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Borneo Journal of Medical Sciences
Faculty of Medicine and Health Sciences
Universiti Malaysia Sabah
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Tel: +60 88 320000 Ext 5620
Fax: +60 88 320928
Email: bjms@ums.edu.my

PUBLISHER

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EDITORIAL

Influenza!! A hidden Burden of Disease in Malaysia

M. Tanveer Hossain Parash[1, 4*], Sadia Choudhury Shimmi[1], Mohammad Saffree Jeffree[3], Mohd Yusof Ibrahim[3], Kamruddin Ahmed[2, 4]

[1] Department of Biomedical Science and Therapeutics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[2] Department of Pathobiology and Medical Diagnostics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[3] Department of Community and Family Medicine, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah Kota Kinabalu, Sabah, Malaysia
[4] Borneo Medical and Health Research Centre, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah Kota Kinabalu, Sabah, Malaysia

*Corresponding author's email: parash_cmc@ums.edu.my

Influenza, a contagious respiratory illness presented with sudden onset of fever, sore throat, cough – usually unproductive, runny nose, headache, aching muscles and severe malaise, caused by influenza viruses that infect the nose, throat, and lungs. Yearly, influenza infection leads to an estimated 3 to 5 million cases of severe illness and 250,000 – 500,000 deaths[1]. In children under 5 years, an estimated 28,000 – 111,500 deaths accountable to influenza occur annually, with 99% of the number occur in developing countries[2]. Information on childhood influenza is scarce in Malaysia.

Vaccination is the most potent measure to inhibit influenza infection and its complications[3]. In Malaysia, the H1N1 pandemic in 2009 has increased consciousness of the general people and medical personnel about influenza[4]. Most of the information were obtained from Kuala Lumpur with no information obtained from Sabah. In 2009, Seroprevalence rate of seasonal H1N1 was 14.7% and of H3N2 was 21%. Among the Malaysian population, influenza A Infection was common[5].

The available data is not enough to draw a complete picture of the predominant circulating strain of the influenza virus in Malaysia at any one time. In 2018, a preliminary study was conducted using Prolast Flu One kits to investigate the prevalence of influenza among the students of Universiti Malaysia

Sabah. The study revealed that prevalence of influenza was 8.1%. The prevalence for both Influenza A and Influenza B was equal (4.05%) which is a unique finding of this study compared to other studies, where influenza A was more common occurring strain than influenza B[6]. This finding identifies that the present scenario of influenza prevalence is varying with the previous. It is perceptible that influenza causes a pronounced and under recognized burden of disease in Malaysia.

The void in knowledge of the burden of influenza in Malaysia is diverse. Timely and adequate efforts are essential to fill these gaps. Vaccines and antivirals are potent interventions that can be adopted to reduce the burden.

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REVIEW ARTICLE

Recent Advances in Breast Cancer Diagnosis Entering an Era of Precision Medicine

Christopher Lim[1*], Aung Mra[1], Chin Suliong[2], Challa Venkata Rao[1], Tun Aung[1], Jony Sieman[1], Win Tin[1], Maher F. Sefein Beshay[1], Alvin Oliver Payus[3], Meryl Grace Lansing[3], Mohammad Saffree Jeffree[4], Fairrul Kadir[4], Firdaus Hayati[1,4]

[1] Department of Surgery, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[2] Department of Medical Education, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[3] Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[4] Dean's Office, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

*Corresponding author's email: christopherlim@ums.edu.my

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Keywords: breast cancer/neoplasm, cancer/neoplasm staging, diagnostic imaging, precision medicine

ABSTRACT

This article will cover some of the most recent advances in the diagnosis of the world's most common cancer in women, namely, breast cancer as we enter the era of precision medicine. The authors will discuss the differences between East and West pertaining to the incidence and mortality rates, the types of breast cancer and the revised staging criteria of breast cancer according to the American Joint Committee on Cancer (AJCC) Staging Manual, 8th edition. In addition, the advances of newer imaging modalities are presented and compared with traditional ultrasonography and mammography.

INTRODUCTION

Worldwide, breast cancer remains the most common cancer in women. According to WHO (2015) 1,700,000 new cases were diagnosed each year and 520,000 died from the disease[1]. In the USA alone (2017), 252,710 cases were added and 40,610 died from the disease. This translates into something like 1 in every 8 women in the USA will be diagnosed with breast cancer each year[2]. In Malaysia, the Ministry of Health's National Cancer Registry reported the Age Adjusted Incidence Rate to be 47.3/100,000. Simply put, 1 in every 20 women in Malaysia will be diagnosed with breast cancer each year. This rate is highest in Malaysians of Chinese descent followed by Indians and Malays. The mortality rate is exactly reversed among the three ethnic

groups leaving a lot of fertile ground for epidemiologists to sort through[3].

Is there a difference between breast cancers seen in the West compared to those seen in Southeast Asia? While the disease shares many common features, there are obvious differences. The peak incidence is 40 – 50 years in the East and 60 – 70 years in the West. The incidence is increasing in the West but the mortality is decreasing. However, in the East, both incidence and mortality are increasing. Most of the cancers seen in the West are at earlier stages compared to later stages in the East.

In 2018, the Age of Precision Medicine is upon us. The first and most common example of this modality of treatment was started in 2005 when Trastuzumab was used in treating HER positive breast cancer [4, 5]. Precision Medicine is the prescription of a specific therapy based on that individual's molecular characteristics. Recent breakthroughs in pharmacogenetics and pharmacogenomics have allowed us to predict which treatment(s) will be safe and effective and which are not. This model is changing the way we think and manage diseases and will only expand as our understanding and technology advances[6, 7, 8].

Traditionally, chemotherapy works by killing cells that are replicating rapidly. Precision medicine works differently by slowing and/or stopping the growth and even metastasis of cancer at the cellular level by interfering with cellular signalling and/or metabolic pathways. This method of treatment will improve outcome as well as decrease side effects[9].

While precision medicine is making great strikes in the treatments of melanoma, lung and genitourinary malignancies, its impact on breast cancer is still limited to clinical trials involving metastatic Triple Negative Breast and HER positive breast cancers. That too will change in the not too distant future.

The observations of a causal relationship between BRCA 1 & 2 mutations and increased risks for breast and ovarian cancers have increased the demand for genetic testing[10]. However, the conventional methodology is costly as well as time consuming. A new next-generation sequencing (NGS) technique is now available that supersedes conventional approach. This method is more accurate, less time consuming and more affordable[11, 12].

TYPES OF BREAST CANCERS

Traditionally, breast cancer is histologically classified as ductal or lobular assuming that the tumours arise from ductal and lobular epithelium respectively. However, it is now believed that both categories of tumours actually arise from cells in the terminal duct lobular unit (Figure 1A). The terms ductal carcinoma and lobular carcinoma now describe their histomorphologic likeness to normal ducts and lobular spaces respectively (Figure 1C). In combination, these two accounted for over 90% of breast cancers. The remainder is a mixture of other types like papillary, medullary and metaplastic.

A more informative and recent classification is molecular sub-typing of breast cancer. Studies are being done on how molecular subtypes of breast cancer may be useful in planning treatment and developing new therapies. Molecular and genetic information from tumour cells are used to determine the complicated profile of each subtype.

Most studies divide breast cancer into 4 major molecular subtypes (Figure 1B):

- Luminal A
- Luminal B
- Triple negative/basal-like
- HER2 type (human epidermal growth factor receptor 2 type)

Luminal A and Luminal B tumour cells look like those of breast cancer cells that start in the inner (luminal) cells lining the mammary ducts. However, there are differences between the two molecular subtypes.

Luminal A tumours tend to be estrogen receptor-positive (ER-positive), HER2 receptor-negative (HER2-negative) and Tumour grade 1 or 2. About 30 – 70 per cent of breast cancers are luminal A_tumours. Of the 4 subtypes, luminal A tumours tend to have the best prognosis and to a moderate extent high survival rates and fairly low recurrence rates[13].

Luminal B tumours tend to be ER-positive. They may be HER2-negative or HER2-positive. About 10 – 20 per cent of breast cancers are luminal B tumours. Women with luminal B subtype breast cancer tend to have fairly high survival rates, but not as high as those with luminal A tumours[13].

Triple negative breast cancers are oestrogen receptor-negative (ER-negative), Progesterone receptor-negative (PR-negative) and HER2-negative. Out of several subsets of triple negative breast cancer one variety is known as basal-like. Basal-like tumours have cells that look similar to those of the outer (basal) cells surrounding the mammary ducts which contract for milk ejection (Figure 1B). About 15 – 20 per cent of breast cancers are triple negative/basal-like. Triple negative/basal-like tumours behave aggressively and have a poorer prognosis compared to the ER-positive subtypes (luminal A and luminal B tumours) [13].

The molecular subtype HER2 type tumours are HER2-positive but a few percentages are HER2-negative (Figure 1D). HER2 type tumours tend to be ER-negative, PR-negative, Lymph node-positive and poorer tumour grade. About 5 – 15 per cent of breast cancers are HER2 type. HER2 type breast cancers that are HER2-positive can be treated with anti-HER2 drugs such as trastuzumab (Herceptin)[13].

Breast cancer pathogenesis and histologic vs molecular subtypes

Adapted from The McMaster Pathophysiologylogy Review (MPR)[14]

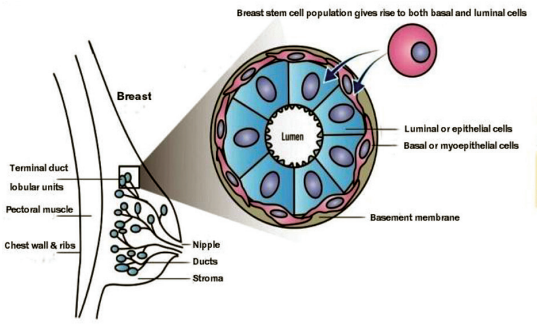

Figure 1A Cross section view of mammary duct in terminal duct lobular unit. All breast cancer lesions arise from the terminal duct lobular unit.

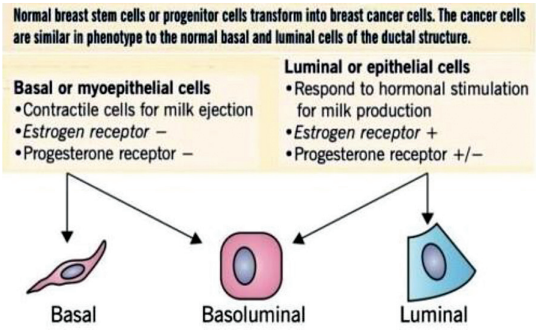

Figure 1B Cancer cell phenotype

Histological subtypes	Ductal	Lobular
Preinvasive cancer 25% Cells limited to basement membrane	Ductal carcinoma in situ (DCIS) 80% May spread through ducts and distort duct architecture 1% progress to invasive cancer per year Usually unilateral	Lobular carcinoma in situ (LCIS) 20% Does not distort duct architecture Same genetic abnormality as ILC – E-cahderin loss 1% progress per year Can be bilateral
Invasive cancer 75% Extension beyond the basement membrane	Invasive ductal carcinoma (IDC) 79% Usually from DCIS precursor Cause fibrous response, producing a palpable mass on examination Metastasis through lymphatics and blood	Invasive lobular carcinoma (ILC) 10% Usually from LCIS precursor Minimal fibrous response, presents less often with palpable mass Metastasis through abdominal viscera to GI, ovaries, uterus Almost always ER+

Figure 1C Histological subtypes

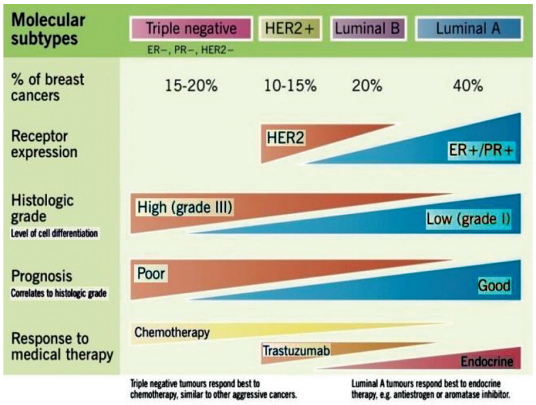

Figure 1D Molecular subtypes

IMPORTANT CHANGES MADE IN 8TH EDITION OF AJCC CANCER STAGING MANUAL[15]

Table 1 Shows the incorporation of TNM staging into the AJCC prognostic stage groups

ANATOMIC STAGE/ PROGNOSTIC GROUPS			
Stage 0	Tis	N0	M0
Stage IA	T1*	N0	M0
Stage IB	T0	N1mi	M0
	T1*	N1mi	M0
Stage IIA	T0	N1**	M0
	T1*	N1**	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2	M0
	T1*	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

T1* and N1** changes are discussed below.

Starting in 2018, the American Joint Committee on Cancer (AJCC) Staging Manual 8th edition requires inclusion of prognostic factors such as ER, PR, HER2 in breast cancer staging. In addition, whenever appropriate genomic tests like Oncotype-Dx, Mamma Print, Endopredict, PAM 50 Prosigna, and Breast cancer index are also to be included[15, 16]. Inclusion of multigene panels have resulted in changes in T1* category downstaging some tumours regardless of T size, and placing them in the same prognostic category as T1a-T1b N0 M0. These tumours will be staged using the AJCC prognostic stage group table as Stage I[16] (Table 1).

Isolated tumour cells clusters (ITC) in regional lymph nodes can be identified by haematoxylin and eosin (H&E) or immunohistochemistry (IHC). ITC that are 0.2 mm or less is classified as pN0(i+). The pN1 category includes pN1mi micrometastases which is defined as node deposits of tumour cells 0.2 – 2 mm. pN1a is defined as 1 – 3 nodes with at least 1 node with a deposit greater than 2 mm. pN2a is defined as 4 – 9 positive nodes and pN3a is 10 or more positive nodes. pN1b is defined as a positive internal mammary sentinel node with a deposit greater than 0.2 mm in the absence of axillary node metastases. pN1c is a combination of pN1a and pN1b[16].

Previously, any nodal involvement was considered N I or Stage II. Lobular carcinoma in situ is removed from the staging system because it is not a malignancy but a risk factor. It is no longer considered Tis. With these changes, it is anticipated that in the coming years, more Stage I breast cancers will be seen and less of Stage II and III.

