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EDITORIAL

Anxiety Disorders and Suicidal Behaviours

MZ Azhar
Dept Of Psychiatry, Faculty Of Medicine and Health Sciences,
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

In this issue of the journal there are three papers in the area of clinical psychiatry dealing with help seeking behaviours, parasuicides and anxiety in hospital patients. In these articles discussions were made to associate events and symptomatology to some specific measurements such as quality of life, specific behaviours, etc. However there have been increasing questions asking to look at certain symptoms or comorbidity to associate with worsening symptoms which have not been adequately touched in the articles in this issue. The work currently being done in several centres is related to anxiety disorders and risk for suicidal ideation and attempts which is always a major issue in psychiatry. It has been shown that suicidal ideation and suicide attempts are highly prevalent in the community and are strong risk factors for completed suicides.

POSSIBLE ASSOCIATION?

It has been well established that the risk factor for suicide is the presence of mental disorders, especially mood disorders, substance use disorders, and schizophrenia. The controversy is whether anxiety disorders are also risk factors for suicide. Many cross sectional community studies [1] and clinical studies have demonstrated that anxiety disorders are associated with suicidal ideations and attempts as well as completed suicides. Among the anxiety disorders, panic disorder [2] has received the greatest attention. But what is forgotten is that in clinical practice, if we see enough patients, we will realize that anxiety disorders are highly comorbid with other anxiety disorders and tend to cluster together so studies ought to look at whether anxiety disorders as a group have an impact on suicidal behaviour after adjusting for other types of mental disorders especially mood and substance abuse disorders.

POSSIBLE ETIOLOGICAL MECHANISMS?

Theoretically there are a number of possible explanations for the relationship between anxiety disorders and suicidal behaviour. The most obvious is the direct effect of the high levels of anxiety itself together with worry and fear leading to routes of seeking escape from suffering by considering a suicidal act. Other explanations could be the indirect effect of comorbidity with other mental disorders which could be complication of the anxiety disorders such as substance abuse or mood disorders. Other possible mechanisms could be the etiological factors of anxiety such as childhood trauma, genetic factors, personality factors like neuroticism, impulsivity, self-criticism among others, may explain the increased risk for suicidal behaviour. The biological theory of anxiety such as the neurotransmitter theory that postulates the low levels of hydoxyindolacetic acid in the cerebro spinal fluid may link anxiety disorders to suicidal behaviours. Also not forgetting, chronic anxiety can lead to many other factors such as poor social support, traumatic events, loss of job, loved ones; etc may also lead to suicidal behaviour.

STUDIES?

There have been attempts at studying these issues from the early nineties [3] but then they were looking at depression as the main factor but found that comorbidity with anxiety is a stronger risk factor then depression alone. Slowly there were few studies looking at the same issue of comorbidity but with small sample sizes. One study in Germany pointed out the same finding with regards to comorbidity of anxiety and depression and not depression itself is a risk factor for suicide and they look at lifetime-anxiety-disorder diagnosis, a further improvement from the earlier study. However there are very few studies that are population based, prospective, and longitudinal that would provide better results. There has been one study [4] that look at a two year longitudinal follow-up and it is population based done in the Netherlands that I believe is a good study to look at and perhaps get clinicians to pay more attention to this issue. They use data from the Netherlands Mental Health and Incidence Survey which is a large Dutch population survey.

Their findings from this large population survey are very interesting and should provoke more clinical research in this area. Among all the respondents with suicidal ideation at baseline, 52.4% had at least one anxiety disorder. Among all respondents with suicidal attempts at baseline 64.1% had at least one anxiety disorder. After adjustments they found that the presence of at least one anxiety disorder diagnosis at baseline was significantly associated with lifetime

*Corresponding Author: *azharmz@medic.upm.edu.my*

suicidal ideation and attempts. In multivariate analyses, they found panic disorder, agoraphobia without panic and simple phobia (but not obsessive compulsive disorder, generalized anxiety disorder or social phobia) was significantly associated with lifetime suicidal attempts even after adjusting for other mental disorders and sociodemographic variables.

The main finding of the study was that the presence of an anxiety disorder at baseline was a risk factor for subsequent onset of suicidal ideation and attempts.

IMPLICATION?

The findings contribute to resolving the issue of whether anxiety disorders are risk factors for suicidal behaviour. The fact that anxiety disorders are highly under-recognized and under treated in the community and primary care, these findings suggest that untreated anxiety disorders might be missed opportunities for preventing suicidal behaviour. Will early recognition and early intervention reduce likelihood of suicidal behaviours is a good follow-up study for clinicians.

CONCLUSION

Suicidal behaviour is a complex process because of numerous interrelated factors, and although this write up suggests that anxiety disorders play an important role, the mechanism of the increase in suicidal behaviour associated with anxiety disorders remains to be determined.

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Acute Gastroenteritis Among Indigenous Paediatric Patients – A Descriptive Study in a Rural District Hospital, Sarawak

[1]WL Cheah*, [2]PY Lee, [1]SAR Syed Alwi, [1]K Kamarudin, [1]H Albela, [1]EH Lau, [1]O Noraini & [1]WA Siti Sanaa

[1]Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak
Lot 77, Section 22 KTLD, Jalan Tun Ahmad Zaidi Adruce, 93150 Kuching, Sarawak, Malaysia.
[2]Faculty of Medicine and Health Sciences, Universiti Putra Malaysia
43400 Serdang, Selangor Malaysia

ABSTRACT

Introduction: Acute gastroenteritis (AGE) is one of the frequent causes of hospitalization in children under the age of five, particularly in a rural setting. This study was conducted to determine the epidemiology of acute gastroenteritis in indigenous children admitted to a rural district hospital in Sarawak. **Methods:** A retrospective review of indigenous paediatrics cases of acute gastroenteritis admitted to the ward of Serian District Hospital, a rural district hospital in Sarawak, between the years 2006-2007. The data was collected from the patients' case notes, obtained with permission from the hospital management. Data was entered and analyzed using SPSS version 16. **Results:** During the study period, 234 indigenous children with acute gastroenteritis were admitted with the highest prevalence in 2006 (53.4%). The findings showed higher prevalence was found in children aged 3 years and below (76.5%) and male (56.4%) The minimum duration of hospital stay is 1 day, and the maximum stay is 5 days. The clinical findings showed that the majority of the cases presented with vomiting, diarrhea, dry mouth and tongue, sunken eye, with the majority (76%) reported having mild dehydration. The most common treatment used is oral rehydration solutions (85.4%), followed by intravenous bolus or drip (82.3%), paracetamol (79%) and antibiotic (36.2%). Peak incidence of admissions was between November to January. About 38.5% of the AGE cases admitted were found to be underweight (weight-for-age below -2SD). **Conclusion:** The findings indicated children aged 3 years and below are the most vulnerable to AGE and malnutrition could be one of the predisposing factors. The peak incidence during the raining season at the end of the year indicated a possible relationship between AGE and seasonal type of virus infection. Prevention in the form of proper hygiene at the household level probably will prove to be useful.

Keywords: Acute gastroenteritis; indigenous; children; Malaysia

INTRODUCTION

Acute gastroenteritis (AGE) is one of the frequent causes of hospitalization in children under age of five in the hospital setting, both in Malaysia and throughout the world, especially in developing countries[1, 2]. It is estimated that approximately 440,000 annual deaths in children <5 years of age worldwide are due to diarrhea related illnesses, with rotavirus as the main cause[1]. Due to the inability to cope with greater body fluid loss and immature renal tubular re-absorption process, children tend to face greater risk of being criticall ill and their illness can cause death or irreversible prognosis[3]. Recent studies suggest that as global deaths from childhood diarrhea decreased during the past 2 decades, the proportion of hospitalization due to diarrhea may have not changed much. In fact, the mortality caused by this disease contributes significant health costs to the government. This is reflected in a recent study by Lee *et al.*[2] who estimated that the financial burden of providing inpatient care for rotavirus GE in Malaysian children was US$1.8 million (range US$0.6 million 7.5 million) annually. The findings further revealed that the median cost of providing inpatient care for an episode of rotavirus GE was US$211.91 (range US$68.50–880.60). This figure is rather high as compared to per capita health expenditure provided by the Malaysian Government in 2002 – US$71.47.

In view of the importance of understanding the epidemiological aspect of AGE, this report aims to establish a preliminary profile among the indigenous AGE cases reported in the paediatric ward in a rural district hospital in Sarawak. The report describes the information obtained from the case notes over a period of 2 years from 2006-2007.

*Corresponding author: *wlcheah@fmhs.unimas.my*

MATERIALS AND METHODS

The study was conducted in the Serian District Hospital, located 1.6 km from Serian, a town and the capital of the Serian District in Samarahan Division, Sarawak. It is located about 65 km from Kuching, the capital of Sarawak. The population as reported in the year 2000 population census was 84,800 with 65% of the population being Bidayuh, followed by Iban, Chinese and Malay. Serian District Hospital has a serving history of 32 years with 84 beds. The paediatric ward consists of 34 beds.

Data on indigenous AGE cases in the paediatric ward of Serian District Hospital from the year 2006-2007 was obtained from the Medical Record Section of the hospital. A prior permission was obtained from the hospital management and all information was treated with confidentiality. All the cases included in this study were of children 12 years old and below. Information obtained from the case notes consisted of patients' details on admission, age, gender, race, weight during admission, duration of hospitalization, presenting signs and symptoms, investigation findings and management of patients. Categorization of anthropometry status in the form of z-score was based on World Health Organisation (WHO) Anthro version 3.01[4] for 0-60 months. For classification of age group ranged from 5-10 years, 2007 WHO Reference[5] was used. Between 10-12 years old, CDC growth charts 2000[6] were used.

All data was entered and analyzed using Statistical Package for Social Science (SPSS) version 16. Descriptive analysis was done and presented in various forms.

RESULTS

Between the 1st of January 2006 and the 31st of December 2007, 234 children (0.08-11 years) were hospitalized in the paediatric ward due to AGE. The length of hospitalization ranged from 1 to 5 days, with a mean of 1.86 ± 0.93 days. Approximately 39.7% of patients stayed in the hospital for 1 day, whereas 0.9% stayed for 5 days. Of these, 56.4% were boys and 70.9% were Bidayuh. Approximately 14.1% of the AGE children were severely underweight (<-3SD). Information on characteristics of the patients is presented in Table 1.

Table 1 : Information on Patients (n=234)

	No	%	Mean	Range
Age (year)				
0-0.5	19	8.1	2.45	0.08-11
0.6-3	160	68.4		
4-6	39	16.7		
7-9	12	5.1		
10-12	4	1.7		
Male				
Male	132	56.4		
Female	102	43.6		
Race				
Bidayuh	166	70.9		
Iban	68	29.1		
Duration of Hospitalization (day)				
1	93	39.7	1.86	1-5
2	93	39.7		
3	37	15.8		
4	9	3.8		
5	2	0.9		
Weight-for-age				
Severe Underweight (<-3SD)	33	14.1		
Moderate Underweight (<-2SD)	57	24.4		
Mild Underweight (<-1SD)	76	32.5		
Normal	68	29.1		

The most common admission month was from November to January (15.0 – 17.9%), and admission rates was lowest in March and April (2.6% each). The details are presented in Figure 1.

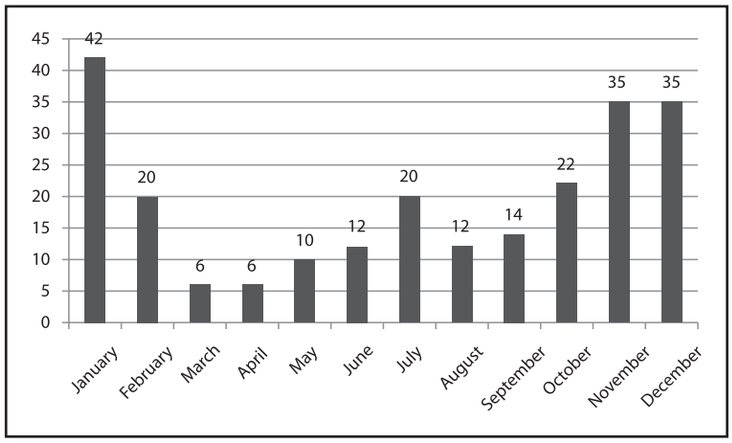

Figure 1 : Number of Acute Gastroenteritis cases according to Month

The distribution of signs and symptoms during the admission are presented in Table 2. Sunken eye, dry mouth and tongue were the most common signs. Most of the patients (85.2-89%) complained of diarrhea and vomiting during the admission. The majority of the patients (79-85.4%) were treated with oral rehydration salt, IV bolus/drip and paracetamol. Only one third (36.2%) were given antibiotic, possibly the cases were due to bacterial infections. Based on the signs and symptoms of dehydration, the physical examinations revealed that the majority (73.9%) of patients had mild dehydration, and 26.1% had moderate or severe dehydration.

Table 2 : Signs and Symptoms of the AGE cases and type of treatment (n=234)

	No	%
Signs		
No signs of dehydration	57	24.4
Drowsiness	53	22.6
Sunken eye	67	28.6
Dry mouth and tongue	67	28.4
Tachypnoea / tachycardia	39	16.5
Reduced skin turgor	30	12.7
Symptoms		
Diarrhea	199	85.2
Vomiting	208	89.0
Fever	140	59.9
Poor oral intake	141	60.4
Treatment		
ORS	200	85.4
IV bolus or IV drip	193	82.3
Paracetamol	185	79.0
Antibiotic	85	36.2

Table 3 : Severity of the AGE cases (n=234)

Severity	No	%
Mild dehydration	173	73.9
Moderate dehydration	50	21.4
Severe dehydration	11	4.7
Total	234	100%

DISCUSSION

The findings of this study indicated that the majority of the AGE patients were from age group 3 years and below, consistent with other studies done in Singapore[7], New Zealand[8] and Italy[9] where the majority of the AGE rotavirus related hospitalization were from the same age group. The most affected age group was those from 6-23 months. This finding is consistent with a study done in urban area in Malaysia[10]. Those who were below 6 months were less affected probably due to protective effect of maternal antibodies in infants[11] and the antibody obtained from breastfeeding[12]. As for gender, there was only a slight difference between male and female, although studies have shown mix difference between the groups[13].

In terms of nutritional status, more than two third of the AGE cases were reported to be underweight. It is known that the interaction between malnutrition and infection is the leading cause of morbidity and mortality in children[14]. Infections, particularly AGE can make malnutrition worse and poor nutrition can increase the severity of infectious disease. As the findings were based on the case report at the point of hospital admission, the relationship between malnutrition and infection is inconclusive.

Unlike a study done locally by Hung *et al.*[10], this study shows that the greatest burden of AGE was between November to January (raining season) - a seasonal pattern. Another study done in urban area in Malaysia, showed peak incidence of admissions was between January to March, and September to October[14]. Evidences done in other countries indicated only regions with a temperate climate have a seasonal pattern[8, 9], and usually the admissions occur more in late winter and early spring. Nevertheless, this is a potential area for further study.

The most common signs and symptoms of AGE in this study were similar with other studies[8, 9]. The main limitation of this study is comparison between children with and without rotavirus infection cannot be done as such information was not available in the medical records. Other studies in Malaysia have shown that Rotavirus was the commonest pathogen identified for the causes of AGE in young children[14, 15, 16]. Studies[8, 9] have shown that children with rotavirus-positive AGE were more likely to have dehydration as compared to those who are rotavirus-negative. This is common as rotavirus-positive AGE cases are considered as a more severe form of illness[8, 9]. In terms of severity of dehydration, 24.2% of the children in our study had moderate or severe dehydration on admission. This is rather consistent with another study done in an urban area in Malaysia where 17% had moderate or severe dehydration and the commonest pathogen are rotavirus[15]. In term of management of AGE in the district hospital, the mean duration of stay of 1.86 days is slightly shorter than studies done in other urban hospitals in Malaysia[14, 17]. This could be due to the fact that cases admitted to a rural district hospital may be less severe compared to cases admitted to a tertiary hospital. For the treatment with intravenous fluid therapy, 85.4% of patients received intravenous fluid therapy which is consistent with other studies in Malaysia[15]. Antibiotic were prescribed in 38.5% of the patients in our study. Although we do not have information on the type of pathogen involved in this study, most cases of acute gastroenteritis in children are viral, self-limited, and need only supportive treatment. Antibacterial therapy should be restricted to specific bacterial pathogens and disease presentations[18]. However, empiric therapy may be appropriate in the presence of a severe illness with bloody diarrhea and stool leucocytes, particularly in infancy and the immunocompromised[18].

The limitation of this study is that only children seeking healthcare at the district hospital were included. Therefore generalization of these findings cannot be applied to others who seek treatment elsewhere. In a district hospital setting, many clinicians do not depend on stool investigation or rapid antigen detection for diagnosis. Only during an outbreak or when patients present with bloody diarrhea and high fever, such tests are done. Therefore, information on types of pathogen involved in this study was not available for further analysis.

In conclusion, this study has clearly demonstrated that AGE cases in indigenous children are compatible to their non-indigenous peers in urban setting. One common finding indicated that children aged 3 years and below are the most vulnerable to AGE and malnutrition could be one of the predisposing factors. The peak incidence during the raining season at the end of the year indicated a possible relationship between AGE and seasonal type of virus infection. Prevention in the form of proper hygiene at the household level probably will prove to be useful.

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Anxiety Disorders and Quality of Life among Patients with Hematological Cancer in a Malaysian Hospital

[1]D Priscilla*, [2]A Hamidin, [2]MZ Azhar, [2]KON Noorjan, [1]MS Salmiah & [4]K Bahariah

[1]Department of Community Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[2]Department of Psychiatry, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[3]Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Objective: The purpose of this study is to access the prevalence of anxiety disorders and quality of life factors among hematological cancer patients in a Malaysian hospital. **Methods:** This study used a cross-sectional research design. It was conducted at the Ampang Hospital in Kuala Lumpur, a tertiary referral center for hematological cancer. Anxiety disorders were diagnosed using the Mini International Neuropsychiatric Interview (MINI); quality of life was assessed using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) questionnaire. **Results:** A total of 105 hematological cancer patients participated in the study, which constituted a response rate of 83.3%. The prevalence of anxiety disorders in our sample ranged from 1% to 24.8%. Overall, compared to patients without anxiety disorders, hematological cancer patients with anxiety disorders reported impaired quality of life in regards to emotional functioning, cognitive functioning, insomnia, dyspnoea, nausea and vomiting, appetite loss and constipation ($p<0.05$). **Conclusion:** The findings of this study support the notion that, like other cancer patients, hematological cancer patients are vulnerable to developing anxiety disorders that could impact their overall quality of life. Therefore flexible cancer treatments should include a referral to a health professional or psychiatrist who is able to address a patient's mental health status and quality of life.

Keywords: Anxiety disorders, hematological cancer, quality of life

INTRODUCTION

Cancer diagnosis affects the lives of many patients, and the burden can be overwhelming for patients with psychiatric disorders. Cancer, particularly hematological cancer, can have a strong impact on a patient's mental health status.[1-2] Hematological cancer affects the blood cells or lymphatic system, which seriously impacts the lives of patients. Hematological cancer exists in various forms; leukemia, lymphoma and multiple myeloma are three different types of blood cancer.[3] In peninsular Malaysia, a total of 21,773 people were diagnosed with cancer. Lymphoma (4.2%) and leukemia (3.6%) in males and lymphoma (2.4%) in females were among the ten most frequently diagnosed cancers in peninsular Malaysia.[4]

Anxiety is a common psychiatric disorder diagnosed among cancer patients.[1-2, 5] The prevalence of anxiety disorders in hematological cancer patients has been found to be approximately 8.2%.[2] Many international western studies suggest that hematological and other cancer patients are at a higher risk of developing comorbid psychiatric disorders, especially anxiety.[2, 5-6] Anxiety covers a broad spectrum of disorders, which includes panic disorder without agoraphobia, panic disorder with agoraphobia, agoraphobia without history of panic disorder, specific phobia, social anxiety disorder (SAD), obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), acute stress disorder and generalized anxiety disorder (GAD). Each anxiety disorder has a specific definition and symptoms, but all are related to anxiety.[7]

Hematological cancer is not only a life-threatening disease, but it also can adversely affect a patient's quality of life, particularly with regard to the level of functioning and clinical symptoms.[8-9] Thus, patients with hematological cancer appear to be at high risk for having both an anxiety disorder and poor quality of life.[1]

Given the important relationship between anxiety disorders and quality of life in patients with hematological

*Corresponding author: *daspriscilla@yahoo.com*

cancer, it is important to know the prevalence of anxiety disorders (and the quality of life) of Malaysian hematological cancer patients. To our knowledge, no Malaysian data have been published on this topic, nor have there been any official attempts to fully understand the phenomena.

