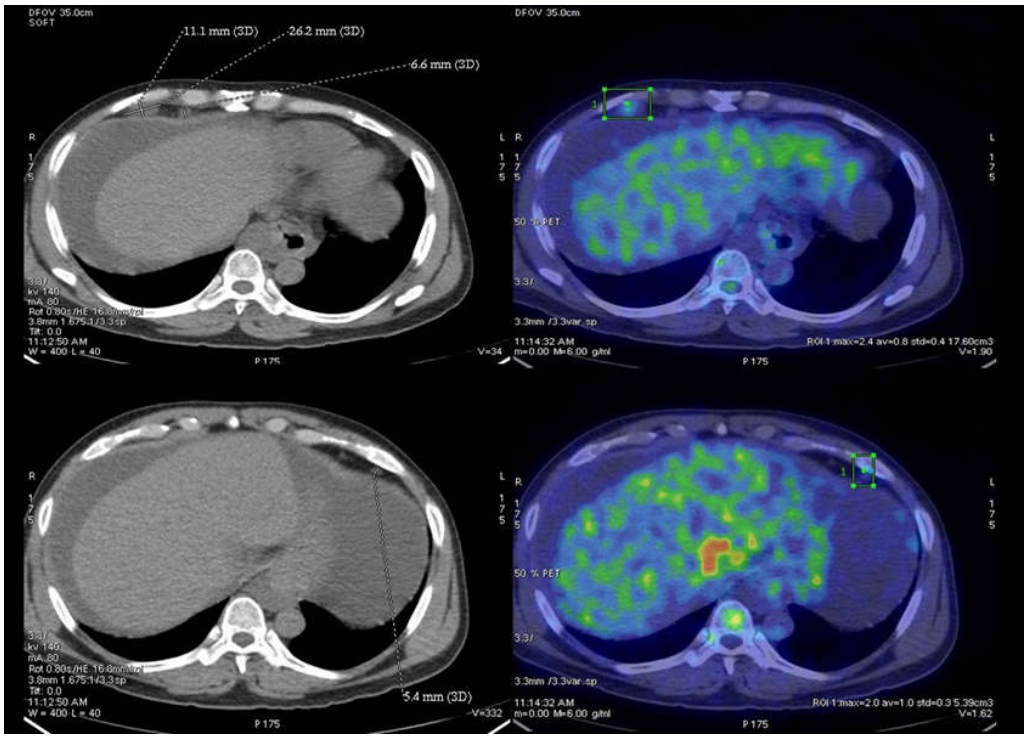

Figure 3: Fused axial images of FDG PET-CT showing mild metabolically active peritoneal nodules.

markers such as epithelial membrane antigen, calretinin, Wilms' tumour-1 protein, cytokeratin 5/6, antimesothelial cell antibody-1 and mesothelin may be positive in MPM [1,7]. However, the challenges would be in cases where there is only low yield or inadequate sampling taken for interpretation. Hence, for some patients despite the limitations, imaging may have a role in aiding the diagnostic work-up by complementing the histological findings with the abnormalities seen on scan.

CT scan is the commonest imaging tool being utilised in oncology as it is widely available. However, generally radiological presentation of mesothelioma is non-specific and it is challenging as well as not possible to distinguish a

benign from a malignant process and a primary from a metastatic disease [1]. It was previously documented that the usual findings of MPM would appear as a solid enhancing soft-tissue mass within the mesentery, omentum or peritoneum, with nodular peritoneal and omental masses may be seen in early phase of the disease progression followed by confluent plaque-like masses and eventually omental caking as the disease evolved [2]. Ascites may also be present [1,2]. It has been highlighted that in this ''wet'' type of peritoneal mesothelioma with ascites, CT scan may reveal widespread small nodules and ascetic fluid, but no dominant mass [11].

Literature review revealed only little published information available on the manifestations of MPM on ultrasound, MRI as well as FDG PET-CT imaging [1,4]. On the other hand, FDG PET-CT for pleural mesothelioma has been described as being useful in the disease staging and pre-operative evaluation especially in detecting intrathoracic and extra-thoracic lymphadenopathy, meta-static disease as well as predicting the prognosis, evaluating treatment response and detecting recurrence [12,13]. The basis for this is that PET-CT scan has the abil-ity to detect high glucose metabolism of tumour cells following the administration of FDG which is a radioactive -labelled glucose analogue. Interesting to note that FDG PET-CT has also been used to guide and determine the most appropriate biopsy site in cases of pleural mesothelioma [12,13].

A retrospective study of 24 cases with suspected peritoneal carcinomatosis revealed that FDG PET adds to conventional imaging in the disease staging of peritoneal carcinomatosis apart from being a useful diagnostic tool when peritoneal biopsy was either unavailable or inappropriate [14]. In another publication, FDG PET-CT done for a patient with unknown primary malignancy was shown to facilitate the diagnosis by demonstrating a lesion in the greater omentum to be metabolically active, although with limitation in detecting subcentimeter metastatic pleural nodules [4]. As for our patient, the mass at the porta hepatic region was metabolically active, associated with multiple peritoneal and nodal deposits in the absence of any abnormal radiotracer uptake in the thorax or other solid organs to suggest FDG-avid distant metastasis or other possible primary malignancy. These PET-CT scan findings which were representative of malignant mesothelioma in the abdomen had corresponded to his clinical diagnosis.

CONCLUSION

MPM is a rare malignancy that can be difficult to diagnose and treat. In this case report, the FDG PET-CT had demonstrated findings that corresponded to the clinical diagnosis of malignant mesothelioma in the abdomen with no significant scan evidence of distant metastasis or other possible sites of primaries. FDG PET-CT has a promising part to be utilised in the management of MPM. It may have a supplementary role not only in the disease staging but also in aiding the diagnostic work-up such as determining the most appropriate site for biopsy and excluding other possible site of primary malignancy.

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Original Article

TAPHONOMIC STUDY OF ADULT *SUS SCROFA DOMESTICA* IN EQUATORIAL CLIMATE IN SARAWAK, MALAYSIA.

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ABSTRACT

This was the first taphonomic study conducted in Sarawak, Malaysia. In this research, two adults female Sus scrofa domestica weighed 77 kg each were killed by machete at the heart region. In order to mimic a real human body, clothes made of pure cotton was put on the subjects. Pure cotton clothes are mostly worn by Malaysian population due to hot and humid climate. Both the carcases were placed in separate locations labelled as A and B in a jungle and taphonomic changes were recorded. All stages of decomposition namely the fresh stage, bloated stage, active decay stage, advanced decay stage and remains stage were observed and documented. Daily observation on both the remains was recorded. The ambient temperature, internal body temperature, temperature of larvae mass, body surface temperature, soil surface temperature and humidity of air were recorded daily. Time for each stage of decomposition was determined. During the decomposition process, insects and larvae appeared in each stage of decomposition process were collected and preserved for reference. Photos and videos were taken on each subject throughout the research. The hot and humid climate in Sarawak accelerates the decomposition process. The time taken for both bodies to reach remains stage was nine days. Both the cotton clothes were still preserved until the end of research. In conclusion, it took 9 days for Sus scrofa domestica to be fully skeletonised, on the ground, in an equatorial climate of Sarawak, Malaysia.

Keywords*: taphonomic study; Sus scrofa domestica; cotton clothes; decomposition process; equatorial climate*

INTRODUCTION

Taphonomic study on adult *Sus scrofa domestica* in hot and humid climate in Malaysia was studied to mimic the real human body decomposition. Study on human decomposition is not eligible for ethical approval, therefore an alternative was used for that purpose. In Malaysia, data on taphonomic changes concerning the time range is not available. Most of the research were concentrating on insect succession pattern [1, 2]. Based on that drawback, a research was conducted in Bintingor, Sarawak to find out the decomposition rate for on -the- ground body disposal. It is quite common for murder cases to have the body being dumped in the rural area or open field in the jungle.

This study was the pioneer research on taphonomic study in Sarawak. Sarawak is located in equatorial climate in Malaysia, having a high and stable temperature throughout the year. The annual mean temperature of Malaysia is 27.3 °C except for Genting Highlands and Cameron Highlands which have lower annual mean temperature of 18.6 °C [3]. In this research model, two female adults *Sus scrofa domestica* weighed 77 kg each was selected due to its size that resembles the adult human body. *Sus scrofa domestica* as a human body surrogates is an acceptable manner for scientific research [4]. It is known that, adults *Sus scrofa domestica* can be used as an analogue for human body due to its characteristic such as lack of fur, having the same weight with a normal human body, and easily obtain from the market [5-7]. Apart from that, the decomposition process for *Sus scrofa domestica* was found to be almost the same with the human body. The decomposition time for each stage was almost the same with human body under the same environmental condition [7].

This taphonomic study can generate a complete set of data which includes pattern of insect succession, post-mortem interval determination based on the decomposition rate, effects of cotton clothes on the decomposition process, effects of equatorial climate on the research study and

comparison of research study between West Malaysia and East Malaysia in the aspects of decomposition rate. The outcome of this study can benefit the enforcement body in the investigation of crime scene involving a human body as well as the researchers in this field.

MATERIALS AND METHOD

This study was conducted in the morning on 26 May 2009. Two adults *Sus scrofa domestica* carcases with weigh of 77 kg each were used in this study. The subjects were killed by piercing at the heart region with a machete, as advised by the ethical committee. After that, cotton clothes were put onto both the carcases. Later, the carcases were transferred into a jungle, on the ground of two different sites labelled as Site A and Site B. The distance of the sites was 400 footsteps away from each other. Fencing (120 cm × 70 cm × 48 cm) was built around the carcases to protect them from wild animals. In this study, cotton clothes was put onto the adult *Sus scrofa domestica* due to the preference of Malaysian to wear sweat-absorbent material like cotton for daily attire.

Observations were made for 26 days with 3 visits per day except on the first day. The first-day visit was in the morning (0800 hrs), the second visit was in the noon (1200 hrs) and the third visit was in the afternoon (1800 hrs). First day observation was conducted 4 times (1100 hrs, 1300 hrs, 1800 hrs, and 2100 hrs) to evaluate the changes of the carcasses in details. Climatological data such as ambient temperature and humidity were recorded for 26 days by using digital thermometer and hygrometer. Ground temperature, body surface temperature, internal temperature and maggot mass temperature were taken in each visit. Adult flies were caught by using sweep net. Some of the fly larvae were collected using forceps and put into 70% ethyl alcohol for preservation [8]. The specimens were processed for storage and kept as a record in the Forensic Science Department, Faculty of Allied Health Sciences, National University of Malaysia.

RESULTS AND DISCUSSION

The air humidity in the jungle of Bintangor, Sarawak for the 26 days ranged from 69% to 93% (mean 83.54 %, ± 6.21%) at Site A while ranged from 73% to 95% (mean 85.27%, ± 6.00%) at Site B. Three times of raining were recorded during the study (day-1, 9 and 13).

The ambient temperature ranged from 27.1°C to 32.4°C (mean 30.17°C, ± 1.34°C) at Site A while ranged from 27.0°C to 31.9°C (mean 29.85°C, ± 1.32°C) at Site B. Ground surface temperature recorded ranged from 26°C to 28°C (mean 27.38°C, ± 1.32°C) at Site A while ranged from 27°C to 31°C (mean 28.46°C, ± 1.30°C) at Site B. Maggot mass temperature varied from 33°C to 48°C (mean 43.14°C, ± 4.85°C) at Site A while ranged from 28°C to 51°C (mean 42.43°C, ± 7.79°C) at Site B. Internal temperature ranged from 27°C to 50°C (mean 39.23°C, ± 7.34°C) at Site A while ranged from 27°C to 55°C (mean 37.73°C, ± 8.27°C) at Side B.

Five stages of decomposition process were observed in the study. The stages were classified as fresh, bloated, active decay, advanced decay and remains (Table 1). All of the decomposition processes took a certain period of time. From the graph (Figure 1 & 2), there was a higher temperature of maggot mass temperature and internal temperature.

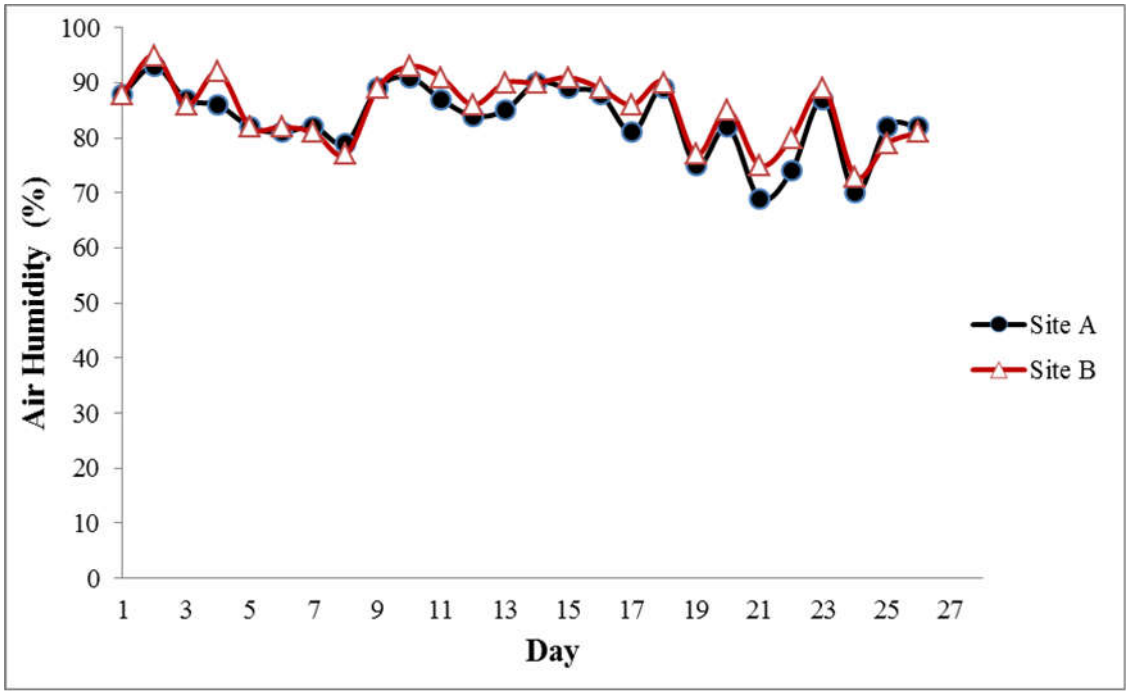

Figure 1: Air humidity in Bintangor, Sarawak

Table 1: Stages of decomposition

Stage	*Sus scrofa domestica* A	*Sus scrofa domestica* B
	Day	Day
Fresh	1	1
Bloated	2-3	2-3
Active decay	4-6	4-6
Advanced decay	7-8	7-8
Remains	9-26	9-26

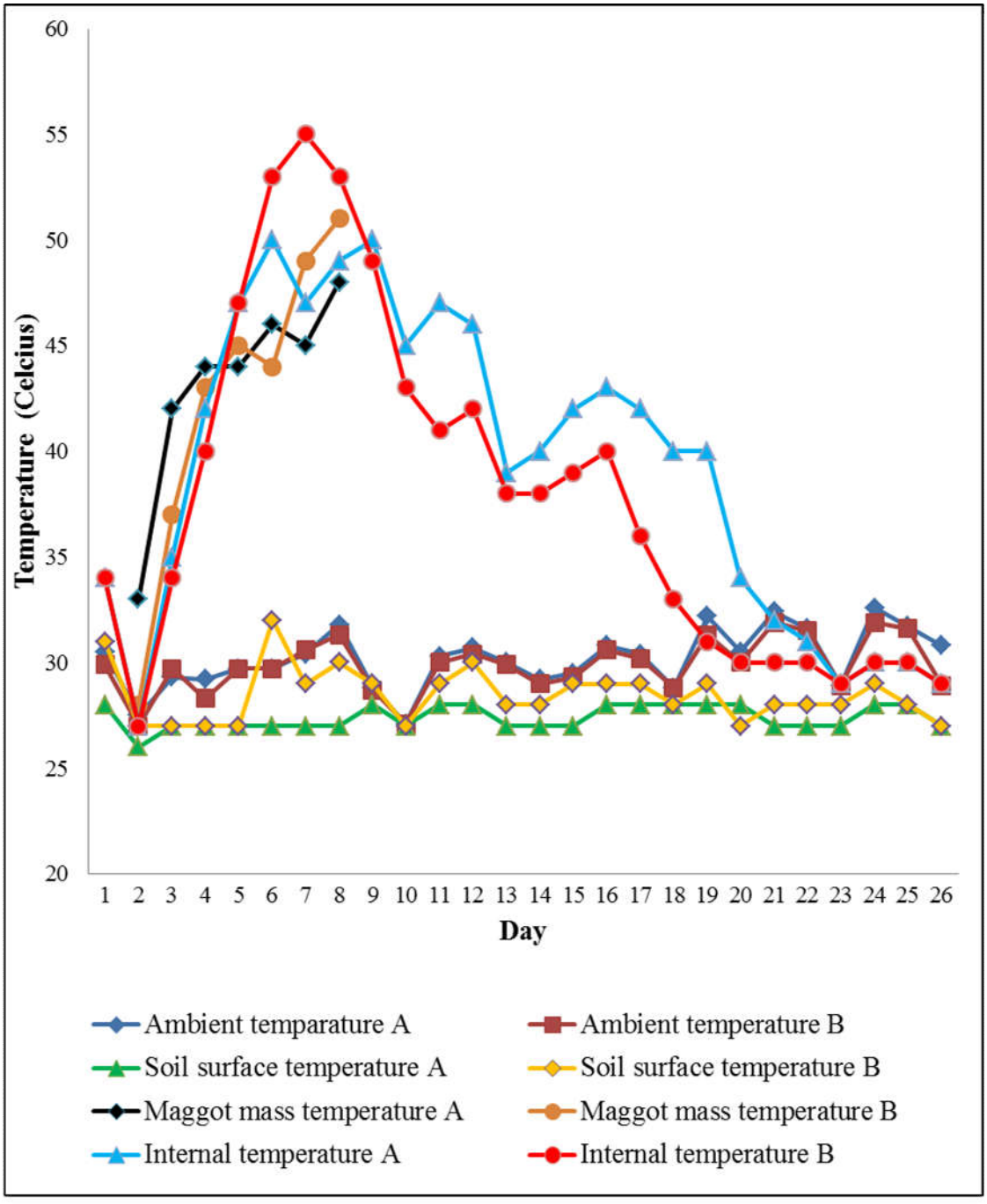

Figure 2: Data from site A and site B

DISCUSSION

In this study, two adults *Sus scrofa domestica* displayed the five stages of the decomposition process namely fresh, bloated, active decay, advanced decay and remains. Each stage had emerged at certain time period as showed in Table 1. The cotton clothes on the carcas had little or no significant effects on the decomposition process. This phenomenon could be explained through the research done in West Malaysia [2]. Only slight colour changes were noted, from original blue to brownish in colour due to contamination by putrefaction fluid. Generally, the textile material would undergo a different rate of decomposition and greatly depends on the composition, colouring, and treatment done on the textile [4]. The cotton clothes cannot withstand the acidic environment. The putrefaction process would alter the surroundings and eventually promote the microorganism activity, increase the pH and redox reactions [9]. The clothes in the study were still well preserved due to the blue synthetic colour used on the clothes. The coloured-synthetic pigment could prevent the clothes from being decomposed [10].