With implementation of these changes, breast cancer treatment has entered the era of precision medicine where treatment is tailored for each patient based on genomic profiling of her breast cancer and its response to biopharmacologic manipulation.

CURRENT AND FUTURE STATUS OF BREAST IMAGING

Current modalities of breast imaging include Mammography, Breast Ultrasound, Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Thermography, Positron Emission Tomography (PET-CT, PET/MRI) and Breast Elastography, a new sonographic imaging technique. While mammography remains the Gold Standard, there are limitations associated with its use. One main issue is the lack of sensitivity in young women with dense breasts. However, the introduction of 3D Mammography (Tomosynthesis) is changing this old paradigm that young woman undergoes ultrasound but not mammographic imaging. Below are averages of sensitivity and specificity of the various modalities (Table 2).

Table 2 Shows the averages of sensitivity and specificity of the various modalities of imaging

Imaging modality	Sensitivity	Specificity
Mammography	~72%	~47%
MRI	~93%	~61%
Ultrasound	~61%	~87%
PET	~77-100%	~69-80%
CT	~71%	~83%
Thermography	~39%	~82%
3D (Tomosynthesis)	~100%	~75%

With 3D Mammography, the breast is placed between two compression plates (Figure 2). A robotic arm will move the scanner in an arc over the breast and over 60 multiple images are then taken. The procedure takes 10 – 20 minutes. The dose is slightly higher than standard 2D digital mammography. The software is similar to that used in CT and 3D reconstructed images are then ready for the radiologist to read. This method eliminates tissue overlap, increases the number of invasive cancer detected and initiates the number of women being recalled for further testing. A new Software called C-View Hologic allows Tomosynthesis to acquire both 2D and 3D images at the same time, hence decreasing further exposure[17].

Figure 2 3D mammography[17]

Automated Breast Ultrasound (ABUS)

Figure 3 Automated breast ultrasound (ABUS)

Photo Courtesy of U-Systems[17]

The automated breast ultrasound uses a transducer that is placed over the breast lightly and in about 10 minutes, 3D images of the **entire** breast are acquired automatically compared to the traditional hand-held method which obtains 2D images and takes over 30 minutes (Figure 3). This device is operator-independent. When used in conjunction with mammography it increases sensitivity to 97%. According to a University of Chicago study when ABUS was combined with mammography, there was an absolute increase of 31% in detection rate of breast cancer in asymptomatic women with dense breasts and a normal mammogram[17].

Photoacoustic Microscopy (PAM) or Photoacoustic Tomography (PAT)

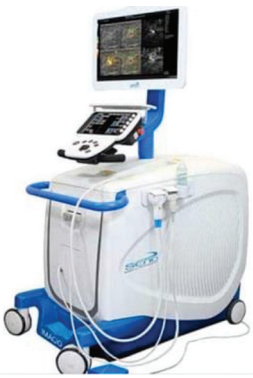

Figure 4 SENO Medical's Imagio Opto-Acoustic Breast Imaging System proves a strong predictor of malignancy[17]

This new hybrid technology combines light and sound to achieve a high optical as well as spatial resolution of the breast tissue structure as well as function (Figure 4, Figure 5). This modality can be used to follow progression and resolution of breast cancers following therapy. Potentially it can give the surgeon real intraoperative information as to the adequacy of his margins of resection[17].

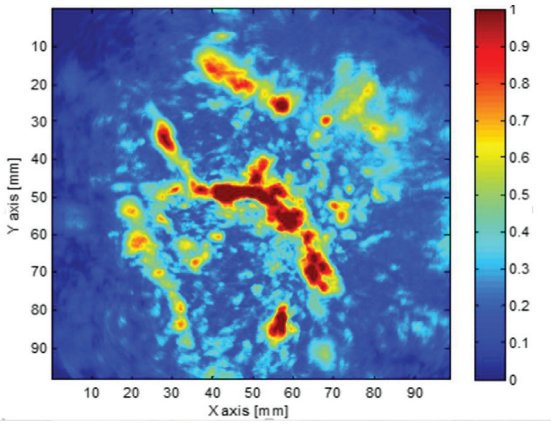

Figure 5 Photoacoustic Imaging of breast cancer Optical Imaging Laboratory, University of Michigan[17]

Molecular Breast Imaging (MBI)

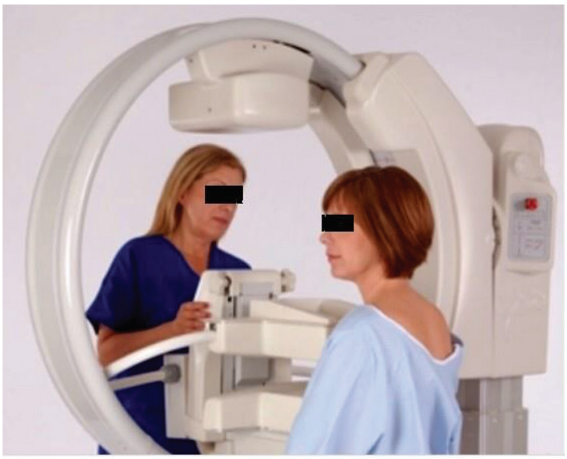

Figure 6 Molecular Breast Imaging (MBI)[17]

This new Nuclear Medicine technique employs injecting Technetium-99m Sestamibi intravenously about 5 minutes before scanning the breast under light compression between two plates (Figure 6). Overall sensitivity is 90% but only 82% if the tumour is <1.0 cm and much less if below 5.0 mm in size. MBI does detect 2 – 3 times more breast cancers in mammography dense breasts and in those women with an increased risk for cancer[17] (Figure 7).

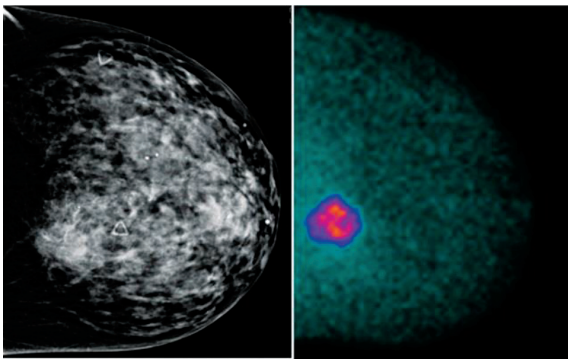

Figure 7 Using this new technology (MBI) a palpable mass that was difficult to see on mammography (left) is clearly visible on molecular breast imaging (right). (Images courtesy of Dr Beatriz Adrada)[17]

Breast Elastography

Elastography is an ultrasound imaging technique that evaluates the **stiffness** of a breast lesion. It is an attempt to differentiate between benign versus malignant breast lesions. The hope here is to reduce the number of unnecessary benign breast biopsies. Currently there are **two** techniques used:

A. Straw Elastography

It is a qualitative, fast, real time method but influenced by the size and characteristics of the lesions. This is also operator-dependent.

B. Shear Wave Elastography

It is a quantitative, more accurate method but is also limited in the assessment of a very **stiff** lesion. While this technique is used to complement traditional mammography and ultrasonography, its stand-alone sensitivity and specificity has yet to be determined[18].

CONCLUSION

This article discusses recent advances in the diagnosis of breast cancer, the differences between the incidences and mortality rates between the West and the East, the different types of breast cancers, the recent revision of the staging criteria of the American Joint Committee of Cancer Staging Manual (8th edition), and the new advances in the various medical imaging modalities. With these developments in diagnosis, it is hoped that treatment will be more targeted and personalized, thus improving health outcomes of women with breast cancer in the era of precision medicine.

CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this article.

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ORIGINAL ARTICLE

Effects of Dyes on Pulmonary Functions in Male Spray Dye Workers in Dhaka, Bangladesh

Nahid Sultana[1*], Md. Shahidullah[2]

[1] Department of Community Medicine, Dhaka National Medical College, Dhaka, Bangladesh
[2] Department of Neurology, Bangobanbhu Sheikh Mujib Medical University, Dhaka, Bangladesh

*Corresponding author's email: dr_nahidsultana@yahoo.com

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Keywords: spray dye workers, pulmonary functions, Dhaka

ABSTRACT

There are many steel product manufacturing industries in Bangladesh where chemical dyes are used for coating the steel made product. The spray dye workers are continuously exposed to chemical dyes. Prolong exposure of these dyes can cause pulmonary disorders of the workers. This cross-sectional study was done to evaluate pulmonary functions of male dye workers. This study was carried out in the Department of Community Medicine in Dhaka National Medical College from September 2013 to September 2014. Out of 60 subjects, 30 apparently healthy male spray dye workers were taken from different steel manufacturing factories as study group and 30 apparently healthy male subjects who are not exposed to spray dye were taken as control. Sample was taken by convenient type of non-probability sampling. Auto spirometer (AS-507) was used to measure the lung functions of both groups. Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), peak expiratory flow rate (PEFR) and FEV1/FVC% of all subjects were recored by using a digital spirometer. Unpaired *t*-test were performed for analysis of data. The mean percentage of predicted value of FEV1, FVC and FEV1/FVC% were significantly lower ($p < .001$) in spray dye workers than control group. From the result of this study it can be concluded that spray dye may have harmful effects on some pulmonary functions.

INTRODUCTION

Poor health , safely and waste management with no control over the length and frequency of exposure may pose several health hazards to

textile workers. Occupation health authorities arround the world have established safety regulations and /or guidelines to limit workers exposures through safe practices and personal protective equipment (PPE)[1]. An appropriate attitutute towards the health risks associated with exposure to dyes, depends on knowledge about the danger and harmful effects fo dyestuff. Millions of workers are exposed to dyes in different occupation but they have very little knowledge or no knowledge at all about the harmful effects of dyes. Hydrocarbon are organic compounds that are mainly derived from petroleum and used as solvent with different colour. These substances are volatile, serious toxicity and death can be associated with hydrocarbon exposure through inhalation and ingestion. Pulmonary toxicity is the most common health hazard. Hydrocarbon pneumonitis is caused by destruction of alveolar and capillary membranes as well as alteration of surfactant function and production, this eventually can lead to acute respiratoty distress syndrome[2].

Due to extensive surface area, high blood flow and free communication with external environment lungs are more susceptible to occupational pulmonary diaseases. On prolong exposure which may turn to lung fibrosis[3]. The pulmonary function test (PFT) have opened a new era towards scientific approach in diagnosis, prognosis and management of pulmonary disorders. The normal value ranges for pulmonary functions tests will be adjusted for the subjects age, height, sex and sometimes race. Research reports on the effects of spray dyestuff on pulmonary functions are very scarce. Different types of oxide colours like red oxide, blue oxide, silver oxide and hydrocarbon solvent were used in steel manufacturing factories. Those oxide contain free radicals and generally more reactive than non-radicals[4]. In this study, dye workers exposed to those oxides were included.

This study aims to investigate pulmonary fuction in workers dealing with dyestuff to observe the changes in lung function upon prolong exposure.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Community Medicine, Dhaka National Medical College from September 2013 to September 2014. This research work was approved by the Ethical Committee of Dhaka National Medical College. Total 30 appraently healthy male spray dye workers exposed to dyestuff for at least 6 months, age range 20 – 40 years were taken as study group. They were selected from different steel manufacturing and dyeing industries in old Dhaka City. Another 30 apparently healthy, age and socio-economically matched male non-spray dye workers were taken as control for comparison. A detailed medical and family history of all worker was recorded in a prefixed questionaire. Those who had history of any respiratory or cardiac problem were excluded. Among 60 respondents 54 (90%) were smoker, 26 (48.15%) were belong to study group and 28 (51.85%) were control group. Dye worker but non-smoker was not available, thay is why almost similar number of smoker control group was taken and mean value of their lung functions were compared. Smokers were defined as currently smoking at least one cigarette daily. Thorough clinical examination of each subject were done. Height, weight were measured to calculate BMI and for assessing lung function, FVC, FEV1, PEFR and FEV1/FVC % were measured at normal room temperature by using a digital spirometer (AS-507). For statistical analysis, unpaired *t*-test was perform by using SPSS-20 version.

RESULTS

Mean age of study and control group were (24.40 ± 3.57) and (23.43 ± 3.41) respectively. Daily working hours of the study group and control group were mentioned in Table 1. Among the study group, 13 (43.33%) workers worked for 8 – 10 hours per day, 9 (30%) worked for 10 – 12 hours per day and 8 (26.66%) worked for 6 – 8 hours per day. All subject of control group worked for 8 – 10 hours per day.

Table 1 Working hours per day of study group (*n* = 30) and control group (*n* = 30)

	Working hours/ day	**Frequency**	**Percentage**
Study group	6 – 8 8 – 10 10 – 12	8 13 9	26.66% 43.33% 30.0%
Control group	8 – 10	30	100%

Data expressed daily working hours of study group and control group. Data are expressed as mean ± SD, *n* total number of subject, study group: workers exposed to dyestuff and control group: workers non-exposed to dyestuff.

BMI (body mass index) of study group and control group were (17.02 ± 1.64) and (21.51 ± 2.6) respectively (Figure 1).

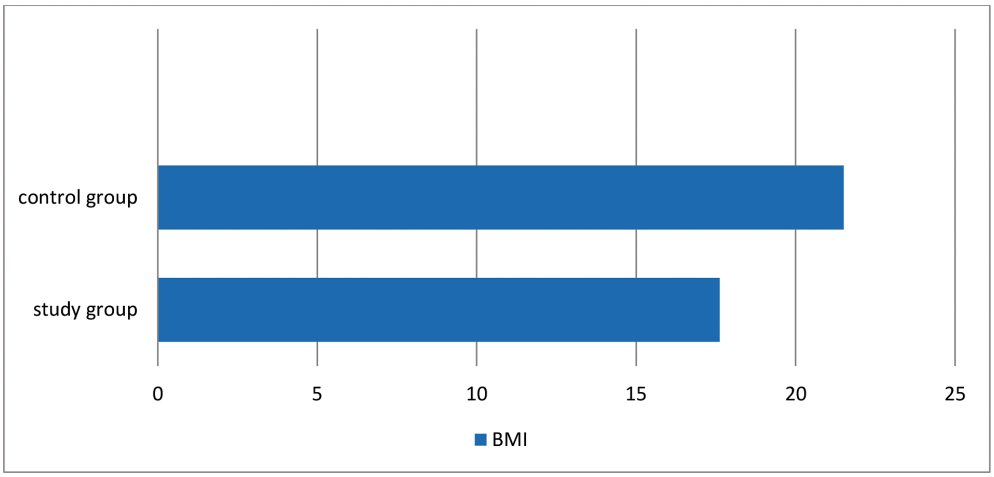

Figure 1 Mean BMI in study group and control group

The measured values of FVC, FEV1, PEFR and FEV1/FVC% were significantly lower in workers exposed to spray dyes than the control group (Table 2).