This study was conducted to fill this gap using a sample of hematological cancer patients from a Malaysian tertiary referral center. The data derived from this sample were used to determine the prevalence of various anxiety disorders and its effect on quality of life for these patients.

METHODOLOGY

This hospital-based study was conducted at the Ampang Hospital in Kuala Lumpur, Malaysia. Ampang Hospital is a tertiary referral center for hematological cancer in Malaysia. Data were collected for a period of 8 months, from May to December 2009; a cross-sectional study design was used. Sample size estimation calculated using the single proportion formula, $n= Z^2 P (1-P) / d2$.[10] The Z value is determined as 1.96 with $\alpha= 0.05$ level of significance. A previous study done by Prieto *et al.*, found the prevalence of anxiety among hematological cancer patients was 8.2%.[2] Hence, P equals to 0.082 was determined to be used in the formula. Symbol d in the formula is denoted for precision[10] and the d value was set at 5% or d=0.05 in the study. Hence from the formula a total of 116 patients were required in the study; $1.96^2 \times 0.082 (1-0.082) / (0.05)^2 =116$ patients. Additional 10 patients has been added making a total sample of 126 hematological cancer patients.

The Ethical Committees of the Ministry of Health and the Faculty of Medicine and Health Sciences, University Putra Malaysia, approved the study. All patients who were admitted to the hematology wards during this period were approached for participation. Informed consent was obtained from all who agreed to participate in the study. Eligible criteria for participation were: (1) hematological cancer patient; (2) able to communicate in English, Malay, Mandarin or Tamil; (3) at least 15 years of age; and (4) fit and conscious. Socio-demographic profiles of the subjects were obtained through a socio-demographic questionnaire. Clinical statuses of patients were confirmed through medical records.

Anxiety disorder diagnoses were confirmed using modules from the Mini International Neuropsychiatric Interview (MINI), version 6.00.[11] Modules used included: D - panic disorder; E - agoraphobia, F - social anxiety disorder or current social phobia [SAD]; G - obsessive compulsive disorder (OCD); H - post-traumatic stress disorder (PTSD) and N - generalized anxiety disorder (GAD). The MINI was developed based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria and the International Classification of Diseases (ICD-10). The disorders were determined based on "yes" or "no" answers to the questions on the MINI. Interviewers for this research were trained by senior psychiatrists who were certified to use the questionnaire. The questionnaire has been rated positively for sensitivity and specificity, with ratings of 96% and 88%, respectively.[11]

This study also utilized the European Organization for Research and Treatment of Cancer Quality of Life (EORTC QLQ-C30) questionnaire, version 3.0, which has been pre-tested and validated.[12-13] This disease-specific questionnaire is used to evaluate the quality of life of cancer patients. The questionnaire consists of five functioning scales (physical, role, cognitive, emotional and social functioning), three symptoms scales (fatigue, pain and nausea/vomiting), a global health status scale and six single item scales (dyspnoea, insomnia, appetite loss, constipation, diarrhoea and financial difficulties). The EORTC QLQ-C30 has 30 questions in total. Of these, 28 questions were rated using a 4-point scale (1= "Not at all", 2= "A little", 3= "Quite a bit" to 4= "Very much") and 2 questions were rated using a 7-point scale, ranging from "very poor" (1) to "excellent" (7).[12] Final scale scores are computed based on a scoring manual, and they range from 0 to 100. Higher scores on the functional and the global health status scales represent a high or healthy level of functioning and high global health status; higher scores on the symptoms and single item scales represent a high level of symptoms or problems.[12, 14]

The Statistical Package for Social Sciences (SPSS) program, version 17.0, was used to conduct Mann-Whitney U-tests to make comparisons between the quality of life for patients with and without anxiety disorders. One-tailed tests with a significance level of $p<0.05$ were used for the analyses.

RESULTS

One-hundred and twenty-six patients were approached for participation in our study between May and December 2009. The final sample included 105 patients, which made for a total response rate of 83.3%. Reasons for declining study participation among the patients approached included: (1) refusal to participate (n=6); (2) non-hematological cancer diagnosis (n=13) and (3) difficulties understanding the questionnaire (n=2).

The mean age of the study participants was 40.43 years (95% CI, 37.36-43.49), with a range of 15.00 to 77.82 years. Male patients had a higher mean age (43.61) than female patients (37.53). This difference was statistically significant ($t = 1.991$, $df = 103$, $p=0.049$).

The majority of the patients who participated in the study were female (52.4%). In terms of ethnicity, the majority of the patients who participated in the study were of Malay ethnic descent (60%), followed by Chinese descent

(24.8%). Those with Indian descent (13.3%) and the other ethnic groups (1.9%) represented a small proportion of the study population. In terms of diagnosis, 23.8% of the patients had non-Hodgkin lymphoma, 22.9% had acute myelogenous leukemia, 14.3% had acute lymphoblastic leukemia, 10.5% had Hodgkin lymphoma and 28.5% had other hematological cancer diseases.

Table 1 shows the prevalence of anxiety disorders (current episode) among hematological cancer patients. Approximately one-quarter (24.8%) of the sample had agoraphobia without history of panic disorder, 10.5% had GAD, 7.6% had SAD, 2.9% had panic disorder with agoraphobia, 2.9% had PTSD, 1.9% had OCD and 1% had panic disorder without agoraphobia.

Table 1: Prevalence of anxiety disorders (n=105)

Anxiety Disorders	NO		YES	
	N	%	N	%
Panic disorder with Agoraphobia	102	97.1	3	2.9
Panic disorder without Agoraphobia	104	99.0	1	1.0
Agoraphobia without history of panic disorder	79	75.2	26	24.8
Social Phobia (Social Anxiety Disorder)	97	92.4	8	7.6
Obsessive Compulsive Disorder	103	98.1	2	1.9
Post Traumatic Stress Disorder	102	97.1	3	2.9
Generalized Anxiety Disorder	94	89.5	11	10.5

Mann-Whitney U tests were conducted to compare the quality of life of participants with or without anxiety disorders. Table 2 presents between-group differences in the quality of life mean rank score as a function of anxiety disorders. Patients with panic disorder with agoraphobia had a significantly lower cognitive functioning score (p= 0.014), a significantly higher dyspnoea score (p=0.017) and reported significantly less pain (p=0.045). Patients with agoraphobia without a history of panic disorder were found to have lower emotional functioning (p=0.043) and cognitive functioning scores (p=0.05) compared to patients without the disorder. Hematological cancer patients with SAD had significantly lower scores in emotional functioning (p<0.001), cognitive functioning (p=0.002), and social functioning (p=0.015) and reported a significantly higher level of financial difficulties (p=0.010). Patients with OCD had significant impairment in role functioning (p=0.050), emotional functioning (p=0.017), cognitive functioning (p=0.031), and global health status (p=0.048) compared to patients without the disorder. Patients with OCD were also found to show more symptoms of nausea and vomiting (p=0.023) and appetite loss (p=0.023) compared to other patients.

Patients with PTSD were found to have significantly poorer social functioning (p=0.043) and more severe dyspnoea symptoms (p=0.007) than respondents without the diagnosis. Hematological cancer patients with GAD had significantly lower scores in physical functioning (p=0.034), role functioning (p=0.007), emotional functioning (p<0.001), and cognitive functioning (p=0.017) and had significantly higher scores in insomnia (p=0.016), constipation (p=0.006) and financial difficulties (p=0.010). Finally, patients with panic disorder without agoraphobia did not significantly differ from patients without the disorder in their quality of life scores.

Overall among the quality of life domains, the highly negative effect of anxiety disorders were significant for 6 domains for patients with OCD and GAD, 4 domains for patients with SAD, 2 domains for patients with panic disorder with agoraphobia and agoraphobia without panic disorder and 1 domain for patients with PTSD.

Table 2: Between-group differences in the quality of life mean rank score as a function of anxiety disorders (n=105)

	QOL	PF	RF	EF	CF	SF	FA	NV	PA	DY	SL	AP	CO	DI	FI
No- Panic Disorder with Agrophobia	53.38	53.17	53.54	53.55	54.10	53.77	52.78	52.85	53.84	52.08	53.19	52.23	52.45	53.50	52.57
Yes- Panic Disorder with Agrophobia	40.17	47.33	34.50	34.33	15.67	26.67	60.33	58.00	24.50	84.33	46.67	79.33	71.83	36.00	67.50
z	-0.750	-0.328	-1.106	-1.083	-2.209	-1.546	-0.427	-0.312	-1.695	-2.132	-0.398	-1.586	-1.148	-1.191	-0.870
p-value	0.227	0.372	0.135	0.140	*0.014	0.061	0.335	0.378	*0.045	*0.017	0.346	0.057	0.126	0.117	0.193
No- Agoraphobia without history of Panic disorder	54.39	54.91	51.68	55.92	55.75	53.06	51.95	52.80	55.14	52.54	52.78	52.03	54.58	54.02	50.61
Yes- Agoraphobia without history of Panic disorder	48.77	47.19	57.02	44.12	44.65	52.83	56.19	53.60	46.50	54.38	53.65	55.94	48.21	49.90	60.27
z	-0.828	-1.126	-0.804	-1.724	-1.652	-0.034	-0.622	-0.124	-1.293	-0.315	-0.137	-0.593	-0.977	-0.725	-1.458
p-value	0.204	0.130	0.211	*0.043	*0.050	0.487	0.267	0.451	0.098	0.377	0.446	0.277	0.165	0.234	0.073
(No) SAD	53.84	53.54	53.56	56.36	55.49	54.82	51.98	52.74	53.70	52.53	52.45	51.81	52.63	53.42	51.08
(Yes) SAD	42.81	46.44	46.19	12.25	22.75	30.94	65.38	56.13	44.50	58.75	59.63	67.38	57.50	47.88	76.25
z	-0.997	-0.637	-0.682	-3.957	-2.998	-2.169	-1.206	-0.326	-0.847	-0.655	-0.697	-1.449	-0.459	-0.601	-2.335
p-value	0.160	0.262	0.248	***0.000	**0.002	*0.015	0.114	0.372	0.199	0.256	0.243	0.074	0.323	0.274	*0.010
(No) OCD	53.68	53.11	53.66	53.87	53.76	53.51	52.51	52.23	53.34	52.93	52.80	52.20	52.36	53.33	52.91
(Yes) OCD	18.00	47.50	19.00	8.00	14.00	26.75	78.00	92.50	35.50	56.75	63.25	94.00	86.00	36.00	57.50
z	-1.663	-0.259	-1.652	-2.120	-1.875	-1.252	-1.183	-2.000	-0.846	-0.207	-0.523	-2.006	-1.635	-0.967	-0.219
p-value	*0.048	0.398	*0.050	*0.017	*0.031	0.105	0.119	*0.023	0.199	0.418	0.301	*0.023	0.051	0.167	0.413
(No) PTSD	53.77	53.02	53.47	53.49	53.64	53.86	52.73	52.98	52.77	51.92	52.44	52.95	52.59	53.50	52.57
(Yes) PTSD	26.67	52.33	37.00	36.50	31.17	23.67	62.33	53.83	60.67	89.67	72.00	54.83	67.00	36.00	67.50
z	-1.540	-0.039	-0.957	-0.957	-1.292	-1.722	-0.543	-0.052	-0.456	-2.495	-1.194	-0.110	-0.854	-1.191	-0.870
p-value	0.062	0.485	0.170	0.170	0.098	*0.043	0.294	0.480	0.324	**0.007	0.116	0.456	0.197	0.117	0.193
(No) GAD	54.12	54.85	55.45	58.22	55.11	54.63	51.51	52.92	53.80	52.46	50.98	53.59	50.55	53.32	50.70
(Yes) GAD	43.45	37.18	32.09	8.41	34.95	39.09	65.77	53.68	46.18	57.64	70.23	48.00	73.91	50.27	50.70
z	-1.113	-1.828	-2.494	-5.158	-2.130	-1.629	-1.483	-0.085	-0.809	-0.629	-2.160	-0.601	-2.543	-0.381	-2.349
p-value	0.133	*0.034	**0.007	***0.000	*0.017	0.052	0.069	0.466	0.210	0.265	*0.016	0.274	**0.006	0.352	*0.010

*p < 0.05; **p < 0.01; ***p < 0.001

SAD, Social Anxiety Disorder; OCD, Obsessive Compulsive Disorder; PTSD, Post Traumatic Stress Disorder; GAD, Generalised Anxiety Disorder; QOL, Global quality of life; PF, Physical Functioning; RF, Role Functioning; EF, Emotional Functioning; CF, Cognitive Functioning; SF, Social Functioning; FA, Fatigue; NV, Nausea And Vomiting; PA, Pain; DY, Dyspnoea; SL, Insomnia; AP, Appetite loss; CO, Constipation; DI, Diarrhoea; FI, Financial difficulties

DISCUSSION

Data from the present study provide several important implications for understanding the impact of various anxiety disorders on patients' quality of life. The findings of this study support the notion that, like other cancer patients, hematological cancer patients are vulnerable to anxiety disorders, and these anxiety disorders subsequently impact a patient's quality of life.[2, 5-6, 8-9]

In this study, the prevalence of anxiety disorders in Malaysian hematological cancer patients varies from 1% to 24.8%. The prevalence breakdown by type of anxiety disorder in our sample is: agoraphobia without history of panic disorder (24.8%); GAD (10.5%); SAD (7.6%); panic disorder with agoraphobia (2.9%); PTSD (2.9%); OCD (1.9%) and panic disorder without agoraphobia (1%). An earlier study by Prieto and colleagues found that the overall prevalence of anxiety disorders in hospitalized hematological cancer patients was approximately 8.2%.[2]

Previous studies have shown that cancer patients with anxiety disorders had a significantly poorer quality of life compared to patients without the disorder.[8-9] Similarly, Pamuk and colleagues (2008) showed that hematological malignancy patients with a diagnosed anxiety disorder had lower global quality of life, higher symptomatology scores and poorer cognitive, emotional and social functioning than those without a diagnosed anxiety disorder.[1] A study among advanced cancer patients found that the functional scales (role functioning, emotional functioning, and cognitive functioning) and the symptom scale (insomnia) were the most significant predictors of quality of life followed by dyspnoea, sleep disturbance and appetite loss. The study indicates the cancer patients have impaired social, emotional and global quality of life.[9] Quality of life impairment, especially in functioning score, also has been found to be associated with anxiety and depression in hematological cutaneous lymphoma cancer patients.[15]

In the present study, the impact of each type of anxiety disorders on quality of life domains was analyzed separately. First, we found that the prevalence of panic disorders in patients with hematological cancer ranged from 1% to 2.9%, depending on the panic disorder subtype. This includes panic disorder with agoraphobia (2.9%) and panic disorder without agoraphobia (1.0%). Panic disorder is defined as experiencing a state of intense apprehension with symptoms including shortness of breath, palpitations, discomfort, choking sensations and fear of losing control.[7] The prevalence rates found in our sample are consistent with a previous study of hospitalized hematological cancer patients for stem cell transplantation, which reported a prevalence rate of 1.4%.[2] However, other studies have found a prevalence rate for panic disorder of 20.75% in advanced inpatients, including both hematological and other types of cancer.[6] This difference may be accounted for by differences in methodological settings and compositions of the study populations.

Panic disorder with agoraphobia

Compared to hematological cancer patients who do not have panic disorder with agoraphobia, hematological cancer patients with panic disorder with agoraphobia had significantly lower scores on the EORTC-QLQ-30 cognitive functioning scale and pain symptoms scale and significantly higher scores on the dyspnoea symptom scale. This indicates the patients with panic disorder with agoraphobia have impaired cognitive functioning and more symptoms of dyspnoea. Previous studies have reported similar results related to impaired cognitive functioning of hematological cancer patients.[1] Patients with panic disorder with agoraphobia reported fewer pain symptoms; however, previous studies among hospitalized cancer patients found that patients without anxiety or depression have less pain interference than patients who have anxiety, depression or both diagnoses.[16] To our knowledge, no published studies have investigated the dyspnoea rates of cancer patients with or without anxiety; thus, comparisons with previous studies cannot be made.

Agoraphobia without panic disorder

Another type of anxiety disorder is agoraphobia. Patients with agoraphobia tend to avoid situations or places where escape or help might be impossible; the disorder is similar to having a panic attack or panic-like symptoms.[7] The prevalence in our study of agoraphobia without a history of panic disorder (24.8%) appears higher than other studies among other cancer patients, which have found a prevalence rate of 2.7% in patients aged 55 to 74 years old. In this previous study, the odds ratio of cancer patients developing agoraphobia was 0.40 among those aged 15 to 54 years and 5.94 among those aged 55 to 75 years old.[5]

Analyses showed that hematological cancer patients with agoraphobia without a history of panic disorder had significantly lower scores on the EORTC-QLQ-30 functional scales (cognitive function, emotion function) compared to respondents without this diagnosis. This finding is consistent with previous studies, which found that cancer patients were impaired in their emotional[9] and cognitive functioning.[1]

Social Anxiety Disorder (SAD)

Social phobia, or social anxiety disorder (SAD), is characterized by avoidance behaviors related to exposure to social

or performance situations.[7] The present study found a higher prevalance rate for social anxiety disorder (7.6%) than was reported in a study of hematological cancer patients receiving stem cell transplantation treatment (1.8%).[2] This study also revealed that hematological cancer patients with SAD had significantly lower scores in emotional functioning, cognitive functioning and social functioning and had significantly higher financial difficulties. This finding is consistent with previous findings that have found impaired emotional functioning,[9] cognitive functioning[1] and social functioning[1, 9] in cancer patients. The finding of higher levels of financial difficulties in the present study also is consistent with other Malaysian studies. Studies have found that finances are one of the main problems faced by cancer patients, especially those in the transplantation treatment, because only two government hospitals subsidize the cost of treatments. Other organizations (such as universities, welfare and non-governmental organizations) help patients by fundraising to cover the cost of treatments, but patients often still face financial difficulties.[17]

Obsessive-Compulsive Disorder (OCD)

OCD is characterized by obsessions that cause anxiety and/or compulsions that serve to neutralize anxiety. In the present study, the prevalence rate of OCD was 1.9%. In terms of quality of life, hematological cancer patients with OCD had significantly lower role functioning, emotional functioning, cognitive functioning and global health status scores than patients without OCD. They also had significantly higher scores on the physical symptoms scale (nausea and vomiting, as well as appetite loss). This finding is consistent with previous studies which found impaired emotional functioning,[1, 9] cognitive functioning and poor global health status.[1]

Post traumatic stress disorder (PTSD)

PTSD is characterized by patients re-experiencing traumatic situations with increased arousal and avoidance. The prevalence of PTSD in the present study was 2.9%. However, this prevalence rate was far lower than the prevalence rate of 17% of PTSD found in a previous study of hematological cancer patients.[18]

Hematological cancer patients with PTSD had significantly higher dyspnoea scale scores and lower social functioning scale scores compared to patients without the disorder. The finding of impaired social functioning is consistent with previous studies.[1]

Generalized anxiety disorder (GAD)

GAD is defined as persistent and excessive anxiety and worry for a period of at least 6 months. The prevalence of GAD in this study (10.5%) was higher than the prevalence rate of 1.8% reported by Prieto and colleagues.[2] Analyses showed hematological cancer patients with GAD were more likely to have lower functional scale scores (physical functioning, role functioning, emotional functioning, cognitive functioning) and lower symptoms scale scores (insomnia, constipation and financial difficulties) compared to patients without the disorder. The present study is consistent with earlier studies investigating emotional functioning[1, 9] and cognitive functioning.[1] Insomnia has been found to be a significant predictor of quality of life.[9] Another study among Hodgkin lymphoma patients found that impaired physical health was also a predictor of quality of life; patients who rated poor physical health rated their overall quality of life as worse compared to the general population. Hodgkin lymphoma patients who have financial difficulties also rated their mental health status poorly compared to the general population. Inability to work may account for the complications in physical and mental health in patients with Hodgkin lymphoma.[19]

LIMITATIONS

This study has a number of important limitations that need to be considered. First, the method of assessing anxiety and quality of life used in this study limited the symptoms reported to the patient's recall at the time of the interview. The numbers of patients eligible for participation in this study were also limited by the patient's physical status and their ability to respond. Finally, some self-rated questionnaires were answered by the respondents themselves or were read to them to clarify their responses. These three limitations should be noted and considered when interpreting results.

CONCLUSION

This research has provided us with some insight into the prevalence of various types of anxiety disorders (as well as the quality of life) among Malaysian hematological cancer patients. The findings demonstrate that the rates of comorbid anxiety disorders for Malaysian hematological cancer patients are similar to those for hematological cancer patients in other countries.[1, 9] This study also found that different types of anxiety disorders impacted a patient's quality of life domains differently. Among anxiety disorders, only patients diagnosed with OCD reported poorer global health status. If anxiety disorders are left untreated, it may impair the patient's cancer management systems; therefore, patients with anxiety should be referred to their health professional or psychiatrist in order to improve their mental health status and quality of life.