The air humidity in Bintangor, Sarawak is high and always above 65% during the study (Figure 1). Air humidity was higher in the morning and afternoon if compare to noon due to heat from the sun. Since Bintangor in Sarawak displayed Equatorial climate characteristic, it will accelerate the decomposition process of *Sus scrofa domestica*. The carcases have 60% to 80% water inside their body tissue. During the decomposition process, there was a competition of water between the decomposition and drying process [11]. In an extremely humid condition, decomposition process will slow down due to saturated water in the tissue [12]. On the other hand, humidity was positively correlated with the insect's activity [13]. Further research had proven that rain has little or no effects on the insect's activity [13]. In this study, the adult flies were reduced greatly during the rain but the larvae were still active. During the rain, adult flies were hiding inside the clothes worn by the carcases and behind the leaves.

The taphonomic study in Bintangor, Sarawak showed the ambient temperature of Site A and Site B were high, stable and displayed same data distribution pattern (Figure 2). Ambient temperature is vital and influential in decomposition process [12, 13]. Thus, faster rate of decomposition was observed in high ambient temperature [14]. The ambient temperature of Bintangor, Sarawak was always over 25°C during the study, thus promoting faster decomposition rate on both the carcases. The ground surface temperature at both sites did not fluctuate drastically according to the ambient temperature. Generally, the ground surface temperature is always cooler due to shading effect from the surroundings trees. So far there is no research being conducted and proven that there is a correlation between decomposition process and ground surface temperature.

Maggot mass temperature was recorded from the beginnings since the emergence of the first instar larvae which initially feeds on fluid exuded from the carcas. The first instar larvae was usually found at the mouth region which filled with bloody fluid due to the internal trauma [15]. On the other hand, the maggot mass temperature had no different with the ambient temperature but increased drastically during the emergence of the third instar larvae [14]. The maggot mass temperature of Site A and Site B reached around 50°C (Figure 2) during advanced decomposition stage (Table 1). Maggot mass temperature is always higher than ambient temperature [14]. Increased decomposition rate is directional proportional to the increased temperature [13]. In addition, the clothing worn by the carcas was providing heat to the eggs and larvae [15]. Maggot mass temperature increased drastically due to increased rate of decomposition process and biological activity [16]. Maggot mass temperature started to increase on the second day which was the bloated stage for the remains. No maggot mass temperature was recorded on the ninth day which was the remains stage commenced. This phenomenon was due to depletion of tissue for maggot consumption and all maggots had fully developed into adult flies. New maggot emergence was impossible due to lack of interest of adult flies to lays eggs on the remains.

Apart from that, internal temperature of both *Sus scrofa domestica* was 55°C which was the highest during the advanced decay stage (Figure 2). This temperature was equal to the maggot mass temperature. Thus, the active decomposition process had taken place. Furthermore, increased rate of decomposition and biological activity will directly elevate the temperature [16]. From this study, the internal temperature started to decrease on 9th day (Figure 3) signalling beginning of remains stage and decreased maggot activity. The decomposition process was decreased due to depleted soft tissue for further autolysis. The amount of insect presence was greatly reduced. According to Payne (1965), to determine starting point of remains stage immediately after the advanced decay stage is difficult. However, the starting point of remains can be determined by the surrounding flora or weeds around the carcases. On the other hand, no growing of flora or weeds was clearly observed in the study conducted in Bintangor, Sarawak. Thus, the guideline of observing the starting point of remains stage cannot be applied in this study. However, the internal temperature and maggot mass temperature are the most appropriate way to determine each of the decomposition stage.

CONCLUSION

In conclusion, the taphonomic study in Bintangor, Sarawak showed that five stages of decomposition (fresh, bloated, active decay, advanced decay and remains) were clearly defined and each of the stage took certain period of time. The cotton clothes had little or no significant effect on the decomposition process as the ambient temperature effect outweighed the clothing effect. The equatorial climate in Bintangor, Sarawak had increased the rate of decomposition process. The decomposition process of carcas in West Malaysia and East Malaysia (Bintangor, Sarawak) is almost the same which took 9 days to skeletonise *Sus Scrota domestica* of 77kg.

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Original Article

OBSERVATIONS ON THE MORPHOLOGY AND LIFE CYCLE OF *Lambornella stegomyiae* (CILIOPHORA: TETRAHYMENIDAE)

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ABSTRACT

The morphology and life-cycle of Lambornella stegomyiae, a facultative parasitic ciliate of larval Aedes albopictus, were described. Parasitic and free-living ciliates multiply by transverse binary fission. Free-living ciliates occasionally undergo conjugation. Infection in larval mosquitoes may be transtadially transmitted to adult stages when larval hosts do not succumb to the infection. Infected adults are fertile. Besides fertile eggs, female mosquitoes deposit ciliates and infected eggs during oviposition. Results suggest that L. stegomyiae may survive through droughts in infected Ae. albopictus eggs and as desiccation resistant cysts.

Keywords: *Lambornella stegomyiae*; morphology; life-cycle; *Aedes albopictus*

INTRODUCTION

Lambornella stegomyiae is a facultative parasitic ciliate of tree -hole and container-breeding mosquitoes. The ciliate was first reported from *Aedes scutellaris* larvae collected in Kuala Lumpur, Malaysia by Lamborn in 1921 and was described by Keilin (1921) [1,2]. It was subsequently reported as *Tetrahymena pyriformis* from Singapore [3], as *T. stegomyiae* from the Philippines [4], as *Glaucoma pyriformis* from North Rhodesia [5,6], as *T. stegomyiae* from South Africa [7], and as *T. stegomyiae* from the Soviet Union [8-10].

The latest description of *L. stegomyiae* was based mainly on specimens collected from South Africa [11]. There is no detail description on *L. stegomyiae* from other parts of the world. The present study provides additional information on the life-cycle of the ciliate collected in Penang, Malaysia, and the morphology of some stages in its development.

MATERIALS AND METHODS

L. stegomyiae used in the present experiment was collected from artificial containers in Penang. Infected *Aedes albopictus* larvae were brought back to the laboratory and prepared for *in vivo* cultures. A laboratory strain of *Ae. albopictus* was used as host to *L. stegomyiae* throughout the experiment. The mosquito was collected in Penang in 1985 and has since been maintained in the laboratory at Universiti Sains Malaysia at 27^{o}C and 80% relative humidity.

In vivo cultures of *L. stegomyiae* were set up in Petri dishes (2 x 9 cm) in the laboratory. A fourth instar mosquito larva infected with ciliates was placed inside a Petri dish with 40 ml of culture medium. This medium was prepared by mixing equal amounts of boiled field water and deionized water. Tetramin^{R} fishfood was used as food for the larva and was added when necessary. Usually the ciliates multiply in the hemocoel of the larva until they fill the whole hemocoel. At the end of their development in the larval hemocoel, the ciliates break out from the mosquito larva. The ciliates are then ready for reinfection. At that stage, the larva was teased to free the rest of the ciliates from the hemocoel and 10 newly hatched first stage mosquito larvae were added into the Petri dish. No food was administered during the first 24 hr after the larvae were exposed to the ciliates. Lack of food retards growth and delays molting of the first instar larvae and thus increases the chance of infection. Stages of development of ciliates were observed fresh in live hosts or fixed in 70% alcohol under a compound microscope (100-1000x).

The silver carbonate method was used to stain the infraciliature and the nuclei of the ciliates [12]. Ciliates that break out from their hosts after completing their parasitic

cycle were first transferred into transparent screw-capped plastic bowls (9 x 9 cm) containing 200 ml culture medium and allowed to live as free living ciliates for 48 hr. The ciliates were then picked, stained and observed under a compound microscope (100-1000x).

Infected mosquito larvae that did not succumb to the infection and pupated were transferred into a bowl in a mosquito cage (30 x 30 x 30 cm) and allowed to emerge. Sugar solution was introduced into the cage as food for the adult mosquitoes. The bowl was removed once all the pupae had emerged. Adult mosquitoes were then blood-fed on white mice. A Petri dish containing 40 ml culture medium was introduced for oviposition. The Petri dish was examined daily for ciliates under a compound microscope (100 - 1000x).

RESULTS

Life-cycle and sexuality

The development of *L. stegomyiae* was observed in 30 *Ae. albopictus* larvae in 3 Petri dishes, each containing 10 larvae. Observations were made at 4 hr intervals. Formation of cuticular cysts was first observed 4 hr after the larvae were exposed to ciliates (Fig.1). The largest number of cuticular cysts was observed at 12 hr after exposure. The number of free living ciliates in the Petri dishes decreased with time and can hardly be found at 16 hr. Once attached to the cuticle, the ciliates within the cuticular cysts went on to penetrate into the hemocoel of the mosquito larvae. The first ciliate was observed in the hemocoel 12 hr after exposure. By 16 hr most ciliates had penetrated into the hemocoel and they were seen as small rounded forms slowly rotating in about the same spot. The empty cysts were left attached to the outside of the larval cuticle. Between 16 to 92 hr after exposure, the ciliates transformed into large, sluggish but actively dividing ameboid forms. Multiplication was by binary fission. From 88 hr, the ameboid forms began to retransform back into rounded forms. The locomotion of these rounded forms was more active than the ameboid forms. Multiplication was sustained at a rapid rate and by 188 hr, the ciliates filled the entire hemocoel of the host. At 136 hr, the ciliates began to transform into swift swimming pear-shaped ciliates. At 212 hr, the pear-shaped ciliates were seen escaping from dead mosquito larvae into the surrounding medium. Ciliates were also seen escaping from live mosquito larvae. Some punctured larvae lived for several hours before dying.

Ciliates that broke out from the mosquito larvae continued to multiply asexually and sexually in the culture medium. Asexual reproduction was by transverse binary fission with the division plane cutting across the kineties (Fig.3). The fission began with the division of the micronucleus followed by the division of the macronucleus. The division was accomplished by a transverse constriction. Sexual reproduction was achieved through conjugation (Fig. 4). Pairs of ciliates were seen to remain fused anterior-laterally for hours.

***Morphology of ciliates* (n=25)**

The general shape of the free living ciliates from *in vivo* cultures (fixed in 70% alcohol) was pear-shaped (Fig. 5). The buccal overture was round or oval and located ventrally in the anterior fourth of the body. The preoral suture was located to the left of the buccal overture. Mean body length 70.3 µm (range 48.6-89.5); mean body width 50.5 µm (range 29.5-65.7); macronucleus and micronucleus single; mean width of macronucleus 25.6 µ m (range 18.1-34.3); mean width of micronucleus 4.7 µm (range 3.8-6.7); median number of kineties 38 (range 31-43); median number of postoral meridians 4 (range 2-5).

Infection in adult mosquitoes

Six adult male and 6 adult female mosquitoes emerged from pupae that did not succumb to the infection. The

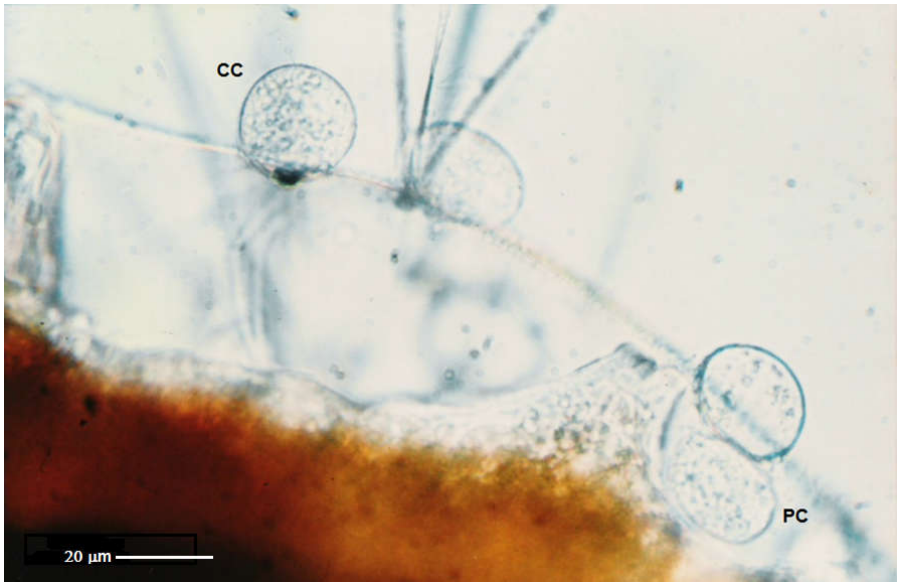

Figure 1: Cuticular cysts (CC) of *Lambornella stegomyiae* on cuticle of first-instar larva of *Aedes albopictus*. Note the newly penetrated ciliate (PC).

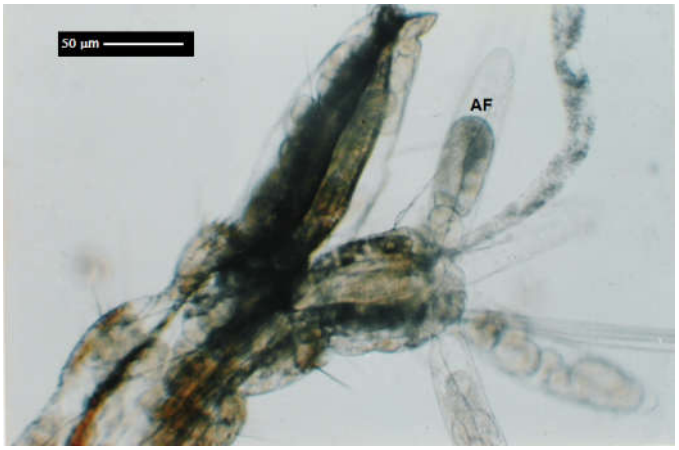

Figure 2: Ameboid forms (AF) of *Lambornella stegomyiae* in the anal papilla of *Aedes albopictus* larva.

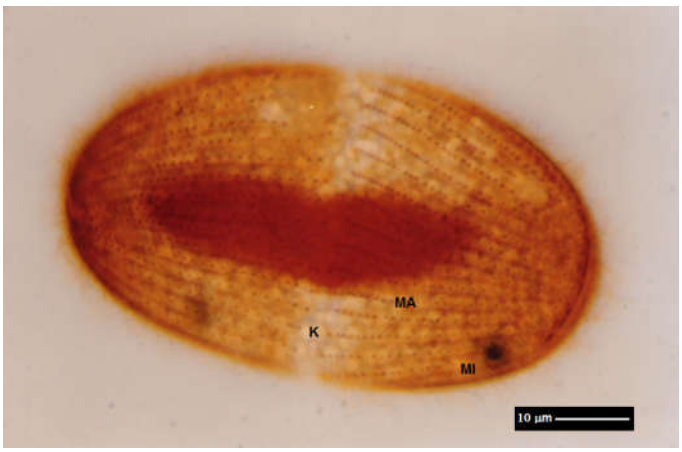

Figure 3: Silver carbonate preparation of a *Lambornella stegomyiae* trophont undergoing binary fission. Fission began with the division of the micronucleus (MI) and followed by the macronucleus (MA). The fission plane cuts transversely across the kineties (K).

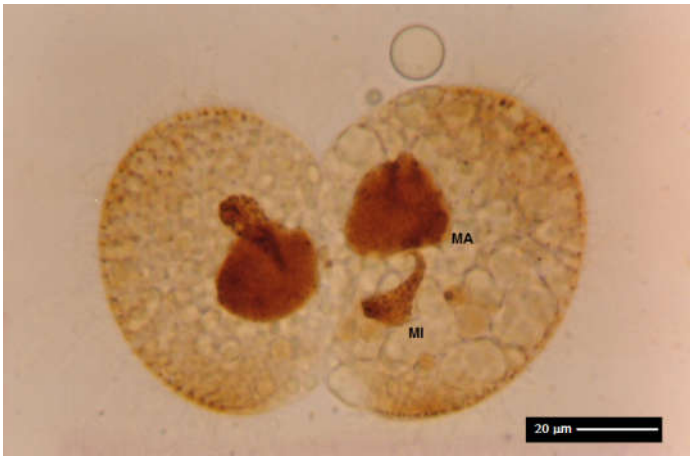

Figure 4: Silver carbonate preparation of a pair of conjugating *Lambornella stegomyiae*. Note the macronuclei (MA) and the dividing micronuclei (MI).

Figure 5: Silver carbonate preparation of *Lambornella stegomyiae* trophont showing the undulating membrane (UM), the tripartite adoral zone of membranelles (AZM), preoral suture (PS), macronucleus (MA) and micronucleus (MI).

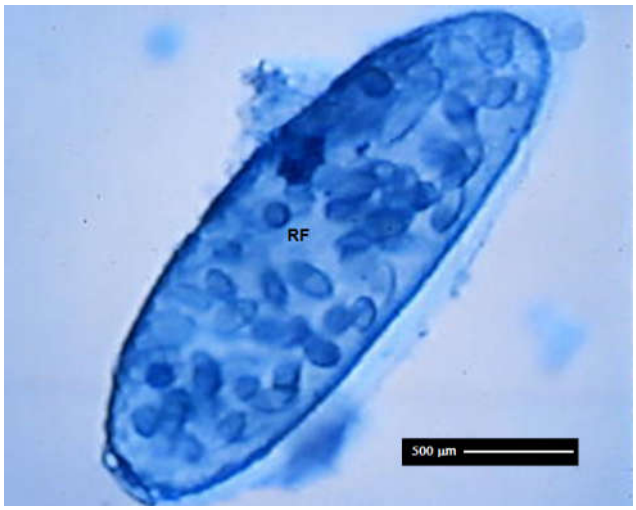

Figure 6: Rounded forms (RF) of *Lambornella stegomyiae* in an *Aedes albopictus* egg.

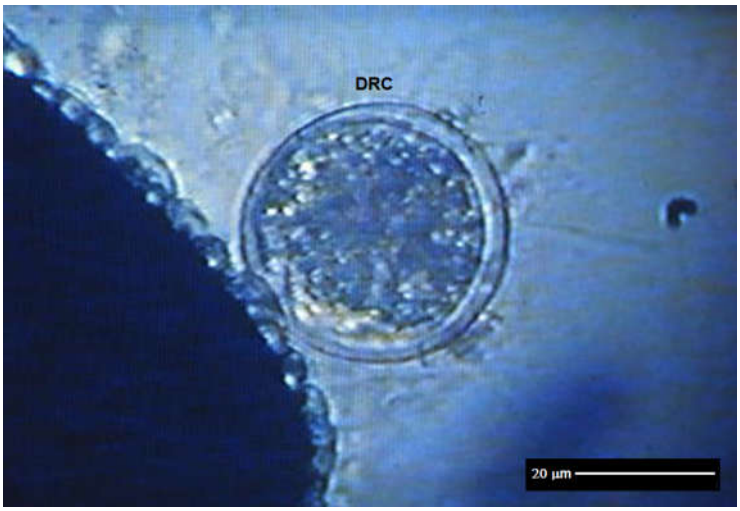

Figure 7: Desiccation resistant cyst (DRC) of *Lambornella stegomyiae* next to an *Aedes albopictus* egg.

infected adult female mosquitoes laid fertile eggs in the Petri dish placed in the mosquito cage. When the eggs were dried and hatched, the larvae were free from ciliates. Two eggs were infected with ciliates (Fig. 6). Both were almost completely filled with rounded and pear-shaped ciliates. No cuticular cysts were found on the egg shells. The eggs were ruptured for closer examination of the ciliates. Some uninfected eggs in the Petri dish had cuticular cysts. However, none of the ciliates from the cysts penetrated into the eggs. When the eggs hatched, the ciliates remained in the cysts on the egg shells. Examination of the culture medium in the Petri dish showed the presence of free living ciliates. Infection was attained when first stage mosquito larvae were exposed to the ciliates. Two desiccation resistant cysts were found at the bottom of the Petri dish (Fig. 7). The cysts were spherical with a diameter of 40 μm. The single ciliate in the cyst could be seen sluggishly rotating within the transparent cyst wall.