Table 2 Percentage of predicted value of FVC, FEV1, PEFR, FEV1/FVC%

Parameter	Study group (*n* = 30)	Control group (*n* = 30)
FVC	63.70 ± 25.26***	100.63 ± 13.48
FEV1	58.03 ± 25.33***	106.60 ± 11.48
PEFR	45.70 ± 31.13***	93.07 ± 9.50
FEV1/FVC%	94.12 ± 21.40***	111.63 ± 7.45

*** = $p < 0.001$

Data are expressed as mean ± SD, unpaired *t*-test was done, *n* = total number of subjects, study group: workers exposed to dyestuff and control group: workers not exposed to dyestuff.

DISCUSSION

Hydrocarbon are used as thinner with different dyes in many steel manufacturing industries. The pulmonary system is most commonly affected by hydrocarbon through inhalation or aspiration.

Different types of oxides contain free radicals, which are more reactive than non-radicals[4]. A number of studies had demonstrated that free radicals are responsible for injurious and inflammatory response in the airways[5]. Exposure to the gases which contaisn free radicals at higher concentration causes characteristics lesions of the respiratory tract due to accumulation of macrophages in the alveoli and damage to the type-I alveolar epithelial cells which line the alveolar sacs[6].

Hydrocarbon pneumonitis is caused by destruction of alveolar and capillary membranes as well as alteration of surfactant function and production, this eventually can lead to acute respiratoty distress syndrome[2]. In this study lung fuction were measured in both control and study group.

Mean measured value of FVC, FEV1, PEFR and FEV1/FVC% were significantly lower in exposed group than control group. These findings are similar with those of other researchers[3, 7,8].

In a hot humid workplace, the dyes affect both allergens (e.g. reactive dyes) and irritants (e.g. H_2S, SO_2 and nitrogen oxides) to increase in the respiratory system. Another study showed the frequency of acute and chronic respiratory symptoms was significantly higher among workers in the exposed group than in the control group. Means of FVC and FEV1 of pre-shift spirometry were lower than control ($p < 0.001$). Across-shift spirometry showed significant reduction of FVC ($p < 0.001$), FEV1 ($p < 0.001$), FEF 25 – 75% ($p = 0.05$) and FEF 25% ($p = 0.007$) in dyeing workers compared to the control group[9].

Eugenija Zuskin et al. found in their study that the prevalence of respiratory problems were more in case than control group. The spirometric parameters were found decreased in male after work shift and force expiratory flow (FEF) 25 – 75%, (FEF) 50% and (FEF) 25% were found significantly decrease in female workers[10]. Another study done in Turkey textile dyeing factories which comprised 106 exposed and unexposed workers which revealed that the mean expiratory flow rate (FEF) 25 – 75% of the exposed worker[11]. Various flow rates (FEF 25%, FEF 50% and FEF 75%) were found reduce in workers who working in carpet industries[12].

CONCLUSION

From the result of this study it can be concluded that spray dye may have harmful effects on some pulmonary functions.

CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this article.

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ORIGINAL ARTICLE

Comparison of the Activities among Three *SUL* Genes Present in Uropathogenic *Escherichia Coli*

Yun Mei Lai[1], Myo Thura Zaw[1], Tin Sabai Aung[1], Win Tin[2], Zaw Lin[1]

[1] Department of Pathobiological and Medical Diagnostics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[2] Surgical Based Department, Faculty of Medicine and Health Science, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

*Corresponding author's email: zawlin@ums.edu.my

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ABSTRACT

The three plasmid borne alternative dihydropteroate synthetase (DHPS) genes namely *sul1*, *sul2* and *sul3* genes were heterologous in amino acid sequence and have about 40 – 50% identity. However, they have same DHPS activity with disc diffusion zone size of 6 mm with suphamethoxazole disc in our previous study. *Sul1*, *sul2* and *sul3* genes were observed in sulphamethoxazole resistant uropathogenic *Escherichia coli* (UPEC). In this study, all the three genes were cloned into *E. coli* host and minimum inhibitory concentration (MIC) was investigated for each *sul* gene to compare the activities of *sul* genes. The MIC values of *E.coli* containing *sul2, sul1* and *sul3* genes inserted recombinant plasmid were observed to have 18.5 mg/ml, 18 mg/ml and 17.5 mg/ml respectively as mean value of five experimental results. Although comparable MICs were obtained as a result, the MIC value was highest in *E. coli* carrying *sul2* gene indicating that this DHPS enzyme activity of *sul2* was strongest among three *sul* genes.

INTRODUCTION

In the folic acid synthesis, DHPS is an important enzyme. Sulphonamides is similar in structure of para amino benzoic acid for binding of DHPS. This inhibited dihydropteroate synthetase activity[1]. Sulphonamides are the important antimicrobial agent for treatment of *E. coli* infections such as urinary tract infection (UTI). Mutations in DHPS gene in the chromosome results in resistance to sulphonamides in gram

positive bacterial, whereas the acquisition of plasmid-borne alternative DHPS gene caused resistance in gram negative bacterial including *E. coli*. Affinity of *sul* gene product to sulphonamides is low[2 - 4]. There are three plasmid-borne alternative DHPS genes namely *sul1*, *sul2* and *sul3* genes.

Sul1 is always observed on large conjugative plasmids carrying class 1 integrons. In the early report, *sul2* was commonly located on small non-conjugative plasmid but the updated study showed it to be present on large conjugative plasmid[5]. *Sul3* gene was first reported in pigs at Switzerland and it was consequently present in humans globally. *Sul3* gene has been observed in non-classic class 1 integron which was present on plasmid [5, 6]. This gene was first time reported in clinical UPEC isolate in 2003 at Sweden[7]. The previous report observed that *sul2* gene was the most common gene found in *E. coli* whereas *sul1* gene was also common followed by *sul3* gene which was rarely present in *E. coli*[8].

Sul1 and *sul2* from *E. coli* share 57% DNA level identity, and their origin remains unknown, as their sequences are totally different from all the known chromosomal DHPS genes from *E. coli* and other bacteria[9]. *Sul3* gene was 40.6% homologous in amino acid sequence to *sul2* from *E. coli* plasmid RSF1010[10], and 40.9% identical to *sul1* from *E. coli* plasmid R388[11, 12]. Although these three genes do not share amino acid identity, these were observed to have same alternative DHPS activity. However, potency of each *sul* genes was not well understood. The aim of this study is to test the potency of *sul1*, *sul2* and *sul3* gene on the sulfamethoxazole resistance activities. This is the first report to investigate the sulphonamides resistant activities of each *sul* gene.

MATERIALS AND METHODS

PCR Amplification of Complete DNA Sequence of *sul* Genes (*sul1*, *sul2* and *sul3*)

Gene bank data analysis was done for *sul* genes and the primers were designed to amplify the open reading frame of complete *sul* genes. The primer sequences were stated in Table 1 together with the size of amplicon (the open reading frame of genes). *Sul* genes were amplified under the PCR cycling condition listed in Tables 2. *Sul* genes were amplified from sulphonamides resistant UPEC and verified by DNA sequencing.

Table 1 Primer sets applied for the amplification of *sul* genes

Target gene	Sequence of Primers (5′ – 3′)	Size of amplicon (bp)
Sul1 WS	F: 5′ –ATG GTG ACG GTG TTC GGC –3′ R: 5′ –CTA GGC ATG ATC TAA CCC T –3′	840
Sul2 WS	F: 5′ –ATG AAT AAA TCG CTC ATC A –3′ R: 5′ –TTA ACG AAT TCT TGC GGT –3′	816
Sul3 WS	F: 5′ –ATG AGC AAG ATT TTT GGA ATC G –3′ R: 5′ –CTA ACC TAG GGC TTT GGA TAT T –3′	792

WS - whole sequence

Table 2 PCR conditions performed for the amplification of *sul* genes

	Sul1 WS	*Sul2 WS*	*Sul3 WS*
Initial	95°C for 5 minutes	95°C for 5 minutes	94°C for 5 minutes
Cycles	35	35	30
Denaturation	95°C for 30 seconds	95°C for 30 seconds	94°C for 30 seconds
Annealing	55°C for 30 seconds	55°C for 30 seconds	58°C for 30 seconds
Extension	72°C for 30 seconds	72°C for 30 seconds	72°C for 30 seconds
Final extension	72°C for 7 minutes	72°C for 7 minutes	72°C for 7 minutes
Stop	Hold at 4°C	Hold at 4°C	Hold at 4°C

Cloning of *sul* Genes into TA Cloning Vector

The PCR products were then cloned into TA cloning vector using a TArget Clone™ kit (TOYOBO, Tokyo, Japan). The reaction mixture for ligation was prepared and ligation was incubated at 24°C for 10 minutes according to the instruction of the manufacturer.

Transformation of Recombinant Plasmids

The recombinant DNA was transformed into the Competent Quick DH5α *E. coli* (TOYOBO, Osaka, Japan). Transferred 3 µL of recombinant DNA was transferred into 100 µL of competent cell and then the mixture was incubated in ice for 30 minutes. The mixture was heat shocked for 30 seconds in 42°C, transferred the tube into ice again and incubated for at least 2 minutes. The mixture was added into 900 µL of SOC (Super Optimal broth with Catabolite repression) medium and incubated at 37°C for 1 hour. One hundred µL of the mixture was then plated on MHA (Müller-Hinton agar, Becton Dickinson, USA) plates containing ampicillin and sulphamethoxazole, and incubated overnight at 37°C. Among the colonies on MHA agar, 6 colonies were selected for PCR to confirm *sul* genes were inserted in the TA vector.

Minimum Inhibitory Concentration (MIC) Determination of *E. coli* Containing Recombinant Plasmid Inserted with *sul1*, *sul2* and *sul3* Genes

MIC determination was performed according to the guidelines of Clinical laboratory Standards Institute[13]. Inoculum was prepared by inoculated a single colony of the *sul* gene positive transformed *E. coli* into 3 mL MHB (Müller-Hinton broth, Becton Dickinson, USA) and incubated at 37°C for 2 hours. The inoculum was adjusted with MHB to 5×10^5 CFU/mL. Adjusted inoculum was added to the vials containing MHB and sulphamethoxazole solution in the volume stated in Table 3.

Table 3 Content of serial Mueller Hinton Broth for M.I.C. determination

Final concentration of the mixture in 1 mL	Volume added (µL)		
	Sulphamethoxazole	**MHB**	**Inoculum**
0.0 mg/mL	–	500.0	500
5.0 mg/mL	25.0	475.0	500
6.0 mg/mL	30.0	470.0	500
7.0 mg/mL	35.0	465.0	500
8.0 mg/mL	40.0	460.0	500
9.0 mg/mL	45.0	455.0	500
10.0 mg/mL	50.0	450.0	500
12.0 mg/mL	60.0	440.0	500
12.5 mg/mL	62.5	437.5	500
13.0 mg/mL	65.0	435.0	500
13.5 mg/mL	67.5	432.5	500
14.0 mg/mL	70.0	430.0	500
14.5 mg/mL	72.5	427.5	500
15.0 mg/mL	75.0	425.0	500
15.5 mg/mL	77.5	422.5	500
16.0 mg/mL	80.0	420.0	500
16.5 mg/mL	82.5	417.5	500
17.0 mg/mL	85.0	415.0	500
17.5 mg/mL	87.5	412.5	500

18.0 mg/mL	90.0	410.0	500
18.5 mg/mL	92.5	407.5	500
19.0 mg/mL	95.0	405.0	500
20.0 mg/mL	100.0	400.0	500
25. 0 mg/mL	125.0	375.0	500

Absorbance at 600 nm was measured before and after overnight incubation by using Multiskan™ GO Microplate Spectrophotometer (Thermo Scientific, Waltham, USA). The experiment was repeated for five times and each measurement was repeated five times. The growth of the competent *E. coli* with recombinant inserted were determined by the change of absorbance measured. Competent *E. coli* without recombinant insertion was used as the growth control.

RESULTS

Amplified *sul* genes were verified by DNA sequencing and the sequences were deposited in NCBI GenBank. Accession number of *sul1*WS is MH765657, while *sul2*WS and *sul3*WS are MH765655 and MH765653, respectively.

Growth curve was plotted from the change of absorbance and average value of five times measurement was used. Among all three *sul* genes, sul2 needed 18.5 mg/mL of sulfamethoxazole to inhibit the growth while *sul1* and *sul3* required 18.0 mg/mL and 17.5 mg/mL respectively (Table 4). The growth was dropped drastically in the concentration of sulfamethoxazole at 12.0 mg/mL for all three genes (Figure 1). Competent *E. coli* without recombinant inserts did not grow in MHB containing sulfamethoxazole. All transformed and untransformed competent *E. coli* were grown in the sulfamethoxazole-free MHB.