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Functional Specialisation and Effective Connectivity During Self-paced Unimanual and Bimanual Tapping of Hand Fingers: An Extended Analysis Using Dynamic Causal Modeling and Bayesian Model Selection for Group Studies

[1]Ahmad Nazlim Yusoff *, [1]Aini Ismafairus Abd Hamid, [1]Khairiah Abdul Hamid, [2]Wan Ahmad Kamil Wan Abdullah, [1]Mazlyfarina Mohamad & [1,3]Hanani Abdul Manan

[1]Functional Image Processing Laboratory (FIPL), Diagnostic Imaging & Radiotherapy Program, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

[2]Department of Radiology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

[3]Medical Imaging Department, Faculty of Therapeutic Sciences, Masterskill University College of Health Sciences, G-8, Jalan Kemacahaya, Batu 9, 43000 Cheras, Selangor

ABSTRACT

Introduction: This multiple-subject fMRI study continue to further investigate brain activation within and effective connectivity between the significantly (p<0.001) activated primary motor area (M1), supplementary motor area (SMA) with the inclusion of BA44 during unimanual (UNI_{right} and UNI_{left}) and bimanual (BIM) self-paced tapping of hand fingers. **Methods:** The activation extent (spatial and height) and effective connectivity were analysed using statistical parametric mapping (SPM), dynamic causal modeling (DCM) and the novel method of Bayesian model selection (BMS) for group studies. **Results:** Group results for UNI_{right} and UNI_{left} showed contra-lateral and ipsi-lateral involvement of M1 and SMA. The results for BIM showed bilateral activation in M1, SMA and BA44. A larger activation area but with lower percentage of signal change (PSC) are observed in the left M1 due to the control on UNI_{right} as compared to the right M1 due to the control on UNI_{left}. This is discussed as due to the influence of the tapping rate effects that is greater than what would be produced by the average effects of the dominant and sub-dominant hand. However, the higher PSC observed in the right M1 is due to a higher control demand used by the brain in coordinating the tapping of the sub-dominant hand fingers. Connectivity analysis indicated M1 as the intrinsic input for UNI_{right} and UNI_{left} while for BIM, the inputs were both M1s. During unilateral finger tapping, the contra-lateral M1 acts as the input center which in turn triggers the propagation of signal unidirectionally to other regions of interest. The results obtained for BIM (BIM_{left} and BIM_{right}) however yield a model with less number of significant connection. M1-M1 connection is unidirectional for UNI_{left} and UNI_{right} originating from contra-lateral M1, and is inhibited during BIM. **Conclusion:** By taking into consideration the presence of outliers that could have arisen in any subject under study, BMS for group study has successfully chosen a model that has the best balance between accuracy (fit) and complexity.

Keywords: **Primary motor area, Supplementary motor area, BA44, Bayes rule, Statistical Parametric Mapping**

INTRODUCTION

In spite of a vast number of research conducted in studying how uni- and bimanual motor action are coordinated by the brain[1–3], questions still arise about the exact mechanism underlying the existence of activation clusters in the contra-lateral as well as in the ipsi-lateral regions and their functional relationships. These pertaining, in particular, to the height and spatial extent of activation in the respective motor associated areas as well as the strength and direction of the connections between those areas. With the inception of several novel computational neuroscience approaches in studying connectivity i.e. structural equation modeling (SEM)[4] and dynamic causal modeling (DCM)[5], the number of works conducted in studying brain dynamic escalated dramatically, focusing not only among the areas in one region but also with the areas in the other brain regions[1-3].

A comprehensive assessment of uni- and bimanual hand movements covering the aspects of functional specialisation and effective connectivity has been reported[1]. They studied the dynamic intra- and interhemispheric interactions among the motor regions by means of functional magnetic resonance imaging (fMRI) and dynamic causal modeling (DCM). They found that the uni- and bimanual types of hand movement did modulate the neural coupling within the motor

*Corresponding author: *nazlim@fskb.ukm.my*

network. The excitation and inhibition of neural activity found in their study were evidences of dynamic interplay of various motor regions, in particular the primary motor cortex (M1), supplementary motor area (SMA) and pre motor cortex (PMC). They also suggested that the SMA represents the key structure in promoting or suppressing the activity in the cortical motor network during both types of movement. With the aid of DCM, they had successfully modeled the intrinsic connectivity among motor regions modulated by sensory input that are generated via visual instructions. Their works however concentrated only on the connectivity between motor related regions which were activated at corrected alpha value. The use of a high threshold will certainly exclude regions of mild activation regardless of the existence of their connection with the primary motor regions. As a result, significant connections between areas of mild responses with those significantly high may be unintentionally left unattended.

Another novel work on motor activation and network in human[3] reported that the dominant hemisphere is responsible in initiating the control over bilateral movement. They also discovered that bilateral activation is not the sum of the right and left unilateral activation from which it was later indicated that the left and right unimanual movements differ significantly in terms of the activation of and connectivities between the areas involved. They finally concluded that by using SEM, as opposed to other study[1] that made use of DCM, individual subject and group network identification has been made possible.

SEM however, rests on the assumptions that the interaction between independent variables is linear and the data are instantaneous and conservative. Since the observed blood oxygenation level dependent (BOLD) signal is time-series which rendered the data non conservative and that there is always a possibility that the interaction between any two regions is non linear, BOLD signal can only be explained by combining both the expressions for the neuronal and hemodynamic levels[6] as encapsulated in DCM. Therefore, DCM is the preferred approach for fMRI data. Implementing DCM will result in a complete model for fMRI, from which it is understood that the effective connectivity expressed at the level of neurodynamics, will cause changes in the observed hemodynamics or BOLD signal. A detailed explanation on the comparison between SEM and DCM in modeling functional integration has been given elsewhere[7].

It has been established that the primary motor area (M1) in the precentral gyrus (PCG), the supplementary motor area (SMA) and premotor cortex (PMC) are involved in movement preparation and execution of motor action[8]. However, motor coordination is not limited only to those three areas especially when modulatory inputs come into play. For example, areas that are involved in processing sound, speech and language such as BA41, BA44 and BA45 may also be activated during a motor activation task if the instruction is verbally or auditorily given. Having that in mind, establishing a reliable model of how these areas interact is crucial for a wider understanding of the mechanism underlying motor function. This was also found to be useful for both healthy subjects and patients[1]. The modeled interaction will certainly find its importance since the present knowledge on functional specialisation and organization of human brain is limited and still lacking[9].

Many previous finger tapping studies[1, 10 & 11] relied on systematically contained instructions visually or auditorily given to the subjects. The externally triggered stimuli will then evoke responses not only in motor areas but also in areas related to vision, hearing and cognition. This will certainly complicate the study of connectivity between motor areas but will enhance the networks further outside motor regions. More information will be gained since motor areas could also be connected to other associated areas in the cortex. In this study, the subjects were instructed to perform self-paced tapping of right, left or bimanual hand fingers, at moderate tapping force and speed, cued by auditory instruction given in a very short duration.

This study is a continuation of our previous work on single[12] and multiple subjects[12-15]. In this study, the brain functional specialisation and integration were investigated on multiple subjects with regards to the activation in the cerebral motor cortices evoked by unimanual and bimanual finger tapping which were robustly done by the subjects. First, group analyses were conducted by means of random effects analysis (RFX) and inferences based on the group responses were made onto the whole subject at a relatively low significant level ($\alpha = 0.001$) uncorrected for multiple comparisons. The motivation is to search for motor areas and other areas not related to motor areas but are also activated with significant connectivity with the motor areas. Brain activation at low significant level has also been previously reported[16-18]. A low alpha threshold is thought to be suitable for group studies that consist of a relatively high between-subjects variability.

Secondly, the connectivity measure between regions of interest (ROIs) were studied and evaluated by implementing the dynamic causal modeling (DCM) to model interactions among neuronal populations at cortical level. Prior to input determination, full connectivity models with various inputs are initially constructed for UNI_{right}, UNI_{left} and BIM based on the ROIs defined in the RFX and estimated using DCM. The estimated models are then compared in a Bayesian framework. Finally, the most probable model with predetermined input was then reduced to a model with significant connections that would represent the intrinsic couplings during unimanual and bimanual tapping of hand fingers for all subjects.

As opposed to our previous study[13] that uses Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC)[9] in selecting the most optimum model, this study uses a novel BMS approach for group study in

searching for a model that has the best balance between accuracy (fit) and complexity and a model that best represent the observed BOLD signal. More importantly, the results obtained from group BMS studies, while being able to reproduce results of group Bayes factor (GBF) and positive evidence ratio (PER) as explained in our previous study[15], are reported[19] to be able to take into consideration the presence of outliers that could have arisen in any subject under study. While GBF is very sensitive to outliers (magnitude of differences across subjects) and PER can only describe the qualitative reproducibility of model comparison over subjects, BMS analysis for group study is the preferred approach in model comparison involving multiple subjects.

METHODS

Subject

Functional magnetic resonance imaging (fMRI) examinations were performed on 16 right-handed subjects (4 males and 12 females). The subjects were given informed consent and screening forms as required by the Institutional Ethics Committee (IEC). The subjects were interviewed on their health condition prior to the scanning session. Prior to the fMRI scans, the subjects' handedness was tested using the Edinburgh Handedness Inventory[20]. The subjects were also told not to move their head during the scan to avoid serial correlation and drift. Head movement will also cause artifacts on functional images due to the voxels that are not correctly registered (or moving) during the scan resulting in significant changes in signal intensity of that particular voxels over time[21]. The immobilising devices were used together with the head coil in order to minimise head movement.

fMRI Scans

Functional magnetic resonance imaging (fMRI) examinations were conducted using a 1.5-tesla magnetic resonance imaging (MRI) system (Siemens Magnetom Vision VB33G) equiped with functional imaging option, echo planar imaging (EPI) capabilities and a radiofrequency (RF) head coil used for signal transmission and reception. Gradient Echo - Echo Planar Imaging (GRE-EPI) pulse sequence with the following parameters were applied : repetition time (TR) = 5 s, acquisition time (TA) = 3 s, echo time (TE) = 66 ms, field of view (FOV) = 210 × 210 mm, flip angle = 90^{o}, matrix size = 128 × 128 and slice thickness = 4 mm. Using the midsagittal scout image (TR = 15 ms, TE = 6 ms, FOV = 300 × 300 mm, flip angle = 30^{o}, matrix size = 128 × 128 and magnetic field gradient = 15 mT/m) produced earlier, 35 axial slice positions (1 mm interslice gap) were oriented in the anterior-posterior commissure (AC-PC) plane. This covers the whole brain volume. In addition, high resolution anatomical images of the entire brain were obtained using a strongly T1-weighted spin echo pulse sequence with the following parameters : TR = 1000 ms, TE = 30 ms, FOV = 210 × 210 mm, flip angle = 90^{o}, matrix size = 128 × 128 and slice thickness = 4 mm[12].

Experimental paradigm

The subjects were instructed on how to perform the motor activation task and were allowed to practice prior to the scanning. The subjects had to tap all four fingers against the thumb beginning with the thumb-index finger contact and proceeding to the other fingers in sequence which would then begin anew with contact between thumb and index finger. This study used a robust self-paced finger movement. The tapping of the fingers would approximately be two times in one second (using an intermediate force between too soft and too hard). A six-cycle active-rest paradigm which was alternately and auditorily cued between active and rest was used with each cycle consisting of 10 series of measurements during active state and 10 series of mesurements during resting state. The tapping of the fingers were done unimanually (UNI_{left} or UNI_{right}) or bimanually (BIM) in an alternate fashion[12]. Each functional measurement produces 20 axial slices in 3 s (one image slice in 150 ms) with an inter-measurement interval of 2 s. The measurement starts with active state. The imaging time for the whole functional scan was 600 s (10 minutes) which produced 120 × 20 = 2400 images in total. High resolution T2*-weighted images were obtained using the voxel size of 1.64 mm × 1.64 mm × 4.00 mm.

Post processing of the fMRI data

All the functional (T2*-weighted) and structural (T1-weighted) images were analysed using a personal computer (PC) with a high processing speed and large data storage. The MATLAB 7.4 – R2007a (Mathworks Inc., Natick, MA, USA) and Statistical Parametric Mapping (SPM5 and SPM8) (Functional Imaging Laboratory, Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College of London) software packages were used for that purpose. The raw data in DICOM (.dcm) format were transformed into Analyze (.hdr, .img) format and preprocessed by means of SPM5. Functional images in each measurement were realigned using the 6-parameter affine transformation in translational (*x, y* and *z*) and rotational (pitch, roll and yaw) directions to reduce artifacts from subject movement and in order to make within and between subject comparison a meaningful way. After realigning the data, a mean

image of the series is used to estimate some warping parameters that map it onto a template that already conforms to a standard anatomical space (EPI template provided by the Montreal Neurological Institute - MNI). The normalisation procedure used a 12-parameter affine transformation[22]. The images were then smoothed using an 8-mm full-width-at-half-maximum (FWHM) Gaussian kernel. Activated voxels were identified by the general linear model approach by estimating the parameters of the model and by deriving the appropriate test statistic (*T* statistic) at every voxel. Statistical inferences were finally obtained on the basis of random effects analysis (RFX) and the Gaussian random field theory. The inferences were made using the *T*-statistic at significant level (α) = 0.001, uncorrected for multiple comparisons. A detail description on spatial pre processing can be found elsewhere[21].

Region of interest (ROI) Analyses

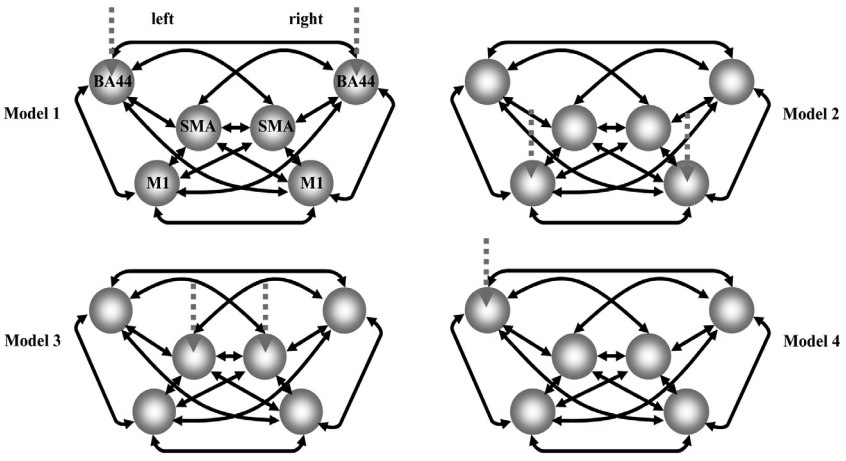
The cortical brain regions which are found to be significantly activated during the finger tapping task are defined using the Anatomy toolbox[23] at $\alpha < 0.001$, uncorrected for multiple comparisons. The selected regions of interest (ROIs) in this study are the primary motor area (M1), supplementary motor area (SMA) and the opercular part of inferior frontal gyrus or BA44. The peak coordinates of the respective ROI on the statistical parametric maps (SPMs) produced from RFX were taken as the anatomical landmark and the corresponding coordinates for the individual subject. The anatomical constraints for the RFX coordinates as suggested in a previous study[1] are; 1) the M1 coordinates had to be located in the precentral gyrus/sulcus, 2) the SMA coordinates had to be in the dorsal medial wall within the inter-hemispheric fissure. For the coordinates of BA44, they had to be in the area bounded caudally and dorsally by the agranular frontal area 6, dorsally by the granular frontal area 9 and rostrally by the triangular area 45. A spherical volume of 4-mm radius with the ROIs' peak coordinates (Table 1) as the center are defined and named as the left M1 (M1-L), left SMA (SMA-L), left BA44 (BA44-L), right M1 (M1-R), right SMA (SMA-R) and right BA44 (BA44-R). Group's percentage of signal change (PSC) relative to the baseline for all ROIs was extracted from the 4-mm radius sphere using MarsBar toolbox for SPM[24].

Dynamic causal modeling (DCM)

Dynamic causal modeling (DCM) was implemented in evaluating the effective connectivity between the ROIs within and between the right and left hemispheres. A detail explanation of the underlying mathematical and biophysical concepts can be found elsewhere[5] but the basic principle is presented here. DCM is a study of the dynamic of interaction among neuronal populations at cortical level. This is done by modeling the interaction as a dynamic input-state-output system. DCM can be described by the following multivariate bilinear differential equation.

$$\dot{z}_t = \left(\boldsymbol{A} + \sum_{j=1}^{M} u_t(j)\boldsymbol{B}^j\right) z_t + \boldsymbol{C}u_t \qquad (1)$$

In Equation (1), $\boldsymbol{A}$ is the matrix that represents the fixed or context-independent strength of connections between the modeled regions (intrinsic couplings) and the matrices $\boldsymbol{B}^j$ represent the modulation of these connections. The matrix $\boldsymbol{C}$ is free of z_t but its role is to model the extrinsic influences of inputs on neuronal activity. In the absence of u_t, $\dot{z}_t = \boldsymbol{A}z_t$, which implies that the only existing connectivities are that of the intrinsic couplings between the regions of interest (ROIs)[5, 9, 25].



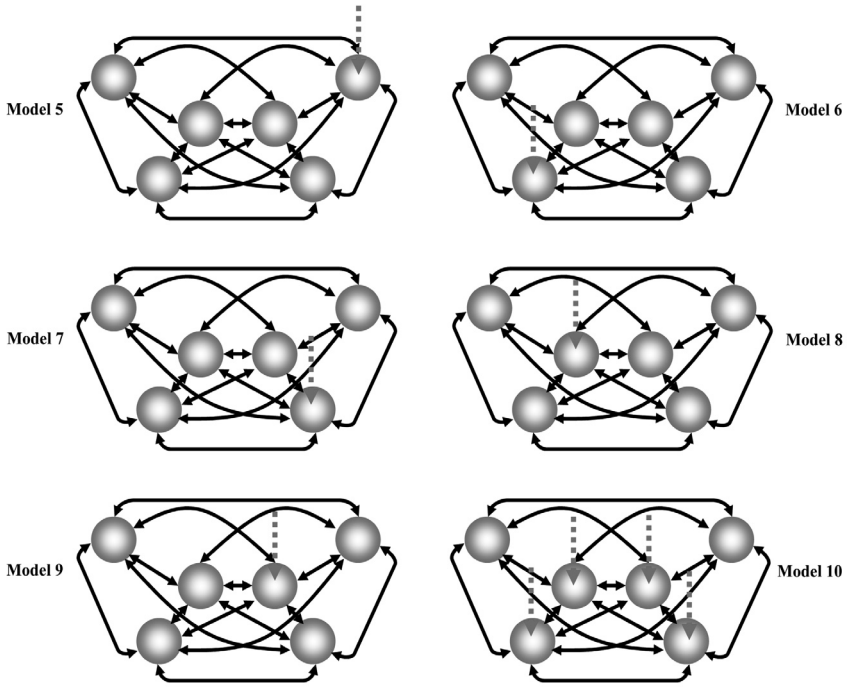


Figure 1: Full connectivity model used in dynamic causal modeling (DCM) in determining the input center for UNI_{right}, UNI_{left} and BIM. The labels for the ROIs are only shown in Model 1. The red dotted arrows represent the input(s) while ↔ represents bidirectional connection

Table 1 a): RFX coordinates for the left and right M1, SMA and BA44 that are obtained from UNI_{right}, UNI_{left} and BIM,

	RFX Coordinates					
	M1-L	M1-R	SMA-L	SMA-R	BA44-L	BA44-R
UNI_{right}	-32 -22 50	-	-6 6 48	-	-54,2,32	-
UNI_{left}	-	38 -20 62	-	6 2 54	-	56,8,32
BIM	-32,-22,50	34,-20,50	-6,6,48	8,8,46	-56,6,22	64,8,22

Table 1 b): The actual coordinates used in the construction of DCMs

		RFX Coordinates					
		M1-L	M1-R	SMA-L	SMA-R	BA44-L	BA44-R
Subject 1	UNI_{right}	-32,-22,50	46,-26,60	-6,6,48	6,2,54	-56,6,30	56,8,32
	UNI_{left}	-32,-22,52	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	10,8,44	-56,6,22	64,8,22
Subject 2	UNI_{right}	-32,-22,50	36,-18,64	-6,6,48	6,2,54	-54,2,32	56,8,32
	UNI_{left}	-32,-22,50	38,-20,62	-6,6,48	6,2,56	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	8,8,46	-56,6,20	64,8,22
Subject 3	UNI_{right}	-32,-22,50	38,-20,60	-6,6,48	8,2,54	-54,2,32	56,8,32
	UNI_{left}	-40,-24,58	38,-20,62	-6,6,48	6,2,54	-54,4,34	56,12,34
	BIM	-32,-22,50	32,-20,50	-4,4,48	8,8,44	-56,8,20	62,10,24