DISCUSSION

Three distinct forms of *L. stegomyiae viz*., spherical, ameboid and pear-shaped have been observed during the development of the ciliate in the hemocoel of larval *Ae. albopictus*. Different forms of *L. stegomyiae* in larval mosquitoes had earlier been reported by Muspratt (1945). He observed two types of ciliates - a rapidly multiplying form and a larger slowly multiplying form in *Aedes* and *Culex* mosquitoes in Livingstone, Northern Rhodesia. He later concluded that the 2 types of ciliates were actually different forms of the same ciliate *Glaucoma pyriformis* (probably *L. stegomyiae*) but in mosquito hosts of differing susceptibility [6]. While different forms may be attributable to differing host susceptibility, we found that morphological transformations always occur during the development of the ciliate in its natural host.

L. stegomyiae multiplies by binary fission during its parasitic and free living existence. Conjugations were observed only among free living ciliates. As conjugations were rare and occurred with low frequency, only a few pairs of conjugants were observed and only one pair was successfully stained. The fate of the exconjugants were not known. All conjugating pairs were seen attached anterior-laterally (the anterior ends of the ciliates were more strongly tapered than the posterior). There is reason to suppose that the ciliates adhere in a typical ciliate fashion at the oral region.

Lamborn (1921) described the shape of *L. stegomyiae* from Kuala Lumpur as pear-shaped [1]. Keilin (1921) examined Lamborn's collection preserved in formalin and described the shape of the ciliates and as generally elongately oval but occasionally pear-shaped [2]. Corliss and Coats (1976) examined preserved samples of *L. stegomyiae* from South Africa and Kuala Lumpur and reported that the ciliates were somewhat spindle-shaped with mean body measurements of 78 x 22 μm [11]. The shape of *L. stegomyiae* within any single sample may vary from pear-shaped to spindle-shaped. We observed some variation in the shape of the ciliates in our samples but they were generally pear-shaped. It is possible that Corliss and Coats based their description mainly on *L. stegomyiae* from South Africa which might have been spindle-shaped. Furthermore, *L. stegomyiae* described by Corliss and Coats had fewer number of kineties (30) than those described in the present study (38) although they are of about the same length.

Although the oviposition behavior of the infected adult mosquitoes was not followed, there is reason to believe that ciliates were deposited by adult female mosquitoes during oviposition. Egerter *et al.* (1986) reported that females of *Ae. sierrensis* infected with *L. clarki* were parasitically castrated [13]. The females exhibited oviposition behavior by which ciliates were actively dispersed. However, *Ae. albopictus* females infected with *L. stegomyiae* in the present study were not castrated and were able to lay fertile eggs.

Ciliates survive occasional drought seasons in desiccation resistant cysts and in mosquito eggs. The eggs must have been infected while they were inside the mosquito and before the egg shells were deposited as no cuticular cysts were found on them. Once deposited, *Ae. albopictus* eggs were impenetrable to the ciliates. Surviving in mosquito eggs has advantages over surviving in desiccation resistant cysts. The ciliates multiply in mosquito eggs like they normally do in larval mosquitoes. The infected eggs hatch simultaneously with embryonated eggs and immediately infect the first stage mosquito larvae. Infection and multiplication in mosquito eggs is important for the survival of the ciliates. Tropical mosquitoes like *Ae. albopictus* develop rapidly during their larval stages. The larval instars may only take about a week. Thus parasitic ciliates like *L. stegomyiae* must find a mechanism by which they can quickly infect the mosquito larvae so that they can complete their life-cycle before the larvae pupate.

ACKNOWLEDGMENTS

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Case Report

PERFORMING PHACOEMULSIFICATION WHILE STANDING FOR PATIENT WHO IS UNABLE TO LIE FLAT

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ABSTRACT

Phacoemulsification in patients who are unable to lie flat can be challenging for ophthalmic surgeons. Difficulty in positioning the head can lead to inadequate surgical exposure, and awkward position of the body may lead to unnecessary high vitreous pressure and subsequent posterior capsule rupture. We describe a standing phacoemulsification technique for bilateral cataract in a patient who is morbidly obese, positioned at 30 degrees inclination in a reverse Trendelenburg position with hyperextension of the neck.

Key words: *phacoemulsification, reverse Trendelenburg position, morbid obesity*

INTRODUCTION

Patients who are unable to lie flat during cataract surgery due to various causes such as cervical kyphosis, morbid obesity and obstructive sleep apnea (OSA) present a challenge to the ophthalmic surgeon. This condition can also complicate the surgery as the patient will assume improper positioning and making the surgeon uncomfortable [1]. Obese patients particularly are prone to have increased intracranial venous pressure and intraocular pressure from raised intra-abdominal pressure due to central obesity on lying flat [2]. They are at increased risk of intra operative complications such as posterior capsular tear due to high vitreous pressure and in return, shallow anterior chamber [3]. Furthermore, the risk of anaesthetic complications is higher making them unsuitable for general anaesthesia. Other issues such as associated comorbidities, difficult intravenous access and post-operative care also need to be considered when subjecting these patients to surgery [3].

Phacoemulsification with the surgeon in a standing position for patients who are unable to lie supine has been reported and proposed by a few surgeons [4]. Obesity and respiratory problems account for about 60% of the cases [3, 5]. Unfortunately, the operating room microscope is in an awkward angle of approach in patients who are seated or in a partially reclined position. Forward movement of the posterior capsule and vitreous due to gravitational pull can occur when the head is upright. Together with shallowing of the anterior chamber, this caused an increased risk for posterior capsule rupture and vitreous loss [6].

We report a case in which phacoemulsification was performed for bilateral cataract in an obese patient with OSA who cannot lie flat and was seated at 30 degree position and his neck hyper extended.

CASE REPORT

A 56 years old obese man with underlying OSA requiring bilateral positive airway pressure (BiPAP) machine during sleep had significant bilateral posterior subcapsular cataract in both eyes. The best corrected visual acuity was 3/60 in the right eye and 6/60 in the left eye. He was scheduled for bilateral cataract extraction using the standing phacoemulsification technique (STP) by a single surgeon on different occasions.

Intraoperatively, the patient was seated on a dental chair reclined at 30 degrees. This position has been described as reverse Trendelenburg position (RTP) from previous authors [3]. The head rest was levelled so that the patient's neck is hyperextended allowing the face to be on a flat plane beneath the operating microscope (Figure 1). Both surgeries was done using the Zeiss operating microscope (Carl Zeiss, Oberkochen, Germany) and the surgeon was standing at a temporal approach from the patient. The microscope pedal was controlled by the surgeon (Figure 2).

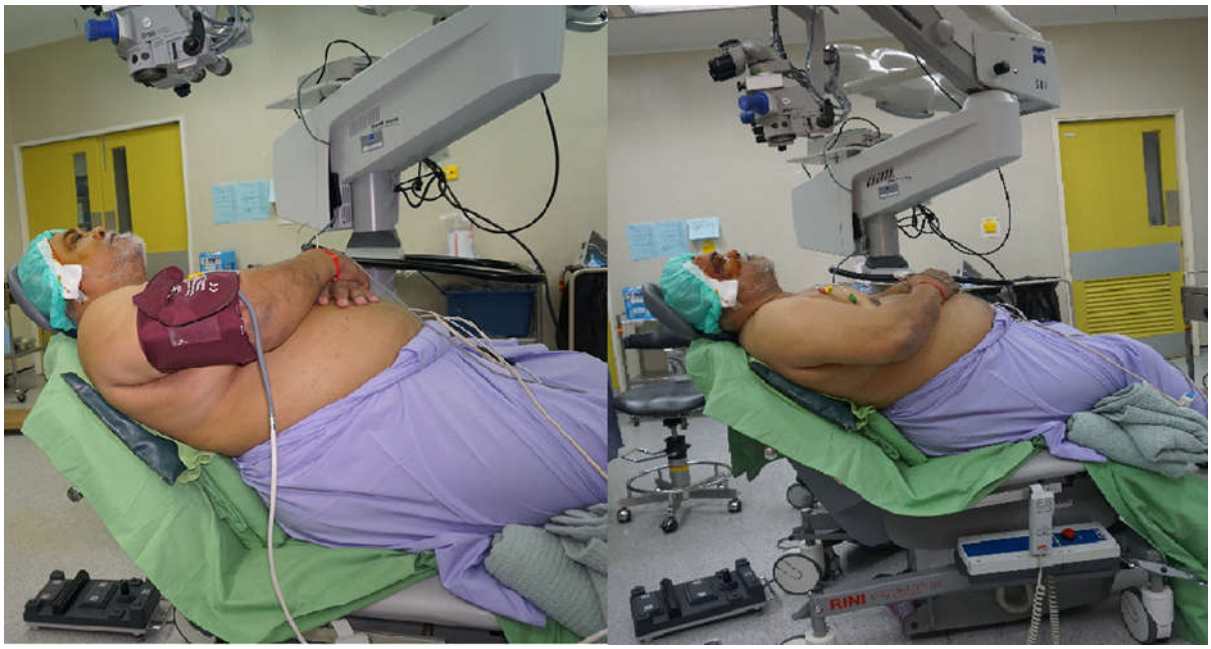

Figure 1 Patient at 30 degree reclined position (Left), with the face levelled to a flat plane beneath the microscope (Right)

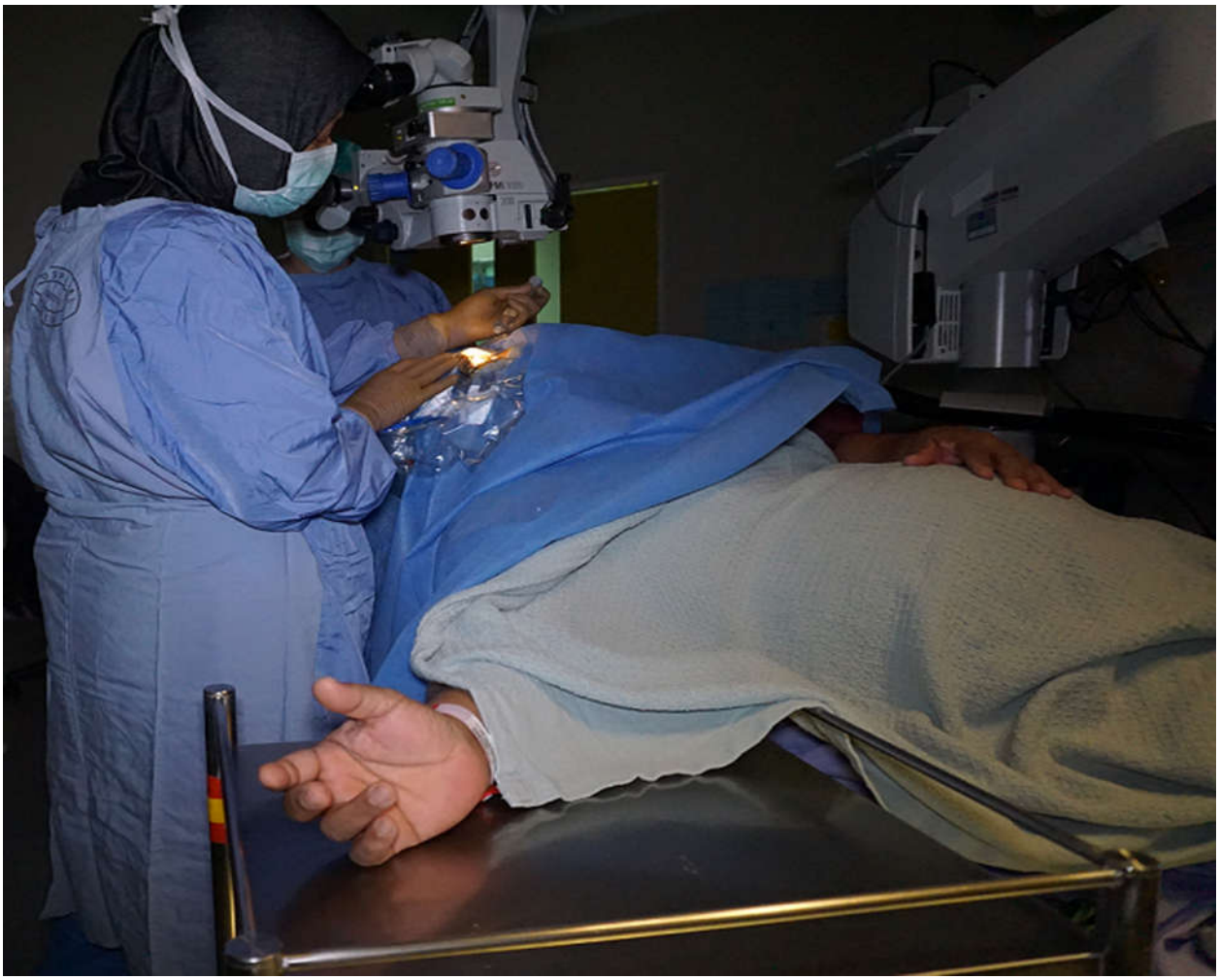

Figure 2: Surgeon in standing position with right foot controlling the microscope pedal

The surgery was performed under subtenon local anaesthesia using a temporal clear corneal approach. The rest of the surgery was performed in the usual manner and was uneventful. The post-operative course went smoothly. At five weeks post procedure, his visual acuity was 6/9 in the right eye and 6/12 in the left eye, and the patient was satisfied with this vision.

DISCUSSION

Modern day cataract surgery via small incisional phacoemulsification has become the procedure of choice. Medical conditions and neck deformities restrict patients from lying supine to achieve optimum positioning during conventional seated surgery. Morbid obesity can further impose intra operative complications such as posterior capsular tear and vitreous loss due to high vitreous pressure [2].

Modifications in certain steps of the usual phacoemulsification technique have enabled surgeons to perform cataract surgery in difficult patients. Similar outcomes in visual acuity between standing surgery and the conventional seated surgery have been reported. SPT showed superb safety profile with no intra operative complications [7]. Mansour et al reported standing phacoemulsification in reverse Trendelenburg position (RTP) in four eyes of 3 morbidly obese patients with no complications in all aptients [3]. However, other authors have reported posterior capsular tear requiring anterior vitrectomy and retained cortical matter with the standing technique that was done using headlamp illumination and magnification with a loupe [5].

As opposed to other reports where cataract extraction was done via loupe magnification or the microscope pedal was controlled by a third person [4, 5], the surgery in our patient was performed using the standard operating microscope with the surgeon standing and controlling the pedal herself. We did not encounter any serious complications using this technique.

For obese patients undergoing cataract surgery, this technique in fact might be advantageous. Lowering of intracranial venous pressure and vitreous pressure can be achieved by 15 to 30 degree RTP in morbidly obese individuals. This position can tremendously improve alveolar-arterial oxygen difference to baseline limit. Furthermore, the 30 degree RTP is thought to be the best position for anaesthesia in morbidly obese patients [3].

Intra operative shallowing of the anterior chamber due to high vitreous pressure can be overcome by the use of an anterior chamber maintainer [3]. Shallowing of the anterior chamber in SPT is mainly due to the effect of gravity in seating position, pushing the posterior capsule and vitreous body forward [6]. In this patient, although he was seated, his hyper extended neck allowed his head to be positioned on a flat plane beneath the operating microscope to achieve a good red reflex. Furthermore, with the neck in hyperextended position, the effect of gravity towards the eyes can be ameliorated and the risk of posterior capsular tear reduced.

CONCLUSIONS

Standing phacoemulsification technique offers an alternative method to the modern day cataract surgery. It gives a new hope for cataract removal in difficult patients that cannot undergo conventional seated surgery even under general anaesthesia. Further studies may determine the safety profile and visual outcomes of this technique in comparison to the conventional seated phacoemulsification. However, we recommend this technique to be performed by an experienced senior surgeon. Early removal of visually significant cataract should be considered in difficult patients with problematic comorbidities.

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Original Article

A 5 YEARS RETROSPECTIVE STUDY ON POST-MORTEM CASES INVOLVING MOTOR VEHICLE ACCIDENTS (MVA) IN HOSPITAL PULAU PINANG

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ABSTRACT

Motor Vehicle Accidents (MVA) is one of the common cases that was brought to Forensic Department for post-mortem examination. Injuries due to MVA had been very serious and fatal. Based on this, a retrospective study was conducted on post-mortem cases involving MVA cases starting from 1st January 2008 until 31st December 2012 at Jabatan Perubatan Forensik, Hospital Pulau Pinang. This study was conducted to determine the demographic data with regards of gender, race, age, and common cause of death involving MVA victims. A total of 604 cases obtained from post-mortem record books. The result showed that males victims (85.3%) were higher compared to female (14.7%). Categorization based on race indicates that Chinese (45.4%) dominated the other races followed by Malay (32.9%), Indian (15.4%), and others (6.3%). Those aged between 21-30 years (20.4%) had the highest incidence among other age groups. The most common cause of death due to MVA was head injury (56.6%). In conclusion, post-mortem examination on dead body due to MVA should not be underestimated. Proper attentions must be taken to prevent fatal injuries among MVA victims.

***Key Words**: post-mortem, motor vehicle accidents*

INTRODUCTION

Motor Vehicle Accidents (MVA) is one of the common cases was brought to Forensic Department for post-mortem examination. Each year, many victims have died due to accidents especially during festival seasons. The victims are not only Malaysians, but also include foreigners. According to Malaysian Institute of Road Traffic Research (MIROS) [1], there were more than 400,000 of road accidents and more than 6,000 death in the year 2012 only. There was an increasing pattern of death due to road accidents in Malaysia even though there were many campaigns conducted through-out the year. They also predicted that number of fatalities in 2020 will be up to 10,716 deaths. More efforts are needed and effective interventions should be made in order to reduce death among MVA victims. Therefore, a study was conducted on post-mortem cases involving MVA in Hospital Pulau Pinang.

MATERIAL AND METHOD

A retrospective study of 5-years duration from 2008 until 2012 was conducted in Jabatan Perubatan Forensik, Hospital Pulau Pinang. The data was collected from post-mortem record books. Inclusive criteria were including Motor Vehicle Accidents (MVA) cases and only those who were brought in dead and examined in Jabatan Perubatan Forensik, Hospital Pulau Pinang.

Data collected were including gender, race, age, and cause of deaths among MVA cases and analysis was done using SPSS 20.0.