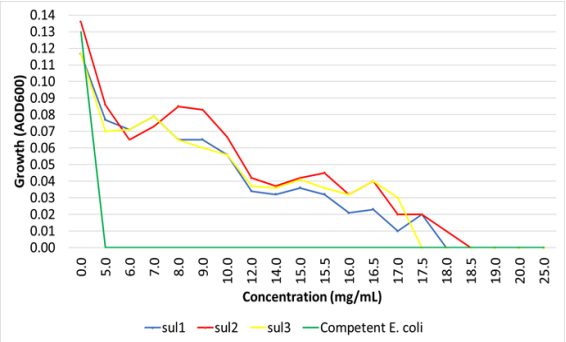

Figure 1 Graph for the growth of the competent *E. coli* with carried of different *sul* genes in different concentration of sulfamethoxazole

Table 4 Growth of the *E. coli* containing recombinant plasmids with different *sul* genes in different concentration of sulfamethoxazole

Concentration	Growth (A_{OD600})			
	Sul1	**Sul2**	**Sul3**	**Competent *E. coli***
0.0 mg/mL	0.12	0.14	0.12	0.13
5.0 mg/mL	0.08	0.09	0.07	0.00
6.0 mg/mL	0.07	0.07	0.07	0.00
7.0 mg/mL	0.08	0.07	0.08	0.00
8.0 mg/mL	0.07	0.09	0.07	0.00
9.0 mg/mL	0.07	0.08	0.06	0.00
10.0 mg/mL	0.06	0.07	0.06	0.00
12.0 mg/mL	0.03	0.04	0.04	0.00
14.0 mg/mL	0.03	0.04	0.04	0.00
15.0 mg/mL	0.04	0.04	0.04	0.00
15.5 mg/mL	0.03	0.05	0.04	0.00

16.0 mg/mL	0.02	0.03	0.03	0.00
16.5 mg/mL	0.02	0.04	0.04	0.00
17.0 mg/mL	0.01	0.02	0.03	0.00
17.5 mg/mL	0.02	0.02	0.00	0.00
18.0 mg/mL	0.00	0.01	0.00	0.00
18.5 mg/mL	0.00	0.00	0.00	0.00
19.0 mg/mL	0.00	0.00	0.00	0.00
20.0 mg/mL	0.00	0.00	0.00	0.00
25. 0 mg/mL	0.00	0.00	0.00	0.00

DISCUSSION

Antibiotics resistance acts by different mechanism at the genetic level. Fuoroquinolone resistance occurs mainly by mutations at the quinolone resistant determining region (QRDR) in *gyrA* and *parC* genes of chromosomal level. However, plasmid mediated quinolone resistance (PMQR) was also present e.g. *qnr* genes. Mutations at chromosomal level gave rise to high level resistance when compared to PMQR. Mutations at chromosomal level have consistent mutations in two genes which are mutations at amino acid 83 and 87 of gyrA gene and mutations at amino acid number 80 and 84 in *parC* gene[14].

However, in case of sulphonamides resistance, mutation at *folP* gene at chromosomal level is relatively rare in Gram-negative bacteria including *E. coli* so that research in that field is uncommon whereas plasmid borne alternative DHPS gene, *sul* genes, were distributed widely and level of drug resistance is high[8]. Although there are three *sul* genes in *E. coli*[8] and other Gram-negative bacteria up to now, these are heterologous at the amino acid level with consequent difference at nucleotide level[12] whereas their potency of drug resistance was nearly the same as shown by disc diffusion method[8]. Since the difference in amino acid levels is about 40 – 50%[12], it is worthwhile to study the comparison of the potency levels of the *sul* genes, although we know that there is not much difference in the potency between these genes based on the results of disc diffusion.

In the wild type *E. coli* strains, the size of the plasmids, the copy number of plasmids and other associated proteins in the host and the plasmid affect the level of expression and influence the drug resistant activity of each *sul* gene. To compare easily, we tried to clone each *sul* gene into same vector in the study and the recombinant plasmids were transformed into the same *E. coli* host for further expression of each gene. The activity of each DHPS enzyme was then compared for MIC by means of OD at Absorbance 600 and subculture method on culture plate. To reduce the experimental error, the same procedure was performed for five times and the mean value was taken as result and the plot was drawn with the concentration of sulfamethoxazole and OD at Absorbance 600.

In the previous study, there was no variant in *sul1* and *sul3* genes while *sul2* gene has 2 variants and also the most commonly distributed *sul* genes in *E. coli* isolates[8]. Therefore it may be necessary to test the other variant of *sul2* gene to draw the firm conclusion. However, we can conclude *sul2* gene has the strongest drug resistant activity in comparison with other two *sul* genes.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this article.

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CASE REPORT

Metastatic Squamous Cell Carcinoma of Sternum: An Oncological Curiosity

Jia Ying Soo[1], Nur Ayub Mohd Ali[2], Aishath Azna Ali[1], Firdaus Hayati[3*], Nornazirah Azizan[4], Andee Dzulkarnaen Zakaria[5], Zainal Adwin Zainal Abidin[6], Mohd Ramzisham Abdul Rahman[2]

[1] Department of Surgery, Universiti Kebangsaan Malaysia Medical Centre, Cheras, Kuala Lumpur, Malaysia
[2] Division of Cardiothoracic Surgery, Departments of Surgery, Universiti Kebangsaan Malaysia Medical Centre, Cheras, Kuala Lumpur, Malaysia
[3] Department of Surgery, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[4] Department of Pathobiology and Medical Diagnostic, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[5] Department of Surgery, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
[6] Department of General Surgery, Surgical Sciences Cluster, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia

*Corresponding author's email: firdaushayati@gmail.com

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Keywords: squamous cell carcinoma, metastasis, sternum

ABSTRACT

Skeletal metastasis is a frequent complication of cancer resulting in significant morbidity as well as mortality. We highlight a case of a 73-year-old gentleman with metastatic squamous cell carcinoma of the sternum. He denied dysphagia, shortness of breath, goitre, and presence of chronic non-healing ulcer. He was anaemic and carcinoembryonic antigen (CEA) was 18.7. Chest radiograph on lateral view showed a suspicious cortical irregularity. Computed tomography (CT) scan of thorax revealed an aggressive sternal lesion with soft tissue component. Ultrasound-guided biopsy was performed and the biopsy was consistent with metastatic squamous cell carcinoma. Squamous cell carcinoma has a predilection to metastasize via haematogenous spread, but direct extension of tumour into the bone is not frequently seen. Finding the primary cause is utmost importance either via imaging modalities or invasive procedures. Isolated secondary lesion is extremely rare but unfortunate among defaulters. We discuss its diagnostic work-up and treatment options conserved to manage this condition.

INTRODUCTION

Skeletal metastasis is a diagnostic dilemma to the attending surgeon as well as the oncologist. It can result in significant morbidity and mortality if prompt diagnosis cannot be achieved. The prevalence of bone metastasis is estimated to account of 70% of all malignant bone tumours with majority happen in breast and prostate cancer. Bone metastases most commonly affect the axial skeleton[1]. Metastatic

tumour of the sternum is rare with paucity of published articles regarding the incidence of sternal metastasis[2]. When it happens, it can lead to loss of the biomechanical support of the sternum-rib-thoracic spine complex and risk of fracture causing acute kyphosis and neurological injury[2].

Bone metastases are haematogenous in onset. Blood flow is high in the red marrow as compared to yellow marrow accounting for the predilection of metastases for those sites, commonly to the axial skeleton[3]. Our case showed a very unusual manifestation of initial presentation of bone metastasis with absences of symptoms or signs of primary origin.

CASE PRESENTATION

A 73-year-old gentleman presented with 3-month history of sternal pain after falling from a motorbike. He was previously well. During the initial trauma, he was treated with analgesia and discharged home uneventfully. However, due to persistent pain, he sought for a second opinion. He had a 20-pack/year history of cigarette smoking. There was no history of chronic cough, fever, or night sweats. He denied dysphagia, shortness of breath, non-healing ulcer, and neck swelling. On examination, there were no obvious masses, deformities, ulcer, or discolouration seen at the sternum. Full detailed physical examination of heart, lungs and abdomen including per rectal examination revealed no abnormality. There was no lymphadenopathy as well.

Work up revealed anaemia with haemoglobin level of 9 g/dL with normal serum electrolyte, renal and liver functions. However, the level of carcinoembryonic antigen (CEA) was elevated with result of 18.7. A chest radiograph on AP view did not demonstrate any lung lesions (Figure 1A) but on lateral view, it showed a suspicious cortical irregularity (Figure 1B).

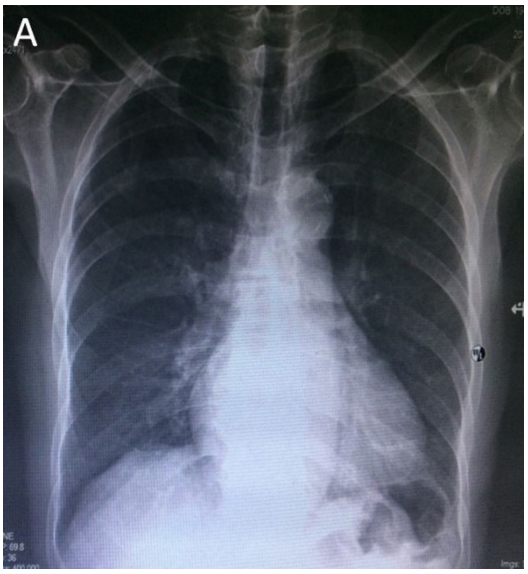


Figure 1A Chest radiograph showing as normal finding from PA view

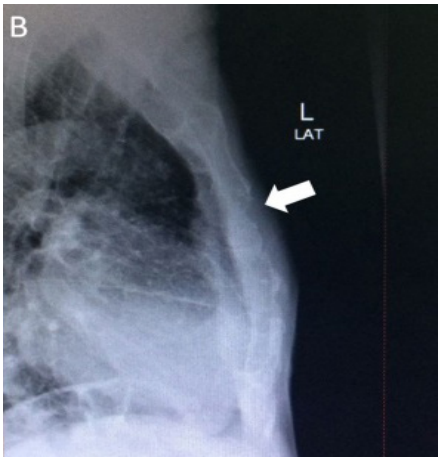


Figure 1B Chest radiograph from lateral view showing abnormal cortical outlines of the sternum (white arrow)

Computed tomography (CT) scan of thorax revealed an aggressive sternal lesion with soft tissue component measuring 4.1 × 6.8 × 6.4 cm (Figure 2A) with absence of oesophageal or lung lesion (Figure 2B and Figure 2C). It was more prominent on 3-dimensional (3D) view (Figure 3). Ultrasound-guided biopsy was performed and the biopsy was consistent with metastatic squamous cell carcinoma (Figure 4A and Figure 4B). In view of the elevated CEA level, a colonoscopy was performed, but to our surprise, it was a normal finding. We were deciding for more advanced imaging such as PET scan, but he unfortunately lost to follow up.

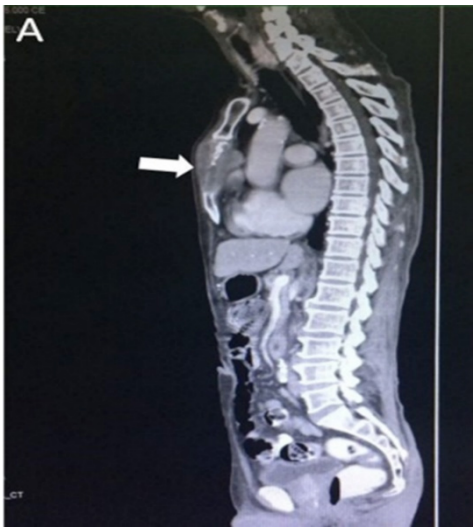
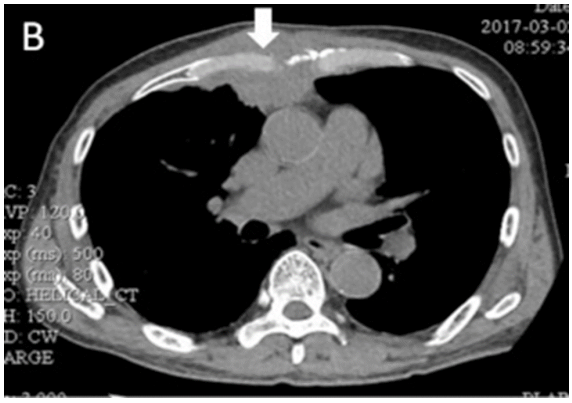
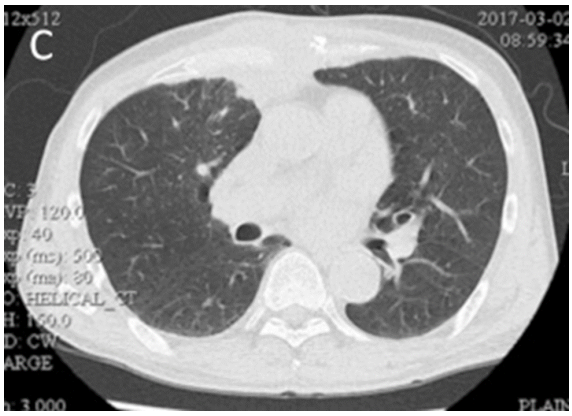


Figure 2A Sagittal view of CT of thorax revealing an aggressive lytic lesion of the body of sternum (white arrow)



2B



2C

Figure 2B Axial view showing sternal lesion (white arrow) with absence of oesophageal cancer or dilated oesophagus

Figure 2C Lung window view revealing normal appearance of the bilateral lung fields

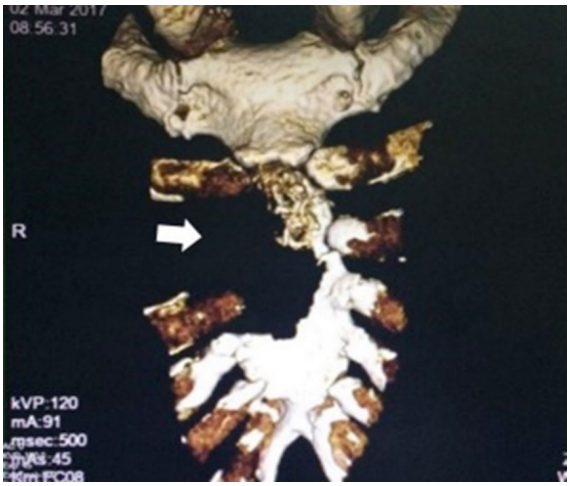


Figure 3 3D reconstruction of CT of thorax showing an aggressive lesion of sternum (white arrow)

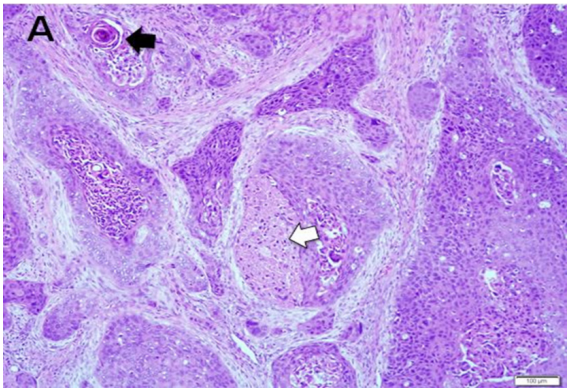


Figure 4A Section from the tumour showing sheets and islands of malignant cells with marked desmoplastic stroma. There are also presence of necrosis (white arrow) and keratin pearl (black arrow) (H&E, original magnification ×10).