Continuation

Table 1 b): The actual coordinates used in the construction of DCMs

		RFX Coordinates					
		M1-L	M1-R	SMA-L	SMA-R	BA44-L	BA44-R
Subject 4	UNI_{right}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	UNI_{left}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	0,6,52	4,8,50	-56,8,22	62,10,20
Subject 5	UNI_{right}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	6,2,54
	UNI_{left}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	8,6,46	-56,6,24	64,14,24
Subject 6	UNI_{right}	-32,-22,50	40,-24,60	-6,6,48	8,2,54	-56,4,30	52,6,30
	UNI_{left}	-30,-18,50	38,-20,62	-6,6,48	6,2,52	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	8,8,46	-52,2,28	64,8,20
Subject 7	UNI_{right}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	UNI_{left}	-36,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	10,8,44	-56,6,22	62,8,14
Subject 8	UNI_{right}	-32,-22,50	34,-18,66	-6,6,48	6,2,54	-54,2,32	56,8,32
	UNI_{left}	-32,-24,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	8,8,46	-56,6,22	64,8,22
Subject 9	UNI_{right}	-32,-22,50	48,-20,62	-6,6,48	6,2,54	-54,4,32	56,10,26
	UNI_{left}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	12,6,48	-54,6,22	68,0,18
Subject 10	UNI_{right}	-32,-22,50	34,-16,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	UNI_{left}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	8,8,46	-56,6,22	64,8,22
Subject 11	UNI_{right}	-32,-22,50	34,-10,68	-6,2,48	0,-8,52	-56,6,32	58,8,32
	UNI_{left}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	8,8,46	-56,6,22	64,8,22
Subject 12	UNI_{right}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	UNI_{left}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	8,8,46	-56,6,22	64,8,22
Subject 13	UNI_{right}	-32,-22,50	36,-14,62	-6,4,48	8,2,52	-54,2,32	56,8,32
	UNI_{left}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,48	10,8,46	-56,6,20	62,6,22
Subject 14	UNI_{right}	-32,-22,50	38,-20,62	-6,6,46	6,2,54	-54,2,32	58,8,30
	UNI_{left}	-32,-22,50	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,32
	BIM	-32,-22,50	34,-20,50	-6,6,46	8,8,44	-58,8,24	62,8,24
Subject 15	UNI_{right}	-32,-22,50	38,-20,62	-6,6,48	8,0,54	-54,2,32	56,8,32
	UNI_{left}	-30,-22,52	38,-20,62	-6,6,48	6,2,54	-54,2,32	56,8,30
	BIM	-32,-22,50	34,-20,50	-6,6,48	8,6,46	-56,6,22	64,8,22
Subject 16	UNI_{right}	-32,22,50	38,-16,60	-6,6,48	6,2,54	-54,2,32	56,8,32
	UNI_{left}	-36,-26,58	38,-20,62	-4,8,50	6,2,54	-52,2,34	54,10,34
	BIM	-32,-22,50	34,-20,50	-6,6,48	8,8,46	-56,6,22	64,8,22

Full connectivity models with their respective input assumed to be through unilateral and bilateral M1, SMA and/or BA44, were constructed using the above mentioned ROIs (Figure 1). Model construction for BIM used the ROIs' coordinates obtained from RFX. For UNI_{left} and UNI_{right}, the coordinates obtained via RFX are combined together during model construction. The coordinates are shown in Table 1(a). These coordinates were used for all subjects during the construction of VOIs to test for the existence of the effects at uncorrected $\alpha = 0.1$. The final coordinates for model construction are shown in Table 1(b). The models were tested onto all subjects for all UNI_{left}, UNI_{right} and BIM conditions. The estimation procedure was carried out using DCM on the assumption that the interactions between all ROIs are linear. Prior to estimation, the models are specified by including slice timing in the DCMs[6]. The TR for this

study is 5 s which means that the EPI pulse sequence acquires slices at different time over the 5-s duration. Since DCM was not informed about this relatively long TR, it assumed that all slices are acquired simultaneously. As a result, one may obtain unacceptable DCM values if this long time interval is neglected and slice timing is not included in the DCMs. Furthermore, the location of the chosen ROIs in this study is quite distant from each other if measured in the direction perpendicular to slice orientation, rendering the slice timing important in DCM.

The models shown in Figure 1, which have been estimated for UNI_{left}, UNI_{right} and BIM for all subjects were compared using Bayesian model selection (BMS) for group studies. Model comparisons were separately done for UNI_{left}, UNI_{right} and BIM to determine the most probable input centers for the full connectivity models.

The intrinsic connectivity values of the most probable full connectivity models that have been determined for each UNI_{left}, UNI_{right} and BIM were analysed to identify the most probable connection. This is done by first, justifying the connectivity values and their posterior probability for each connection and averaging the values over 16 subjects. Second, the average values for all the thirty connections were tested against 0 by means of Statistical Packages for Social Sciences (SPSS) at significant level (α) = 0.05/[30 connections] (95% CI). Third, for any connection that is significant, it must be presented by at least 8 subjects with posterior probability larger than 0.9 so that it can be finally concluded that it is significant and the connectivity exist in at least half of the total number of subject under study. The most probable connectivity models for UNI_{left}, UNI_{right} and BIM are then suggested. Finally, the One-Way ANOVA was used on the most probable model for UNI_{left}, UNI_{right} and BIM to search for the existence of any significant difference at $\alpha = 0.05$ in the mean intrinsic connectivity among those connections that are significant by using the respective connections as factors and the connectivity values as the dependent list. Tukey post-hoc analysis for multiple comparison at $\alpha = 0.05$ will be used if there exist at least one pair of connections that shows significant difference in their connectivity values.

RESULTS

Demographical results

The average subjects' age and its standard deviation are 22.31 ± 2.65 years old. Five of the subjects were Chinese while the rest are Malays. All subjects were confirmed to be healthy and right-handed with the average laterality index of 76.25 (in the range of 4th right).

RFX analyses

Figure 2 shows the statistical parametric maps (SPMs) obtained from random-effects analysis (RFX) indicating contra-lateral and ipsi-lateral brain activations due to (a) UNI_{right}, (b) UNI_{left} and (c) BIM (BIM_{left} and BIM_{right}). The SPMs are overlaid onto Colin27T1_seg.img template. The crossing of the hair-lines indicates the point of maximum intensity which occurred at (-32,-22,50) and (38,-20,62) in the left and right hemispheres for UNI_{right} and UNI_{left} respectively. For BIM, the peak coordinates obtained from RFX are (-32,-22,50) in the left and (34,-20,50) in the right hemispheres. These coordinates have been confirmed to be located in the respective M1 regions in the left and right hemispheres and are comparable to the M1 coordinates obtained in a previous study[1]. In order for several ipsi-lateral regions to be visualised, the SPMs shown in Figure 2 are thresholded at $\alpha = 0.01$. Some significant clusters ($p < 0.001$) with their MNI coordinates at the point of maximum intensity due to UNI_{right}, UNI_{left} and BIM, as well as the respective anatomical areas in which the activation occurs are shown in Figure 3 (for BIM only) and summarised below.

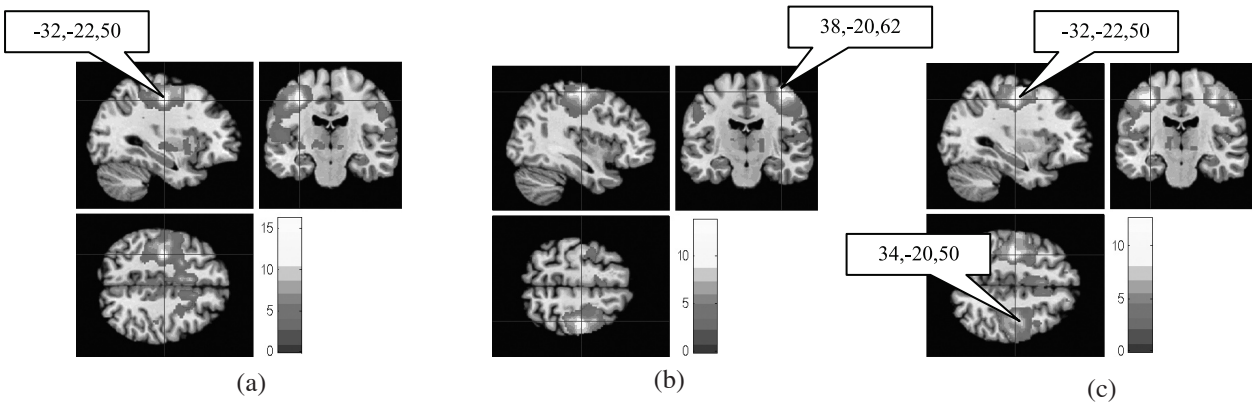


Figure 2: Statistical parametric maps (SPMs) obtained from random-effects (RFX) analysis (n = 16, $t > 2.60$, $p < 0.01$ uncorrected) showing brain activation due to (a) UNI_{right}, (b) UNI_{left} and (c) BIM overlaid onto structural brain images. Color codes represent increasing t value from red to white

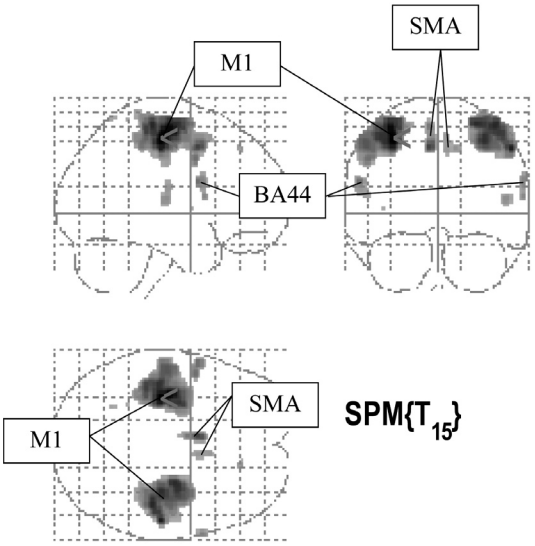


Figure 3: The SPMs obtained from RFX on all subjects for BIM at uncorrected α = 0.001 showing significant activation in the left and right M1, SMA and BA44

For UNI_{right}, 9 significant clusters survive a height threshold of uncorrected $\alpha = 0.001$ and a spatial threshold of 10 voxels. In this study, the clusters with the number of activated voxel of less than 10 are believed to be generated by factors not included in the experimental paradigm such as aliased biorhythm and mild responses of the brain during the experiment. There are a total of 4164 activated voxels ($t > 3.73$) in the main cluster which covers parts of the left post and precentral gyrii and left SMA. The eight highest peaks are at Talairach-MNI coordinates of (-32,-22,50), (-42,-18,52), (-42,-24,52), (-26,-16,64), (-36,-12,64), (-42,-36,46), (-46,-16,48) and (-6,6,48). The results indicate that 29.8% of the main cluster is in the left BA6 (27.8% activated), 9.7% of cluster is in the left BA2 (44.6% activated), 7.8% of cluster is in the left BA3b (50.9% activated) and 7.1% of cluster is in the left BA4p (52.5% activated).

For UNI_{left}, 4 significant clusters survive the uncorrected height threshold of $\alpha = 0.001$ and a spatial threshold of 10 voxels. The main activation cluster in the precentral gyrus consists of 5 maxima. Their Talairach-MNI coordinates are (38,-20,62), (42,-24,62), (36,-32,60), (46,-16,58) and (48,-22,58). A number of 2012 voxels are activated ($t > 3.73$); 35.0% of cluster is in the right BA6 (16.0% activated), 13.5% of cluster is in the right BA1 (32.7% activated), 10.2% of cluster is in the BA3b (22.5% activated) and 8.7% of cluster is in the right BA4a (15.2% activated).

For BIM, 9 major clusters of activation are revealed under a significant level of uncorrected $\alpha = 0.001$ ($t > 3.73$) and a spatial threshold of 10 voxels, occurring in the left and right precentral and postcentral gyrii, left and right SMA, right superior frontal gyrus, right Heschl's gyrus and right rolandic operculum. The primary cluster which is believed to be due to the control on right hand fingers has 5 maxima covering the left post and precentral gyrii which are centered at Talairach-MNI coordinates of (-32,-22,52), (-36,-16,60), (-30,-8,62), (-30,-12,64) and (-40,-32,60) respectively. This cluster has 2200 activated voxels ($t > 3.73$) from which 29.9% of the main cluster is in the left BA6 (14.9% activated), 14.5% is in the left BA1 (34.6% activated), 10.5% of cluster is in the left BA2 (25.8% activated) and 9.9% of cluster is in the left BA4p (39.1% activated). On the other side of hemisphere, the secondary cluster that resulted from left hand fingers coordination, has also 5 maxima, all located in the right pre and postcentral gyrii and right superior frontal gyrus. The maxima are centered at Talairach-MNI coordinates of (54,-22,46), (42,-28,62), (28,-12,64), (34,-22,52) and (46,-26,56). A number of 2142 voxels are activated in the main cluster ($t > 3.73$) from which 35.7% of this cluster is in the right BA6 (17.4% activated), 14.0% in the right BA1 (36.1% activated), 9.2% of cluster in the right BA3b (21.6% activated) and 8.5% in the right BA4a (15.9% activated).

BIM has also resulted in the activation of the left and right SMA which correspond to the third and fourth major clusters. For the left SMA, 81.5% of cluster is in the left BA6 (3.0%) activated. It has 2 maxima at Talairach-MNI coordinates of (-6,6,48) and (-6,-2,62). A number of 175 voxels are activated in this cluster ($t > 3.73$). For the right SMA, 83.8% of cluster is in the right BA6 (1.1% activated). The cluster also has 2 maxima at Talairach-MNI coordinates of (8,8,50) and (8,14,54) with a number of 60 activated voxel ($t > 3.73$).

Another interesting activation during BIM are indicated by symmetrical characteristic of clusters 7 and 9. Cluster 7 consists of 38 activated voxels ($t > 3.73$) which occur in the right precentral gyrus (64,8,22) and right Rolandic

operculum (64,10,12). It was found that 39.5% of cluster is in the right BA44 (1.7% activated) and 8.9% of cluster is in the right BA6. Whereas for cluster 9 (27 activated voxel; t > 3.73), 98.1% of cluster is in the left BA44 and 1.9% of cluster is in the left BA6. This cluster occurs in the left precentral gyrus (-56,6,22).

Conjunction analysis

The results obtained from the analysis of conjunction ($\alpha = 0.1$) on the present UNI_{right} and UNI_{left} datasets indicate that all subjects show common activation areas in the primary motor area. For UNI_{right}, 3 activation clusters are detected in the left postcentral gyrus and precentral gyrus. The main cluster which has 64 activated voxels ($t > 1.28$) with the point of maximum activation at (-34, -22, 54), shows that 55.1% of cluster is in the left BA4p (6.3% activated), 25.6% of cluster in the left BA4a (1.3% activated), 10.7% of cluster in the left BA6 (0.2% activated) and 8.6% of cluster is in the left BA3b (0.9% activated).

For UNI_{left}, the analysis of conjunction at significant level of $\alpha = 0.1$, reveals 1 cluster of activation which is in the right precentral gyrus. The cluster consists of 95 activated voxels ($t > 1.28$) and has 5 maxima with the highest two at (36, -20, 62) and (40, -14, 56). 89.9% of the cluster is in the right BA6 (1.9% activated), 8.2% is in the right BA4a (0.7% activated), 0.5% of cluster is in the right BA4p (0.1% activated) and 0.3% of cluster is in the right BA3b (0.1% activated).

The results for conjunction analysis on BIM reveal 3 significant clusters at $\alpha = 0.1$ which are located in the left and right precentral gyrus. For cluster 1, there are 18 activated voxels with 97.9% of cluster is in the left BA6. For cluster 2, 12 voxels are activated and 82.3% of cluster is in the right BA6. For cluster 3, only 2 voxels are activated and 100% of the cluster is in the right BA6.

Percentage of signal change

The percentage of change in signal intensity (PSC) that had occurred in the left and right M1, SMA and BA44 are tabulated in Table 2 for UNI_{right}, UNI_{left} and BIM. For UNI_{right}, M1-L, SMA-L and BA44-L show higher PSC values as compared to the ipsi-lateral M1-R, SMA-R and BA44-R. The PSC values for UNI_{left} are higher in M1-R and SMA-R as compared to the ipsi-lateral M1-L and M1-R, but the ipsi-lateral and contra-lateral values for BA44 are about the same. For BIM, SMA-L and BA44-L have higher PSC values as compared to SMA-R and BA44-R. However, the PSC for M1-R is slightly higher than the opposite M1-L. As opposed to number of activated voxels, the tapping of the left hand fingers generated higher signal change in M1-L than in M1-R during the tapping of right hand fingers. The effect is however incomparable in SMA and BA44.

Table 2: Percentage of signal change (PSC) in a spherical region about the peak coordinates of the right and left M1, SMA and PMC for UNI_{right}, UNI_{left} and BIM

	Percentage of signal change/%					
	M1-L	M1-R	SMA-L	SMA-R	BA44-L	BA44-R
UNI_{right}	1.650	0.633	0.860	0.670	0.688	0.643
UNI_{Left}	0.713	2.377	0.739	0.793	0.551	0.525
BIM	1.746	1.774	0.629	0.599	0.514	0.471

Dynamic causal models

Group Bayesian model selection (BMS) results for the left, right and bimanual finger tapping over 16 right handed subjects are shown in Figure 4(a), (b) and (c) respectively. The results are obtained by means of fixed (FFX) and random (RFX) effects analysis for BMS. The BMS results clearly show evidence of superiority of Model 7 for UNI_{left} and Model 6 for UNI_{right} as compared to the other 9 models indicating the right M1 as the most probable input center during left hand finger tapping and the left M1 for right hand finger tapping. For BIM, three models show a relatively high probability as compared to the other seven models but are unequally preferred as can be seen in Figure 4(c). The models are Models 2, 6 and 7. From RFX perspective, the winning model among the three models is obviously Model 6. However, since BIM is assumed to be coordinated by the primary motor area in both hemispheres, Model 2 is the most preferable model for BIM. Furthermore, Model 2 is seen to be the most probable model in FFX perspective with a high posterior model probability and relative log evidence. Table 3(a – c) summarises the group (RFX and FFX) BMS analyses for UNI_{left}, UNI_{right} and BIM respectively. All tapping types exhibit constant sum of negative free energy (F) for all models. The Dirichlet parameter estimates (α_d), expected posterior probability (<*r*>) and exceedance probability

(φ) obtained from RFX for BMS and log-evidence and posterior model probability (P) obtained from FFX for BMS show high preference for Model 7 as the most probable model for UNI_{left} and Model 6 for UNI_{right}. These two models will be further analysed in determining the effective connectivity between the ROIs for UNI_{left} and UNI_{right}. As for BIM, Model 2 is the model of choice for further analyses. The decision made in choosing these three models to represent UNI_{left}, UNI_{right} and BIM rests on the results obtained from both the FFX and RFX analyses for BMS as depicted in Table 3 and Figure 4. These models show consistent evidence of optimal models either in FFX or RFX analytical framework.