RESULT

Results showed that post-mortem examination among Motor Vehicle Accidents (MVA) victims in the year 2010 (22.4%) was the highest compared to other years (Figure 1). In terms of gender, males (85.3%) predominantly higher compared to females (14.7%) (Figure 2). Chinese (45.4%) was the highest reported post-mortem cases due to MVA in terms of race followed by Malay (32.9%), Indians (15.4%), and others (6.3%) (Figure 3). The most common age group was between 21-30 years (20.4%) (Figure 4). Head injury (56.6%) was the highest cause of death due to MVA (Figure 5).

Year	Frequency	Percentage (%)
2008	105	17.4
2009	118	19.5
2010	135	22.4
2011	116	19.2
2012	130	21.5
Total	604	100.0

Figure 1: Distribution of Post-Mortem Cases by Duration of 5 Years (2008-2012)

Gender	Year of Death					
	2008	2009	2010	2011	2012	Total
Male	93	96	114	102	110	515 (85.3%)
Female	12	22	21	14	20	89 (14.7%)
Total	105	118	135	116	130	604

Figure 2: Distribution of Post-Mortem Cases of Motor Vehicle Accidents by Gender According to Year

Race	Year of Death					
	2008	2009	2010	2011	2012	Total
Malay	32	39	50	38	40	199 (32.9%)
Chinese	55	50	53	55	61	274 (45.4%)
Indian	15	19	20	17	22	93 (15.4%)
Others	3	10	12	6	7	38 (6.3%)
Total	105	118	135	116	130	604

Figure 3: Distribution of Post-Mortem Cases of Motor Vehicle Accidents by Race According to Year

Age	Year of Death					
	2008	2009	2010	2011	2012	Total
< 1	0	1	1	0	0	2 (0.3%)
1-10	0	1	4	1	1	7 (1.2%)
11-20	14	21	28	24	25	112 (18.5%)
21-30	16	31	24	22	30	123 (20.4%)
31-40	16	14	14	8	14	66 (10.9%)
41-50	17	7	12	14	12	62 (10.3%)
51-60	17	14	20	16	20	87 (14.4%)
61-70	14	15	19	23	15	86 (14.2%)
71-80	11	12	9	7	10	49 (8.1%)
81-90	0	2	4	1	3	10 (1.7%)
Total	105	118	135	116	130	604

Figure 4: Distribution of Post-Mortem Cases of Motor Vehicle Accidents by Age Groups According to Year

Cause of Death	Year of Death					Total
	2008	2009	2010	2011	2012	
Head Injury	64	59	74	73	74	344 (56.6%)
Massive Bleeding	1	0	1	3	1	6 (1.0%)
Multiple Injuries	30	48	45	22	35	180 (29.8%)
Crashed injuries to thorax	5	5	6	9	10	35 (5.8%)
Abdominal Injury	4	3	7	7	5	26 (4.3%)
Neck Injury	1	3	0	2	2	8 (1.3%)
Others	0	0	2	0	3	5 (0.8%)
Total	105	118	135	116	130	604

Figure 5. Distribution of Post-Mortem Cases of Motor Vehicle Accidents by Cause of Death According to Year

DISCUSSION

In this study, male and female ratio among the victims was 4 : 1. More than 80% of males died due to Motor Vehicle Accidents (MVA) during the 5 years (2008-2012) duration. The ratio was in conformity with the previous study conducted in India where there was a marked male preponderance 88.77% and another study conducted in University Malaya Medical Center (UMMC) showed that 57.6% male involved in the fatal MVA [2, 3, 4]. According to Malaysia Labour Force Survey Report 2012 [5], in Pulau Pinang there was 80.9% of working men and 56.5% women. The majority was men could be due to men as the predominantly the earning member of the family. They use road frequently to work thus put them in a higher risk of getting involved in MVA.

The highest post-mortem cases involving MVA was observed among the age group 21-30 (20.4%) followed by 11-20 years (18.5%) and the lowest 0-1 year old (0.3%). Similar age distribution of MVA victims has also been reported in several studies where the age group 20-30 had the highest percentage which is 44.19%; and in Malaysia, those age group 21-50 years account for 59% [5, 7-10]. Teens and young adults are more likely than older drivers to underestimate dangerous situations or not be able to recognize hazardous situations. Factors such as speeding, recklessness, beating red lights, overtaking and indiscriminate lane change coupled with the fact that motorcyclists are exposed make them more vulnerable to accidents.

The race with the highest cases of post-mortem examination involving MVA was Chinese with percentage of 45.4% of all cases. This was followed by Malays (32.9%) and Indians (15.4%) and others (6.3%). According to Department of Statistics Malaysia, Chinese accounts for 41.5% of total Pulau Pinang population followed by Malay (40.9%), Indians (9.9%), and others (0.3%) [11, 12]. Therefore there are higher chances of the victims involved in the MVA in Pulau Pinang to be Chinese, followed by Malays and Indians. However another study conducted in University Malaya Medical Centre (UMMC) revealed that Malay involved in MVA accounts for 60% followed by Indians (21%), Chinese (16%) and other ethnic groups (3%) [5]. The result was different due to population and proportion in races of Pulau Pinang and Kuala Lumpur.

The results showed that there was a marked preponderance of younger age group of Chinese male in the MVA in Pulau Pinang during the five years duration. In the list of cause of death (Figure 5), it can be seen that the most common cause of death in motor vehicle accidents and brought into Hospital Pulau Pinang was due to head injury with a total of 342 cases out of 604 cases. This was followed by multiple injuries (180), crashed injuries to thorax (35), abdominal injury (26), neck injury (8), massive bleeding (6), others (5) and skull fracture (2).

Department of Neurosurgery, Hospital Sultanah Aminah, Johor Bahru stated that severe traumatic brain injury is one of the major cause of death in Malaysia [13]. Head injury is the most common injury to occur towards motorcyclist in motor vehicle accident, in which a study done stated that open wounds and superficial injury to the head make up (69.3%) of the cases followed by upper extremity (27%) and lower extremity (24%) [14]. Another study [15] also shown that there were few differences in types of injuries sustained by riders and pillions though riders had a significantly lower risk of crush injuries of the lower extremity than pillions and overall, (11%) motorcyclist users died, of which (42.8%) died before reaching the hospital [16].

Traumatic brain injury is also the leading cause of death in United States of America based on the statistics from Brain Trauma Foundation of the USA [17]. Brain is the control centre of the body, if there is injury to the brain or the blood supply to the brain is cut off, death can occur rapidly. Probably, this was the reason of head injury being the highest cause of death in motor vehicle accidents in Pulau Pinang.

The number of MVA cases gradually increased from 2008 to 2010 but dipped slightly in 2011. In 2012, the numbers continued to rise again.

There were several limitations about this study. Firstly, this study did not represent the data for the whole Malaysia. This study may only represent Pulau Pinang state. Secondly, although there were many recorded road fatalities in Malaysia, but post mortem was not conducted for all of the cases, especially at east coast of Peninsular Malaysia. This may be, due to religion and culture restrictions. Therefore, it may not represent the real scenario in Malaysia. Besides that, we were not able to show data about vehicle that commonly involved in MVA due to time constraints. It is hoped that this research will be continued and improvised. In the future, it would be great if this kind of research can be conducted all over Malaysia. According to MIROS, they predicted in the year 2020 the number road fatalities will be up to more than 10,000 deaths. Hopefully this research can alert the public to reduce the death rate due to MVA.

CONCLUSION

In conclusion, post-mortem examinations revealed that common cause of death due to Motor Vehicle Accidents (MVA) in Pulau Pinang was head injury with age group of 21-30 years old as the most common age. MVA cases were also seen more in men and Chinese. Thus, road safety education and regulation should be more thoroughly reinforced.

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Case Report

A CASE OF *Acanthocheilonema reconditum* IN A DOG

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ABSTRACT

Acanthocheilonema reconditum is a blood sucking microfilarial parasite of zoonotic importance. It has a wide distribution among the world's continents and can cause cutaneous and subcutaneous problems. This is the first recorded case reported in Malaysia of A. reconditum in a dog, during a routine blood evaluation taken for an export quarantine evaluation at a private veterinary hospital. Incidental skin lesions were present. This species of microfilaria should be considered based on its geographic distribution as a differential for itching and erythema in a dog and due to its zoonotic significance.

Keywords*: Acanthocheilonema reconditum, dog and zoonosis*

INTRODUCTION

Abundance of vectors and favourable climatic conditions in addition to presence of suitable hosts are recognised as key determinants of the success of *Acanthocheilonema reconditum* [1]. Many vector borne parasitic diseases i.e. filariasis, leishmaniass and onchocerciasis are known to cause different cutaneous lesions [2] like pruritic dermatitis, erythema, papules, focal and multifocal alopecia, crusting and nodules [3]. Circulating microfilariae of *Dirofilaria repens, D. immitis* and *A. reconditum* are common microfilarial vector born parasite that have been associated with cardiac and subcutaneous lesions [1]. *A. reconditum has* been described in humans and has been considered as a zoonotic parasitc causing cutaneous disease [4]. *A. reconditum's* life cycle starts with ingestion of the vector (fleas and lice) infested with L3 stage of larva [4]. The adult *A. reconditum* resides subcutaneously and releases microfilariae in blood that is subsequently ingested by the vectors while feeding on the dog [5].

CASE REPORT

A 5 year and 3 months old spayed beagle was presented for a routine export evaluation. From the history, the owner had mentioned that the pet had a short history of gastritis, was active and alert apart from intermittent symptoms of itching and erythema without alopecia. The pet was fed on a heart worm prevention program according to American Heartworm Society [6] and was on routine monthly frontline spot on. Eight month earlier, a blood sample was taken and confirmed on a blood smear examination as negative for microfilaria and other blood parasites. Haematology and biochemistry results were normal using the acid phosphatase staining method [7].

The veterinarian suspected atopic allergy, however the 8-month timeline and the sudden onset of skin lesions was not in line with the pathogenesis of atopic allergy. This patient had been in Malaysia for a number of years and no filaria were detected in the previous blood samples. Skin scrapings, hair pluck and sticky tape evaluations confirmed a low population of *Microsporum gypseum*. Two out of the 3 blood smear impressions confirmed the presence of uncommon microfilaria larvae. The country of destination (South Africa) requested for blood sample report from approved laboratory to clarify the filaria status. Due to export requirements for clarification, the blood sample was submitted to a veterinary diagnostic laboratory at Universit of Pretoria (South Africa), where that filaria *A. reconditum* was confirmed in the blood sample. Treatement was based on filaria treatment protocol according to American Heartworm Society [6]. A dose of ivermectin had been administered also after 2 weeks from starting heartworm protocol. Malacetic shampoo plus chlorhexidine are also given as a broad spectrum action to cover any suspected infection.

DISCUSSION

A. reconditum (Grassi 1889) (Spirurida, Onchocercidae) is a vector-borne parasite of dogs [4]. It is the sole or prevalent inhabitant in dogs. The information about *A. reconditum* is scarce despite its wide distribution in the world. The geographical distribution of *A. reconditum* widely covers the world's regions like the Mediterranean Basin, Middle East, South Africa, South America and Oceania [4, 5]. Intermediate host is a mandatory step to complete the life cycle of *A. reconditum* [5]. Fleas like *Ctenocephalides canis, C. felis, Pulex irritans, P. simulans, Echidnophaga gallinae* and lice like *Heterodoxus spiniger, Linognathus setosus* plays a major role in vectoring *A. reconditum* [4].

Nevertheless, not much data is presently available on *A. reconditum*. It is not yet clear if infective larval stage are transmitted through the ingestion of infected vectors or through vector's bites [5]. In this case under current study, it had been diagnosed positive for *A. reconditum* and atopic allergy. The positive result of *A. reconditum* based on the result of the acid phosphatase staining method [7].

It was not possible for the authors to confirm if the skin itching and erythema were caused by which of them exactly. *A. reconditum* can be treated with the heartworm protocol [6] however further protocol for others parasites and for skin lesions also should be conducted.

CONCLUSION

Microfilaria (*A. reconditum*) in dogs should be considered as one of the differentials during diagnosis of skin lesions, especially with a history of dirofilariasis and/or ectoparasites. Laboratory examinations should be done to exclude and/or confirm the possibility of *A. reconditum*.

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Expert Opinion

MEDICAL SURVEILLANCE ON CHEMICALS HAZARDOUS TO HEALTH IN MALAYSIA

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1. INTRODUCTION

Medical surveillance as defined under the Occupational Safety and Health (Use and Standards of Exposure of Chemicals Hazardous to Health) Regulations 2000, or USECHH Regulations 2000 is "the monitoring of a person for the purpose of identifying changes in health status due to occupational exposure to chemicals hazardous to health" [1]. Chemicals hazardous to health are, as defined by USECHH Regulations 2000, any chemical or preparation which:

a) is listed in Schedule I or II.
b) possesses any of the properties categorized in Part B of Schedule I of the Occupational Safety and Health (Classification, Packaging and Labeling of Hazardous Chemicals) Regulations 1997. This Regulation has been superseded by the Occupational Safety and Health (Classification, Labeling and Safety Data Sheet of Hazardous Chemicals) Regulations 2013, or CLASS Regulations 2013.
c) comes within the definition of "pesticide" under the Pesticides Act 1974 [Act 149].
d) is listed in the First Schedule of the Environmental Quality (Scheduled Wastes) Regulations 1989.

Based on the definition above, "occupational exposure" to these hazardous chemicals is the prerequisite for conducting medical surveillance. In industries, occupational exposure takes place during various tasks carried out by the employees at the workplace. Tasks of mixing chemicals, performing maintenance works or carrying out laboratory tests potentially expose the employees to the hazardous chemicals that they handle. To eliminate or minimize the exposure, various preventive measures known as control measures are used. Gloves, goggles or fume cupboard are among the control measures intended to protect the employees from exposure to the hazardous chemicals. Breach in any of these control measures will potentially expose the employees to the hazardous chemicals, and put them at risk of contracting adverse health effects. Thus, to monitor their health as a result of exposure to the hazardous chemicals, medical surveillance needs to be conducted.

Department of Occupational Safety and Health Malaysia, or DOSH, has provided a guideline on how medical surveillance should be conducted [2]. This guideline outlines the tests to be carried out on 35 chemicals as part of medical surveillance program. However, this guideline lacks the details on why medical surveillance must be conducted. Thus, the author of this article will focus on the issue on *why* medical surveillance shall be conducted rather than *how* to conduct the medical surveillance.

2. CHEMICAL HEALTH RISK ASSESSMENT (CHRA)

Under the USECHH Regulations 2000, CHRA is a written risk assessment that must be carried out by employer prior to allowing any chemical to be used in the workplace [1]. It is a *qualitative* risk assessment based on observation at workplace. The assessment is essentially to evaluate the potential risks to employees as a result of exposure to the hazardous chemicals. For that reason, CHRA will look into the availability and adequacy of control measures at the workplace. Control measures act as barriers against hazardous chemicals from being in contact with the employees. There are four (4) routes where chemicals can get into contact with the employees and subsequently enter into the body. These are through inhalation, skin contact, ingestion and rarely inoculation. Control measures must be adequate to protect the routes of entry.

Apart from that, the CHRA report will recommend to the employers on the necessity to conduct airborne chemical monitoring and/or medical surveillance on their employees. These are essential to *quantitatively* asses the employees' degree of exposure to the hazardous chemicals and the health effects from exposure to the hazardous chemicals. Results of chemical monitoring and medical surveillance are important to compliment the CHRA report, which, as mentioned above, is qualitative in nature. To get a better understanding on the association between CHRA and medical surveillance, the concept of risk, control measures and chemical monitoring is elaborated below.

2.1 RISK

There are many definitions of risk, but generally risk is a factor of consequences and probability. When handling

hazardous chemicals, risk refers to:

i. Adverse health consequences when employees are exposed to the hazardous chemical, and
ii. Probability of employees being exposed to the hazardous chemicals.

Adverse health effect as a result of exposure to the hazardous chemical is due to the fact that each chemical possesses its own *intrinsic property*. These intrinsic properties may exist in many forms; such as carcinogenic, corrosive, irritant, mutagenic, etc. Following exposure, these chemicals are absorbed into the body, metabolized and excreted or excreted unchanged. However, there are some chemicals which are excreted unchanged. Interaction between these chemicals (or its metabolites) with the body can result in adverse health effects.

In industrial practice, each chemical will be assigned hazard rating that ranges from 1 to 5 based on its toxicity exerted by the intrinsic properties [3]. Chemicals with ratings 5 are most hazardous whereas those with ratings 1 are considered non-hazardous. It follows that based on the rating scale; exposure to chemicals with hazard rating 5 should result in more grave consequences than those with lower ratings. Despite that, these intrinsic properties could be altered through processes such as dilution, mixing, or chemical interaction. For example, acetic acid 100% which is corrosive, will be known as vinegar and edible when diluted to 0.4%.

Probability, on the other hand, refers to the chances of an employee being exposed to the hazardous chemicals at work-place. To objectively gauge the probability of exposure, an exposure rating scale ranges from 1 to 5, is used. Any chemical rated with exposure rating of 5 shows that the employee's probability exposure to the chemical is immense. On the other hand, a chemical rated with exposure rating 1 implies that there is practically no contact between the chemical and employee at all, and thus, the probability of employee being exposed to the chemical is negligible.

If hazard rating depends solely on the intrinsic factor of the chemicals, the probability of exposure is otherwise dependent on other factors [3]. These are:

i. Frequency of exposure.
ii. Duration of exposure.
iii. Intensity or magnitude of exposure.

Frequency of exposure refers to the *number of time* spent in handling chemicals. It can be once a week or twice a year. Duration of exposure otherwise refers to the *amount of time* spent for each session of work. For example, the duration can be six (6) out of eight (8) hours per shift or 75% of work hours, or fifteen (15) minutes performing chemical mixing out of an eight (8) hour shift, or 3% of work hours. Even though frequency and duration of exposure appear similar, they are in fact different. Frequency does not elaborate on the amount of time spent with chemicals as what duration of exposure does [3]. For that reason, frequency of exposure is more relevant to acute health effects, while duration of exposure implies effects of chronic exposure. Thus, duration of exposure gives a good indicator for medical surveillance.

From the above, it is clear that the amount of time spent by an employee in handling the chemicals is important in deciding the probability of one exposing him/herself to the hazardous chemicals. The more frequent and/or the longer time an employee spent in handling chemicals hazardous to health, the higher the exposure rating will be. Consequently, he/she will be more likely to be adversely affected by the hazardous chemicals.

The intensity or magnitude of exposure depends on the degree of chemical release and chemicals absorbed by the employee. A chemical with high vapor pressure (e.g. acetone) will be more readily airborne compared to those with low vapor pressure (e.g. diesel). Thus, acetone will be more easily released into the ambience where the employee is working, and will be more readily inhaled by the employees.

2.1.1 RISK MATRIX

To assess the risk posed by each chemical, a risk matrix is used [3]. The risk matrix has two (2) axes – the x-axis represents Exposure Ratings (ER) while the y-axis shows the Hazard Ratings (HR). Figure 1 shows a typical risk matrix used to assign the Risk Rating for any chemical used in the workplace [3].

The risk matrix above is divided into three (3) colored zones; green, yellow and red. Each zone denotes whether the risk posed by the chemical is significant or not. It could be seen that any chemical with both high hazard and/or exposure ratings will be categorized as chemicals with high Risk Rating. The reverse is true for chemicals with low hazard and/or exposure ratings.