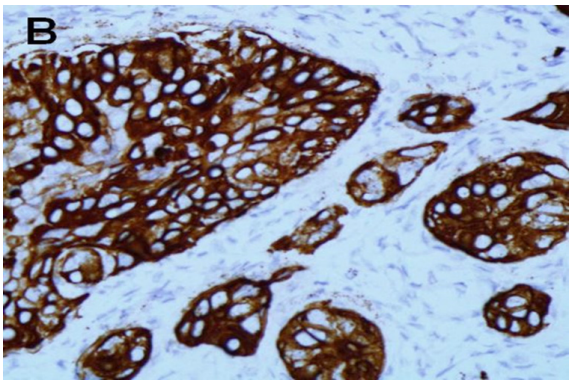


Figure 4B The malignant cells are positive for CK5/6 (Immunohistochemistry, original magnification ×40)

DISCUSSION

When skeletal metastasis is the presenting problem and the primary site is occult, there is a need to identify the primary site as soon as possible. Due to its nature of being a late presentation, many studies on bone metastasis had been on autopsy series. Although skeletal metastasis is common especially vertebral spine, isolated metastatic tumours of the sternum are rare. The common histology are mostly adenocarcinoma or ductal in origin arise from the breast, kidney, gastrointestinal tract, lung, and even thymus[4, 5, 6].Squamous carcinoma metastatic to bone marrow is an exceedingly rare lesion. Although the search for the primary tumour is often time-consuming and difficult, its identification and histologic diagnosis provide valuable information for determining the appropriate treatment.

For our patient, a vigorous search for the primary tumour was undertaken. Combinations of non-invasive as well as invasive modality were carried out to assist the final diagnosis. The histopathological findings of metastatic squamous cell carcinoma, coupled with the CT scan were highly suggestive of the lung as the primary culprit. Metastatic skeletal disease places an enormous burden on patients, secondary to pain, functional impairment, and worsening quality of life. Treatment modalities include systemic (chemotherapy or hormonal therapy) or local (radiotherapy or surgery) therapy which all carry their own benefits and complications.

Resection of sternal malignancies carries formidable surgical challenges. The main difficulty is making a radical full thickness resection and reconstruction of chest wall without compromising the stability and its ventilatory mechanics[7]. Literature reviews of radical or complete chest wall resection are only performed for primary malignancy of sternum or rarely, in metastatic sternum[8]. Resection of a metastasis, therefore, should only be performed if the primary disease has been controlled with no evidence of other metastasis provided the patient is fit to undergo the procedure. Thus, the role of surgery is controversial in this case.

Pain management is also often an important part of management especially pain is the main presenting complaint. Radiotherapy is the standard of care for bone metastasis. It takes time to take effect and may fail to relieve pain in 20 – 30% of patients[9]. Radiation therapy over the sternum is hampered by the proximity to thoracic viscera. It can have devastating complication to the thoracic viscera including cardiovascular disease, acute pneumonitis and oesophageal problems[10]. Minimally invasive and image-guided procedures are gaining wider acceptance in treating these lesions as an adjunct to radiotherapy.

The mechanism of pain relief of bone metastasis after radiofrequency ablation (RFA) and percutaneous osteoplasy includes (a) bone stabilization and prevent micro-motion; (b) direct tissue toxicity; (c) necrosis of neural tissue; and (d) thermal injury. However, osteoplasty of non-weight-bearing flat bones is rarely reported[10]. Combined RFA and percutaneous osteoplasy appears to be promising for the palliative treatment of both spinal and extra-spinal metastasis. This is evident in a retrospective study by Tian et al. showing effective methods for pain relief and functional recovery in patients with painful extraspinal bone metastases and can significantly improve quality of life[11].

CONCLUSIONS

Palliative treatment is of consideration in the management of this patient who displayed a systemic disease. The combination therapy with full abbreviation radiofrequency ablation and percutaneous osteoplasty is feasible, effective, and safe. It is a promising technique for the treatment of painful bone metastases and can improve quality of life. Combination therapy can be considered in this patient if analgesia fails.

CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this case.

CONSENTS

Written informed consent was obtained from the patient to publish the case. A copy of written consent is available for review by the Chief Editor.

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CASE REPORT

Isolated Hypophosphataemia Mimicking Cerebrovascular Accident

Hanizah Ngadiron[1], Razrim Rahim[1], Firdaus Hayati[2*], Nornazirah Azizan[3], Affirul Chairil Ariffin[1]

[1] Faculty of Medicine, Universiti Sains Islam Malaysia, Kuala Lumpur, Malaysia
[2] Department of Surgery, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[3] Department of Pathobiology and Medical Diagnostic, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

*Corresponding author's email: firdaushayati@gmail.com

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Keywords: hypophosphataemia, cerebrovascular accident, blood serum

ABSTRACT

Hypophosphataemia occurs in an abnormally low serum phosphate level. Three main mechanisms are postulated: decreased intestinal absorption, increased renal excretion, and extracellular shifts to intracellular compartments. It is potentially a fatal disease if not intervene. The management is merely treating the underlying disorder, giving phosphate supplement and requiring close biochemical monitoring. The incidence of symptomatic isolated hypophosphataemia is extremely rare. In this case report, a 33-year-old man presented with three days history of dysphagia, inability to complete sentences and generalized muscle weakness. He developed blurred vision especially upon exposure to bright light. He had a history of single parathyroidectomy for parathyroid adenoma 2 years ago. Physical examinations were unremarkable. Laboratory investigations were normal except for phosphate level of 0.30 mmol/L. Intravenous KH_2PO_4 with a dosage of 10 mmol was administered in slow bolus in 3 hours. His symptoms resolved slowly after correction. Although isolated hypophosphataemia is rare but need to recognize the symptoms and signs of hypophosphataemia and treat accordingly.

INTRODUCTION

Phosphate is the most abundant intracellular anion in the body. It is essential for several biological processes including energy production, formation of cellular structure, and tissue repair. Hypophosphataemia develops when there is an abnormally low level of phosphate in the blood. It is often missed due

to non-specific features which can lead to a considerable morbidity as well as mortality if neglected. It is imperative to obtain a thorough history with complete physical examination in achieving an accurate diagnosis. Although it is rare, the incidence of hypophosphataemia can occur in as many as 3% of hospitalized patients and as high as 30% of patients admitted to intensive care unit[1].

Mild and moderate hypophosphataemia are usually asymptomatic. Major clinical sequelae usually occur only in severe hypophosphataemia. Fortunately, this extreme form is very unusual especially in hospitalized patients. Hypophosphataemia in hospitalized patients has many causative factors. These include alcoholism, gram-negative sepsis, hyperalimentation, diabetic ketoacidosis, primary hyperparathyroidism, gastrointestinal malabsorption, as well as medications such as diuretics, insulin, corticosteroids, phosphate-binding antacid and epinephrine[2]. In this case report, we highlight the case of symptomatic isolated hypophosphataemia.

CASE PRESENTATION

A 33-year-old man presented to emergency department with three days history of dysphagia, inability to complete sentences and generalized muscle weakness. He developed blurred vision especially upon exposure to bright light. Upon further questioning, he was in a motivation camp a week prior to this presentation. He claims to develop reduced oral intake during the camp. He had a history of single parathyroidectomy for parathyroid adenoma 2 years ago. The surgery was eventful though it was complicated with transient hypocalcaemia. However, he defaulted his subsequent follow-up. Fortunately, he did not develop any electrolytes imbalance.

On examination, he was alert and conscious. His vital signs were stable. He had difficulty to speak which clinically was consistent with dysarthria. Surprisingly, the systematic reviews were unremarkable with intact neurological examinations. Arterial blood gases and electrocardiogram was normal. Laboratory investigations were unremarkable except for phosphate level of 0.30 mmol/L.

In view of his unusual presentation, he was planned for computed tomography (CT) of the brain to exclude cerebrovascular disease which was negative. A decision of phosphate correction was made due to isolated phosphate abnormality. Intravenous KH_2PO_4 with a dosage of 10 mmol was administered in slow bolus in 3 hours. His symptoms resolved slowly after correction. Repeat phosphate level was 0.51 mmol/L. He was discharged with early follow-up at nearby clinic for phosphate monitoring.

DISCUSSION

Hypophosphataemia usually occurs in response to the reduced function affected by systemic conditions. This most likely happened to the patient due to a decreased intestinal absorption caused by dietary restriction during camping that he went a week before onset. The categorization of hypophosphataemia can be determined biochemically with its level. It is divided into mild (0.6 – 0.8 mmol/L), moderate (0.3 – 0.6 mmol/L) and severe (<0.3 mmol/L) form[3].

It is important to consider the possibility of an isolated event in view of unequivocal findings. Body weakness is common but it can affect any specific muscles. Other symptoms include dysphagia, diplopia, and dysarthria. Although patient developed dysphagia, the short duration negates the possibility of mechanical or functional oesophageal diseases. Hence, a central neuromuscular cause is suspected. It is essential to remember that various presentations of neuromuscular dysfunction can occur. They include a simple

paraesthesia and profound mental alteration. Hypophastaemia has been reported involving respiratory muscle paralysis which mimics Guillain-Barre syndrome[4]. Another case reported a child presented with diabetic ketoacidosis (DKA) and seizure which was not resolved with optimization of the endocrine function. The child was proved to developed hypophosphataemia related seizure rather than being affected by the DKA status[5]. A systematic review by Ariyoshi N et al. reported a negative relationship between severe hypophosphataemia and left ventricular dysfunction leading to cardiomyopathy and arrhythmias. Consequently, patient may also present with failure symptoms which may posed a challenge especially in ischemic heart disease patient[6].

Hypophosphataemia does not automatically mean that replacement therapy is indicated. Recognizing and establishing the cause of the hypophosphataemia can give proper guidance on how we are going to treat the patient. Sometimes treating the underlying causes can automatically correct the phosphate level[7]. Asymptomatic patient with moderate hypophosphataemia may be considered for oral phosphate supplementation provided that the enteral route is feasible. Oral phosphate supplement can be given at the usual dose of 500 mg BD. Intravenous phosphate medication is requisite in patients with severe hypophosphataemia, symptomatic moderate hypophosphataemia, and none feasible enteral route. Either potassium phosphate or sodium phosphate injection may be used for replacement. The recommended regime involves administration of 0.08 mmol/kg per body weight over 6 hours for severe hypophosphataemia without obvious clinical manifestation while 0.16 mmol/kg per body weight infused over 2 – 6 hours in life threatening condition[8]. Oral therapy is safer compared to intravenous route. Rapid correction is safe but the magnitude of response can be unpredictable. The faster the therapy, the more likely the side effects will occur.

CONCLUSION

Although it is rare, recognizing symptoms and signs of hypophosphataemia is crucial after ruling out other alternative diagnoses. In this patient, we have decided to treat the hypophosphataemia, with resultant resolution of symptoms.

CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this case.

CONSENTS

Written informed consent was obtained from the patient to publish the case. A copy of written consent is available for review by the Chief Editor.

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CASE REPORT

Non-Cirrhotic Portal Hypertension: A Case of Bleeding Gastro-Oesophageal Varices with Non-Cirrhotic Liver Reported in East Malaysia

Alvin Oliver Payus[1*], Justin Leow Wen Hsiang[2], Liew Sat Lin[1], Malehah Mohd Noh[1]

[1] Medicine Based Department, Faculty of Medicine and Health Science, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[2] General Medicine Department, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia

*Corresponding author's email: dralvinpayus@ums.edu.my

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Keywords: non-cirrhotic portal hypertension, normal liver, variceal bleeding

ABSTRACT

Non-cirrhotic portal hypertension (NCPH) is clinically defined as the presence of portal hypertension in the background of non-cirrhotic liver. It is diagnosed by the findings in ultrasound of the hepatobiliary system and also oesophagogastroduodenoscopy (OGDS) that consistent with that of a portal hypertension, but otherwise has a relatively normal liver function and echotexture. The treatment mainly focuses on primary and secondary prophylaxis of variceal bleeding both pharmacologically like non-selective beta-blockers and octreotide, and non-pharmacologically like endoscopic band ligation of varices and sclerotherapy. In advance cases, sometimes surgery such as Porto-systemic shunt or splenectomy may be required especially in patients with uncontrolled variceal bleeding or with symptomatic hypersplenism. Here we report a case of a young man who presented with upper gastro-intestinal bleeding, which was initially thought from a bleeding ulcer but was found to be secondary to oesophageal and gastro-oesophageal varices. Apart from having mild ascites, he has no other features of portal hypertension. His liver biochemistry and echotexture were also normal. Unfortunately, the patient was lost to follow up while he was still in the early stage of investigating the condition. The purpose of this case report is to share an uncommon occurrence of NCPH in East Malaysia, where liver cirrhosis predominates the aetiology of portal hypertension. Also, to the best of our knowledge, there is a very limited reporting of a similar case in this region.

INTRODUCTION

Portal hypertension often developed in the background of liver cirrhosis. It is the end result of multiple conditions associated with increased resistance in portal circulation and ultimately leads to life-threatening complications such as variceal bleeding, splenomegaly and ascites[1]. However, portal hypertension can occur in a non-cirrhotic liver in a condition that can be broadly grouped as Non-Cirrhotic Portal Hypertension (NCPH). NCPH frequently associated with a wide range of vascular changes in the portal system. Unlike those due to liver cirrhosis, it is associated with normal or mildly elevated hepatic venous pressure gradient[2]. The leading conditions of NCPH are classified anatomically as pre-hepatic, hepatic, and post-hepatic[3] according to the site of resistance in portal circulation. For the diagnosis of NCPH, it is essential to rule out other causes of chronic liver diseases such as viral hepatitis B and C, chronic alcoholism, non-alcoholic fatty liver disease, autoimmune hepatitis, hereditary causes like hereditary hemochromatosis and Wilson's disease, and also drug induced causes as well. The principal of management is aimed to prevent and treat complications which expected to occur from portal hypertension. Therefore, prompt diagnosis of the disorder is very important for initiating the correct management.