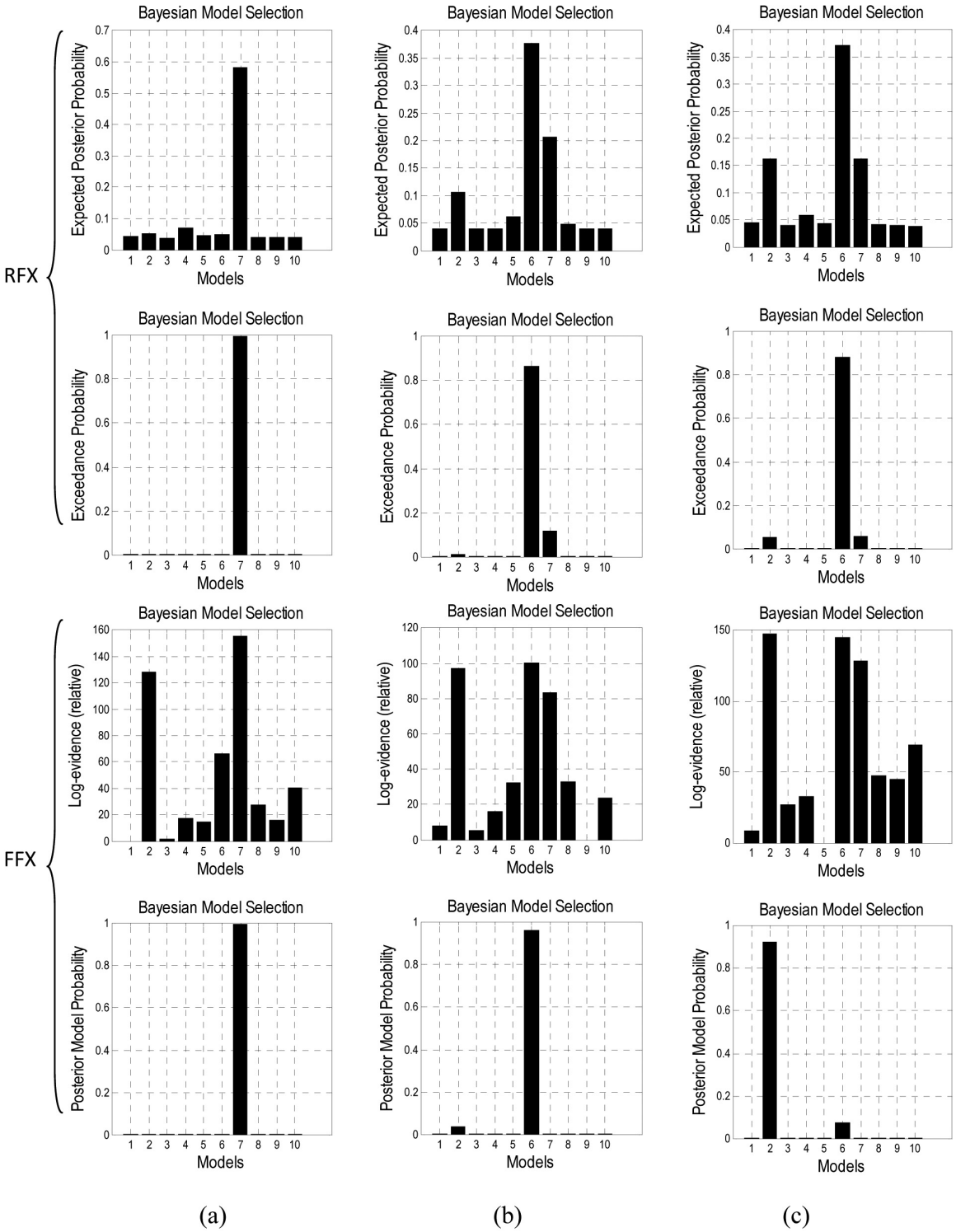


Figure 4: BMS histograms for a) UNI_{left}, b) UNI_{right} and c) BIM obtained from RFX (top) and FFX (bottom) showing preferences on models 7, 6 and 2 respectively

Table 3: BMS RFX and FFX results for a) UNI_{left}, b) UNI_{right} and c) BIM for the ten models obtained across the 16 subjects under study

(a)

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9	Model 10
$-\Sigma F$ (x 10^4)	3.0542	3.0413	3.0540	3.0524	3.0527	3.0475	3.0386	3.0514	3.0526	3.0502
α_d	1.0945	1.3519	1.0027	1.7885	1.2282	1.3057	15.1320	1.0272	1.0462	1.0231
$<r>$	0.0421	0.0520	0.0386	0.0688	0.0472	0.0502	0.5820	0.0395	0.0402	0.0394
φ	0	0.0001	0	0.0002	0.0001	0.0001	0.9995	0	0	0
Log-evidence	0	128.00	1.65	17.50	14.40	66.60	156.00	27.90	15.80	40.30
P	1.27E-14	1.20E-12	1.27E-14	1.27E-14	1.27E-14	1.27E-14	1.0000	1.27E-14	1.27E-14	1.27E-14

(b)

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9	Model 10
$-\Sigma F$ (x 10^4)	3.0360	3.0271	3.0363	3.0352	3.0336	3.0268	3.0285	3.0336	3.0368	3.0345
α_d	1.0287	2.7788	1.0390	1.0196	1.5950	9.8164	5.3925	1.2614	1.0304	1.0382
$<r>$	0.0396	0.1069	0.0400	0.0392	0.0613	0.3776	0.2074	0.0485	0.0396	0.0399
φ	0.0006	0.0118	0.0007	0.0006	0.0021	0.8659	0.1159	0.0011	0.0006	0.0007
Log-evidence	8.14	97.10	5.36	16.30	32.50	100.00	83.50	32.80	0	23.50
P	1.22E-14	0.0363	1.22E-14	1.22E-14	1.22E-14	0.964	4.19E-8	1.22E-14	1.22E-14	1.22E-14

(c)

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9	Model 10
$-\Sigma F$ (x 10^4)	3.0253	3.0114	3.0235	3.0229	3.0262	3.0117	3.0133	3.0214	3.0217	3.0192
α_d	1.1527	4.2075	1.0212	1.5117	1.1185	9.6556	4.2332	1.0628	1.0224	1.0143
$<r>$	0.0443	0.1618	0.0393	0.0581	0.0430	0.3714	0.1628	0.0409	0.0393	0.0390
φ	0.0010	0.0549	0.0007	0.0021	0.0009	0.8821	0.0561	0.0008	0.0008	0.0007
Log evidence	8.42	147.00	26.90	32.80	0	145.00	128.00	47.40	44.80	69.20
P	1.17E-14	0.9234	1.17E-14	1.17E-14	1.17E-14	0.0766	5.25E-9	1.17E-14	1.17E-14	1.17E-14

Intrinsic connectivity

A detailed analysis conducted on Model 7 for UNI_{left} reveals intrinsic connectivity values resembled by the elements of the A matrix shown in Equation (1). The A matrix is a 6 × 6 matrix in which each matrix element represents the fixed or context-independent strength of connections between the modeled regions (intrinsic couplings). As can be seen in Figure 1, there are 30 possible connections with 30 intrinsic connectivity values for each model. For Model 7, the intrinsic input has been determined to be through the right M1. To test for the significance of the intrinsic input and connectivity values, the average values of the input and intrinsic connectivity for all connections for each subject is entered into a one-sample t-test with '0' as target value. All the inputs (M1-R) and majority of the connections are

found to be significant ($p < \alpha = 0.05/30$ connections $= 1.7 \times 10^{-3} \approx 0.002$; 95%CI). Insignificant ($p > 0.002$) connections are M1-L→M1-R, BA44-L→SMA-R, BA44-R→M1-R, BA44-R→SMA-R, SMA-R→M1-R, SMA-R→BA44-L and SMA-R→BA44-R. However, not all connections that are significant show posterior probability value higher than 0.9 which is the cut-off value for a connectivity between any two regions to be considered as significant in a Bayesian framework. The connectivities are small whenever their posterior probability is less than 0.9. In this study, for any high probability and significant connections to be accepted, it must be seen to occur in at least eight subjects. Connections that are significant and with a high occurence probability for UNI_{left} are M1-R→M1-L, M1-R→ BA44-L, M1-R→ BA44-R, M1-R→SMA-L, M1-R→SMA-R, which are evident in the majority of the subjects.

It can be concluded that during UNI_{left}, connectivity in the brain is represented by the unidirectional M1-R→M1-L, M1-R→BA44-L, M1-R→ BA44-R, M1-R→SMA-L, M1-R→SMA-R connections. The average intrinsic input (through M1) and intrinsic connectivity for connections obtained from Model 7 for UNI_{left} for all subjects together with their statistics are tabulated in Table 4(a).

Similar analyses were conducted on Model 6 for UNI_{right}. All the inputs (M1-L) and majority of the connections are again found to be significant ($p < \alpha = 0.05/30$ connections $= 1.7 \times 10^{-3} \approx 0.002$; 95%CI). There is no connection that is not significant ($p > 0.002$). For UNI_{right}, connections that are significant and with a high occurence probability are M1-L→M1-R, M1-L→BA44-L, M1-L→BA44-R, M1-L→SMA-L, M1-L→SMA-R, which are evident in the majority of the subjects. It can be concluded that during UNI_{right}, connectivity in the brain is represented by the unidirectional M1-L→M1-R, M1-L→BA44-L, M1-L→BA44-R, M1-L→SMA-L, M1-L→SMA-R connections. The average intrinsic input (through M1) and intrinsic connectivity for connections obtained from Model 6 for UNI_{right} for all subjects together with their statistics are tabulated in Table 4(b).

Table 4: The intrinsic input and connectivity values and their statistics for a) UNI_{left}, b) UNI_{right} and c) BIM obtained from high probability significance connections that had occurs in the majority of the subjects

(a)

	Input	Intrinsic connectivity				
	M1-R	M1-R to M1-L	M1-R to BA44-L	M1-R to BA44-R	M1-R to SMA-L	M1-R to SMA-R
Ave	0.2034	0.1759	0.1483	0.1224	0.1653	0.1300
SD	-0.1098	-0.0937	-0.0995	-0.0902	-0.0837	-0.1002
p	2.19E-06	1.87E-06	2.62E-05	7.02E-05	1.01E-06	1.09E-04
t	7.409	7.505	5.959	5.426	7.899	5.192
Occurence	15 subjects	11 subjects	11 subjects	11 Subjects	12 subjects	11 subjects

Ave = average, SD = standard deviation

(b)

	Input	Intrinsic connectivity				
	M1-L	M1-L to M1-R	M1-L to BA44-L	M1-L to BA44-R	M1-L to SMA-L	M1-L to SMA-R
Ave	0.1711	0.2427	0.1624	0.1823	0.196	0.1544
SD	0.0517	0.096	0.0585	0.0806	0.0672	0.0826
p	1.10E-09	4.29E-08	1.25E-08	1.83E-07	6.39E-09	1.96E-06
t	13.253	10.117	11.098	9.051	11.661	7.476
Occurence	16 subjects	13 subjects	14 subjects	13 subjects	14 subjects	11 subjects

Ave = average, SD = standard deviation

(c)

	Input		Intrinsic connectivity				
	M1-L	M1-R	M1-L to BA44-L	M1-L to SMA-L	M1-L to SMA-R	M1-R to SMA-L	M1-R to SMA-R
Ave	0.1252	0.1733	0.119	0.1362	0.1304	1.281	0.1271
SD	0.0373	0.0269	0.0572	0.0482	0.0772	0.0487	0.0815
p	9.13E-10	8.01E-03	5.25E-07	9.88E-09	5.17E-05	2.59E-08	1.58E-05
t	13.432	3.055	8.325	11.292	6.76	10.51	6.241
Occurence	16 subjects	16 subjects	8 subjects	10 subjects	8 subjects	9 subjects	9 subjects

Ave = average, SD = standard deviation

For BIM, the above mentioned analyses were implemented on Model 2. The input M1-L was found to be significant ($p < 0.002$) while M1-R was not ($p > 0.002$). Both inputs however indicate high occurrence probability in all subjects. All intrinsic connections were found to be significant ($p < 0.002$) except for the SMA-R→SMA-L connection which has a p value of 0.008. For BIM, connections that are significant and with a high occurence probability are M1-L→BA44-L, M1-L→SMA-L, M1-L→SMA-R, M1-R→SMA-L, M1-R→SMA-R, which are evident in at least half of the number of subjects. It can be concluded that during BIM, connectivity in the brain is represented by the unidirectional M1-L→BA44-L, M1-L→SMA-L, M1-L→SMA-R, M1-R→SMA-L, M1-R→SMA-R connections. The average intrinsic input (through M1-L and M1-R) and intrinsic connectivity for connections obtained from Model 2 for BIM for all subjects together with their statistics are tabulated in Table 4(c). Figure 5 summarises the intrinsic connectivity model for UNI_{left}, UNI_{right} and BIM.

The results obtained from One-Way ANOVA indicate no significant difference among the significant connections for UNI_{left} ($F = 0.938$, $p = 0.447$) and BIM ($F = 0.894$, $p = 0.489$). However, for UNI_{right}, significant difference was found to exist ($F = 3.204$, $p = 0.017$) between M1-L→M1-R and M1-L→BA44-L ($p = 0.037$) as well as between M1-L→M1-R and M1-L→SMA-R ($p = 0.037$) connections after performing Tukey post-hoc test for multiple comparison.

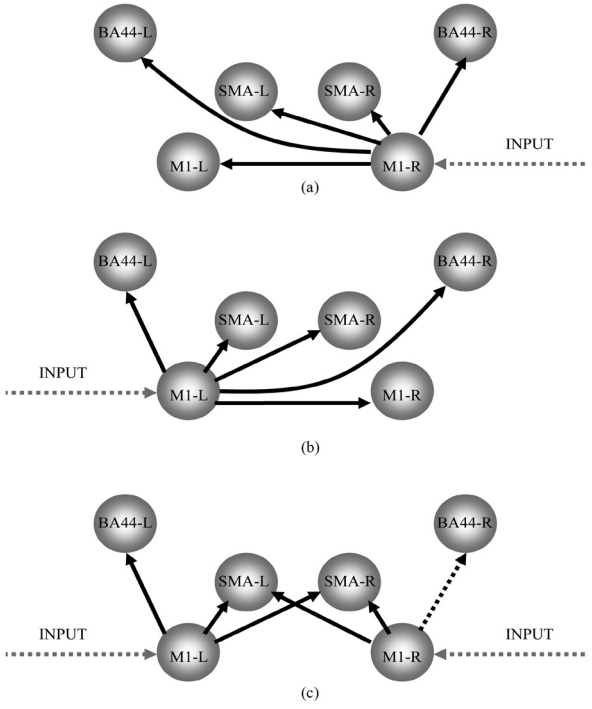


Figure 5: Intrinsic connectivity models for a) UNI_{left}, b) UNI_{right} and c) BIM

DISCUSSION

Individual subject activation

The activation patterns obtained from individual subject are not perfectly the same in terms of activation area and intensity despite the fact that all subjects performed similar task. For example, not all subjects activate a particular brain region and any one subject may experience activation in many different areas than others and with different activation magnitudes. The differences between the results obtained from individual analysis on all subjects clearly show the subject-specific effects which are not always the same from subject-to-subject due to the intrinsic variability in each particular subject. These could be due to the differences in the blood oxygenation level dependent (BOLD) signal intensity that is captured from each subject and may also arise due to subjects' different brain sensitivity when the task is performed, since the vasodilatory signal, cerebral blood flow (CBF), cerebral blood volume (CBV) and the quantity of deoxyhemoglobin which govern the height and spatial extent of the activation in the brain, differ significantly in all individuals. The variability is thought to be intrinsic in nature since precautions in reducing the effects from confounding factors have been taken into consideration so as to ensure that the fMRI experiment performed on all subjects is as similar as possible. Another possible source of variability is the inconsistency of the force and pace used by the subjects to tap their fingers, despite the training and tutorial given to the subjects prior to the fMRI scans. Previous studies[26 & 27] indicate that brain activation in cerebral motor cortices does depend on tapping frequency as well as tapping force. However, since the objective of this study was mainly focused on self-paced type of movement, large differences in tapping force and frequency between subjects are expected to occur among subjects, hence robustness, and will be taken into consideration in the interpretation of results. The fact that subjects' movements are not contributing to the observed activation is acceptable since those movement related effects, in particular the translational (*x, y* and *z*) as well as rotational (pitch, roll and yaw) motions have been excluded in generating the contrast images.

RFX and conjunction results

From Figure 2 and the summarisation of the SPMs results given previously, it is quite interesting to see that the left side of the brain (triggered by the tapping of the right hand fingers) shows a larger number of activated voxels as compared to the right side of the brain (triggered by the left-hand finger tapping). For BIM, the number of activated voxel in the left hemisphere is only slightly higher than in the right hemisphere and the activated voxels in the left hemisphere is much less as compared to that in the left hemisphere due to UNI_{left}. These results are in contrast to that obtained from our previous study on a single right-handed male subject[12], despite the fact that all the subjects are right handed, but are in consistent with the other study[13] conducted on seven right-handed female subjects. This explains how reliable a multiple subject analysis is, in making inference over a population. Moreover, group results indicate the existence of ipsi-laterality accompanying the expected contra-laterality.

The analyses conducted were focused on three bilateral anatomical regions from which two are known to be involved in controlling motor movement; the primary motor cortex in the precentral gyrus (named as M1) and SMA which is also known to be involved in planning complex movements and in coordinating movements involving both hands[3]. The other area is BA44. The reasons behind the inclusion of BA44 are 1) it is symmetrically activated at α = 0.001 especially for BIM, 2) due to one of its functions which is speech and language processing and 3) to enable study on the connectivity between motor regions and other regions not related to motor coordination but have significant activation. Speech and language areas should have reveal certain extent of activation in this study since the interchange between UNI_{left}, UNI_{right} and BIM tapping is done via verbal instruction using the intercom i.e. "START LEFT", "STOP LEFT" or "START RIGHT", "STOP RIGHT".

The M1, SMA and BA44 were found to be activated at different significant level in all of the participating subjects but the coordinates of the activation peak differ by a few millimeters from subject to subject. Differences in activation can also be observed when comparing between the left and right hemisphere regions. Group RFX results for BIM shown in Figure 3 clearly indicate a larger spatial extent of activation in the left SMA and BA44 as compared to the right, while the spatial extent of activation for M1 is quite symmetrical. The larger spatial extent of activation for left BA44 as compared to the right can be easily understood since the processing of speech and language are more likely to occur in the left hemisphere. However, a similar effect that occur on SMA is not known and needs further clarification.

The typicality of the effects of the unilateral and bilateral tapping of hand fingers in all subjects was investigated using conjunction analysis. Conjunction analysis[21] provides a way to locate common features of functional anatomy between subjects under the same experimental condition. The results obtained from the analyses of conjunction on the present UNI_{right}, UNI_{left} and BIM datasets indicate that all subjects show common activation areas in precentral gyrus (M1). Due to the relatively high variability among the subjects under study, the SPM results generated at significant level of α = 0.1 indicate significant activation only at voxel level. Both the set and cluster level inferences about the activation clusters revealed insignificant brain activation. Conjunction analyses results therefore confirm the central

role of M1 in coordinating the three finger tapping tasks used in this study.

In our previous study on a single right-handed male subject[12], we found that the activated primary motor areas in the right hemisphere due to UNI_{left} showed a higher signal intensity and larger activation area as compared to that in the left hemisphere due to UNI_{right}. We also found that the right hemisphere exhibited larger activation area during BIM. The findings obtained from our single subject study are in good agreement with a multiple subject fMRI study on unilateral and bilateral sequential movement in right-handers[26]. They found that the right hemisphere showed more activation than the left hemisphere in both unilateral and bilateral task at two tapping frequencies. They also concluded that faster movement rates will cause higher activation both in terms of signal intensity and number of activated voxel, the so called "rate effects". Their interpretations are that right-handers expend more effort to perform with their non-preferred hand. A stronger activation pattern in the right hemisphere is the result of trying to perform with a system that is slightly less competent with the implication that the more skilled and competent system will expend less effort and will therefore provide a weaker activation. As for the rate effects, they concluded that faster movement involves the recruitment of more motor units and will therefore activate a greater number of voxels. Their findings had later be reconfirmed[10].

However, in this study and in our separate study on seven right-handed female subjects[13], we found that the average responses obtained from FFX (not shown) and RFX indicate higher height (signal intensity) and spatial (activation area) extent of activation in the left hemisphere for unilateral type of finger tapping. As mentioned earlier, this study used a robust self-paced finger tapping. Prior to the fMRI scan, the subjects were told that they need to tap their fingers two times in one second using an intermediate force between too soft and too hard. However, since all the subjects are right-hand dominant, there would be a tendency for the subjects to tap their preferred hand fingers faster than their non-preferred hand fingers, resulting in the rate effects. Based on the interpretation given above, it seemed that the influence of the rate effects is greater than the effects that would be produced by the average effects of the dominant and sub-dominant hand, hence greater spatial activation in the left hemisphere. A larger activation area could also be due to the tendency of these right-handers to press their fingers harder against the thumb using their dominant hand fingers, whereby a larger force will activate a larger area with higher intensity. Interestingly, in contrast to the spatial extent of activation, the height extent or PSC for M1 obtained in this study is higher in the right hemisphere (due to UNI_{left}) as compared to the PSC measured in the left hemisphere (due to UNI_{right}), see Table 2. This finding is in contrast to the number of activated voxels which is higher for UNI_{right} as compared to UNI_{left}. PSC is defined as the relative signal change within a cytoarchitectonic area evoked by the different experimental conditions, which reflects the involvement of that particular area in a specific task[23]. It is simply the ratio between the condition-specific signal change and the mean signal during the session. In relation to the discussion above, it can be assumed that tapping rate does not influence the height extent of activation as it does on the spatial extent of activation. As a result, the higher PSC observed in the right hemisphere is due only to a higher control demand used by the brain in coordinating the tapping of the sub-dominant hand fingers.

For BIM, the height and spatial extents of activation are almost similar between the left and right M1, SMA as well as BA44. Group results indicate relatively small differences in the number of activated voxel and percentage of signal change as can be seen in Table 2 and Figure 3. The findings are consistent with a previous study[1] which indicate symmetrical spatial extent of activation in M1 and SMA. The effects observed for bimanual however are different from the resultant effects of combining between UNI_{left} and UNI_{right}. Thus, for a bimanual type of finger tapping, it can be concluded that the effects obtained are not the sum of the effects produced individually by UNI_{left} and UNI_{right} as reported[3].