The intrinsic property of a given chemical will determine its degree of toxicity and this is reflected by the assigned hazard rating. In practice, the hazard rating remains unchanged. However, exposure rating can be possibly reduced by implementing appropriate control measures. To illustrate this, imagine if a worker performing a cleaning task bare handedly using a solution with hazard rating 4. This will definitely exposes his skin to the solution and his exposure rating will be 5. However, by wearing appropriate gloves, his skin will not be in contact with the solution, and dramatically, his exposure rating is reduced from 5 to 1. Thus, based on the risk matrix above, his initial Risk Rating of 5 (HR 4, ER 5), has gone down to 2 (HR 4, ER 1). In addition, the Risk Rating has shifted from red to green zone (from "danger" to "safe"). This implies that despite using the same chemical, the risk could be possibly reduced by lowering the exposure rating. The aim is to place all the chemicals into the green zones where risk is not significant and thus, no medical surveillance is required for the employees handling the chemicals.

Another way of achieving the same low Risk Rating is by reducing the hazard rating. This means that the chemical used must be substituted with another less hazardous chemical. Chemical dilution is another possibility to achieve a lower hazard rating. However, in industrial

		EXPOSURE RATINGS (ER)				
		1	2	3	4	5
HAZARD RATING (HR)	1	RR=1	RR=2	RR=2	RR=2	RR=3
	2	RR=2	RR=2	RR=3	RR=3	RR=4
	3	RR=2	RR=3	RR=3	RR=4	RR=4
	4	RR=2	RR=3	RR=4	RR=4	RR=5
	5	RR=3	RR=4	RR=4	RR=5	RR=5

RR = Risk Rating

RISK NOT SIGNIFICANT | RISK SIGNIFICANT CATEGORY 1 | RISK SIGNIFICANT CATEGORY 2

Figure 1: Risk Matrix chart used in assigning Risk Rating for chemical. (Source: DOSH, 2000)

practice, substitution or dilution of chemicals is rarely an option.

2.2 CONTROL MEASURES

Control measures are all the steps taken to prevent or minimize risks [4] . Each of the control measures that will be discussed below is aimed in preventing contact between the chemicals hazardous to health and the employees handling them. In trying to control the identified risks, the measures taken should be in a certain hierarchy or order of priority and an assessment of the adequacy of the control measures need to be made.

2.2.1 Elimination of the hazardous chemicals

Elimination ranks the top in control measures. By eliminating the hazardous chemical in any process, exposure will obviously cease. Thus, no medical surveillance is required for the employees. However, one should bear in mind that certain chemicals which has been eliminated, for example Crocidolite, which is a type of asbestos, will only manifest its adverse health effect after many years, and in some cases, after thirty years. This exception example shows that despite elimination, the affected employee might have to undergo lifelong medical surveillance.

2.2.2 Substitution of the chemicals hazardous to health with a less hazardous chemical

The aim of this control method is to use another chemical of lower toxicity in place of the present chemical. As pointed out by the risk matrix above, this method will effectively shift the hazard rating of the chemical from high to a lower rating. If a suitable chemical is used, the shift will result in the chemical to be placed in the green zone – which means that the particular chemical will not pose a significant risk when handled.

2.2.3 Total enclosure

Total enclosure ensures that the chemicals used are contained and thus, preventing it from escape. Practically, there will be no contact between contained chemical and the employee handling it. Examples are formaldehyde which is supplied in containers or natural gas (which contains Mercury), that runs in a pipeline.

2.2.4 Isolation of the process releasing hazardous chemical

Isolation means removing the source of chemicals from the worker's working environment. It can either be a physical barrier separating the workplace from the employees, or the use of automated process where contact with chemicals will be unlikely.

2.2.5 Use of engineering control equipment

Local exhaust ventilation or LEV is engineering control equipment which will exhaust out chemical contaminants before they come into the employees' breathing zone. A breathing zone is essentially a hemisphere forward of the shoulders within a radius of approximately six to nine inches from the mouth and nose [5]. This control measure removes contaminants at source. A fume cupboard used to carry out laboratory tests is an example of LEV.

2.2.6 Administrative measures

Adoption of safe work practices and procedures are among administrative measures that can be used as control measure. Safe work system is a formal work procedure that results from systematic examination of a task in order to identify all hazards. It defines safe methods to ensure that hazards are eliminated or risks are minimized [4]. By abiding to the safe work practices and procedures, one can reduce the exposure to the chemicals hazardous to health.

2.2.7 Use of Personal Protective Equipment (PPE)

Use of PPE is usually seen as a last resort approach in achieving effective control methods due to problems associated with workers' compliance. In order to be effective, PPE must be properly and continuously worn when handling the chemicals. Respirators, gloves and goggles are among the PPE used to provide protection against chemical exposure.

2.3 CHEMICAL EXPOSURE MONITORING

Chemical Exposure Monitoring is a method used to quantify the concentration of a particular airborne chemical in the workplace. There are two (2) types of monitoring, namely personal and area chemical monitoring. Personal sampling results represent the exposure to the individual who was actually wearing a sampling device. Area samples are taken in a fixed location and results may represent the potential risk from airborne contaminants or physical agents to workers in that area [6]. The result obtained is compared to the Permissible Exposure Limit or PEL of the chemical. As a general rule, if the result is equal or exceeds the Action Level or AL of the chemical, medical surveillance is warranted. AL is typically one-half the value of PEL [7, 8]. It is important to stress that only results of personal chemical monitoring should be considered for medical surveillance. This is due to the reason that PEL values are only applicable to personal chemical monitoring and there is no PEL value for area chemical monitoring.

In interpreting the result, it must be made aware that the result is a sample of chemical concentration of "that particular day" only. Variation in workplace temperature, humidity, ventilation, alteration in process or change in control methods might give a totally different result from the result obtained on the sampling day.

3. INDICATIONS FOR MEDICAL SURVEILLANCE

Based on the discussion above, there are multiple factors that must be taken into consideration before subjecting employees for medical surveillance. In principle, there must be elements of chemical exposure which resulted in significant risk when handling the hazardous chemicals.

3.1 Adequacy of control measures

Control measures are the means to protect employees from being in contact with or exposed to hazardous chemicals. Inadequate control measure will result in escape of the hazardous chemicals into the ambience and risks the employees to be exposed to the chemicals. As a general rule, medical surveillance is warranted if assessment shows that the chemicals are not adequately controlled. Thus, control measures for each chemical as documented in the CHRA report must be reviewed before deciding if there is a need for medical surveillance to be conducted. Decision on conducting medical surveillance based on the control measures is illustrated by Figure 2.

Elimination and substitution of hazardous chemicals means that the chemicals used are no more in use or have been replaced with less toxic chemicals. At one glance, there is no requirement for medical surveillance to be conducted. However, medical surveillance must be considered if the employee has been exposed to the eliminated or substituted chemical before. This is because many adverse health effects will only manifest after years of exposure, despite the fact now the chemicals have since been discarded. Asbestosis which is caused by Crocidolite, cancer-causing asbestos, is a classic example where occupational disease is detected after the employee has probably retired, and no longer exposed to the chemical.

Local Exhaust Ventilation, or LEV, is a control measure used to remove hazardous chemicals at source. In order for LEV to function effectively, various LEV parameters such as face and duct velocities must always fall within range of the recommended values. These values are the standards set by the Professional Engineer during construction and commissioning of the LEV. Failure to obtain these values during LEV inspection and examination means that the LEV is suboptimal.

It is important to know that different chemical requires different face velocity in order for the LEV to exhaust it effectively. For example, face velocity to remove light hydrocarbon would differ from the velocity required to remove silica dust. Thus, each LEV is designed specifically for a particular process and for a particular chemical. If the same LEV is used for different chemicals and processes, the protection might be inadequate. Subsequently, the employees are at risk of being exposed to the hazardous chemicals when they use this LEV. Thus, in such cases, medical surveillance program should be considered.

There are situations where hazardous chemicals can only be controlled using lower hierarchies of control measures. These are the administrative and use of PPE methods. For these chemicals, the author is in the opinion that the employees would still have to undergo medical surveillance – despite the fact that the chemicals are "said to be adequately controlled". The reason is simple. Employees' compliance with these lower hierarchies of control measures is always questionable. A study in Africa on herbicide sprayers indicated that low PPE compliance persists despite workers' awareness of herbicide exposure risks [9].

Based on the control measures, the author of this article would like to propose on how to link between control measures and requirement to conduct medical surveillance.

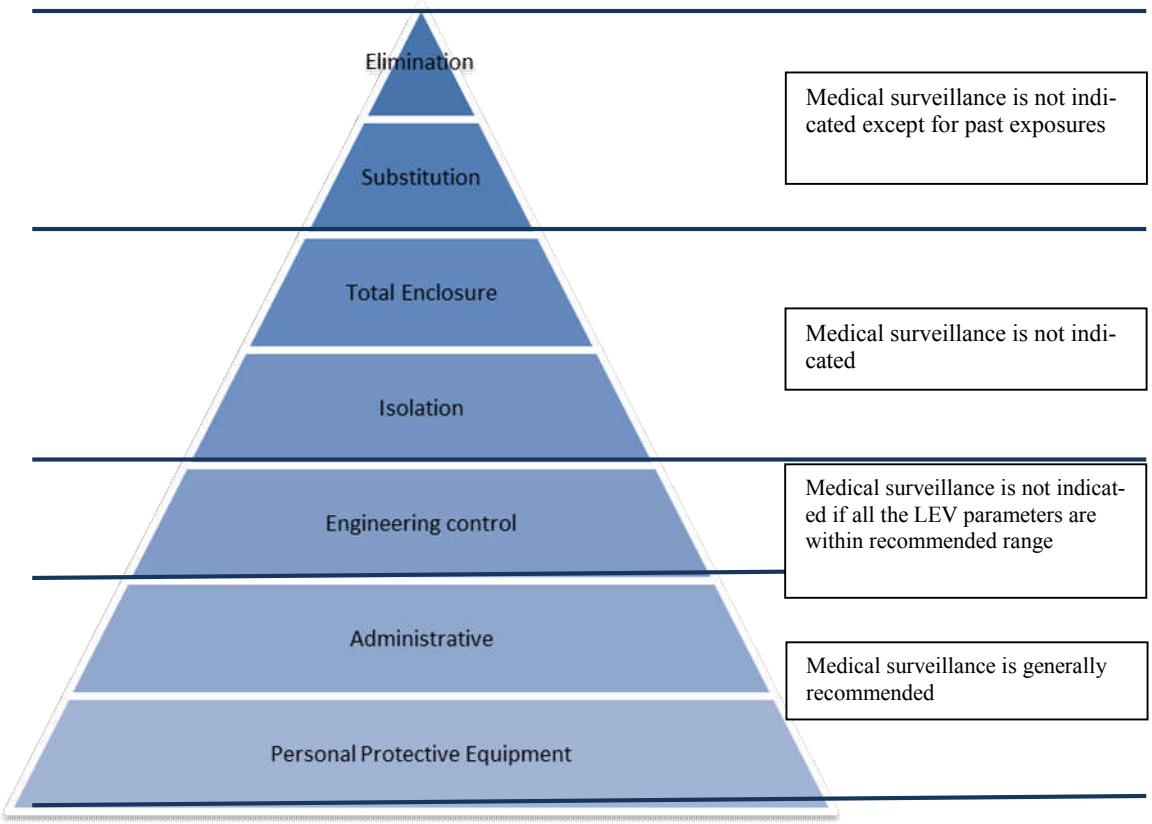

Figure 2: Proposed decision for conducting medical surveillance based on the control measures

		EXPOSURE RATING (ER)				
		1	2	3	4	5
HAZARD RATING (HR)	1					
	2					
	3					
	4					
	5					

NO REQUIREMENT FOR MEDICAL SURVEILLANCE

MEDICAL SURVEILLANCE SHOULD BE CONSIDERED

MEDICAL SURVEILLANCE SHOULD BE STRONGLY CONSIDERED

Figure 3: Proposed decision for conducting medical surveillance based on risk ratings.

3.2 Risk rating

Risk rating of any chemical is obtained using the matrix as described in Figure 1 above. The risk here refers to the health risk as a result of exposure to the hazardous chemicals. Obviously, the hazardous chemicals that fall into the green zones are of low risks (RR = 1 or RR = 2) can be regarded as of "low toxicity" and thus, generally, no medical surveillance is indicated for employees exposed to these chemicals. Despite that, it must be stressed that here is no such "safe chemical", but rather "safe handling of chemicals".

Generally, hazardous chemicals that fall into the yellow or red zones would require medical surveillance. This is because the toxicity of hazardous chemicals and/or the exposure to them will be considered as high. However, since medical surveillance involves recurrent longitudinal examinations and data analysis over time, only chemicals that produce *chronic rather than acute health effects* should be considered for medical surveillance. Chemicals that produce acute health effects (such as corrosive acids) pose safety rather than health issues.

To illustrate the association between risk ratings and requirement for medical surveillance, the same risk matrix chart as in Figure 1 is used below. It could be seen that chemicals with risk ratings of 1 and 2 will fall into the green zones and do not require medical surveillance. However, other chemicals with risk ratings of 3, 4 and 5 that will either be in the yellow or red zones shall be considered for medical surveillance. This is because the risk is considerably high. The author thus would like to propose that the medical surveillance consideration shall be based on the risk ratings as depicted in Figure 3.

3.3 Results of Personal Chemical Exposure Monitoring

Chemical sampling is only made possible if the airborne chemical is released into the employees' breathing zone. Thus, whatever chemical that has been sampled will represent the amount of chemical that is presumed to be inhaled by the employees. For that reason, the chemical concentration inhaled must be below a certain cut-off point to ensure that the employees are "safe" and this cut -off point is known as Permissible Exposure Limit or PEL. In addition to PEL, another important value of chemical concentration obtained from Personal Chemical Exposure Monitoring is the Action Level, or AL. AL is typically half (1/2) the value of PEL [10]. AL triggers many preventive and protective measures to be taken, and this includes conducting medical surveillance [10] .

As a general principle, employees whose results of personal chemical exposure monitoring equal, or exceed the AL, should be subjected to medical surveillance. Despite that, this does not mean that employees whose chemical exposures are below AL would not be subjected to medical surveillance. It is important to realize that chemical monitoring device will only sample airborne chemicals. Chemicals that are absorbed through skin such as hydrocarbons, or those which are ingested such as lead will not be sampled. As a result, the results of personal chemical exposure concentration might be falsely low because other routes of exposure were not considered. For that reason, it is important to conduct a walkthrough survey to observe the hygiene practice at workplace.

The chemical exposure monitoring result obtained must be compared with the Assigned Protection Factor or APF for a respirator before determining whether the employee has been adequately protected against chemical inhalation or not. A high personal chemical exposure monitoring result does not mean that the employee must undergo medical surveillance if the APF provided by the respirator is adequate. For example, the APF of a full face respirator is 50 [11]. Thus the respirator will be able to protect the employee wearing it even when the chemical concentration outside the mask is 50 times the PEL, provided that the user has undergone fit testing and use the respirator when at work. Respirators worn without the employee being fit tested risk the chemical might be inhaled through a loose-fitting respirator. The same goes with wrongly prescribed cartridge, or poorly maintained respirator.

It is important that one should not be "distracted" by the result of area chemical monitoring, but instead, focuses on the result of personal chemical monitoring. Unlike personal chemical monitoring, area chemical monitoring result does not have any PEL. Thus, it will be inappropriate to make any inference from it.

3.4 Availability of methods to perform medical surveillance

Medical surveillance is aimed to detect the earliest possible biological changes in an employee's health through continuous monitoring. The biological changes must be proven to be caused or associated with the hazardous chemicals absorbed by the body. Thus, there must be available methods to quantify the amount of hazardous chemical absorbed *and* the biological changes as a result of the absorption.

3.4.1 Biological Exposure Indices

Following exposure, hazardous chemicals will be absorbed into the body. These chemicals will undergo metabolism and excreted. Measuring the concentration of these chemicals will reflect the amount of chemicals absorbed in the body. The chemicals can either be the parent chemical or its metabolite – and both are known as determinants. An example involving the parent chemical is where blood lead concentration is measured to monitor the employees' exposure to lead. However, urine hippuric acid, instead of urine/blood toluene, will be measured to evaluate exposure to toluene. In this example, hippuric acid is the metabolite of toluene. The measured concentration of the determinants will be compared to a standard known as Biological Exposure Indices, or BEI.

BEI represents the level of determinant that is most likely to be observed in specimen collected from healthy workers. These healthy workers have been exposed to

chemicals to the same extent as workers with inhalation exposure at the Threshold Limit Value, or TLV [12]. TLV "refers to airborne concentrations of chemical substances and represent conditions under which it is believed that *nearly all* workers may be repeatedly exposed, day after day, over a working lifetime, without adverse health effects" [12]. In Malaysia, TLV is known as PEL.

Each chemical has its own specific and unique BEI value. While laboratory results below BEI imply that the employees' exposure to the chemicals is low or negligible, results that exceed BEI do not necessarily mean that the employees' health has been adversely affected. Rather, this simply means that the control measures are inadequate or have been breached. In addition, BEI is not intended to be used as an index of occupational disease despite most BEIs have correlation with TLV [12]. For that reason, Biological Effect Monitoring or BEM must be performed to complement the results obtained for the BEIs [13].

3.4.2 Biological Effect Monitoring

While BEI is an indicator of adequacy of control measures, BEM is the measurement and assessment of early biological effects caused by absorption of chemicals [13]. It normally involves measuring *biochemical responses* such as measuring increases in urinary protein following exposure to Cadmium, a heavy metal known to be nephrotoxic [2]. By doing this, the adverse health effects of the chemicals on a particular organ can be assessed quantitatively. Other examples include conducting lung function test to look for features of restrictive lung disease on employees exposed to Silica dioxide at quarries. Or, performing peripheral blood count on offshore employees exposed to Benzene to look for abnormal blood cells as Benzene is a confirmed leukemia-causing agent [14].

Since medical surveillance is a component of secondary prevention, early detection of disease is crucial. Thus, it is important that the tests perform are specific to the organs that are affected by the chemicals. Review of the Safety Data Sheet (SDS) of the particular chemical is a must to determine the target organs affected by the chemical. Otherwise, there will be a great chance that the opportunity to detect the earliest occurrence of the disease will be missed.

4. DISCUSSION

Use of chemicals in industries is inevitable nowadays. Many of these chemicals are hazardous to health. Thus, employees are always at risk of exposure to these chemicals at workplace. Various control measures are designed to protect employees from such exposure, and these control measures must be strictly adhered. However, inadequate or breach in the control measures will result in loss of protection, and the employees will be at risk of contracting occupational disease or occupational poisoning.

It is important that the employees who are exposed or at risk of being exposed to hazardous chemicals to undergo medical surveillance to monitor their health. This is aimed to detect the earliest changes in the employees' health so that appropriate corrective measures can be taken and preventive measures instituted. This include removing the affected employees from the workplace through the action of Medical Removal for Protection, or MRP [1]. In addition, medical surveillance is a form of audit of the workplace. Result of the findings is a reflection of the workplace hygiene. Improvement of the workplace condition must be made if medical surveillance shows that the employees' health has been adversely affected.