CASE PRESENTATION

A 25-year-old man with no known medical illness presented with progressive abdominal pain and distention, and passing blackish stool for the past 2 weeks prior to admission, which was accompanied by symptoms of anaemia. He also had reduced appetite and loss of weight for the past one year. Otherwise, he has neither history of haematemesis nor any other bleeding elsewhere, and he denied having any fever, altered bowel habit, night sweats or jaundice. There is no significant family history. On examination, patient was well and comfortable. He was cachectic and pale, and there was mild ascites present. Otherwise, there were no jaundice, no lymphadenopathy, no pedal oedema, and no stigmata of chronic liver disease. The abdomen was soft and not tender, and there was no palpable mass or organomegaly felt. As for his investigations, his haemoglobin was 3.7g/dl, microcytic and hypochromic picture and his reticulocyte count was 4.2%. The platelet count was normal and the coagulation time was not prolonged. His renal and liver function test (Total Bilirubin: 5.9umol/L, Alanine Aminotransferase (ALT): 12u/L, Alkaline Phosphatase: 94u/L) was normal, and his albumin: globulin ratio was reduced (albumin: 20 g/L, globulin: 32g/L). There was no cardiomegaly but the right costophrenic angle was blunted on radiographic imaging of the chest. Echocardiography was done and it was normal. Subsequently he was subjected to Oesophagogastroduodenoscopy (OGDS) and it showed Grade I Oesophageal Varices (Figure 1) and Gastro-oesophageal Varices grade 2 with Stigmata of Recent Haemorrhage (Figure 2). Endoscopic sclerotherapy was performed to secure the bleeding.

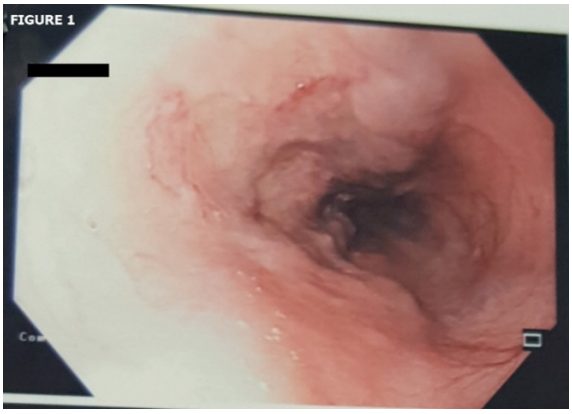

Figure 1 Oesophagogastroduodenoscopy shows Grade 1 Oesophageal Varices

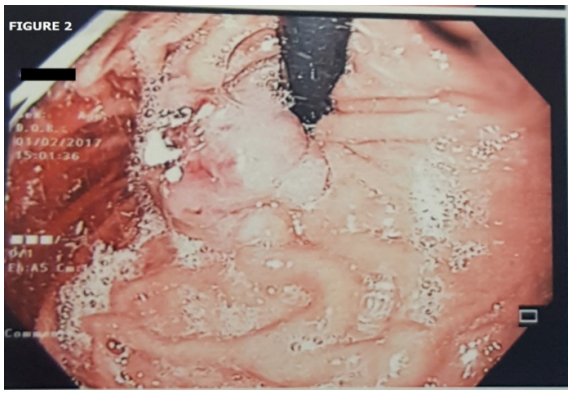

Figure 2 Oesophagogastroduodenoscopy shows Gastro-Oesophageal Varices with stigmata of recent bleed.

As it was initially thought the UGIB was due to bleeding ulcer, the patient was started on intravenous (IV) Pantoprazole 8 mg hourly infusion and planned for 72 hours. However, after the OGDS showed variceal bleeding, he was then given IV Terlipressin 2 mg bolus, then 1 mg 4 hourly for 24 hours. He was also given IV Ceftriaxone 1 gm once daily, and was started on oral Propranolol 20 mg twice daily thereafter. He was also transfused 4 pints of packed red cells. The repeated Hb was 8 g/dL. Preliminary investigations for liver cirrhosis such as HBsAg, Anti-HCV, HIV antibody, stool microscopy for Schistosoma egg and others were all negative. Ultrasound of the hepatobiliary system showed enlarged liver, dilated hepatic sinusoids and minimal ascites, but otherwise the liver parenchymal structure was normal (Figure 3), and the portal vein was patent on Doppler (Figure 4). Patient was consulted for liver biopsy. However, he defaulted and was lost to follow up.

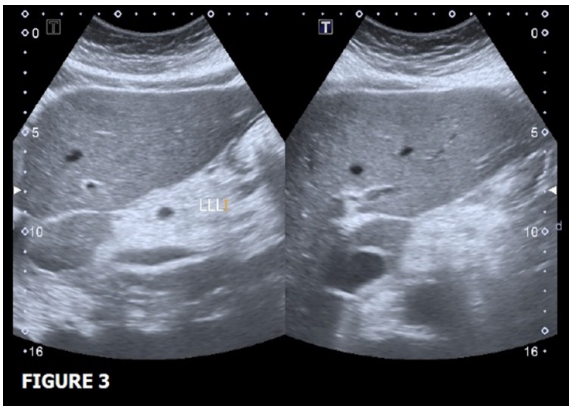

Figure 3 Ultrasound scan of the hepato-biliary system shows mild hepatomegaly.

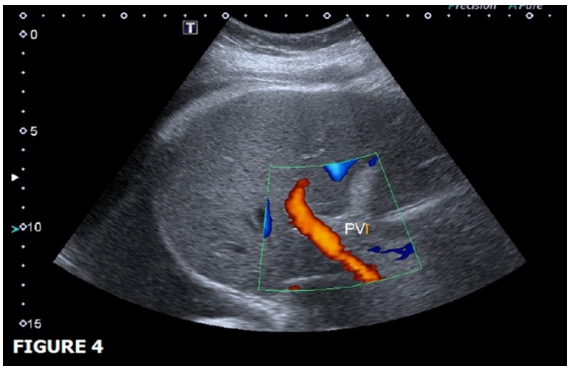

Figure 4 Doppler ultrasound scan of the portal vein shows normal patency of the portal vein.

DISCUSSION

Portal hypertension is a clinical condition defined by pathological increase in the pressure of the portal venous system with the gradient between portal vein and inferior vena cava more than 5 mmHg[1]. This usually occurs as a result of increased resistance in the portal circulation and ultimately lead to the formation of extensive network of portosystemic collaterals in multiple sites that divert portal blood to the systemic circulation and bypassing the liver[2]. In most cases, the resistance occurs within the liver (as in the case of liver cirrhosis). But there are some cases where it can also occur from pre-hepatic site (like portal vein thrombosis) or post-hepatic site (like Budd-Chiari syndrome). As portal hypertension progresses, splanchnic blood flow will increase as a result of local

release of humoral vasodilatory mediators, such as vascular endothelial growth factor, prostacyclin, nitric oxide and others that will cause splanchnic vasodilation and angiogenesis. These mediators will also affect the systemic circulation and lead to hypotension and vascular underfilling, which triggers the release of endogenous systemic vasoactive substance and plasma volume expander by renal sodium retention, and lead to increased cardiac output[2]. The haemodynamic changes in both splanchnic and systemic circulation will ultimately lead to the main complications of portal hypertension, like bleeding varices causing upper gastrointestinal bleeding (UGIB), ascites, hepatic encephalopathy, hepatorenal and hepatopulmonary syndrome[1,2]. All of these complications are essentially a sequalae of the abnormal collateral formation and hyperdynamic splanchnic and systemic circulations.

Liver cirrhosis is the most common cause of portal hypertension worldwide[2]. But portal hypertension can also develop in the background of normal functioning liver, in a group of condition collectively referred to as noncirrhotic portal hypertension (NCPH). In NCPH, the hepatic venous pressure gradient is often normal or only mildly elevated and usually lower than the portal venous pressure. The causes of NCPH are primarily vascular in origin and generally can be classified according to anatomical location of blood flow resistance, such as pre-hepatic, hepatic, and post-hepatic. The hepatic causes are further subdivided into pre-sinusoidal, sinusoidal and post-sinusoidal[3]. Although NCPH only account less than 10 per cent of cases in the Western world, it is the leading causes of portal hypertension in other parts of the world. NCPH is clinically defined by the presence of features of portal hypertension, for example like splenomegaly, varices and ascites with preserved liver functions and patent hepatic and portal veins. It is usually diagnosed by ultrasound of the hepatobiliary system and esophagogastroduodenoscopy (OGDS) findings consistent with portal hypertension, but normal liver function and histology.

NCPH is said to affect predominantly the low socio-economic population of the developing world. But it is also reported in higher strata population from all parts of the world[4]. Epidemiologically, it constitutes approximately 10 – 30% of patients with variceal bleed worldwide[5]. And it is thought that NCPH is largely under-diagnosed particularly from the parts of the world where it is rare. Overall NCPH is estimated to contribute 14% of portal hypertension in adults[6].

The management of NCPH are geared at preventing and treating the complication that may arise as a sequalae of portal hypertension, for example like variceal bleeding. Therefore, early recognition and prompt diagnosis of these conditions is very important so that the appropriate therapeutic measure which is to reverse the natural course of the disease or prophylactically to prevent disease progression can be initiated. As data are still very limited regarding the best approach to management of this particular condition, patients are usually treated in the manner similar to those of due to cirrhosis.

The prognosis is fortunately good whereby only minority of cases will develop recurrent attacks of uncontrolled bleeding, portal vein thrombosis, and also hepatopulmonary syndrome. Long-term survival of well-treated oesophagogastric varices and properly-timed shunt surgery is nearly 100 and 80%, respectively[7]. Liver functions will usually remain well preserved until very late, but 20 – 33% patients will develop parenchymal atrophy with subsequent decompensation. The commonest causes of death are bacterial infection (31%), followed by progressive liver failure (25%), uncontrolled variceal bleeding (17%), and intestinal infarction (8%)[8, 9].

In this article, we report a patient who presented with UGIB which at first was thought due to peptic ulcer disease. However, his OGDS found gastro-oesophageal varices. Apart from having mild ascites, he has no other signs of chronic liver disease. Upon further test noted the liver biochemistry and echotexture were normal. His portal vein was also patent. The next step of investigation for this patient is to do liver biopsy to confirm no cirrhotic changes over the liver and also to look for histological changes consistent with NCPH such as prominent sclerosed wall of portal vein and its branches, obliteration of small portal venules, also to find site of venous obstruction, if any. He was supposedly to be monitored regularly with Doppler ultrasound at six monthly intervals to check the patency of the portal vein, and also secondary prophylaxis for variceal bleeding with non-selective beta-blockers, but unfortunately, he defaulted follow up. Therefore, this case report suffers one limitation where the patient was lost to follow up before the diagnosis is known. However, the objective of this case report is to illustrate the uncommon occasion of UGIB secondary to portal hypertension in a relatively normal liver function and parenchymal structure.

CONCLUSION

In conclusion, this case report served to remind the reader that portal hypertension does not occur exclusively as a complication of liver cirrhosis, but it can also happen due to multiple other causes with the background of normal liver function and architecture.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this case.

CONSENTS

Written informed consent was obtained from the patient to publish the case. A copy of written consent is available for review by the Chief Editor.

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CASE REPORT

Strongyloides Hyperinfection Syndrome in an Immunosuppressed Patient

Cheo Seng Wee[1], Tan Yee Ann[2], Low Qin Jian[3]

[1] Department of Internal Medicine, Hospital Lahad Datu, Sabah, Malaysia
[2] Department of Internal Medicine, Hospital Queen Elizabeth, Sabah, Malaysia
[3] Department of Internal Medicine, Hospital Sultanah Nora Ismail, Batu Pahat, Johor, Malaysia

*Corresponding author's email: cheosengwee@gmail.com

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ABSTRACT

Strongyloides stercoralis **is an intestinal nematode which is endemic in tropical and subtropical countries. The global prevalence of *Strongyloides* is unknown. Strongyloidiasis is found more frequently in the socioeconomically disadvantaged, in institutionalized populations, and in rural areas. The spectrum of disease varies. It may cause asymptomatic infection, mild eosinophilia or hyperinfection syndrome in the most severe form. Here we reported a case of *Strongyloides* hyperinfection syndrome in an immunosuppressed patient. This patient is a 54-year-old man with myasthenia gravis on long term azathioprine and prednisolone. He presented with fever associated with diarrhoea and was in septic shock. His blood culture was positive for *Klebsiella pneumoniae*. *Strongyloides stercoralis* larvae were detected in his sputum and stool sample. He was diagnosed to have *Strongyloides* hyperinfection and was treated with subcutaneous ivermectin. He recovered well. Our case demonstrated the association of *Strongyloides* hyperinfection with superimposed gram-negative sepsis as a consequence of prolonged immunosuppression. A high index of suspicion is needed in approaching patient with risk factors of hyperinfection syndrome.**

INTRODUCTION

Strongyloidiasis is caused by *Strongyloides stercoralis*. Strongyloidiasis is often asymptomatic. Eosinophilia and larvae in stools being the only indication of infection[1]. Opportunistic disseminated strongyloidiasis

is an important cause of morbidity and mortality in immunocompromised patients. In Malaysia, cases of *Strongyloides* hyperinfection have been reported previously[2]. Risk factors for *Strongyloides* hyperinfection include congenital immunodeficiency, malignancy, malnutrition, immunosuppression, alcoholism and haematopoietic stem cell transplantation[3]. In general, immunosuppression is defined as suppression of body immune system to fight off infection, prevent graft rejection or as treatment of autoimmune diseases. It may occur as a result of disease or deliberately by immunosuppressive drugs such as azathioprine, methotrexate, cyclophosphamide, etc.[2].

CASE PRESENTATION

A 54-year-old man with underlying myasthenia gravis on long-term prednisolone and azathioprine, hypertension and well-controlled diabetes mellitus presented with fever associated with diarrhoea for 3 days. He was on 2 years of azathioprine and prednisolone for his myasthenia gravis and 1 year of metformin for his diabetes prior to this. On arrival to emergency department, he was in shock, blood pressure was 60/37 mmHg. His pulse rate was 104 beats per minute and his temperature was 37°C. His respiratory and abdominal examination were unremarkable.

Initial blood investigation showed anaemia, haemoglobin of 8.7 g/dl, leukocytosis of 20 $\times$ 10^9/L. His renal profile was impaired with urea of 6.2 mmol/L and creatinine of 118 **µmol**/L. He had metabolic acidosis with pH of 7.41 and bicarbonate of 14.4 mmol/L (Table 1). He had low albumin level with albumin of 9 g/L and his C-Reactive Protein (CRP) was 87 mg/L. His chest radiograph was normal. His provisional diagnosis was Gram-negative sepsis in septic shock. He was then given boluses of intravenous crystalloids, noradrenaline and intravenous meropenem.