The results depicted in Figure 2, and Table 2 clearly revealed significant activated areas in the opposite hemisphere to the contra-lateral hemisphere. For UNI_{right}, ipsi-lateral activation occurs in the right postcentral gyrus, right Rolandic operculum, right precentral gyrus, right middle frontal gyrus, right superior frontal gyrus, right SMA and right middle cingulate gyrus. For UNI_{left}, the ipsi-lateral areas are left precentral gyrus, left SMA and left middle frontal gyrus. The existence of ipsi-lateral activation in motor cortex has been widely reported and discussed[1, 3, 28]. It shows evidence of involvement of ipsi-lateral areas in coordinating motor movement. One of the observed effects related to ipsi-lateral activation is inhibition whereby increased neuronal activation in motor area of one hemisphere suppresses neuronal activity of the same area in the opposite hemisphere. Inhibitory has been shown to be either in terms of activated volume or percentage of signal change[28]. In terms of functional specialisation, inhibition is not observable in this study since tapping style is kept constant. However, as can be seen from Figure 2 and Table 2, ipsi-laterality did occur in both M1 and SMA and the effects are asymetrical and these show possible evidence of inhibitory of activation in the ipsi-lateral areas.

Effective connectivity

It has been established that the primary motor area (M1) in the precentral gyrus (PCG) and the supplementary motor

area (SMA) in the medial dorsal wall are involved in movement preparation and execution of motor action. While M1 and SMA are known to be responsible in triggering and initiating motor related movements, SMA has a special function of being able to coordinates interlimbs movements spatially and temporaly especially during bilateral execution[11]. It is evident from Figure 2 that both areas are also involved even in unilateral types of movement suggesting the existence of interhemispheric connectivity between these areas. The inclusion of the left and right BA44 in this study was motivated by their significant activation on uncorrected (α = 0.001) SPMs. It is hypothesized that the left and right BA44 were also connected to the motor areas during the execution of self-paced motor task since the tapping instructions were given verbally via headphones i.e involving speech and language.

In the present study, we investigated the intrinsic couplings only between M1, BA44 and SMA of the right and left hemispheres. The pre-motor cortex (PMC), another important area in motor coordination is not included in the present study due to the inconsistency of the activation in the respective area for all subjects, even at a lower significance level, resulting in lack of activation in group results. This could be due to the nature of task done by the subjects that does not involve the integration of sensory information which is one of the functions of PMC.

Biophysically plausible time-series models that reasonably represent interacting cortical regions can be constructed based on Equation (1). The models may consist of all of the three intrinsic couplings, modulatory and extrinsic inputs or may consist of only the intrinsic couplings and extrinsic inputs, depending on the experimental design[5]. In this study, we use only the extrinsic inputs and intrinsic couplings. Due to experimental limitations, contextual or modulatory input was not included to be estimated by DCM in the present study since no such stimulus was given to the subject so that there is at least one area in the brain that will be influenced by contextual input.

Based on Equation (1) and the activation obtained in Figure 2 and 3, we constructed ten biologically and physically plausible models that consisted of M1, BA44 and SMA in both hemispheres as shown in Figure 1. We hypothesised that the input will either be through M1, BA44 or SMA, unilaterally or bilaterally. To determine which region or regions that will most probably act as the input, our assumption was that any one region is fully connected to any other regions. Therefore, one may see that there are many other alternative models that can be constructed using the right and left M1 and SMA as processing centers, with a large number of mathematically possible connections. However, we limited this study to the ten biologically physically plausible models that we believed would be able to explain the intrinsic couplings between M1, BA44 and SMA in both hemispheres during UNI_{left}, UNI_{right} and BIM.

DCM uses a fully Bayesian approach in estimating and selecting the most probable model among the competing models. According to Bayes' rule, the posterior distribution is equal to the likelihood times the prior devided by the evidence[9] or $p(\theta|y\ ,m) = [p(y|\theta\ ,m)\ p(\theta|m)]/p(y|m)$. Taking logs for both sides; $\log p(\theta|y\ ,m) = \log p(y|\theta\ ,m) + \log p(\theta|m) - \log p(y|m)$. The expression $p(y|m)$ is the probability of obtaining observed data y given a particular model m, also named as model evidence while $p(\theta|m)$ is the probability of obtaining DCM parameters θ given a particular model m which is named as prior. The expression $p(y|\theta\ ,m)$ is the probability of obtaining observed data y given DCM parameters θ and a particular model m also named as likelihood and $p(\theta|y\ ,m)$ is the probability of obtaining DCM parameters θ given data y and a particular model m also named as posterior distribution.

The results obtained from Bayesian model selection (BMS) for group RFX and FFX studies are shown in Table 3 and Figure 4 for comparison. The constant values of the sum of negative free energy (ΣF) for UNI_{right}, UNI_{left} and BIM indicate a perfect balance between accuracy and complexity[19] for all models shown in Figure 1. The fact that the values are almost the same for all types of movement also reflects a good fitting of the models to the observed data regardless of the types of movement. The related equation is $F = \langle \log p(y|\theta\ ,m) \rangle_q - \mathrm{KL}[q(\theta), p(\theta|m)]$[19]. Accuracy or the log likelihood is the first term on the right side of the equation which explains the probability of obtaining observed data y given DCM parameters θ and a particular model m. Complexity is reflected in the second term which contains the amount of information that can be obtained from the data with regards to the parameters of a model.

The Dirichlet parameter estimates (α_d), the expected posterior probability ($<r>$) and the exceedance probability (φ) are all the parameters used in BMS analyses to rank models at group RFX level. The Dirichlet parameter estimates is a measure of effective number of subjects in which a given model generated the observed data. The sum of all α_d is equal to the number of subjects plus the number of compared models. The exceedance probability φ_k is the probability that a given model k is more likely than any other model to give the observed experimental data. If φ_k obtained for model k from K models is 0.95 (or 95%), one can be 95% sure that the favoured model has a greater posterior probability $<r>$ than any other tested models. As can be seen in Table 3, the sum of φ is unity. The histograms in Figure 4 (top) graphically indicate the expected posterior probability and the exceedance probability for all models. Both two quantities for Model 7 and Model 6 for UNI_{left}, UNI_{right} are comparatively higher than any other models. From Table 3, it can be seen that all the values of α_d, φ and $<r>$ agree very well that both the UNI_{left} and UNI_{right} are best represented by Model 7 and Model 6 respectively. Furthermore, these two models have also shown firm evidence in FFX perspective with a high posterior model probability (P) in getting the respective log evidences, see Table 3 and Figure 4 (bottom). However, for BIM, even though the RFX for BMS results indicate Model 6 as the most probable

model, it is not the preferred model in FFX perspective. FFX for BMS instead, has choosen Model 2 as the most probable model for BIM with the log-evidence of 147 and posterior model probability of 0.9234 as opposed to 145 and 0.0766 for Model 6. In view of the model structure used in this study that is assumed to be identical across subjects[29], the results obtained from FFX for BMS are more reliable. Therefore, Model 2 is the model of choice for BIM. More importantly, the results obtained from group BMS studies whether in RFX or FFX perspectives, have been reported[29] to be able to take into consideration the presence of outliers that could have arisen in any subject under study. Thus BMS analysis is the preferred approach in model comparison involving multiple subjects.

Model 7 and Model 6 which have been proven to be the winning models among the ten competing models for UNI_{left} and UNI_{right} have six ROIs that are fully connected to any other ROI. The values depicted in Table 4(a) and (b) represent the acceptable average extrinsic input and intrinsic couplings between the ROIs for Model 7 and Model 6. As mentioned previously, this study excluded contextual or modulatory input. Therefore, the acceptable intrinsic coupling values in Table 4(a) and (b) are basically the element of **A** matrix in Equation (1), while the values in the input column are the element of matrix **C**. Also shown in the tables are the statistics obtained from one-sample t-test to test whether the average values for the connections over the 16 subjects are significant or not against the condition of no connection, i.e. '0'. The effect size (t value) and p values indicate that all inputs and connections are significant. However, an effective connectivity between any two ROIs can be accepted if its value is relatively high with a posterior probability greater than 0.9[5]. For UNI_{right} and UNI_{left}, not a single connection has all subjects with posterior probability larger than 0.9. However, connections that have more than half of the number of subjects with posterior probability equal or larger than 0.9 are M1-L→M1-R, M1-L→BA44-L, M1-L→BA44-R, M1-L→SMA-L, M1-L→SMA-R for UNI_{right} and M1-R→M1-L, M1-R→BA44-L, M1-R→ BA44-R, M1-R→SMA-L, M1-R→SMA-R for UNI_{left}. Therefore, only these connections are considered for the construction of the most probable model for UNI_{right} and UNI_{left}. The models are schematically shown in Figure 5(a) and (b) for UNI_{left} and UNI_{right} respectively.

From this robust finger tapping study that involves 16 healthy young male and female right-handed adults, it can be summarised that during the unilateral tapping of left hand fingers, M1-R will act as the input centre, controlling the movement by triggering the synaptic signal unidirectionally to M1, BA44 and SMA contra- and ipsi-lateral to it. Similar transmission of signal is observed for UNI_{right} from which M1-L is found to be the input centre.

For BIM, significant connections found for M1-L→BA44-L, M1-L→SMA-L, M1-L→SMAR-R, M1-R→SMA-L and M1-R→SMA-R are evident in at least half of the number of subjects under study. This final intrinsic connectivity model (Figure 5 (c)) which is obtained from Model 2 is supposed to be symmetrical in nature. However, connectivity from M1-R to BA44-R (represented by dotted arrow) is found to be significant (posterior probability equal or larger than 0.9) in only four subjects. The input centers for BIM are the left and right M1. It is interesting to see from the results that M1-R-M1-L foward and backward connectivity is inhibited during BIM while it occurs unidirectionally during UNI_{left} and UNI_{right} from the contra-lateral to the ipsi-lateral regions. The inhibition of M1-M1 connection during BIM suggests that the primary motor area contra-lateral to the side of movement is not influenced by the one in the ipsi-lateral side in coordinating BIM type of movement. The synaptic signals triggered in any one M1 however, are sent to SMA in both sides of the brain during the coordination. It can also be observed that the left and right SMA and BA44 are not mutually connected during BIM and this can also be seen either during UNI_{left} or UNI_{right}.

From Figure 5, one can notice that no backward connection exist in all types of finger tapping conducted by this group of subjects. All connectivities originate from M1 contra-lateral to the side of movement for UNI_{left} and UNI_{right} and from both the left and right M1s for BIM. For unimanual type of finger tapping, the regions of M1 ispsi-lateral, left and right BA44 and left and right SMA receive information form M1 contra-lateral in maintaining the coordination of motor task. This includes information needed in maintaining the pace and force of tapping, maintaining the side of movement untill further instruction by understanding the previous instruction and maintaining the stability of tapping. For bimanual tapping of hand fingers, similar kind of coordination is thought to occur but with several inhibition of connectivity as compared to unimanual type of tapping, as mentioned previously. This again indicate that connectivity observed for BIM is not the sum of the connectivity for UNI_{left} and UNI_{right}.

For UNI_{left} and BIM, the difference in the strength of connection obtained from One-Way ANNOVA analyses between the connections that are significant is relatively small and not significantly different from one another. Whereas for UNI_{right}, significant difference is indicated in two out of ten comparisons. This behaviour of cortical network is analogous to the distribution of current at a junction in a parallel electrical circuit that has a comparable amount of load in each junction whereby the same amount of electrical charges flowing through each junction under the same potential difference. It is assumed that the input center, which is M1 served as the hub, producing and propagating the synaptic signal of the same magnitude to all other ROIs, from which the connectivity is said to occur under the same potential difference, hence the coordination of UNI_{left}, UNI_{right} and BIM.

CONCLUSION

The studies of functional specialisation and effective connectivity between activated regions in the brain evoked by

experimental manipulation i.e. unilateral tapping of hand fingers (UNI_{right} and UNI_{left}), has been made possible using noninvasive functional magnetic resonance imaging (fMRI) technique and a novel analyses of statistical parametric mapping (SPM), dynamic causal modeling (DCM) and Bayesian model selection (BMS) for group studies. The tapping rate effect was found to be greater than what would be produced by the average effects of the dominant and sub-dominant hand. The higher PSC observed in the right M1 however, is due to a higher control demand used by the brain in coordinating the tapping of the sub-dominant hand fingers. Ten biophysically plausible models constructed based on low-threshold activation of specific regions of interest (ROIs) were estimated and compared in a powerful Bayesian framework. The results of Bayesian model selection (BMS) for group studies that point to the most probable model for UNI_{left}, UNI_{right} and BIM with M1 as the input, indicating the reliability of the methods and reproducibility of the results. The primary motor area M1 in both hemispheres was determined to be the input centre during the unimanual and bimanual tapping of hand fingers. Significant connections between the ROIs are M1-L→M1-R, M1-L→BA44-L, M1-L→BA44-R, M1-L→SMA-L, M1-L→SMA-R for UNI_{right} and M1-R→M1-L, M1-R→BA44-L, M1-R→ BA44-R, M1-R→SMA-L, M1-R→SMA-R for UNIleft. For BIM, significant connection exist for M1-L→BA44-L, M1-L→ SMA-L, M1-L→SMAR-R, M1-R→SMA-L and M1-R→SMA-R. All significant connections are unidirectional in nature, i.e. no backward connection exist in UNI_{right}, UNI_{left} or BIM. The analyses implemented in this study reveal not only the specialisation of M1, BA44 and SMA during the coordination of unimanual and bimanual movement of hand fingers but also the couplings of interaction between the two regions within and between hemispheres. While M1-M1 connection has been found to be unidirectional for UNI_{right} and UNI_{left}, it is inhibited during BIM. The SMA-SMA and BA44-BA44 connections shows no evidence of significant connectivity for all conditions. On average, it can be said that for connections that are significant, there exist no significant difference between them.

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Help-seeking Pathways for In-patients with First-episode Psychosis in Hospital Kuala Lumpur

[1]CK Phang*, [2]M Marhani & [3]AA Salina

[1]Department of Psychiatry, Universiti Putra Malaysia (UPM)
[2]Department of Psychiatry, Universiti Kebangsaan Malaysia (UKM)
[3]Department of Psychiatry & Mental Health, Hospital Kuala Lumpur (HKL)

ABSTRACT

Introduction: Help-seeking pathway in psychiatry is the important link between the onset of a mental disorder and mental health service provision. Understanding of the help-seeking pathway can help us to device more effective strategies for early detection and treatment. **Objectives:** To determine the help-seeking pathways and treatment delaying factors of in-patients with first-episode psychosis in Hospital Kuala Lumpur (HKL). **Methods:** This is a hospital-based cross-sectional descriptive study of 50 in-patients with first-episode psychosis in HKL. Structured Clinical Interview for DSM-IV - Clinical Version for Axis I Disorders (SCID-CV) was used for establishing diagnosis. Socio-demographic data, information on help-seeking pathways, and treatment delaying factors were determined through face-to-face interview and semi-structured questionnaires. **Results:** The number of non-psychiatric help-seeking contacts prior to first consultation with psychiatric service ranged from 0 to 10. The mean (± SD) number of contacts was 2.3 (± 2.6), and median was 1 (IQR = 0 to 3). About a third of them (32%) had three or more non-psychiatric contacts. The most common point of first non-psychiatric contact was with traditional healer 24 (48%), followed by general practitioners 12 (24%), and only 14 (28%) of them sought help directly from psychiatric service. The most common reason reported for delay in seeking psychiatric treatment was, "not aware that changes were related to mental illness" (74%). **Conclusions:** History of contacts with traditional healers was common among in-patients with first episode psychosis in HKL. Treatment delay was mainly contributed by factors related to lack of awareness on psychosis. More strategic mental health education program is needed for early detection and treatment of psychosis.

Keywords: Help-seeking pathway, first-episode psychosis, traditional healer

INTRODUCTION

Help-seeking pathway in psychiatry is an important link between the onset of a mental disorder and mental health service provision. Understanding of the help-seeking pathway can help us to device strategies to improve detection and treatment of psychiatric disorder. The pathway to care commonly involves a progression from non-psychiatric services or even non-medical services to psychiatric services, with general practitioners having a potentially important role in the early recognition and referral of individuals with early psychosis. The pathways to care are variable, depending on the nature of the health care system and its financial and structural characteristics. There is a significant prevalence of 'never-treated psychosis' particularly in developing countries [1].

In a pathway to care study of 86 patients with schizophrenia spectrum disorders in Canada, a total of 194 contacts were made. The mean number of contacts per person was 2.3 and the range of contacts was from 1 to 6. The majority of contacts were made to emergency services (32.5%) and family physicians (22.7%). The majority of contacts were initiated by the patient (44%) and the family (34%). Health care professionals initiated 12% and friends, police and teachers accounted for the remaining 10%. The number of contacts it took to receive appropriate treatment varied. 33 were successful after one contact, 31 after two contacts, 18 after three contacts, eight after four contacts, 1 after five contacts and 3 after six contacts. The most common contact that resulted in successful treatment was emergency services (52%). Others were family physicians (18%), psychiatrists (18%), psychologist (8%) and family and friends (4%). The family most often initiated successful contacts (44%) followed by patients themselves (25%) and health care professionals (20%). Friends, teachers and the police accounted for the remaining 11% [2].

The pathways to appropriate care following the onset of a psychotic episode can be long, indirect and inefficient.

*Corresponding author: *pckar@yahoo.com*

A study in United Kingdom examined the pathways into the hospital system for people experiencing a first psychotic episode from a family's perspectives. It was found that: i) Appropriate services were not available to relatives when they were required, ii) Multiple contacts were made prior to admission, iii) Relatives usually made appropriate contacts initially but when these proved unsuccessful, they were forced to turn to more unusual contacts, and iv) Police contact was distressing for relatives but they were grateful when it led to hospital admission [3].

In a first-episode psychosis study in Australia [1], it was found that: i) The mean number of helping contacts with a variety of professionals and non-professionals prior to referral to a specialist center was 4.9 (range 1-17), ii) 55% of people had 4-6 contacts, iii) 16% had more than 6 helper contacts, iv) 36% of initial help seeking contacts were with a general practitioner (primary care physician) and 50% had seen a general practitioner at some stage prior to referral to the specialist center, and v) 50% were probably psychotic by the time they first sought help and another 37% were either manic or depressed.

In a pathway to care study of 182 patients with first-episode psychosis referred to early intervention for psychosis service in New Zealand, an average of 3.87 (SD = 6.81) attempts at help-seeking behavior were made in the 6 months prior to referral (range = 0-42). The majority (70%) of the sample had contact with a general practitioner or psychiatric outpatient clinic in the 6 months prior to referral. But these services were low sources of referral (7.7% and 16%, respectively). Most referrals (64%) came from inpatient services [4].

In a study at All India Institute of Medical Sciences (AIIMS) in New Delhi on patients with acute psychosis [5], data gathered from the outpatient psychiatric clinic showed that the first contact with helping agent was with traditional or religious healers (70%). Only 10% first sought help from the psychiatric service. The main reason for current consultation was onset or exacerbation of odd or threatening behavior (86%). The mean time interval between onset of these behaviors and treatment seeking was 14 days. Campion & Bhugra [6] studied the treatment methods and rituals performed by religious healers who were consulted by 45% of psychiatric patients before presenting to a psychiatric outpatient clinic in southern India, and noted that the medical literature seldom mentions traditional healers despite their importance as the first line of treatment in developing countries.

In a pathway to care study of patients with first-episode schizophrenia in 2 hospitals in Japan, Keio University Hospital (54 patients) and Oizumi Mental Hospital (29 patients), the referral pathways were compared. 40 patients (74.1%) came to the university hospital and 12 patients (41.4%) came to the mental hospital. At the mental hospital, nine patients (31.0%) had been admitted because of a legal obligation, and six (20.7%) had been referred through public health centers. None of the patients had been referred to either of the services by general practitioners. The main reason for seeking treatment was psychiatric symptom aggravation (59.3%) at the university hospital and acting out (64.3%) at the mental hospital [7].

In a study of pathway to psychiatric care for 35 patients with first-episode psychosis in Hong Kong [8], the mean number of help-seeking contacts before treatment was 1.06. Majorities were initiated by parents (24.3%) and patients themselves (29.7%). The most frequent first contact was through social workers (27.0%) and primary care physicians (27.3%). For the second contact, the most frequent choices were social workers (33.3%) and private psychiatrists (33.3%). The most common reason given for delay in seeking treatment was lack of knowledge about psychosis (74.3% in patients and 54.3% in family members).

In a pathway study of 5 East Asian countries (China, Japan, Korea, Malaysia and the Philippines) involving patients with schizophrenia [9], most Japanese subjects sought care in western medicine. Subject from Korea and China alternated between western medicine and magico-religious therapies or traditional herbal medicine. In the Philipines and Malaysia, the majority of the subjects sought magico-religious therapies first followed by western psychiatric care.