Medical surveillance must be performed correctly in order for it to benefit both the employees and employers. While it monitors the employees' health, the cost that the employers have to bear can be enormous. To illustrate, while one sample of chemical monitoring might be adequate to represent the whole factory, medical surveillance otherwise requires each and every exposed employee to be sampled and examined. Thus, cost for conducting medical surveillance must be justified.

Risk assessment, adequacy of control measures, chemical monitoring results and availability of methods to conduct medical surveillance must be considered before decision to perform medical surveillance is made. These are essential not only to justify the cost factor, but more importantly, the results obtained can be used to gauge the effects of chemical exposure to the employees, as well to reflect the hygiene of the workplace. Table 1 below summarizes on what to look for before conducting medical surveillance.

Once medical surveillance has been conducted, the Occupational Health Doctor or OHD, must then decide whether the employees' health is affected or not by the chemical exposure. If there is evidence that the health has been affected, it is the duty of the OHD to associate the health condition with the hazardous chemicals that they are exposed to. In doing so, there are multiple factors that have to be considered such as gender, age and concomitant disease. Non-occupational exposure is another important factor that must be taken into account. Smoking for example, will increase the level of benzene in body and leads to increased incidence of leukemia in a study conducted recently in Japan [15].

Since medical surveillance is a process that involves monitoring of employees' health, it has to be repeated at scheduled intervals as long as the exposure element is there. In fact, cessation of exposure (elimination or substitution of chemical) might not necessarily mean that medical surveillance should not be conducted. As mentioned, there are diseases that manifest themselves rather slowly. Asbestosis and mesothelioma are only detected after years if not decades of exposure [16]. Thus, a continuous assessment of the employees' health must carry on despite the fact that the exposure has ceased or the employee has retired.

5. CONCLUSION

Medical surveillance is the "last defense" in detecting the earliest biological changes in body so that prompt action can be taken to remove the affected employee from the workplace. It can also be used as an audit tool to evaluate the hygiene aspect of an industry. Decision on whether to conduct medical surveillance is an art of science. Various considerations have to be made as the benefit to the employees must be balanced with the financial implication to the industries. When performed correctly, medical surveillance is a valuable tool in safeguarding the employees' health and prevention of occupational disease or occupational poisoning.

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Case Report

UNDERGRADUATE STUDENTS' LEARNING CURVE IN FORMULATING DIFFERENTIAL DIAGNOSIS OF SUBSTANCE-INDUCED PSYCHOTIC DISORDER

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ABSTRACT

Two cases from undergraduate case studies were presented. The challenges in diagnosis were highlighted. Invariably, the differential diagnosis of substance-induced psychotic disorder is often misleading and instable over time. Therefore, a systematic framework using multidimensional grid was proposed. Finally, the differential diagnoses were discussed.

Keywords: *Substance-induced psychosis, differential diagnosis*

CASE 1

A 31-year-old gentleman with comorbid cannabis abuse for almost 12 years developed sudden onset of panic attack. The first attack occurred when he was chasing cannabis. Due to that episode, he abruptly stopped taking cannabis and complaint of persistent symptomatic illness, such as shortness of breath, palpitation and fear of dying. He is also burdening with personal problems that aggravate his condition. Mental status examination is normal except he has thought preoccupation with anticipatory fear of having another attack. The diagnosis of this case is challenging as it could be panic disorder with comorbid substance abuse, cannabis withdrawal or cannabis use disorder with panic attack. Urine for cannabis, subsequent to the last attack was negative. Biopsychosocial intervention has been implied on him including SSRIs, counseling, breathing and relaxation therapy.

CASE 2

A 44-year-old Malay rubber tapper first presented with mood symptoms 4 years before he was readmitted due to aggressive behavior toward his mother-in-law following the missing of his 5-year old son. Two days prior to admission, the patient acted aggressively by slapping his wife following a jealousy, that she is having an affair with another man. At the same time, he admitted of taking amphetamine. Mental status examination reveals signs of visual hallucination and paranoid (jealous) delusion. Urine drug screening is positive for methamphetamine. The issue at the earliest presentation is regarding the diagnosis of substance-induced psychosis since his urine taken was negative for methamphetamine. As the matter of fact, methamphetamine is washout from the body within 3-4 days; so negative finding does not mean no drugs were taken. Health care laboratory was not able to provide urine strips for drug detection at the earliest presentation. Patient on the other hand, denied any drug abuse and managed to convince the psychiatric team that he really had mental illness without the influence of substance. As another relapse of psychosis had flared up, he then admitted of methamphetamine abuse.

DISCUSSION

Differential diagnosis of substance-induced psychotic disorders could be misleading as the association of drugs and mental disorders could range from substance-induced psychosis, substance-induced mood disorder, substance induce-panic attack, substance withdrawal disorder to substance intoxication. To pinpoint the diagnostic difference between substance-induced psychosis and other psychotic symptoms, the history must clearly delineate time of reference when the patient start and stop taking substances, the duration and severity of substance addiction, type of drug involved and possibly identification of urine or blood markers that is clearly indicative of type of drug abuse.

Reluctance to reveal diagnosis, concealment of the previous diagnosis for cover-up purposes, deceivable act with regard to duration and time of last drug intake and even type of drug abuse, all could lead to misleading diagnosis. As a result, doctors would derive to disingenuous diagnosis, conclusion and management.

Mathias S *et al.* (2008) reviewed leading electronic data-bases (Medline, PubMed) searching for research studies, case reports and case series from 1992 - 2007. They identified 49 articles and presented data on populations diagnosed with substance-induced psychotic disorder.

They concluded after reviewing all those articles that there remains a striking paucity of information on the outcome, treatment, and best practice for substance-associated psychotic episodes [1].

As such, the most definitive method for making this distinction is longitudinal assessment after a period of sustained abstinence from psychoactive substances. It is time consuming and often impractical given the relapsing nature of substance abuse and psychosis.

Bruce J. Rounsaville (2007) suggested more rapid diagnosis could be facilitated by the identification of "markers" or distinctive clinical features that would identify patients with psychotic symptoms as having transient, substance-induced syndromes or enduring independent disorders [2].

Such markers might take the form of biological indices (eg, a genetic profile suggesting schizophrenia), symptom profiles, or features of the psychiatric history.

Secondly, more definitive information could be gathered on the duration of substance-induced psychotic symptoms and syndromes. At present, for purposes of differential diagnosis, "sustained" remission is considered to be around 4 weeks of abstinence. Conceivably, this duration of abstinence may be too short for psychoses induced by some substances (eg, cannabis or hallucinogens) or too long for those induced by others (eg. benzodiazepines) [2].

Stability of diagnosis over time is related to the ability to discriminate between substance-induced psychosis with schizophrenia. More often than not, the diagnosis changes over time based on finding at longitudinal follow-up.

Carol L.M. Caton *et al.* (2007) conducted a 1-year follow-up study of 319 psychiatric emergency department admissions with diagnoses of early-phase psychosis and substance use comorbidity. They observed a change in diagnostic category from substance-induced psychosis at baseline to primary psychotic disorder at the 1-year follow-up in 34 study participants, representing about 25% of those diagnosed with substance-induced psychosis at baseline. These patients had poorer premorbid functioning, less insight into psychosis and greater family history of mental illness than patients with a stable diagnosis of substance-induced psychosis [3].

Richard N. Rosenthal & Christian R. Miner (1997) has discriminated between drug-induced psychosis and schizophrenia. They found that formal thought disorder and bizarre delusions significantly predict a diagnosis of schizophrenia, with odds ratios (OR) of 3.55:1 and 6.09:1, respectively. Their study also showed that suicidal ideation (OR = 032:1), intravenous cocaine abuse (0.18:1), and a history of drug detoxification (0.26:1) or methadone maintenance (0.18:1) demonstrate inverse relationships with a schizophrenia diagnosis [4].

For undergraduate students, possibility of other factors that could influence their diagnostic precision includes language difficulties, cultural differences, the presence of Axis II disorders and patients' cognitive impairment.

Teaching students making psychiatric differential diagnosis involves many facets or dimensions, taking into account the misleading and instability of diagnosis.

I am suggesting that a systematic framework should be developed to derive to a differential diagnosis using multidimensional grid, x-axis being longitudinal history, y-axis severity dimension of symptom profile and z-axis on co-morbidity (Figure 1).

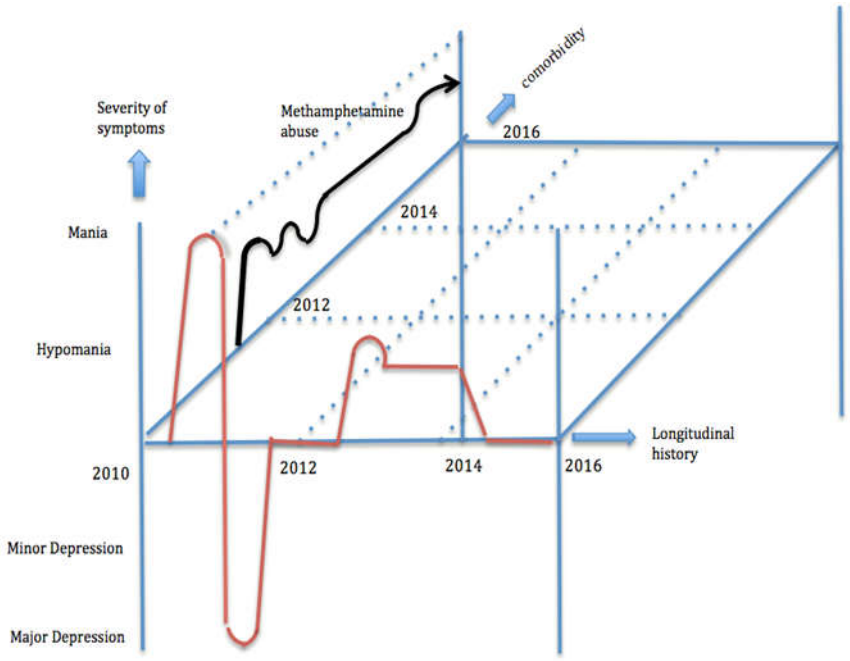

Figure 1: Framework for a differential diagnosis

Multidimensional grid provides 3D visualization on cause and effect of drug abuse (y=effect, z=cause). Symptom formation synchronizes with drug intake behavior strongly suggestive of correlation in some cases. Delay development is not uncommon in cases of low dosage intake and infrequent abuse.

In case of overlapping symptoms, development of symptoms after heavy use and prolonged abuse of substances vs. recent termination the drug intake, reoccurrence of symptoms after reintroduction of drugs, could suggest drug related differential diagnosis.

Understanding of event that could lead to abuse symptom formation may justify the inclusion or exclusion of adjustment disorder.

Rarely, Posttraumatic stress disorder (PTSD) comes to the forefront of differential diagnosis except when the prominent autonomic symptoms, re-experiencing and avoidance colour most of the presentation. Nevertheless, PTSD, by virtue, is a culture-specific diagnosis. As such, abusers with horrible experience of abuse and trauma inflicted by co-abusers or enforcement agency officials might not spare the diagnosis.

Dissociative disorders are not uncommon in view of the symptoms of appear memory loss of certain time periods, events and people as well as the sign of being detached from oneself and distorted perception.

In the Eastern culture, drug-induced mental disorders are often mask by counterfeit explanation of either possession by spiritual beings or imprisonment by an enemy executed through physical mean or through other medium.

CONCLUSION

In conclusion, a systematic framework using multidimensional grid should be utilized as teaching method for making a differential diagnosis for undergraduate students in psychiatry.

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Review Article

ROLE OF HUMAN PAPILLOMA VIRUS IN HEAD & NECK MALIGNANCY

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ABSTRACT

Human papilloma virus has been routinely implicated in the carcinogenesis of uterine cervix, vagina, vulva and anal region. Recently over the past two decades, it has been found that HPV virus is also responsible for the carcinoma of tonsil and base tongue region in the oropharynx. Interestingly the main viral strain that is involved in carcinogenesis of uterine cervix and oropharyngeal region is HPV 16. Currently, the incidence of oropharyngeal malignancy is increasing alarmingly and more than 80% of such malignancies are due to high risk HPV virus infection. So a literature review was done to analyse data on disease prevalence, disease behaviour and treatment outcome of HPV positive Head & Neck malignancy. It was found that unlike non HPV related Head & Neck carcinoma which usually affects older age group, HPV positive head and neck malignancy tend to affect younger age group. Individuals with HPV positive oropharyngeal malignancy tend to have oral sex and have multiple sexual partners. Such sexual practice, explains the reason for the confinement of HPV positive malignancy mainly to the oropharyngeal region in head and neck. Tumour has less aggressive course and they show better response to treatment. Overall the tumour affects younger individuals and shows better treatment response. All these favourable factors have opened up scope for treatment de-intensification, targeted therapy and cancer prevention using HPV vaccine.

Keywords: *Oropharyngeal carcinoma, HPV virus*

INTRODUCTION

Human papilloma virus (HPV) especially the strain 16 and 18 has been known to cause carcinoma of the uterine cervix. However, in the last two decades, various studies have demonstrated the role of HPV virus in carcinoma of head and neck region, especially in oropharynx. In the Head and Neck region, HPV positive squamous cell carcinoma (SCC) commonly affects tonsil and base of tongue region in orophrayngx [1].

All over the world, tobacco products and alcohol remain the leading cause for Head and Neck malignancy. As the public awareness increases, the incidence of tobacco and alcohol related Head & Neck cancer has reduced tremendously especially in the western world. But it is associated with an increasing trend of oropharyngeal malignancy involving tonsil and base of tongue region. More than 90% of such oropharyngeal malignancies are caused by HPV virus [2]. Such rising trend in HPV related oropharyngeal malignancy is attributed to increasing practice of oral sex in the affected individuals. HPV positive oropharyngeal malignancy is increasing alarmingly so knowledge and understanding of HPV positive head and neck squamous cell carcinoma (SCC) have become very essential now.

Human papilloma virus.

Human papilloma virus belongs to the heterogeneous group of small DNA virus of the family Papillomaviridae. They usually affect the basal cells of the stratified epithelium at mucosal or cutaneous sites. More than 90 types of HPV virus have been sequenced so far of which type 16 and 18 is more carcinogenic. This virus has been implicated in the carcinogenesis of cervix, vagina, vulva, penis and anal region [3]. HPV infections are mainly sexually transmitted through direct skin or mucosa contact and represent the most common sexually transmitted infection worldwide. Majority of infections clear spontaneously in 12 to 24 months and clinical progression to invasive carcinoma is a very rare event and occurs only in high grade lesions [4].

HPV induced carcinogenesis.

HPV virus genome has three regions such as a non-coding region (LCR), and two protein coding regions namely early (E) and late (L) region. Genes in the E region especially E6 and E7 are mainly implicated in the carcinogenesis. E6 gene of HPV 16 virus causes degradation of p53 gene in host cell [5]. p53 is considered as a molecular policeman, it induces the cell cycle arrest/apoptosis in response to cellular stress or DNA damage. HPV 16 virus induces carcinogenesis by attacking p53 protein. E7 gene of HPV 16 binds to pRb (retinoblastoma gene), which is in control of G1-S phase transition in cell cycle. Inactivation of pRb leads to uncontrolled cell cycle progression resulting in carcinogenesis [6].

Clinical profile of HPV positive HNSCC.

The demographic profile of HPV positive oropharyngeal SCC is entirely different when compared to the non-HPV related one. Unlike non-HPV related HNSCC, patients with HPV positive oropharyngeal SCC usually belongs to younger age group. They are more likely to

have better dentition, less or no tobacco or alcohol use, a greater marijuana use and greater number of oral sex partners than HPV non related group [7]. They have better performance status and belong to higher socioeconomic status. They can tolerate the treatment better because of less co-morbid factors.

Characteristics of HPV positive HNSCC.

HPV positive oropharyngeal malignancies tend to behave less aggressively when compared to the non-HPV related malignancy. They usually arise from the tonsillar crypts unlike the environment related SCC which usually arise from the surface epithelium [8]. HPV-induced HNSCCs are often described as non-keratinizing, poorly differentiated or basaloid carcinomas, and are diagnosed in earlier T-category with a trend for a more advanced N-category, with cystic degeneration than the HPV-unrelated carcinomas. HPV positivity is associated with better response to treatment and modality-independent survival benefit [3] Thus it becomes very essential to differentiate between HPV positive and non- HPV positive head and neck malignancy.

Management of HPV positive HNSCC.

Standard treatment for oropharyngeal malignancy includes radiotherapy/surgery for stage 1 and stage 2 lesions and concurrent chemo-radiotherapy for stage 3 and stage 4 lesions. The same protocol is being followed for HPV positive oropharyngeal SCC. Many times such an aggressive treatment is often associated with significant long term morbidity in the form of severe swallowing difficulty. It alters the post-treatment quality of life (QOL) significantly [9]. Such QOL issues are undesirable for cancer which is less aggressive with better prognosis and when the affected persons are of relatively younger age group. All these issues have created a scope for treatment de-intensification.

In treatment de-intensification, the focus is to make the treatment less aggressive but without compromising the oncological outcome. Treatment de-intensification is the need of the hour, as the overall treatment related morbidity can be drastically reduced. Treatment de-intensification involves reducing the dose of radiotherapy from the standard 70Gy to 54Gy dosage and substitution of EGFR inhibitor (cetuximab) instead of cisplatin in chemotherapy [10]. Among the surgically treated group, treatment de-intensification involves replacement of routine open surgery by trans-oral robotic surgery (TORS). TORS provides better survival outcome than standard open surgery and causes lesser treatment related morbidity [11]. Some studies show that there is no significant difference between radiotherapy versus surgery in HPV positive oropharyngeal SCC [12]. However, we still need a lot of evidence to recommend treatment de-escalation for HPV positive oropharyngeal SCC at this stage, as we have quite a number of on-going trials on treatment de-intensification which are yet to complete at this time [13].

Prognosis of HPV positive HNSCC:

The better overall survival of HPV-positive patients may depend on their younger age at diagnosis, superior performance status, lower smoking and alcohol related morbidity, the distinct biology of cancer, reduced risk of second primary tumours or a more aggressive treatment strategy. The favourable outcome of HPV-induced SCC may be attributable to enhanced sensitivity to treatment due to a wild-type TP53, allowing an apoptotic response of cancer cells to radiation and chemo-radiation [14].

The role of HPV vaccine.

Two types of HPV vaccines are currently available for protection against cervical and ano-genital cancer namely HPV 4 (protects against HPV 6, 11, 16, 18 strains) and HPV 2 (protects against HPV 16, 18 strains). The quadrivalent HPV 4 vaccine has demonstrated very high vaccine efficacy (>98%) for the prevention of anal, cervical, vaginal and vulvar pre-cancers among vaccine-type-naïve individual [15]. Like HPV4, HPV2 vaccine has very high efficacy (>97%) in the prevention of vaccine-type HPV-related cervical pre-cancers among HPV naïve individuals [16]. The effectiveness of both the vaccines is reduced when the vaccination is given to individuals who are already infected with the vaccine strains. HPV vaccine gives definitive protection against cancer of uterine cervix and ano-genital regions in vaccinated individuals but, it is not sure that whether the same vaccine will give similar protection against oropharyngeal carcinoma. It is also very difficult to study the effectiveness of HPV vaccine in oropharyngeal carcinoma as a lot of other carcinogens and social behaviours have an influence on the outcome.