After 48 hours, his blood culture grew *Klebsiella pneumoniae*, his sputum was positive for *Strongyloides stercoralis* larvae and stool was positive for rhabditidiform larvae (L1). Antibiotic was deescalated to intravenous amoxicillin-clavulanic acid and started on oral albendazole 400 mg and subcutaneous ivermectin. Diagnosis was revised to *Klebsiella pneumoniae* septic shock with *Strongyloides* hyperinfection syndrome. He made good clinical recovery and discharged 2 weeks later. He was prescribed monthly subcutaneous ivermectin for 6 months.

Table 1 Blood investigation of the patient

	Unit	Normal range	Day 1	Day 2	Day 7	Day 8	Day 14
Haemoglobin	g/dL	13 – 18	8.7	10.8	7.6	9	9
Total white blood cell	10^9/L	4.0 – 10.0	20.7	30	8.69	12	12.6
Platelet	10^9/L	150 – 400	102	57	84	358	358
Sodium	mmol/L	135 – 148	129	133	136	137	137
Potassium	mmol/L	3.5 – 5.1	3.8	3.5	2.7	4.1	4.3
Urea	mmol/L	2.8 – 7.8	6.2	5.5	2.1	7	3.8
Creat	**µmol**/L	61 – 110	118	86	61	59	36
pH		7.35 – 7.45	7.41	7.337	–	–	–
PCO2	mmHg	33 – 48	22	34	–	–	–
PO2	mmHg	80 – 100	206	45	–	–	–
HCO3	mmol/L	23 – 29	14.4	14.4	–	–	–
Total bilirubin	**µmol**/l	0 – 17	19	11	10	9	9.1
ALT	IU/L	0 – 41	12	22	35	32	32
AST	IU/L	0 – 40	26	23	34	30	20
ALP	IU/L	40 – 129	171	366	260	203	233
Albumin	g/L	34 – 48	9	12	14	22	22
Globulin	g/L	20 – 35	25	26	25	33	33

DISCUSSION

Strongyloides stercoralis infection was first reported in 1876 in French soldiers on duty in Vietnam[4]. The first report of disseminated infection dates back to 1966, with occurrence of fatal strongyloidiasis with immunosuppression[5]. *Strongyloides* is unique among helminths due to its persistence and ability for autoinfection. Risk factors for *Strongyloides* hyperinfection include congenital immunodeficiency, malignancy, immunosuppression, etc. In our patient, we believe that his risk factor for hyperinfection syndrome was long-term azathioprine and prednisolone for 2 years which rendered him to be immunosuppressed.

The lifecycle of *Strongyloides stercoralis* begins with rhabditiform larvae in the intestine excreted in the stool. The rhabditiform larvae then developed into infective filariform larvae or develop through succeeding rhabditiform stages into free-living adults. It infects adult human by penetrating intact skin[6]. The filariform larvae can enter the circulation, transported to the lung, penetrated the alveolar and swallow into digestive tract of human. Rhabditiform larvae can develop into infective filaform in the gastrointestinal tract and trigger off autoinfection.

The clinical manifestation of strongyloidiasis may vary depending on organs involved. Patient may develop local reaction at the site of entry of the larvae. Respiratory symptoms such as cough, dyspnoea, haemoptysis and tracheal irritation can occur when the larvae migrate through respiratory system[7]. In the gastrointestinal tract, patient may complain of diarrhoea, constipation, abdominal pain and loss of appetite[8]. Sometimes it may cause intestinal obstruction, haemodynamically significant gastrointestinal bleeding or ileus.

In the hyperinfection syndrome, the classic life cycle of *Strongyloides stercoralis* from skin to lungs and gastrointestinal tract is accelerated with increased reproduction leading to excessive worm burden. The clinical symptoms of hyperinfection syndrome can vary. Fever with chills and rigors are not typically

present. We should look for presence of Gram-negative infection when there is fever with chills and rigors[9].The likelihood of developing hyperinfection is increased in patient with impaired cell-mediated immunity[10].

Diagnosis of strongyloidiasis is made by detecting rhabditiform larvae in stool or by serological method[11]. However, the sensitivity of a single stool examination to make the diagnosis is only about 50%[12]. Hence, stool study alone is inadequate for diagnosing hyperinfection syndrome. There have been reports of hyperinfection syndrome with negative screening stool exams and the larvae are excreted intermittently. Other techniques such as Beaermann and formalin-ethyl acetate concentration techniques and Harada-Mori filter paper technique have been used to improve the sensitivity[13].

In term of treatment, thiabendazole has been the treatment of choice in the past. The efficacy of thiabendazole in treating chronic strongyloidiasis is said to be around 67 – 81% if given at a dose of 25 mg/kg twice a day for 3 days[14]. However, in recent days, the recommended choice of treatment is ivermectin with albendazole as alternative. Two single dose of 200 mcg/kg of ivermectin is usually administered on two consecutive days for uncomplicated infection.

The optimal treatment for hyperinfection is still uncertain. Some experts give 3 to 11 doses of ivermectin in disseminated disease and certain experts give a combination of ivermectin and albendazole until patient shows clinical response[15]. Besides that, treatment and screening of *Strongyloides* should be done until faecal cultures are negative for at least two weeks as the autoinfective cycle lasts at least two weeks. The same apply to those who have positive urine or sputum samples.

In term of prevention of hyperinfection syndrome, measures such as wearing shoes and screening of family members will be helpful in endemic area[16]. Patient also should be screened for asymptomatic *Strongyloides* infection, either by serological testing or stool examination[17]. For patient who requires prompt initiation of immunosuppression, empirical treatment for strongyloidiasis may be administered to prevent hyperinfection syndrome.

CONCLUSION

Strongyloides hyperinfection is a treatable condition but can cause potentially life-threatening infection. This present case has illustrated the association of *strongyloides* hyperinfection with superimposed gram negative sepsis as a consequence of prolonged immunosuppression. Clinicians should often have high index of suspicion of possible hyperinfection when treating patients with risk factors. Aggressive treatment is often warranted and immunosuppressant need to be adjusted.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this case.

CONSENTS

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STUDENT'S SECTION

Effects of Hypertension on Cognitive Functions among Rungus Population in Sabah

Jeremy Ling Siew Wei[1*], Liew Seng Yung[1], Ong Sheng Yang[1], Lily Shuzeen Kimin[1], Tamilarasi A/P Sugumaran[1], Pavithran A/L T. Rajanoeran[1], Jennefer Ann[1], Ana Syafiqah[1], Vilasheene A/P S. Vikineswarabalan[1], Ong Jia Ni[1], Nur Syafina[1], Fatin Nurfatihah[1], Nur Athirah[1], Yong Kang Hao[1]

[1] Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

*Corresponding author's email: jeremy0718@outlook.com

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ABSTRACT

Hypertension is the global disease burden and not only a major risk factor for stroke, but is also crucial risk factor for cognitive impairment and subsequently dementia. However, literature highlighting on cognitive functions is scarce in Malaysia. This study aimed to assess the hypertension and cognitive functions among the Rungus population in rural areas of Kudat, Sabah. A community based cross-sectional study was conducted among population aged 18 and above living in two villages of Kudat. A validated Malay version of Mini-Mental State Examination (M-MMSE) was used to assess cognitive function with cut-off point of 22. Socio-demographic data, risk behaviours (e.g. physical activity level, smoking status and alcohol consumption) and clinical characteristics (e.g. blood pressure, body mass index and waist circumference) were collected. Chi-square and regression model were used for data analysis. A total of 183 respondents participated in this study with a mean age of 44.64 ± 17.26. The prevalence of hypertension was 34.97% and mean score of MMSE was 24.19 ± 5.660. Age, education level, body mass index and waist circumference were significantly associated with hypertension. Hypertension was not significantly associated with cognitive impairment but instead significantly associated with lower performance of MMSE and its language domain. Therefore, comprehensive interventions should be emphasized to have better understandings on hypertension and prevent its damage to cognition.

INTRODUCTION

Hypertension, a well-known disease which has been causing major burden not only in Malaysia but also worldwide[1]. In the past decade, the prevalence of hypertension in Malaysia has risen from 32.3% in 2006 to 32.7% in 2011 with a recent decline to 30.3% in 2015[2]. In Malaysia, diseases of the circulatory systems remain the leading cause of death, constituting of 22.77%[3]. Globally, it is estimated to cause 7.5 million deaths worldwide annually, approximately 12.8% of the total mortality worldwide and account for 57 million disability adjusted life years (DALYS) or 3.7% of total DALYS[4].

Hypertension, apart from being a leading risk factor for stroke, is the single most important modifiable risk factor to the cognitive impairment[5] and subsequently dementia[6]. The prevalence of mild cognitive impairment (MCI) among elderly hypertensives range from 16.63% to as high as 50% in population studies in Malaysia[7]. The MCI conversion rate to diagnosis of dementia is about 10% per year, and it is estimated to have a new case of dementia in every seven seconds worldwide[8]. It is forecasted to have 100% increase in the dementia cases between 2001 and 2040[9]. Thus, to have cognitive decline which is evident at the age of 45 years is not so surprising[10].

Hypertension contributes to cognitive decline and dementia in a multifactorial way though the mechanism is not completely understood. Mild cognitive impairment (MCI) is a clinical entity on its own, rather than a part of normal ageing and is a transitional stage between normal ageing and dementia[11]. It is defined as 'cognitive decline greater than expected for an individual's age and education level but that does not interfere notably with activities of daily life'[12]. Growing literature shows that cerebral vessel dysfunction including impaired auto regulation and neurovascular coupling changes[13] and even cerebral atrophy[14] are essential to MCI pathway.

Therefore, to have blood pressure well controlled and avoiding those associated risk factors, it is hypothesized to delay the onset of mild cognitive impairment. In Malaysia, the literature highlighting on cognitive function is scarce[15 – 17]. To our knowledge, there is no previous study done in Northern Borneo and no studies in Malaysia have recruited young adults as the respondents. This is despite the fact that young and older adults are shown to be more prone to blood pressure-related longitudinal cognitive decline[18]. Therefore, this study aimed to assess the hypertension and cognitive functions among the Rungus population in rural areas of Sabah. Mini Mental State Examination (MMSE) was adopted to assess the cognitive function due to its high sensitivity and specificity with the ease of administration and high inter-rater reliability. The outcome from this study may address a baseline data to serve for comparison within the country and understand how blood pressure relates to cognition prior to clinical dementia so that early identification and effective interventions can be designed.

MATERIALS AND METHODS

This was a cross-sectional analytical study conducted in 2 villages of Kudat, Sabah from 13th September to 28th October 2016. Research proposal was approved by committee panel of Community Medicine Department during community medicine posting of year 4. A purposive non-probability convenience sampling method was done where all villagers aged 18 and above who consented and willing to participate. The data was collected using a set of pre-test standardized self-administered questionnaire through face-to-face interview including socio-demographic characteristics (e.g. age, gender, marital status and education level), physical activity level (PAL), smoking status, alcohol consumption, clinical neuropsychology test, waist circumference and body mass index. All interviewers were trained regarding the

study procedures prior to the conduct of the study. The participants were considered having hypertension if: (1) the average systolic BP ≥140 mmHg and/or average diastolic BP ≥90 mmHg on two readings; (2) or the participants reported a history of hypertension; (3) or participants reported taking anti-hypertensive medications[19]. Men with WC ≥85 cm and women with WC ≥80 cm were abdominally obese[20]. BMI was calculated using equation (BMI = weight in kg/ height in m^2). Objective cognitive assessment was done through a validated Malay version of MMSE[21] with maximum score of 30 as shown in Table 1. Interviewers were briefed and trained by experienced psychiatrist. The cut-off points of MMSE was 22 for Malay-speaking population[7, 22].

Table 1 Items incorporated within each domain of MMSE

Domains	Items incorporated within domain
Orientation domain	Temporal orientation
	Spatial orientation
Registration domain	Immediate memory
Attention and calculation domain	Attention and calculation ability
Recall domain	Delayed recall
Language domain	Naming objects
	Repetition
	Language comprehension
	Reading
	Writing
Copying domain	Visual-spatial and executive ability

The data collected was recorded and computed by using Statistical Packages of Social Sciences (SPSS), Window version 23.0. The socio-demographic details and clinical characteristics of the respondents and the mean scores for MMSE and its each domain were tabulated for the descriptive analysis. Numerical data are reported as mean (± SD) while categorical data are reported as the number of cases (percentage). Chi-square and multivariate regression model were used to establish the relationship between hypertension status and its associated factors and also its association with MMSE and its each domain. The results were expressed as odds ratio and confidence interval. *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Out of 509 residents in the two villages, 262 are 18 years old and above. A total of 183 participated in our study, giving 69.85% response rate. Among them, 41.5% were males and 58.5% were females with the mean age of 44.64 ± 17.26 years. Among the interviewed respondents, Rungus (88.5%) was the predominant ethnicity in this study and the remaining are Dusun (1.64%), Chinese (1.09%), Iban (0.55%) and Ubian (0.55%). The age 40 – 49 (24.04%) was the predominant age group while elderly population with age ≥60 was large (21.86%) under the current study (Figure 1). Besides, most were Christians (90.7%), married (77.0%) and had education up to secondary education (40.4%). Also, 74.3% of them were currently working and had mean income of RM390.57 ± 674.57 with 167 (91.3%) of them below poverty line index (less than RM1,080).

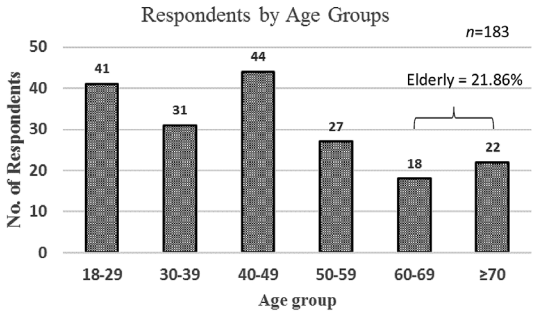

Figure 1 Age distribution among the respondents

In the present study, it was found that 34.97% (*n* = 64) of them were hypertensive while the remaining 65.03% (*n* = 119) were normotensive and female had higher prevalence of hypertension (39.25%) than male (28.95%) as depicted in Figure 2.