In a pathway to care study in Bali, 54 consecutive patients with no prior psychiatric treatment were investigated. Subjects who had sought help from traditional healers were asked to evaluate treatment effect retrospectively according to a 5-point scale. The pathway to psychiatric care was dominated by traditional healers. Of the patients, 47 (87.0%) consulted a healer (mean number 2.9) before visiting the mental hospital. Consultation with the healers was associated with treatment delay. However, of the 137 traditional healers on the pathway, 11 (8.0%) recommended that the subjects go to a mental hospital, and all of them immediately followed the advice. Of the 47 subjects, 14 (29.8%) evaluated the treatment effect as much improved by at least one traditional healer on the pathway, although they ultimately attended the mental hospital. Subjects without psychotic symptoms tended to evaluate the treatment effect as much improved more often than psychotic subjects. Traditional healers function not only as a barrier to reaching psychiatric care, but as either an effective provider of care or a decision-making support for seeking help from psychiatric care for some mental patients in Bali [10].

As for Singapore [11], 24% of the patients with first-episode psychosis had sought consultation with a traditional healer prior to consulting a psychiatrist. A study among Malays in Kelantan, a rural state of Malaysia showed that more than 80% of patients with mental disorders had consulted a traditional healer who also dissuaded these patients from seeking western medical treatment [12].

In a Kuala Lumpur pathway to care study by Koh [13] in University Malaya Medical Center (UMMC), from the 100 patients with first-episode psychosis interviewed, 58% had never sought any form of treatment prior to coming to hospital. 9% had sought help from general practitioners, 14% from traditional healers, 10% from religious mediums and 6% from primary care/specialist clinics. Among those who did not seek any form of treatment prior to coming to hospital, 34% were male patients and 24% were female patients. Traditional healers or religious mediums were the preferred choice for both male (8%) and female (16%) patients. They were also the preferred choice of treatment for Malays (11%), Chinese (6%) and Indians (7%).

There was another local pathway to care study of first-episode psychosis and epilepsy among Malays by Razali & Salleh [14] in Hospital Kubang Kerian, Kelantan. From the 120 out-patients with first-episode psychosis interviewed, 15% sought help directly from the hospital. 10% had sought help from general practitioners, 61.7% from traditional healers and 3.3% from other sources. There was significant treatment delay in patients with first-episode psychosis compared to those with epilepsy.

The objectives of this study are to determine the help-seeking pathways and treatment delaying factors of inpatients with first-episode psychosis in HKL. This has implication in designing better mental health services for early detection and treatment of patients with first-episode psychosis in Kuala Lumpur. This study is unique as it is conducted in a hospital that provides services in a highly urbanized area (i.e. Kuala Lumpur, the capital city of Malaysia), and is gazetted for involuntary psychiatric admissions. Furthermore, psychiatric admissions are totally free of charge as it is fully funded by the government.

METHODOLOGY

Location of study:

This is cross-sectional descriptive study conducted in the Department of Psychiatry & Mental Health, Hospital Kuala Lumpur (HKL). HKL is the largest hospital in the country and Kuala Lumpur is the capital city of Malaysia. HKL offers specialist psychiatric service, and is gazetted for compulsory psychiatric admission. Ethical approval for the study was obtained from the Medical Research & Ethics Committee, National University of Malaysia (UKM). Permission to conduct the study was obtained from HKL, and the study was registered with National Medical Research Registry (NMRR).

Sample:

A convenient sampling was conducted twice a week to identify newly diagnosed in-patients with first-episode psychosis. Patients with first-episode psychosis were those suffering from psychosis for the first time, and sought help for their psychosis from psychiatric service for the first time. The sampling period was a consecutive period of 4 months. The inclusion criteria were all in-patients with first-episode psychosis, including substance-induced psychosis and other organic psychosis. Those with language barrier, no family members around to verify history, and refused consent were excluded. Altogether 50 in-patients were recruited for the study.

Assessment:

The diagnosis of psychosis and different types of psychosis were based on all available clinical information and using the Structured Clinical Interview for DSM-IV Axis I Disorders – Clinical Version (SCID-CV). SCID-CV is a semi-structured interview for making the major Diagnostic & Statistical Manual of Mental Disorders, 4th edition (DSM-IV) Axis I diagnoses [15].

Socio-demographic data, information on help-seeking pathways, and treatment delaying factors were determined through face-to-face interview and semi-structured questionnaire. In order to get more accurate history, the key friend or family members caring for patient prior to psychiatric contact were identified for interview. The researcher was conversant in English, Bahasa Malaysia, Cantonese and Mandarin. The language that was most comfortable to patients and family members was used for the interview and data collection.

Help-seeking pathway variables that were included in the study were: i) Number of non-psychiatric help-seeking contacts, ii) Most frequent point of first non-psychiatric help-seeking contact (i.e. traditional healers, general practitioners, clinical psychologist, counselors, social worker, telephone helpline etc.), iii) Number of help-seeking contacts with traditional healers (i.e. 'bomoh', monk, priest, ayurvedic or homeopathy practitioner, others.), v) The person who initiated the first non-psychiatric and psychiatric help-seeking contact (i.e. patient, friends, family members, employer, others), and iv) Adverse help-seeking pathway experience (i.e. those involving police, violent behavior and compulsory admission).

Factors contributing to delay in seeking psychiatric treatment that were assessed are: i) Not aware that behavioral changes were related to mental illness, ii) Believed that a doctor cannot help the condition, iii) Lack of knowledge about where or how to get help, iv) Lack of financial resources (i.e. for transport, consultation, medicine etc.),

v) Lack of time or nobody is free to accompany patient for treatment, vi) Concern about language barrier in communicating problem to doctor, vii) Concern about stigmatization (i.e. people will laugh or discriminate), viii) Concern about treatment (e.g. pain, side effects or confinement), ix) Considered that symptoms were not serious enough for treatment, x) Believed that symptoms would improve spontaneously, xi) Conflicting opinion with patient (i.e. patient refused to get help), and xii) Conflicting opinion with family members (i.e. family members disagreed to seek help).

Data analysis:

The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 12. Descriptive and non-parametric statistical tests were used for analysis. Statistical significance was set at $\alpha < 0.05$.

RESULTS

Socio-demographic data:

The age of the patients ranged from 15 to 70 years old. The mean (± SD) age was 33.1 (± 14.1) years old. Most of the patients fell into the age group of 21-30 years old (40%). As for gender, 31 (62%) were males, and 19 (38%) females. Most of them were Malay (22, 44.0%) followed by Chinese (14, 28.0%), Indian (6, 12.0%) and others (8, 16.0%). Only seventeen (34.0%) of them were married. As for the pre-hospitalization living arrangement, 48 (92.0%) of them were living with others, and 4 (8.0%) were living alone. Less than half (23, 46.0%) of the patients were employed.

Diagnosis:

Table 1 shows the DSM-IV diagnostic categories of the patients. When regrouped, 19 (38%) of the patients had Schizophrenia Spectrum Disorder (Schizophrenia & Schizophreniform Disorder), 6 (12%) had Mood Disorder (Bipolar I Disorder or Major Depressive Disorder) with psychotic features, 13 (26%) had Substance-Induced Psychosis, and 12 (24%) had other types of psychosis (Brief Psychotic Disorder and other organic psychosis). Three (6%) of the patients had a dual diagnosis of either Schizophrenia Spectrum Disorder or Bipolar I Disorder with substance abuse.

Table 1: DSM-IV Diagnostic categories of patients with first-episode psychosis

Diagnosis	(N = 50)	(%)
Schizophrenia	15	30%
Schizophreniform Disorder	4	8%
Bipolar I Disorder with psychosis	2	4%
Major Depressive Disorder with psychosis	4	8%
Brief Psychotic Disorder	6	12%
Delusional Disorder	1	2%
Substance-Induced Psychosis	13	26%
Other psychotic disorder	5	10%

Help-seeking pathway:

The number of non-psychiatric help-seeking contacts prior to first consultation with psychiatric service ranged from 0 to 10. The mean (± SD) number of contacts was 2.3 (± 2.6), and median was 1 (IQR = 0 to 3). About a third of them (32%) had three or more non-psychiatric contacts. The most common point of first non-psychiatric contact in help-seeking pathway was with traditional healer 24 (48%), 12 (24%) was with general practitioner, and only 14 (28%) of the patients sought help directly from psychiatric service. All the patients were admitted involuntarily under Form A. 23 (46%) of the patients were admitted with police assistance and 27 (54%) had violent behavior on admission. Figure 1 summarizes the key findings on help-seeking pathway of the patients.

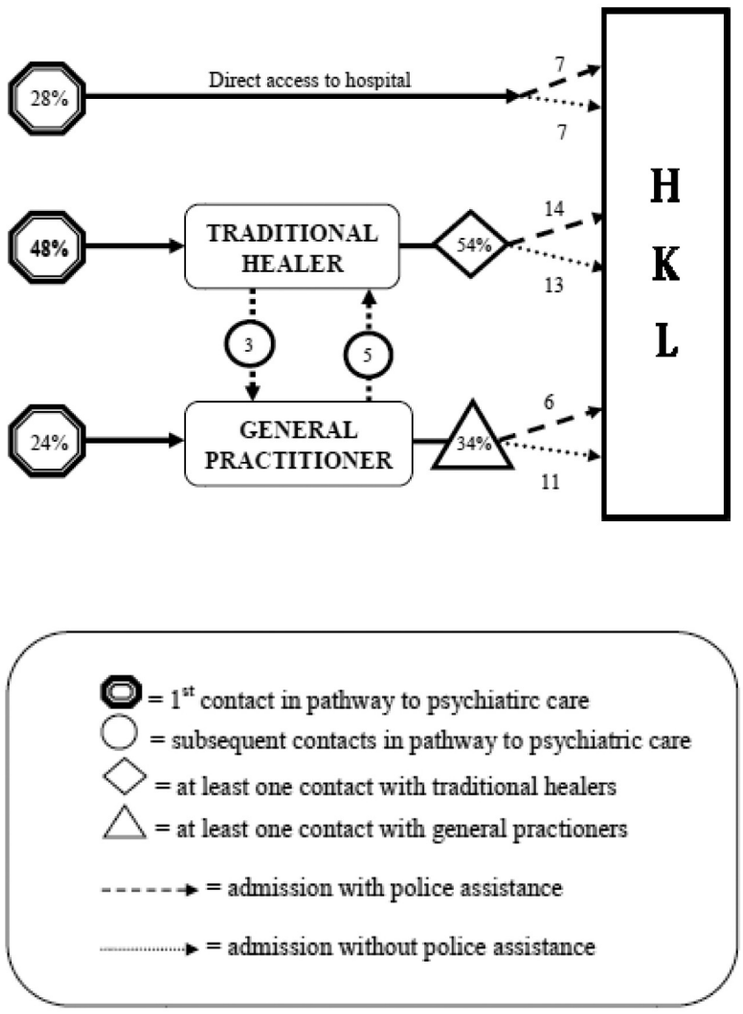

Figure 1: A summary of pathway to psychiatric care in Hospital Kuala Lumpur

Overall, 27 (54%) of the patients had at least one contact with traditional healer prior to their first consultation with psychiatric service. About a quarter of them (24%) had 3 or more contacts with traditional healers prior to consulting psychiatric service. History of contact with traditional healer was not found to be associated with age, gender, ethnic and education level ($P < 0.05$).

Most of the first non-psychiatric contacts in the help-seeking pathway were initiated by patients' family members (35, 70%), followed by public (7, 14%), employer (5, 10%), friend (2, 4%), and patients themselves (1, 2%). As for the first psychiatric contact, most were also initiated by family members (29, 58%), followed by public (14, 28%), employer (4, 8%), patient themselves (2, 4%) and friend (1, 2%). More public initiated first psychiatric contacts compared to first non-psychiatric contacts (28% vs. 14%).

Delay in seeking psychiatric treatment:

Reasons reported by family members for delay in seeking psychiatric treatment are summarized in Table 2. The top 5 reasons reported were, "not aware that changes were related to mental illness" (74%), "conflicting opinion with patient i.e. patient refused to seek psychiatric help" (72%), "believed that symptoms would improve spontaneously" (66%), "considered that symptoms were not serious enough for treatment" (56%), and "believed that a doctor cannot help the condition" (42%). "Conflicting opinion with patient" was significantly associated with history of contacting traditional healer (OR = 4.42, 95% CI = 1.15 to 16.96), and violent behavior on admission ($\chi^2_{1df} = 8.305$, P = 0.004, OR = 7.33, 95% CI = 1.71 to 31.34).

Table 2: Reasons reported by family member for delay in seeking help from psychiatric service

	Reasons for delay*	N (%)
1.	Not aware that changes were related to mental illness.	37 (74%)
2.	Believed that a doctor cannot help the condition.	21 (42%)
3.	Lack of knowledge about where or how to get help.	2 (4%)
4.	Lack of financial resources for transport, consultation, medicine etc.	5 (10%)
5.	Lack of time or nobody is free to accompany patient for treatment.	4 (8%)
6.	Concern about language barrier in communicating problem to doctor.	3 (6%)
7.	Concern about stigmatization – people will laugh or discriminate.	8 (16%)
8.	Concern about treatment e.g. pain, side effects or confinement.	13 (26%)
9.	Considered that symptoms were not serious enough for treatment.	28 (56%)
10.	Believed that symptoms would improve spontaneously.	33 (66%)
11.	Conflicting opinion with patient i.e. patient refused to seek help	36 (72 %)
12.	Conflicting opinion among family members i.e. other family members disagreed with getting help.	8 (16 %)

* Can be more than one reason

DISCUSSSION

In this study, 54% of the respondents had at least one contact with traditional healer prior to first contact with psychiatric service, with about a quarter of them (24%) having 3 or more contacts. In fact, traditional healers were the most popular choice of first non-psychiatric contact (48%). This is in contrast to a local study by Koh in the same city, whereby only 24% of the respondents had sought help from traditional healers prior to contact with psychiatric service [13]. A study in Singapore also showed that only 24% of the respondents had sought help from traditional healer before consulting psychiatrist service [11]. This may be explained by the relatively lower socio-economic status of respondents in this study, and therefore possibly their greater faith in traditional treatments. In-patient psychiatric service in HKL is completely subsidized by the government, thus may naturally attract more patients from the lower socio-economic group. Further studies are needed to validate this association.

Besides that, the relatively more hostile patients in this study could also possibly contribute to this; 54% of the patients were violent, 68% were verbally abusive, and all patients were admitted involuntarily under 'Form A'. So, family members could have difficulty in bringing patients to psychiatric service. In fact this study has shown that history of consulting traditional healer was significantly associated with refusal in seeking psychiatric help. Consulting traditional healers was much easier, as some of the healers were willing to do home visit or even consultation by proxy to offer treatments. This is of course much more convenient, and acceptable to patients and family members. Hence, consulting traditional healers was a more popular choice of first help-seeking contact (48%) as compared to general practitioners (24%) or psychiatric service (28%).

Local studies, [12, 14, 16] and several studies in other Asian countries such as Bali [10], the Philipines [9], and India [5, 6] has evidently supported the popularity of traditional treatments among patients as first line of option for treating mental illness. Mental health services in Malaysia often face competition from traditional healers. All three main ethnic groups in Malaysia have their own version of traditional healers; 'bomoh' for the Malays, 'vaidya' (ayurvedic physician) for the Indians, and 'sinseh' for the Chinese. Typical traditional treatments include the use of holy waters, prayers, religious rituals, herbs, massage etc. The popularity of traditional treatments could be influenced by the social stigma associated with mental illness, and cultural beliefs that accommodate the role of evil spirits, charms and other magico-religious factors in causing mental illness [19]. This is also understandable due to the relative lack of mental health resources and awareness in these countries. This is different from the pathways to care pattern observed in developed countries such as Japan [7], Canada [2], New Zealand [4] and United Kingdom [3] whereby health or social agencies are the more popular point of contacts prior to contact with psychiatric service.

In a developing country like Malaysia, whereby mental health professionals are very limited, we should consider having meaningful collaboration with traditional healers. This is in keeping with the Ministry of Health's policy to integrate traditional and complementary medicine in government hospitals. This had been started in 3 local hospitals; Kepala Batas Hospital in Pulau Pinang, Sultan Ismail Hospital in Johor Bharu, and Putrajaya Hospital for non-psychiatric disorders [17]. Some traditional healers may be helpful to formulate a more holistic concept of psychosis by integrating psycho-spiritual principles. The alternative neo-concept of psychosis may be more easily understandable

and acceptable for patients and family members, especially those from the lower socio-economic background.

Some studies have suggested that traditional treatments can be effective for treating neurosis among patients with mental disorders [12, 18]. Therefore, there can be mutual benefits when traditional healers and psychiatrists consent to collaborate with one another, even for psychotic disorders. Traditional healers can refer psychotic patients for acute management. On the other hand, psychiatrists can refer certain patients after a period of acute psychosis e.g. those with drug induced psychosis to traditional healers for follow-up and psycho-spiritual counseling. This may be better in terms of accessibilities and acceptance of treatment. In this way, workload of psychiatrists may also be reduced, without compromising on the effective care of patients with psychosis.

Direct access to psychiatric service (28%) was only the second most popular help-seeking pathway in this study after consultation with traditional healers (48%). The 3rd common pathway was through private general practitioners or government doctors in primary care clinics (24%). Overall, 34% of the respondents had seen at least one general practitioner prior to contact with psychiatric service. This is more or less similar to those reported in local and developing Asian countries e.g. University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia (15%) [13], Hospital Kubang Kerian, Kelantan, Malaysia (10%), Singapore (26%) [11], Bali, Indonesia (18%) [10], Hong Kong (27%) [9], and India (20%). This is in contrast to those reported in more developed countries e.g. Australia (50%) [1], New Zealand (70%) [4], France (70%) [20], and Germany (66%) [21], whereby awareness on mental illness is better.

As concluded in many other studies, this provides further support that general practitioners and family physicians are important gatekeepers to direct patients with psychosis to psychiatric care. Therefore, the ongoing programs for educating general practitioners on psychosis should be encouraged and strengthen. Besides recognition of psychosis, emphasis should also be on how to skillfully negotiate with patients with psychosis to seek psychiatric help.

The most common reason reported by family members for the delay in seeking psychiatric treatment was "not aware that changes were related to mental illness" (74%). This is consistent with the study in Hong Kong [8] whereby "lack of knowledge about psychosis" was the reason given by 74.3% of patients, and 54.3% of family members. The second most common reason was, "conflicting opinion with patient i.e. patient refused to seek help" (72%). Related to that, 54% of the patients were physically violent, and 68% were verbally abusive on admission; 46% of the patients had to be admitted with police assistance, and all hospitalizations were involuntary admissions. Other common reasons reported were, "believed that symptoms would improve spontaneously" (66%), "considered that symptoms were not serious enough for treatment" (56%), and "believed that a doctor cannot help the condition." (42%). These reasons are more or less similar to those reported earlier by Chiang in Hong Kong [8]. These notable findings suggest that factors related to lack of awareness on psychosis are central reasons for the delay in seeking psychiatric treatment.

These findings on treatment delay have several implications. First, family members need to be educated on recognizing psychosis at an early stage (when it is more easy to manage), and taught skillful ways of negotiating with patients to seek early psychiatric treatments. Second, they also need to know that seeking police help to bring patient for psychiatric treatment when other means have failed, is a wise option in long term, and they do not have to feel guilty about it. Third, dialogue between police officers and psychiatrists should be encouraged to highlight and appreciate the role of police officers in helping patients with psychosis, especially among those with violent behavior.

This study has several limitations. The sample size was small. It involved only in-patients in a highly urbanized area, many patients had substance-induced psychosis (26%), and all patients were admitted involuntarily. Therefore, the findings cannot be generalized to patients in other settings.

CONCLUSION

Contacts with traditional healers prior to consultation with psychiatric service were common among in-patients with first-episode psychosis in HKL. Treatment delay was mainly contributed by factors associated with lack of awareness on psychosis. More strategic mental health education program is needed for early detection and treatment of psychosis.