CONCLUSION

The incidence of HPV positive oropharyngeal malignancy is increasing tremendously. HPV positive malignancy tends to affect a relatively younger age group and has a better prognosis. The disease shows a better response to the currently available standard treatment modalities. Better treatment outcome has created a scope for treatment de-intensification in HPV positive malignancy. The role of HPV vaccine in prevention of HPV related oropharyngeal malignancy is very difficult to understand at this point.

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Original Article

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF *Corchorus capsularis* AND *Stevia rebaudiana* EXTRACTS

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ABSTRACT

The purposes of this study were to determine total phenolic content (TPC) and antioxidant activities of C.capsularis and S.rebaudiana. Methodology: C.capsularis was extracted successively with methanol and chloroform methanol separately while S.rebaudiana was extracted using methanol and chloroform solvent. The plant extracts were used to conduct various antioxidant assays. TPC assay using Folin- Ciocalteu method was used to assess the presence of phenolic compounds in each sample. The extracts samples were also subjected to assess their potential antioxidant activities through DPPH, ABTS and FRAP assay. The present study showed that both methanol extract of C.capsularis and S.rebaudiana had highest TPC, followed by chloroform extract of S.rebaudiana and chloroform methanol extract of C.capsularis. Next, the DPPH radical scavenging assay was found to be higher in the methanol extract of S.rebaudiana at the concentration of 154.67µg/ml. The cation radical scavenging activity, measured by ABTS assay was shown that methanol extract of S.rebaudiana (278µg/ml) had the lowest EC_{50} as compared to the other samples. Interestingly, among the various samples, the methanol extract of S.rebaudiana demonstrated a very significant antioxidant activity in FRAP assay ($p<0.05$). The results of the present study showed that all the extract samples contain significantly high phenolic compounds with superior antioxidant activity as evidence by scavenging of free radical including DPPH and ABTS. In conclusion, it is conceivable that the C.capsularis and S.rebaudiana have shown potential as sources of natural antioxidants.

Keywords*: C.capsularis, S.rebaudiana, TPC, antioxidant activity*

INTRODUCTION

Free radical can be defined as any species that capable of independent existence that contains one or more unpaired electron and this unpaired electron will seek for other electrons in order to become stable [1]. The production of free radicals such as hydrogen peroxide and nitric oxide in our body can cause severe damage to cells. Even though human cells can defend themselves against free radicals via mechanisms of antioxidant systems, the defence mechanisms may not be adequate to protect against excessive free radicals, known as oxidative stress [2]. To compensate this severe effect, World Health Organization recommended the use of natural antioxidants.

Antioxidants can be defined as a substance that may protect cell from damage caused by unstable free radicals by stabilizing the free radicals and prevent some damage that free radicals might cause [3]. Recently, antioxidant from natural sources has gained attention among consumers and the scientific community because of potential and beneficial towards human health. Previous study reported that the consumption of antioxidant constituents reported could protect against oxidative damage which induce degenerative and pathological processes such as ageing and cancer [4]. Thus, antioxidant molecules from medicinal plants have become a major area of interest in scientific research [5].

In Malaysia, *Stevia rebaudiana* Bertoni, commonly known as sugarleaf or sweet leaf, was used traditionally as a source of natural sweetener. *S. rebaudiana* Bertoni is herbaceous perennial plant of the sunflower family (Asteraceae). The herb had been practiced for centuries by Guarani as a cardiac stimulant, for obesity, hypertension and lowered the uric acid level [6]. The leaves and callus of *S. rebaudiana* are commonly used in scientific research as they believed that it contains therapeutic value. In previous study, flavonoids, alkaloids, amino acids, lipids and trace elements have been discovered in the dry extract of leaves of *S. rebaudiana* [7] which are important compounds involving in antioxidant activities.

Corchorus capsularis is a plant belongs to the family of *Tiliaceae,* also konwn as 'kancing baju' among Malays [8]. Its stem, seeds and leaves have a potential medicinal properties which able to be remedied for certain health problems. The leaves are vital component as stimulant, laxative, appetizer. Meanwhile, its infusion helps in treating fevers, constipation, dysentery, liver disorder and dyspepsia [8]. The leaves of this natural

antioxidant have shown the presence of phytochemicals such as flavanoids and tannin [8]. Researchers found that these natural components in *C.capsularis* have therapeutic potential, which possess defence mechanisms to protect against the degenerative diseases associated with marked oxidative damage [8].

Therefore, the aim of this study is to determine the total phenolic content and antioxidant activities of methanol, chloroform and chloroform-methanol extracts of *C.capsularis* leaves. A possible correlation between total phenolic content and antioxidant activities of both plant extracts also have been evaluated. Therefore, several assays such as total phenolic content, DPPH, FRAP and ABTS assays have been used to estimate the phenolic content and antioxidant activities of *S. rebaudiana* and *C.capsularis* leaves.

MATERIALS AND METHODOLOGY

Plant material

The leaves of *S.rebaudiana* were collected from Taman Pertanian Universiti Putra Malaysia, Selangor, Malaysia. *C.capsularis* (SK 1936/11) leaves were obtained from nursery in Pahang. Both plants were identified in Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM) and the voucher specimens have been deposited in Herbarium of Laboratory of Natural Product, UPM.

Chemicals

Sodium carbonate ($NaHCO_3$) was purchased from Systerm®. Ferric chloride ($FeCI_3$) and ferrous sulphate ($FeSO_4$) were purchased from HmbG® Reagent Chemicals. Hydrochloric acid (HCI) was purchased from R & M Chemicals. Both ethanol (C_2H_6O) and chloroform ($CHCI_3$) were purchased from Merck (Darmstadt, Germany). Methanol (CH_4O) was purchased from Friedemann Schmidt (Francfort, Germany). Gallic acid ($C_7H_6O_5$), Folin-Ciocalteu reagent, 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)-ABTS, ammonium persulfate ($(NH_4)2S_2O_8$), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), were purchased from Sigma Aldrich (Germany). Dimethyl sulfoxide (DMSO) ($(CH_3)2SO$) and glacial acetic acid (CH_3CO2H) were purchased from Fisher Scientific Chemical.

Plant extraction

The leaves of *S.rebaudiana* and *C.capsularis* were dried for 72 hours in oven at 40^0C [9]. The dried leaves were ground to get fine powder. For methanol extract of *C.capsularis*, 1 kg of powder was soaked in methanol in the ratio of 1:20 w/v [8]. For chloroform methanol extract of *C.capsularis*, 1 kg of powder was soaked in chloroform methanol solution (2:1 v/v) in the proportion of 1:20 w/v [10]. The process was repeated three times with a fresh volume of solvent. The supernatant of each plant extract was filtered using Whatman filter paper No.1 (Whatman Ltd., England). The pooled extracts of both plants were concentrated using a rotary vacuum evaporator (BUCHI Rotavapor R-220) at 40^0C. Then, it was dried and stored at 4^0C [9].

Determination of total phenolic content

Total phenolic content of the extracts was evaluated by using Folin-Ciocalteu phenol The principle of this method is the ability of reducing the phenol functional group. The reduction of fosfotungstat-fosfomolibdenum complex (Folin-Ciocalteu reagent) by phenolat ion was taken place at basic condition. As the oxidation and reduction reaction of phenolat ion occurred, the yellow colour of reagent was turning into blue. The reduction of this complex reagent was increased when the extracts contain more phenolic compounds [11]. The amount of total phenolic in both plant extract was evaluated as described by method from Ismail *et al*. [12] with minor modification. 1 mg/ml of stock standard solution of gallic acid was prepared. Various concentrations ranged between 0.01 and 0.05 mg/ml were prepared. 1 mg/ml of plant extracts were prepared. Then, 100 µl of plant extract and ascorbic acid were mixed with 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water). The mixture was allowed to stand at room temperature for 5 minutes. 0.75 ml of 6% (w/v) sodium carbonate was added and mixed gently. Again, the mixture was left at room temperature for 90 minutes. The absorbance was read at 725 nm using T60 UV/Vis spectrophotometer and the standard calibration curve of gallic acid was plotted. Ascorbic acid was used as standard.

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity

In DPPH assay, DPPH radical was reacted with an antioxidant compound which that can donate hydrogen, and get reduced. DPPH, when acted upon by an antioxidant, was converted from diphenyl picrylhydrazyl to diphenyl picrylhydrazine. This can be identified by the conversion of purple to light yellow color [13]. The method of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity was estimated as previously described by Blois [14]. The stock solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was prepared by dissolving 2.1 mg of DPPH in 50 ml methanol. It was then incubated for 2 hours in the dark room. Ascorbic acid and plant extracts were prepared at various concentrations (1000 to 15.625 µg/ml). Then, 50 µl of the standard reagents and each concentration of plant extracts were added into 96-well plate. After that, 195 µl of 0.1 mM DPPH solution was added. The reaction mixture was incubated for one hour in the dark condition. Then, the absorbance was measured at 517 nm using microtitre plate reader (Thermo® Multiscan Ascent plate reader). The percentage scavenging activity of the sample on DPPH radical was calculated using the

following equation:

% scavenging activity = [(Abs $_{control}$ – Abs $_{sample}$) / Abs $_{control}$] x100

2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) scavenging activity

ABTS was converted to its radical cation by the addition of ammonium persulfate. This radical cation was blue in colour and absorbed light at 745 nm. During the reaction, the blue ABTS radical cation was converted back to its colorless neutral form [15]. ABTS assay was performed according to the method described by Re *et al.*[16]. The oxidant is generated by persulfate oxidation of 2, 2'-azino -bis (3-ethylbenzthiazoline-6-sulphonic acid) – ($ABTS^{2-}$). ABTS solution (7 mM) was mixed with 2.45 Mm ammonium persulphate to generate ABTS radical cation ($ABTS^{+}$). Then, the mixture was left in dark condition for 12-16 hours. Different concentration (15.625-1000 µg/ml) of plant extract (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol until 1 ml. The absorbance was analysed at 745 nm using T60 UV/Vis spectrophotometer (Tg9 Instrument). Ascorbic acid was used as a standard. The percentage scavenging activity of the sample on ABTS radical scavenging activity was calculated using following equation:

% scavenging activity = [(Abs $_{control}$ – Abs $_{sample}$) / Abs $_{control}$] x100

Ferric –reducing power (FRAP) assay

The principle of the assay was assessed by antioxidant ability to reduce FRAP reagent. It possessed the reducing potential of the antioxidants to react with a ferric trioyridyltriazine (Fe III TPTZ) complex and produced ferrous tripyridyltriazine (Fe II TPTZ) [17]. The FRAP (Ferric reducing antioxidant power assay) method was followed as described by Benzie & Strain,[17] with minor modification. Briefly, a stock solution of FRAP reagent was prepared. Then, FRAP reagent was warmed at 37^0C in water bath (Memmert), before being used. An aliquot of 50 µl plant extract and ascorbic acid of different concentrations were allowed to react. After that, 150 µl of FRAP reagent was added. The absorbance of the mixture at 590 nm was measured using a microtitre plate reader (Thermo® Multiscan Ascent plate reader). Ferrous sulfate ($FeSO_4$, 0-1000 µM in distilled water) was used as standard. The results obtained were shown as the concentration of antioxidants having a ferric reducing ability equivalent to that of µM FeSO4/ mg of dry sample weight.

Statistical analysis

All analyses were performed in triplicate (n = 3). The results were presented as mean ± standard error of mean (SEM). SPSS was used to test any difference in antioxidant activities resulting from this method. Duncan's multiple range test was used to determine significant differences, with $p < 0.05$. Correlations among data obtained were calculated using Pearson's correlation coefficient (r).

RESULTS

The amount of total phenolics, measured by Folin-Ciocalteu method, varied widely in these two plant species and was expressed as Gallic Acid Equivalent (GAE)/100 g dry weight (Table 1). The highest level of phenolic content was found in methanol extract of *S.rebaudiana*, while the lowest was in chloroform

Table 1: TPC and EC_{50} value of *Corchorous capsularis* and *Stevia rebaudiana extracts*

Samples	TPC (mg GAE/100g of dry weight)	EC_{50} of DPPH (µg/ml)	EC_{50} of ABTS (µg/ml)	FRAP (µm $FeSO_4$/mg of dry weight)
Corchorus capsularis				
Methanol	5155.56 ± 408.52^{a}	293.33 ± 108.99^{d}	483.33 ± 92.79^{g}	614.78 ± 74.07^{i}
Chloroform methanol	2475.56 ± 100.39^{b}	763.33 ± 85.70^{e}	-	418.15 ± 15.30^{j}
Stevia rebaudiana				
Methanol	13709.00 ± 585.10^{c}	154.67 ± 8.33^{f}	278.00 ± 5.00^{gh}	1127.86 ± 3.04^{k}
Chloroform	3160.33 ± 51.13^{b}	-	-	239.97 ± 3.85^{l}

Values represent as mean of three replicate determination ± SEM (standard error of mean). Different superscript in a column are significantly different at $p<0.05$.

TPC, total phenolic content; GAE, Gallic acid equivalent; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; ABTS, 2, 2'-Azinobis (3-ethylbenzothiazoline-6-sulphonicacid); FRAP, Ferric reducing antioxidant power; EC_{50}, effective concentration which scavenging 50% of free radicals; $FeSO_4$, ferric sulphate.

methanol extract of *C.capsularis*. As compared between the plant species, methanol extract of *C.capsularis* had the highest TPC than chloroform methanol extract. For *S.rebaudiana*, methanol extract also had higher TPC, and followed by chloroform extract. From the result, it was found that methanol extract was more efficient solvent for extracting the phenolic constituens in both plant species rather than other solvents. Hence, the order of TPC is; methanol extract of *S.rebaudiana* > methanol extract of *C.capsularis* > chloroform extract of *S.rebaudiana* > chloroform methanol extract of *C.capsularis*. ANOVA analysis (Table 1) showed significant difference ($p<0.05$) between TPCs of the samples.

The free radical scavenging effect of both *C.capsularis* and *S.rebaudiana* was assessed using the DPPH assay. Based on the result (Fig.1.), it showed that in both plant species, methanol extracts of *C.capsularis* and *S.rebaudiana* had considerably high DPPH radical scavenging activities as compared to the other extracting solvent. For EC_{50}, the lowest concentration was shown by methanol extract of *S.rebaudiana* (154.67 ± 8.33 µg/ml), followed by methanol extract of *C.capsularis* (293.33 ± 108.99 µg/ml) and chloroform methanol extract of *C.capsularis* (763.33 ± 85.70 µg/ml). There was significant difference ($p<0.05$) between the plant extracts by analysis of ANOVA.

ABTS assay (Fig.2.) was based on the antioxidant ability to react with ABTS radical cation generated in the assay system. Based on the result, it demonstrated that methanol extraction of both plant species had significant antioxidant activity in scavenging ABTS radicals. This was corresponded that high molecular weight phenolics have more ability in quenching ABTS radicals. Comparing between these two plants, *S.rebaudiana* showed more powerful in proving the significant effect of scavenging activity with the lowest

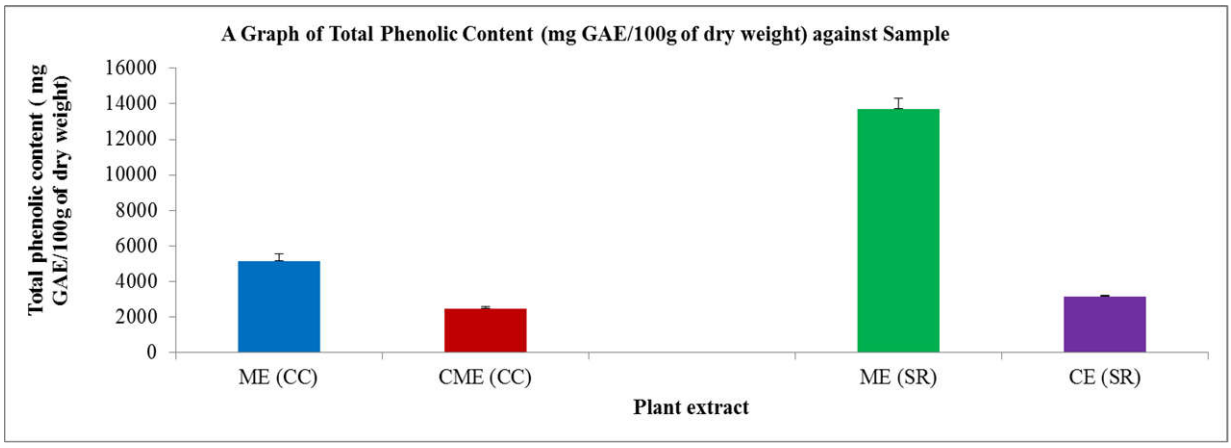

Figure1: Total phenolic content (TPC) assay of two plant species (mg GAE/100g of dry weight). ME (CC), methanol extract of *Corchorus capsularis*; CME (CC), chloroform methanol extract of *Corchorus capsularis*; ME (SR), methanol extract of *Stevia rebaudiana*; CE (SR), chloroform extract of *Stevia rebaudiana*.

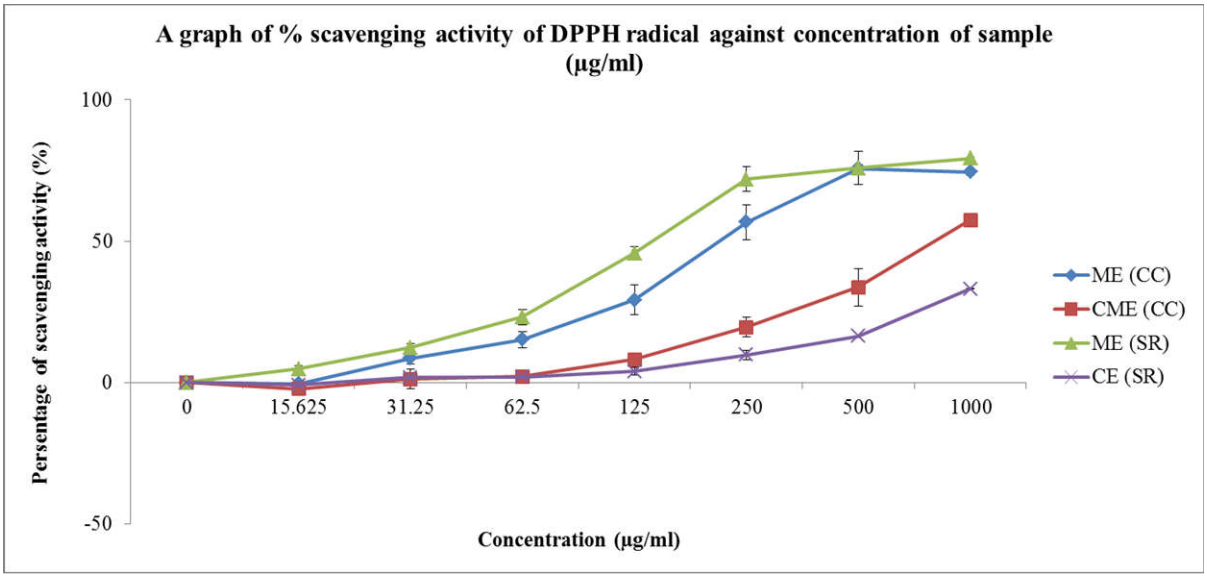

Figure 2: DPPH free radical scavenging activity of two plant species. ME (CC), methanol extract of *Corchorus capsularis*; CME (CC), chloroform methanol extract of *Corchorus capsularis*; ME (SR), methanol extract of *Stevia rebaudiana*; CE (SR), chloroform extract of *Stevia rebaudiana*.