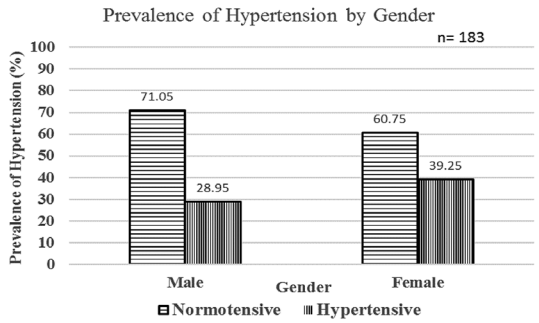

Figure 2 Prevalence of hypertension with gender

With regards to risk behaviours, most of them were neither smoker (77.6%) nor drinking alcohol (91.8%). Majority of them were considered as practising high physical activity level (37.7%) but it was found that 65.0% of them had abnormal BMI and 50.8% were centrally obese (Table 3).

Table 2 Baseline data on MMSE score

MMSE domain	Mean ± Standard deviation
Orientation domain	10.0 ± 9.07
Registration domain	2.54 ± 1.004
Attention and calculation domain	3.35 ± 2.122
Recall domain	1.78 ± 1.279
Language domain	6.73 ± 1.661
Copying domain	0.73 ± 0.444
Total MMSE score	24.19 ± 5.660

Concerning on the cognitive functions, it was found that the total MMSE score among the respondents was 24.19 ± 5.660 which was considered as having normal cognitive function. The median of cognitive score was the highest among the normotensive groups which was 27, 3 scores higher than hypertensive group (Table 2).

Table 3 Association between hypertension and the variables

Variables		Hypertensive status (*n* = 183)		*P* value	OR (95% CI)
		Yes (*n* = 64)	No (*n* = 119)		
Age groups	18 – 29	3 (1.6%)	38 (20.8%)	<0.001**	1
	30 – 39	5 (2.7%)	26 (14.2%)		2.436 (0.535 – 11.091)
	40 – 49	14 (7.7%)	30 (16.4%)		5.911 (1.555 – 22.477)
	50 – 59	16 (8.7%)	11 (6.0%)		18.424 (4.526 – 75.005)
	60 – 69	9 (4.9%)	9 (4.9%)		12.667 (2.840 – 56.489)
	≥ 70	17 (9.3%)	5 (2.7%)		43.067 (9.220 – 201.165)
Gender	Male	22 (12.0%)	54 (29.5%)	0.150	0.631
	Female	42 (23.0%)	65 (35.5%)		(0.336 – 1.183)
Marital status	With partner	54 (29.5%)	87 (47.5%)	0.084	1.986
	Single/ Divorced/ Widowed	10 (5.5%)	32 (17.5%)		(0.904 – 4.364)
Education level	No formal education	30 (16.4%)	16 (8.7%)	<0.001**	9.844 (2.879 – 33.658)
	Primary education	14 (7.7%)	24 (13.1%)		3.062 (0.872 – 10.754)
	Secondary education	16 (8.7%)	58 (31.7%)		1.448 (0.434 – 4.828)
	Tertiary education	4 (2.2%)	21 (11.5%)		1
Education	Education years [Mean ± SD, y]	4.56 (±5.030)	8.83 (±4.732)		–

Working status	Yes	45(24.6%)	91 (49.7%)	0.363	0.729
	No	19 (10.4%)	28 (15.3%)		(0.368 – 1.443)
Household income	Below poverty line index	60 (32.8%)	107 (58.5%)	0.381	1.682 (0.520 – 5.447)
	Above poverty line index	4 (2.2%)	12 (6.6%)		
Smoking status	Yes	11 (6.0%)	30 (16.4%)	0.215	0.616
	No	53 (29.0%)	89 (48.6%)		(0.285-1.330)
Alcohol consumption	Yes	5 (2.7%)	10 (5.5%)	0.889	0.924
	No	59 (32.2%)	109 (59.6%)		(0.302 – 2.829)
Physical activity level	Low	21 (11.5%))	33 (18.0%)	0.417	1.559 (0.733 – 3.317)
	Moderate	23 (12.6%)	37 (20.2%)		1.523 (0.730 – 3.178)
	High	20 (10.9%	49 (26.8%)		1
Body mass index (BMI)	Underweight	5 (2.7%)	6 (3.3%)	0.003*	4.015 (1.038 – 15.533)
	Normal	11 (6.0%)	53 (29.0%)		1
	Overweight	32 (17.5%)	42 (23.0%)		3.671 (1.657 – 8.135)
	Obese	16 (8.7%)	18 (9.8%)		4.283 (1.680 – 10.915)
Waist circumference (cm)	Central obesity	45 (24.6%)	48 (26.2%)	<0.001**	3.503
	Normal	19 (10.4%)	71 (38.8%)		(1.830 – 6.706)

*Significant at $p < 0.05$
**Significant at $p < 0.001$

There were statistically significant relationships between hypertension and age groups ($p < 0.001$), education level ($p < 0.001$), body mass index ($p = 0.003$) and waist circumference ($p < 0.001$) based on Table 3.

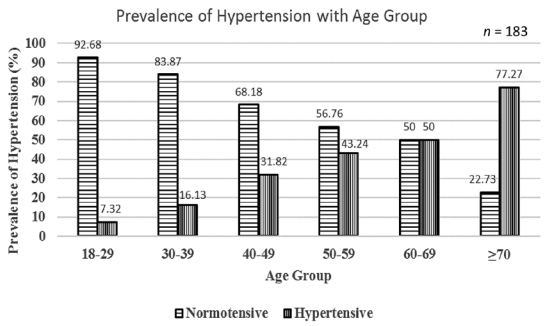

Figure 3 Prevalence of hypertension with age group

For age groups, the proportion of hypertension increased with increasing age. A clear positive association between age groups and hypertension can thus be seen (Figure 3).

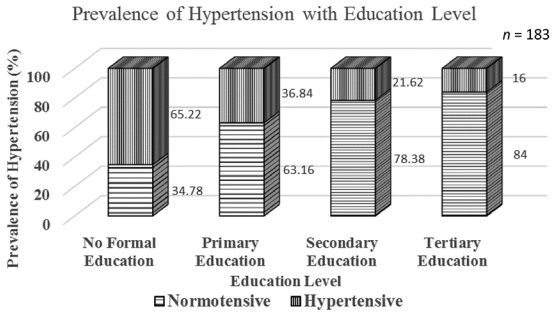

Figure 4 Prevalence of hypertension with education level

In terms of education level, those with tertiary education had the lowest prevalence of hypertension (16%), followed by secondary education (21.62%), primary education (36.84%) and those without formal education (65.22%) as shown in Figure 4.

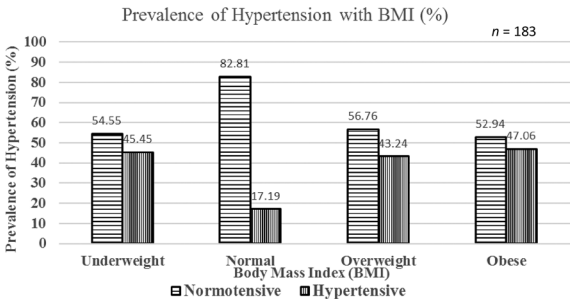

Figure 5 Prevalence of hypertension with BMI

Besides, respondents with abnormal BMI were more likely to have hypertension as depicted in Figure 5. Hypertension prevalence was the lowest (17.19%) among normal BMI group and the highest (47.06%) among the obese group (Figure 5).

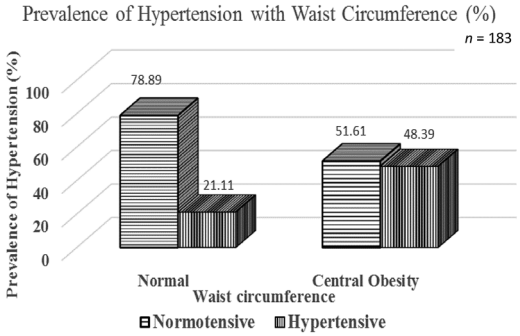

Figure 6 Prevalence of hypertension with waist circumference

Those with central obesity was significantly associated with hypertension (OR: 3.503) when compared to those without central obesity (Figure 6).

Table 4 Association between hypertension and cognitive function

Variables	Cognitive Function		d*f*	*P* value	Adjusted odds ratio	95% confidence interval
	Cognitive impairment	Normal				
Hypertension						
Yes	28 (15.3%)	36 (19.7%)	1	0.052	2.815	0.992 – 7.989
No	13 (7.1%)	106 (57.9%)				

Adjusted for age and education
*Significant at $p < 0.05$

It was found that there was no significant association between hypertension and cognitive impairment ($p = 0.052$) as shown in Table 4.

Table 5: Association between Hypertension and MMSE and its Domains

Variables	Total MMSE	Orientation	Registration	Attention and Calculation	Recall	Language	Copying
Hypertensive status B coefficients (95% CI)	−1.817 (−3.322 – −0.313)	−0.417 (−0.892 – 0.058)	−0.102 (−0.453 – 0.250)	−0.417 (−1.145 – 0.310)	−0.274 (−0.698– 0.150)	−0.652 (−1.104 – −0.200)	0.033 (−0.088 – 0.154)
Sig.	$p = 0.018$*	$p = 0.085$	$p = 0.569$	$p = 0.259$	$p = 0.204$	$p = 0.005$*	$p = 0.588$

Adjusted odds ratio for age and education
*Significant at $p < 0.05$

However, those with hypertension were significantly associated with lower performance on total MMSE. It was thus can be concluded that total MMSE score was 1.817 lower among those who were hypertensive compared to those who were normotensive. Apart from that, those who were hypertensive more likely to have 0.652 lower score in language domain when compared to normotensive respondents (Table 5).

DISCUSSION

The present study aimed to identify the risk factors associated with hypertension and its effect in terms of cognitive function among Rungus population in the rural areas of Northern Borneo. The prevalence of hypertension among the respondents in the present study was 34.97 % which was slightly higher than that of rural population in Malaysia according to a national survey in 2015 (33.5%) [2]. Apart from that, it was found that 21.8% of the study population was 60 years old and above, which was significantly higher when compared to the current elderly population in Malaysia[3]. This study generally demonstrated that the prevalence of hypertension increased with age and the age groups 40 – 49 (OR 5.911), 50 – 59 (OR 18.424), 60 – 69 (OR 12.667), 70 and above (OR 43.067) had significant higher risk of getting hypertension when compared to age group 18 – 29. The same trend was found in a study done in northern west Malaysia and was consistent with other studies[11, 23, 24]. There is a general agreement that with increasing life expectancy, people prone to develop diseases, thus associated with high prevalence of hypertension as in the current study in view of high proportion of elderly population[19 – 22].

Rungus, the population weighed less than 1% in the total population of Malaysia[25], making up 88.5% of the study population under the current study. In this study, education level was significantly associated with hypertension among the respondents and was shown that illiterate had higher risk of hypertension when compared to those with tertiary education ($p < 0.001$, OR 9.844). This was consistent with the findings of previous studies[1, 23]. This was probably due to lack of awareness and knowledge on hypertension, thus reduce the chance of the early diagnosis and early treatment which was supported by high prevalence of not known hypertension among hypertensive respondents (43.75%) in the current study as most hypertension is asymptomatic. These findings indicate the needs of delivery of health education to the low educational level committee.

Another significant association was that both increase in BMI and waist circumference are significantly associated with hypertension which concurred with previous studies[23, 24]. Urbanization and improved socioeconomic status, sedentary lifestyle and unhealthy dietary habits had led to obesity. Malaysia has the highest prevalence of obesity in Southeast Asia and 49% of women and 44% of men in this country were found to be obese[26]. Obese individuals have more fatty tissue that increases their vascular resistance and in turn increases the heart workload to pump blood throughout the body and hypertension results when systemic vascular resistance fails to decrease and unable to cope with the increase of cardiac output[27]. Thus, it was important to emphasize on the weight reduction as it was modifiable and preventable risk factor for hypertension.

It is interesting to mention our study has not only found significant association between high BMI and hypertension, but also lower-than-normal BMI and hypertension which was similar with other studies[28, 29]. In our study, the occurrence of underweight of villagers may exist since childhood, thus it may be the cause of hypertension in their adult life. It was proved that malnutrition during early childhood would lead to underdevelopment of the arterial tree due to low cardiac output and consequently hypertension in adult life. Therefore, concern on malnutrition during development or childhood period could not be emphasized enough.

Alcohol, a well-known risk factor for non-communicable diseases and was shown that excessive consumption was associated with an increased risk of hypertension, stroke and certain cancers[23]. However, the alcohol consumption ($p = 0.889$, OR 0.924) in this study was not significantly associated with hypertension, owing to low proportion of

consuming alcohol (8.2%). It was also found that smokers had lower odds of having hypertension ($p = 0.215$, OR 0.616) as compared to non-smokers which was later proved to be insignificant due to less smokers in the present study despite the well-known facts of smoking increase the risk of pre-hypertension and hypertension.

In the current study, hypertension was not significantly associated with cognitive impairment ($p = 0.052$). Such inconsistencies were likely due to different inclusion criteria, study design, sample size, cognitive assessment method and language limitation. Nevertheless, hypertension was significantly associated with lower performance in total MMSE score ($p = 0.018$, *B* coefficients: −1.817) and language domain ($p = 0.005$, *B* coefficients: −0.652) in the current study.

It was suggested that hypertension-induced microvascular changes situated primarily in frontal system white matter[30], causing language impairment. Another reason could be the usage of Malay version MMSE, which limits the understandings and linguistic proficiency for some elderly respondents.

CONCLUSION

Prevalence of hypertension was high among elderly and low-educated population. Undiagnosed hypertension was also high among hypertensive respondents. Abnormal BMI and central obesity were significantly associated with higher risk of hypertension. With respect to cognitive function, hypertension was significantly associated with lower performance of MMSE and its language domain. A comprehensive interventions and efforts are crucial to have better understandings on hypertension and prevent damage on the cognition.

LIMITATIONS

The sample size was small ($n = 183$), which limits the statistical power to detect the true effect. The age of the population in this study had also great variant compared to previous studies which were more focused on the elderly group of population. There was also limitation in choosing cross-sectional study to study the chronicity and long-term effects of the hypertension. While non-probability convenience sampling was employed in the present study, its vulnerability to severe hidden biases could not be neglected. The validated Malay version Mini Mental State Examination (MMSE) adopted in the current study had language limitations as the main language of some elderly respondents was Rungus. Despite of the limitations, this community based cross-sectional study gives valuable information on the hypertension and cognitive function among Rungus population. Future research should sample larger numbers of persons with controlled, untreated, and uncontrolled hypertension and efforts should be put to generate a Rungus version MMSE to ease the process and obtain accurate results.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this paper.

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