EC_{50} at 278µg/ml. It was continuance to the significant correlation between TPC and ABTS with R^2= 0.475. This was followed by methanol extract of *C.capsularis* with EC_{50} at 483.33µg/ml. In fact, the lowest EC_{50} represents as the good antioxidant. From this study, it showed that analysis of ABTS assay was insignificantly different between these two plant species.

In the FRAP assay, it was measured the antioxidant effect of any substances or extracts in the reaction medium as reducing ability. The antioxidant capacities of both plant extracts were varied significantly, proved by highly positive correlation between TPC and FRAP on both plant extracts. Methanol extract of *S.rebaudiana* showed the highest FRAP antioxidant activity, while chloroform extract of *S.rebaudiana* had the lowest activity. However, comparing on both plant species showed that *S.rebaudiana* was found better to have more ferric reducing or antioxidant power than *C.capsularis*. The order of FRAP activity of respective plant extracts is as follow; chloroform extracts of *S.rebaudiana* > chloroform methanol extract of *C.capsularis* > methanol extract of *C.capsularis* > methanol extract of *S.rebaudiana*. Statistically, these plant extracts were significantly difference in the FRAP activity.

DISCUSSIONS

Nowadays, a lot of plants have become a source of natural antioxidants. The leafy part of plants is believed to contain multiple types of phenolic compounds. These phenolic compounds found in the leafy part are believed to exhibit the antioxidant effect and radical scavenging activity. Besides, phenolic compound also plays important role in preventing several chronic diseases related to oxidative stress and involved in cellular processes such as a redox reaction [18].

In this study, it was found that *S.rebaudiana* and *C.capsularis* plant exhibited antioxidant properties, particularly the leafy part. From the results obtained, it showed that methanol extract of *S.rebaudiana* possessed higher phenolic content, followed by methanol extract of *C.capsularis*, chloroform extract of *S.rebaudiana* and chloroform methanol extract of *C.capsularis*. Methanol extraction has more polarity and is able to extract more polar active compounds. For the chloroform methanol, it is an aqueous solvent, which is able to extract polar and non-polar active compound. While chloroform extraction are able to extract more non-polar active compound.

According to Hsu *et al.* [19], the differences in the phenolic content may be affected by availability of extractable components which depend on functional group that present in the compound. Besides that, the solubility of the antioxidant compounds in the plants also depends on the solvent polarity, extraction time and temperature as well as samples to solvent ratio [20,21]. ANOVA analysis showed that there is a significant difference between TPC's samples ($p < 0.05$). These results suggested that TPCs are varied significantly from one sample to another. Previous study also showed that the methanolic extract of *S.rebaudiana* leaves was rich in flavonoids, alkaloid and tannins [22]. While, the leaves of *C.capsularis* have shown the presence of flavanoids, tannins, steroid, saponin and triterpenes but no alkaloids [8].

The quenching activity of DPPH radicals can be ascertained by the hydrogen-donating ability of antioxidant molecules such as flavanoids, tannins and other active compounds [23]. Statistically, methanol extract is significantly difference from chloroform and chloroform methanol extract, is due to the different ability in scavenging free radical and donating hydrogen to form a stable DPPH molecule. However, some factors such as reaction time, solvent, concentration and used wavelength might influence the reaction of DPPH scavenging activity [24] . Incubation time of DPPH solution might also be one of the factors involved, because short incubation time has been frequently not sufficient to achieve the steady state of the reaction. From the results, it showed that the methanol extract of both plants has high DPPH radical scavenging activity compared to chloroform methanol extract of *C.capsularis* and chloroform extract of *S.rebaudiana*. The methanol extract of *S.rebaudiana* also exhibited low EC_{50} value compared to others. These findings, were similarly in agreement with Misuthisakul *et al*. [25] who found that, low EC_{50} obtained correspond to the highest antioxidant activity of *S.rebaudiana*. In addition, Berg *et al.* [26] reported that the secondary metabolite of *C.capsularis* such as the triterpene saponin extract is known to deliver an antioxidant activity, which exhibit the capability of DPPH radical scavenging activity. The antioxidant activity in the plant reflects the amount of phenolic compounds. This is because phenolic compounds have the ability to absorb and neutralizing free radicals as well as decompose the peroxides [27]. Hence, high phenolic content in the plant reflects the ability in scavenge DDPH radical.

ABTS radical scavenging activity

ABTS assay has the same purpose with DPPH assay as they are evaluating the antioxidant activity by scavenging free radical. Furthermore, the result showed statistically significant and indicates that antioxidant activities of both plant extracts are almost same for methanol extract. Methanol extract of plant were an effective scavenger of ABTS radical as indicated in previous study [16]. However, no EC_{50} value was reported for chloroform methanol of *C.capsularis* and chloroform of *S.rebaudiana*. Therefore, in order to achieve EC_{50} value for both plant extracts, a higher concentration might be needed.

In present study, however, there are several limitations. Firstly, it has to be taken into account that the ABTS assay is in vitro models and do not measure all of antioxidant activities in natural products. Secondly, the antioxidant capacity of plant extract and standard may differ depending on the solvent used. Previous study also reported that methanolic extract of *S.rebaudiana* leaf showed significantly ($p < 0.05$) high antioxidant activity [28]. Hence, from the result obtained, it showed that the methanolic extract of *S.rebaudiana* and *C.capsularis*

exhibited maximal percentage of inhibition activity of free radicals which is a more potent extract compared to chloroform extract of *S.rebaudiana* and chloroform extract of *C.capsularis*.

Ferric reducing ability power

In FRAP assay, the reductant acts by stopping the free radical chain by donation of hydrogen atom, and then responding with the peroxide precursor, thus preventing peroxide formation. The phenolic compounds found in plant may act in a similar activity as reductant. They may donate electrons and reacting with free radicals to convert them to more stable products, thus stop the free radical chain reaction [23]. FRAP assay will assess the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants, which are reductant with half-reaction reduction potentials above Fe^{3+}/Fe^{2+}. It is a hydrophilic antioxidant assay and not well responding to lipophilic antioxidants.

Antioxidant will exert their effect by donating a hydrogen atom to ferric complex and will immediately break the radical chain reaction [29]. Furthermore, previous study reported that ferric reducing bioactive compounds are correlated to antioxidant activity [30]. From the result, it showed that methanol extract of *S.rebaudiana* exhibited higher FRAP value, compared to methanol extract of *C.capsularis*. This showed that methanol extracts of *S.rebaudiana* are more better in reducing the ferric iron to ferrous iron. The ability to reduce Fe (III) is due to donation of a hydrogen atom from phenolic compounds of *S. rebaudiana* leaves which also correlate with the presence of reductant agent [31]. While the FRAP value for chloroform extract of *S.rebaudiana* and chloroform methanol of *C.capsularis* has low FRAP value. This may be due to the less hydrophilic contain of antioxidant properties that is needed in reducing ferric iron.

Correlation between total phenolic content and antioxidant activities

In this study, the total antioxidant activity of *C.capsularis* and *S.rebaudiana* increased with increasing concentration of extracts, indicating the potential of both plants as antioxidants. Relatively, highest total antioxidant activity in plant extracts showed a significant correlation with phenolic contents. This showed that the phenolic contents act as potential antioxidant biomolecules.

There is a highly positive correlation between total phenolic content and FRAP assay of *C.capsularis* (R^2=0. 797). This showed that phenolic contents carried more hydrophilic properties that exhibit antioxidant activity. The significant correlation proved that total phenolic contents act as strong reducing power in FRAP assay. However, there is some lipophilic phenolic content in *C.capsularis* that exhibit antioxidant activities. As the present of lipophilic properties of phenolic content, it acts as a free radical scavenger in DPPH assay and ABTS assay. This is because DPPH and ABTS assay are lipophilic antioxidant assays and increased the antioxidant activities with lipophilic phenol content. Due to that reason, it proved that strong correlation between DPPH and ABTS assay with R^2=0.729. The negative or weak correlation between total phenolic content and DPPH and ABTS assay may be proved that *C.capsularis* extracts carried more hydrophilic phenol as compared to lipophilic phenol content. Hence, the correlation coefficient analysis showed that hydrophilic and lipophilic phenolic content were responsible for antioxidant activities in *C.capsularis*.

As for *S.rebaudiana*, from the result, ABTS and DPPH assays exhibited the highest positive correlation among others (R^2= 0.994). ANOVA result determined that there is significant correlation between DPPH and ABTS ($p < 0.01$). Instead of that, positive correlations also determined from total phenolic content and FRAP value (R^2 =0. 779) which there is a significant correlation ($p < 0.05$). Nevertheless, weak positive correlations were indicated between DPPH and FRAP, TPC and ABTS, TPC and DPPH, as well as ABTS and FRAP. But there is no negative correlation reported.

According to Shukla *et al.* [32], there is a significant and linear relationship between antioxidant activity and phenolic content in *S.rebaudiana* leaf extracts. This indicated that phenolic content is responsible for antioxidant activities of *S.rebaudiana*. In addition, Jahan *et al*. [33] reported that over 100 phytochemical such as tannins and alkaloids had been found in *S.rebaudiana* leaves. This showed that as the total phenolic content increased, the antioxidant activities also increased. Thus, phenolic content reflects the antioxidant activity of the plant that showed a therapeutic potential of antioxidant, which possess the mechanisms to protect against the degenerative disease associated with marked oxidative damage [8].

CONCLUSIONS

From this study, it can be concluded that methanol extract of *S.rebaudiana* and *C.capsularis* possessed high phenolic content that reflects high antioxidant properties. Thus, it is believed that both plants have significant potential to be used as one of the natural antioxidant found in Malaysia as it is believed to exhibit therapeutic potentials in the future. In addition, for further investigation of the plant properties, a method such high performance liquid chromatography (HPLC) can be done to analyse more details related to the active compound involved. Several different assays can also be done to evaluate more about the antioxidant properties of the plants.

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Case Report

CORTICOSTEROID-INDUCED GLAUCOMA IN SEVERE VERNAL KERATOCONJUNCTIVITIS : TUBE OR TRABECULECTOMY

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ABSTRACT

Vernal keratoconjunctivitis (VKC) is a severe form of ocular allergy that affects primarily children and young adults living in areas with warm climates. While VKC is self-limiting, chronic forms in Asian eyes may be at higher risk of permanent visual impairment because of complications such as corneal scarring, cataract formation and glaucoma secondary to corticosteroid therapy. The incidence of glaucoma secondary to corticosteroid therapy is 2–7%, most often requiring filtering surgery. We present a case demonstrating the outcome of surgery in a patient; trabeculectomy in one eye versus Tube/shunt in the other.

Keywords: *Vernal Keratoconjunctivitis, steroid-induced glaucoma, trabeculectomy, Tube/Shunt.*

CASE REPORT

A 20 year old gentleman first presented when he was 10 years old with itchiness and epiphora in both eyes for 2 years duration. Examination revealed giant papillary hypertrophy in the palpebral conjunctiva in both eyes. He was diagnosed with severe vernal keratoconjunctivitis and prescribed topical Olopatadine and Betamethasone 0.1% eye drops in tapering dose over 10 months. Betamethasone 0.1% was replaced with topical Fluoromethalone 0.1% a year later, which was then further replaced by topical Cyclosporine 0.5% in 2008 due to increase in intraocular pressure (IOP) in both eyes.

The patient, however, defaulted follow-up between 2010 and 2015 during which he self-administered Fluoromethalone 0.1% eyedrops on a PRN basis (Pro-re nata). He presented again in December 2015 with left blurring of vision of 3 weeks duration. The intraocular pressure was 48 mmHg in the right eye and 54 mmHg in the left eye. Gonioscopy revealed open angle of grade 4. Funduscopy revealed glaucomatous optic disc with cup-disc ratio of 0.9 in both eyes. The Humphrey visual field showed constricted visual fields in both eyes with mean deviation of -23.87 db and -15.16 db in the right and left eye respectively. Optical Coherence Tomography (OCT) revealed right superior and inferior quadrant thinning and left generalised thinning of the retinal nerve fibre layer.

Intraocular pressure in right eye was 36 mmHg with maximum tolerated medical therapy (3 topical anti-glaucoma drugs and oral Acetazolamide 250mg QID). Glaucoma Drainage Device (GDD), a tube shunt Ahmed Valve was implanted in March 2016 to control the IOP. Trabeculectomy was deemed unsuitable as the eye was too inflamed due to severe vernal conjunctivitis. The postoperative hypertensive phase developed and prolonged requiring anti-glaucoma medication to control the IOP. His final IOP in the right eye was 20mmHg.

The left IOP was also poorly controlled medically with IOP ranging between 50 and 52mmHg. Due to financial constraint, trabeculectomy with intraoperative application of mitomycin C was performed. His IOP maintained at 12mmHg at postoperatively without additional anti-glaucoma medication.

DISCUSSION

Vernal keratoconjunctivitis (VKC) is a severe form of ocular allergy that affects primarily children and young adults living in areas with warm climates [1]. However, the pattern and severity of this disease vary widely, depending on racial and environmental differences; most patients with VKC in temperate countries suffer from seasonal exacerbations, but the disease is often chronic and persistent in warm, tropical climates around Asia [2]. Although most cases of VKC are self-limiting, chronic forms in Asian eyes may be at higher risk of permanent visual impairment because of complications such as corneal scarring, cataract formation and glaucoma secondary to corticosteroid therapy [3].

The reported incidence of glaucoma in patients with VKC receiving corticosteroid therapy is 2–7% [4]. ‘Steroid response’ or corticosteroid-induced ocular

hypertension is due to decreased trabecular outflow causing a rise of IOP [5]. Resultant glaucomatous nerve damage may persist even after corticosteroid therapy is discontinued and the IOP normalizes [6]. Lam *et al.* showed peak IOP to be dose dependent and more quickly developed in children below 6 years of age [7]. However, Ang *et al.* (2012) showed that the risk factors for eyes that have severe VKC with steroid response that eventually requires surgery are related to a longer duration of topical corticosteroid use, higher peak IOP and greater increase in IOP from baseline IOP [8].

In most patients, the average time to steroid response is 6 weeks; although the IOP may still rise after this time period [9]. In patients with severe VKC and steroid response, monitoring of IOP is important throughout the duration of therapy [10]. It is found that both the increase in IOP from baseline and peak IOP were important indicators for increased risk of surgery [8]. The magnitude of IOP rise depends on the potency of topical corticosteroids, with dexamethasone 0.1% the most potent, prednisolone acetate 1.0% followed by fluorometholone 0.1% [11]. On the other hand, Cyclosporine is an immunosuppressive drug that has been shown to reduce corticosteroid dependence in patients with VKC, by blocking Th2 lymphocyte proliferation, histamine release from mast cells and eosinophil infiltration [12]. However, its use did not affect the risk of steroid response and need for surgery [8].

Our patient had a successful trabeculectomy with Mitomycin C (MMC) in the left eye with excellent IOP control. Mitomycin C is an alkylating agent that selectively inhibits DNA synthesis. It has been found to be effective for short-term use if used topically, at low concentrations of 0.01% [13]. By inhibiting both inflammatory cells and fibroblasts, it is effective when steroids or mast-cell stabilizers cannot control symptoms of VKC. Ang et al (2012) showed that MMC 0.02% soaked in surgical sponges applied directly to the bare sclera in the superior fornix during trabeculectomy had a substantial effect on the clinical course of VKC. There was a general improvement of the ocular surface and reduced corneal epitheliopathy post-trabeculectomy with MMC, which led to improvement in BCVA postoperatively [8].

On the other hand, Ahmed Glaucoma Valve implant is often implanted in patients whose glaucoma is inadequately controlled by medical therapy or for whom filtration surgery has been unsuccessful, is contraindicated, or is unlikely to succeed. Multiple studies have shown this device to be safe and effective in lowering IOP [14]. Glaucoma drainage device was offered to the patient initially in view of the possible conjunctival scarring secondary to the underlying severe vernal kerato-conjunctivitis.

In cases where the Ahmed Glaucoma Valve has failed, revision is an option. However, our patient had the prolonged hypertensive phase which often occurs after the implantation of Ahmed glaucoma device. Hypertensive phase is characterized by IOP elevation beyond 21mmHg, occurring anywhere between 1 to 6 weeks postoperatively. It is presumably due to thick-walled bleb over the plate of the implant. Eyes with a hypertensive phase had a higher mean IOP and needed more medications 6 to 12 months after surgery. However, it resolves in minority of eyes [7].

Thieme *et al.* discussed regarding encapsulation of the Ahmed Glaucoma Valve as an early complication in young patients that leads to inhibition of fluid exchange and failure of the procedure. The investigators found that the IOP could be controlled through removal of only the encapsulated blebs in all four of their cases [15]. He also proposed that the valve mechanism was blocked by contracted scar tissue but that the device itself was not affected by the encapsulation. Their conclusion was based on the fact that surgical excision of the capsule immediately led to aqueous flow and a drop in IOP [15].

The Tube Versus Trabeculectomy study showed that Tube shunt surgery was more likely to maintain IOP control and avoid persistent hypotony, reoperation for glaucoma or loss of light perception vision than trabeculectomy with MMC during the first year of follow -up. Both surgical procedures had similar IOP reduction at 1 year, but less supplemental medical therapy was used following trabeculectomy. The incidence of postoperative complications was higher after trabeculectomy with MMC relative to tube shunt surgery, but serious complications associated with vision loss or reoperation developed with similar frequency in both procedures [17]. The reported success rates of Ahmed Glaucoma Valve implants are between 60% and 82% at 2 years [19]. As seen in the Collaborative Initial Glaucoma Treatment Study (CIGTS), trabeculectomy surgery is limited by a suboptimal long-term success rate. The rate of failure has been reported to be as high as 23% to 51% at 5 years and 52% to 59% at around 15 years, even with adjunctive 5-Fluorouracil or mitomycin C [18].

CONCLUSIONS

In Asian eyes suffering from VKC, the risk of corticosteroid-induced glaucoma may be higher due to chronic use of topical corticosteroids. Patients and their parents should be informed regarding the long term side effect of the corticosteroid eyedrops and abuse of the medication should be avoided. Corticosteroid should be added as pulse therapy only in severe cases and not as prolonged use as in this case. While most of these patients may be controlled with topical medications, it is important to identify clinical characteristics, which increase the risk of progression and surgery. There is limited study found comparing the effectiveness of augmented trabeculectomy versus glaucoma drainage device in young VKC patients with steroid induced glaucoma. However, some studies showed significant improvement in the signs and symptoms of VKC

following trabeculectomy with MMC. This could be related to the after-effects of MMC on the ocular surface and it supports the advantages of MMC use in severe, refractory forms of VKC. Future study is warranted in order to identify factors for unfavorable surgical outcomes of Ahmed glaucoma device in steroid induced glaucoma in VKC patients.

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