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Life Events and Parasuicides in Hospital Kuala Lumpur, Malaysia

[1]A Hamidin*, & [2]T Maniam,

[1]Department of Psychiatry, Faculty of Medicine and Health Sciences,
University Putra Malaysia, 43400 University Putra Malaysia, Selangor, Malaysia
[2]Department of Psychiatry, University Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia

ABSTRACT

Objective: The aim of this study is to compare the prevalence of life events among parasuicide patients with the prevalence of similar life events among age, sex and race matched patients with non-chronic medical illness. **Methods:** A hospital-based case-control study using convenience sampling method was conducted in Hospital Kuala Lumpur for a period of three and a half months. A total of 50 patients admitted consecutively after an episode of parasuicide and who fulfilled criteria for entry into the study agreed to participate. For each case one age-, sex- and race-matched control was selected from the list of patients who were admitted to the same hospital for non-chronic medical illness. **Result:** Statistical analysis showed that compared with medically ill patients, parasuicide patients had significantly higher prevalence of threatening life events six months ($p<0.001$) before their act and these life events were significantly concentrated in the last one month before the attempt ($p=0.001$). Among the seven categories of life events, cases had a significant excess of interpersonal problems ($p<0.001$) that included serious problems with a close friend, neighbour or relative, break-up of a steady relationship and separation due to marital difficulties. **Conclusion:** The results suggest that there is a high prevalence of life events among parasuicide patients when compared with medically ill patients especially during the month prior to their admission to the hospital. The data also indicate that there is a significant association between suicide attempts and interpersonal problems.

Keywords: Life events, Parasuicide, Case-controlled, Malaysia

INTRODUCTION

The term parasuicide is defined broadly to describe all non-fatal self-injurious behaviour with clear intent to cause bodily harm though the intention to die is often unclear. It is often used interchangeably, as in this paper, with the term suicide attempt though a strict definition of *suicide attempt* would include some indication of an intent to die. While the rate of parasuicide ranges between 2.6 and 542 per 100,000 in medically treated individuals, the lifetime prevalence from general population surveys is higher ranging between 700 and 1,100 per 100,000.[1] The risk of repetition of this behaviour is also very high with 40% of suicide attempters going on to repeat their act and 13% of them will do so in the year following their attempt.[2]

Suicide attempters commonly have psychosocial difficulties and frequently suffer from mental health problems. They are at high risk for future suicide attempts and completed suicide.[3] The global suicide rate is estimated to be about 16 per 100,000 or one death in every 40 seconds and has become the third leading cause of death among those aged 15-44 years in some countries.[4] Studies have shown that the risk of suicide following parasuicide is estimated around 3% after 10 or more years.[5, 6]

An increased rate of threatening or stressful life events in the recent history of individuals who attempt suicide or die by suicide have consistently been reported in many studies on suicidal behaviour[7] and its occurrence increases to a peak during the month preceding the attempt and is most marked in the week beforehand.[8] Earlier researchers have suggested that the main risk factor for parasuicide might have to do with interpersonal conflict, especially in relation to a partner.[9]

These findings have been supported by Beautrais *et al.* who found that individuals who made serious suicide attempts had elevated rates of life events mainly associated with interpersonal difficulties, work issues, financial difficulties, and legal problems.[7] In research looking at life events in young people, two sets of life events were commonly found to be associated with suicidal behaviour. These include relationship breakdowns; arguments with partners, family or friends; bereavement; and legal, forensic, disciplinary crises (including legal difficulties and charges and being in trouble with police).[10] Researchers have also found that these recent adverse life events are very important factors to determine future suicide risk because they make a moderate contribution to suicide attempt risk

*Corresponding author: *hamidin@medic.upm.edu.my*

with estimated odds ratio (OR) ranging from 1.3 to 15.8 (median=4).[11] In Malaysia, a retrospective study of 134 case notes of the patients who attempted suicide showed that interpersonal difficulties such as marital quarrels and other family conflicts were the prominent precipitating factors in 67.3% of cases.[12]

Although the relationship between life events and parasuicide has frequently been recognized in the local and international literature, we are not aware of any local study on life events using standardized life events questionnaire. Using a standardized questionnaire will ensure more reliable and comprehensive data collection. The aim of this study is to determine the prevalence of threatening life events among parasuicide patients compared to a control group of patients with acute medical illness.

METHODS

This case-control study was conducted in Hospital Kuala Lumpur (HKL), a 2300-bedded hospital and the largest hospital under the Ministry of Health of Malaysia, for three and a half months in 2004. Ethics approval for this study was obtained from the Ethics Committees of the Faculty of Medicine, National University of Malaysia (UKM Medical Centre) and the Ministry of Health.

The definition of parasuicide is based on WHO/EURO Multicentre Study of Parasuicide which defines parasuicide as 'an act with a non-fatal outcome in which an individual deliberately initiates a non-habitual behaviour, that without intervention from others will cause self-harm, or deliberately ingests a substance in excess of the prescribed or generally recognized dosage, and which is aimed at realizing changes that the person desires via the actual or expected physical consequences'.[13]

Sampling was carried out from consecutive series of admissions to the Hospital Kuala Lumpur following episodes of parasuicide. The cases were identified through a daily review of the admission book at the Emergency Department and psychiatric referral book at the Psychiatric Department. In HKL all the patients presented with parasuicide episode were routinely seen in the Emergency Department before they were admitted to the medical wards for further management and referral to the psychiatric clinic for psychiatric evaluation.

Exclusion criteria were: patients aged less than 16 or more than 65 years, not fit to be interviewed, diagnosed with psychotic disorder and not be able to converse in either Malay or English. A total of 50 cases was selected and they were interviewed after their doctors consider they were medically fit, often one or two days before they were discharged.

For each case one age-matched (± 5 years), sex- and race-matched control was selected from the list of patients who were admitted to the same hospital for non-chronic medical illness. The same exclusion criteria were applied to the controls as the cases. Additionally, patients with long-standing chronic illness (information was obtained from the case notes) and past history of parasuicide and mental illness were also excluded from the study. The interview was conducted by the first author who was trained in conducting structured interview and administering questionnaires in a room to provide comfort and privacy to the participants. Prior to the interview, written consent was obtained from the respondents and for the subjects who were under 18 years old consent was obtained from their parents or legal guardian.

To exclude psychotic disorders the Mini International Neuropsychiatric Interview (M.I.N.I)[14] was used. The interviewer was trained to use the M.I.N.I. by an experienced senior psychiatrist in using the instrument. Sociodemographic questionnaire was administered to obtain information about gender, age, ethnicity, marital status, educational level, employment status, past parasuicide history and psychiatric illness of the participants.

To record threatening life events experienced by the respondents we used self-rated English and Bahasa Malaysia versions of the 12-item Life Events Questionnaire (LEQ).[15] LEQ measured threatening life events that occur in the six months prior to the parasuicide. Each item was scored 1 if it was checked and 0 if not. A total score would be the sum of all items. For the purpose of analysis, the 12 common life events were grouped into seven categories: (1) personal issues, (2) family illness or bereavement issues, (3) interpersonal issues, (4) work issues, (5) financial issues, (6) legal issues (serious problems with the law or police) and (7) other life events.[7] The English version of the LEQ has high sensitivity and good test-retest reliability.[15] The English version of LEQ was translated into Bahasa Malaysia and back translated into English to ensure the context of questionnaire was preserved. However the Bahasa Malaysia version of LEQ is yet to be validated. Permission to use all the questionnaires was obtained from the respective authors.

Data from the present study were analyzed using the Statistical Package of the Social Sciences. The comparison between groups was carried out by using Pearson's Chi-square test or Fischer exact test. Yates' correction was used where the expected values were less than 5. Confidence interval and level of significance were set at 95% and 0.05 respectively.

RESULTS

A total of 50 patients with parasuicide and 50 medically ill inpatients aged between 17 and 53 years (median 24.5 years, 26.6 years, S.D. ± 8.7 years) were included in the study. Respondents were mainly women (78%). In term of

ethnicity, more than half (53%) of the respondents were Indians followed by Malays (40%) and Chinese (8%). A majority (70%) of the cases were either single or divorced compared to only 46% of the controls. This difference was significant (p=0.015). Thus most of the parasuicide cases were not married. As for employment status and educational level, the result of analysis did not find any significant differences between the two groups. A fuller account of the characteristic of the respondents, may be found in Hamidin *et al*. [16]

Table 1: A comparison of the number of cases and controls on the presence or absence of life events six months before the study

Life events	No. of cases n (%)	No. of controls n (%)	p value
Present	50 (100%)	31 (62%)	
Absent	0 (0%)	19 (38%)	<0.001
Total	50 (100%)	50 (100%)	

Table 2: A comparison of the number of cases and controls on the occurrence of specific life events six months before the study

Life Event Questionnaire	cases		Controls	
	n	(%)	*n*	(%)
You yourself suffered a serious illness, injury or an assault.	1	(2)	2	(4)
A serious illness, injury or assault happened to a close relative.	4	(8)	3	(6)
Your parent, child or spouse died.	0	0	5	(10)
A close family, friend or another relative (aunt, cousin and grandparent) died.	8	(16)	11	(22)
You had a separation due to marital difficulties.	7	(14)	1	(2)
You broke off a steady relationship.	10	(20)	3	(6)
You had serious problem with a close friend, neighbour or relative.	40	(80)	2	(4)
You became unemployed or you were seeking work unsuccessfully for more than one month.	12	(24)	9	(18)
You were sacked from your job.	3	(6)	2	(4)
You had a major financial crisis.	14	(28)	6	(12)
You had problems with the police and a court appearance.	0	0	0	0
Something you valued was lost or stolen.	8	(16)	5	(10)

Table 3: Comparison of the number of cases and controls on the occurrence of specific category life events six months before the study

	Life Events	cases		Controls		OR (95% CI)	*p*
		N	(%)	*n*	(%)		
1	Personal illness issues	1	(2)	2	(4)	0.5 (0.0,5.7)	1.000
2	Family illness or bereavement issues	11	(22)	18	(36)	0.5 (0.2,1.2)	0.185
3	Interpersonal issues	47	(94)	6	(12)	114.9 (27.1,487.6)	< 0.001
4	Work issues	12	(24)	10	(20)	1.3 (0.5,3.3)	0.810
5	Financial issues	14	(28)	6	(12)	2.9 (1.0,8.2)	0.078
6	Legal issues	0	0	0	0	-	-
7	Other life events	8	(16)	5	(10)	1.7 (0.5,5.7)	0.554

OR = odds ratio

CI = confidence interval

Table 4: Distribution of life threatening life events by month, prior to parasuicide among cases and controls

Month		1*	2	3	4	5	6	Total
No. of life events	Cases n (%)	68 (63.5%)	14 (13%)	6 (5.6%)	4 (3.7%)	2 (1.8%)	13 (12.1%)	107 (100%)
	Controls n (%)	11 (22.4%)	14 (28.6%)	6 (12.2%)	5 (10.2%)	2 (4.1%)	11 (22.4%)	49 (100%)

*p <0.001

Life events in the last six months prior to the study

All the cases reported that they had experienced at least one threatening life event in the past 6 months compared to the controls (62%) (Table1). This difference was highly significant (p<0.001). Table 2 shows among the cases the most commonly reported threatening life events were serious problems with a close friend, neighbour or relative (80%), major financial crisis (28%), became unemployed or unsuccessful in looking for job for more than one month (24%) and broke off a steady relationship (20%). On the other hand, the controls reported bereavement after close family, friend or another relative (aunt, cousin and grandparent) died (22%), became unemployed or unsuccessful looking for job for more than one month (18%) and major financial crisis as their most commonly experienced threatening life events (12%).

For the purpose of analysis the 12 threatening life events were categorized into seven categories (Table 3). In the 6-month period before parasuicide, the cases reported that they had more threatening life events in the categories of interpersonal issues (94%), financial issues (28%) and work issues (24%). In contrast, the controls experienced more life events related to family illness or bereavement (36%) and work (20%). Parasuicide cases had experienced a

significant excess of interpersonal problems ($p<0.001$) compared to the controls. However, no significant differences were observed between the cases and the controls on categorized life events related to personal illness, family illness or bereavement, work issues, financial issues, legal issues and other life events.

Threatening life events one month before the study

Table 4 illustrates monthly distribution of the number of life events six months before the study. During this period the cases experienced 107 life events while the control group had only 49 life events. Of the 107 life events, the majority (63.5% or 68/107) had happened in the last month prior to the parasuicide event. During the same period, the control group experienced only (22.4% or 11/49) life events. This finding showed that the cases had a significantly higher number of life events in the one month before the act ($p<0.001$). Additionally, we noticed an interesting phenomenon in that there was an unexpectedly high frequency of life events in the 6th month prior to parasuicide when it would be expected to be lower than the months closer to the parasuicide. This could be due to subjects' recollection bias resulting in clumping together of life events that had happened prior to the 6 months.

DISCUSSION

The main findings in this study are: a majority of the respondents were young with the mean age of 26.6 ± 8.7. Respondents were mainly women (78%). In terms of ethnicity respondents were mainly Indians (53%) followed by Malays (40%) and Chinese (8%). A majority of the cases were either single or divorced (70% versus 46% of the controls). The parasuicide cases had significantly higher prevalence of threatening life events compared to the medically ill patients during the six-month period before their act. The results of this study also revealed that more than three-fifth of the life events that were experienced by the cases happened in the last one month before the act of parasuicide.

This finding is in agreement with the study by Beautrais[10] that found "people who attempted or committed suicide had higher rates of exposure to adverse or stressful life events in the period preceding their act." This finding is also similar with the earlier study undertaken by Paykel *et al*.[8] which concluded that parasuicides "had greater number of life events in the six months preceding their act and the frequency of occurrence increased to reach a peak in the last month before the attempt." Furthermore, our finding is almost similar to the study that investigated life events associated with parasuicide in the African context conducted at three general hospitals in Kampala, Uganda[17] which found a statistical difference between the cases and the controls on the total number of life events.

Threatening life events in the category of interpersonal difficulties (including relationship breakdowns) were significantly associated with parasuicide in this study. Among the cases the commonly reported threatening life events in this category included serious problems with a close friend, neighbour or relative, broke off a steady relationship and a separation due to marital difficulties This finding is similar to the earlier study by Beautrais *et al*.[7] that found interpersonal difficulties as a significant contributory factor to the risk of suicide attempt. The same pattern was also reported in a case-controlled study of negative life events in Uganda where partner-related interpersonal difficulty such as being emotionally mistreated was found to be significantly associated with parasuicide.[17] Locally, a retrospective study that reviewed medical records of parasuicide cases also found life events such as interpersonal difficulty as prominent reasons for the victims to carry out the act.[12]

Another interesting finding is that the present study was unable to confirm the association between life events related to work issues and parasuicide. This finding is in contrast to the review paper by Platt *et al*.[13] where overwhelming numbers of work issues particularly unemployment was associated with the parasuicide attempt and unemployment increased the risk of parasuicide. However, the result of our study is similar to the conclusion made by the recent review of several case control studies that found unemployment and parasuicide were not significantly related after controlling all the confounding factors.[11]

In addition, this study also found no significant association between life events such as personal illness, family illness and bereavement and parasuicides. One explanation is that our sample consisted mainly of younger people (a majority of the respondents were less than 34 years old). Generally, personal and family illnesses, and bereavement occur less commonly in this age group. This was supported by the previous studies that suggested these categories of life events may change with age; interpersonal difficulties and losses being more significant precipitant factors among younger people while suicidal behaviour in the elderly was found to be related to physical health problems.[18, 19]

Contrary to the finding of Beautrais *et al*.[7] who found life events like legal difficulties and charges, and getting into trouble with police were significant class of life events that were associated with parasuicide, the present study did not find such an association. Possible explanations for the different finding of this study are the small sample size resulting in a lack of statistical power, and that our society being somewhat less litigation conscious may be experiencing less legal problems overall.

The authors acknowledge that this study could not ascertain whether life events that experienced prior to the

parasuicide were independent of or caused by other factors such as sociodemographic factors, childhood and family experiences, personality traits, and psychiatric disorder.[7]

This study provides several important findings to help clinicians and mental health professionals understand the importance of life events in fuelling suicidal behaviour. Firstly, it is important that the management of individuals presenting with parasuicide includes crisis intervention aimed at resolving the precipitating crisis. Since many parasuicides are triggered by interpersonal problems, there is an obvious necessity to offer support and treatment to family members, partners as well as the parasuicide cases themselves to lessen their emotional burden. Thus, emergency doctors and mental health personnel need to be equipped with skills in crisis interventions as well as problem solving techniques.[20]

There is also a need for increased mental health support and involvement immediately after the parasuicide episode as well as after hospital discharge. The intervention such as the use of a token (green card) which allow immediate readmission to the hospital following a suicide attempt is helpful by allowing the patient access to the service where they can communicate their distress without resort to self-harm behavior again.[21] Preventive mental health initiatives such as improving family communication and problem solving especially among patients without major depression may be warranted.[22]

In addition, suicide prevention programs directed toward the general public and school should be promoted as an effective strategy to reduce suicidal behaviour. The school intervention programs that promote prosocial norms such as social competence, decision making, family connections and school bonding have been reported to reduce suicidal thoughts and plans.[23, 24] Previous research study also found empirical support for the long term community suicide prevention programs through education that utilise multiple levels of society commitment to establish community support network was effective in reducing suicidal rates.[25]

Finally, the present finding also suggests the need for investment in educational programs to increase the awareness of the public on the importance of managing stress, conflicts and other factors that are associated with mental health problems. Promoting increased sense of wellbeing by introducing wellness programs and stress management at the work place as well as programs to minimize avoidable life events (change of jobs, relocation and buying a house) or space them to a reasonable time frame can enhance adaptive coping strategies to deal with daily life stressors as well as promote life satisfaction.

We should note several limitations in this study and our findings and conclusions should be interpreted in the light of these limitations. Firstly, this study was based on hospital data, which focuses on medically treated parasuicides. In hospital based study, the finding might be biased towards patients with history of self-poisonings and self-cuttings that require admission and may miss a substantial number of people who do not seek treatment. Secondly, being a cross-sectional study the information was obtained retrospectively. The subjects' report on life events may be influenced by their current or chronic mental state or personality traits. Future studies might need to check the validity of the occurrence of life events reported by the patients with that of significant others to address subjects' recall bias.[7] Thirdly, the modest number of subjects in this study restricts the generalization and strength of the significance of the data. Finally, this study used self-report questionnaire, which, though translated and back translated, was not validated for the population of Malaysia.

CONCLUSION

The study seemed to indicate that prevalence of threatening life events was significantly increased in cases compared to control groups especially in the month prior to parasuicide episode with interpersonal problem has been found to be the significant category of life events that fuels the parasuicide episode. The current research findings point toward some implications for suicide prevention and intervention programs directed toward the public and schools as well as a need for emergency and mental health staff to be equipped with interventional skills to manage parasuicides at Emergency Department as well immediate post-discharge period.

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Prevalence of Hypertension Among Malay Adolescents in Putrajaya Secondary Schools, Malaysia, 2010

L Rampal*, KC Ng, I Nur Izzati, Z Farah Izzati, I Mohammad Nazrul, I Faisal, SY Sharifah Zainiyah

Department of Community Health
Faculty of Medicine and Health Science, Universiti Putra Malaysia,

ABSTRACT

Background: In Malaysia, the prevalence of hypertension amongst adults aged 30 years and above has increased from 32.9% in 1996 to 40.5% in 2004 and to 42.6% in 2006. Information on the prevalence of hypertension among adolescents is lacking. **Objective:** to determine the prevalence of hypertension among Malay secondary school students in Putrajaya. **Methods:** A cross sectional study was carried out in Putrajaya, Malaysia. The sampling frame consisted of a list of all the 12 secondary schools in Putrajaya. Three schools were selected using table of random numbers. All Malay students aged 13 years old to 17 years old from the three selected school students were included in the study. Blood pressure was measured after the respondents had rested for at least 5 minutes using a standard mercury sphygmomanometer. Three blood pressure measurements were taken for each respondent. Systolic blood pressure [SBP] was defined as the average of three SBP readings and diastolic blood pressure [DBP] was defined as the average of three DBP readings. Data was analyzed using SPSS 18. **Results:** The overall mean SBP and DBP were 108.9 mmHg and 63.2 mmHg respectively. The prevalence of pre-hypertension and hypertension among the male was 16.2% and 12.9% respectively as compared to 5.8% and 10.2% respectively in the females. The overall prevalence of prehypertension and hypertension was 11.1% and 11.6% respectively. The prevalence increased with age ($p<0.05$). There was a significant positive correlation between BMI and SBP ($r = 0.52$, $r^2 = 0.27$, $p= 0.001$) and BMI and DBP ($r = 0.38$, $r^2 = 0.15$, $p= 0.001$). The mean SBP was significantly higher in males (111.7 mmHg) as compared to 106 mmHg in females ($p<0.001$). The mean DBP in males (63.5 mmHg) was slightly higher as compared to 62.9 mmHg in females but the difference was not significant. **Conclusions:** Prevalence of hypertension and pre-hypertension is high. There is an urgent need for implementation of a comprehensive CVD prevention program and routine blood pressure measurements should be taken in school children to improve the detection, prevention and treatment of hypertension

Keywords: Prevalence, Hypertension, Adolescents, Putrajaya, Malaysia

INTRODUCTION

Cardiovascular diseases are now responsible for 30% of all deaths worldwide [1-2] . Hypertension is the most frequent treatable risk factor[3]. The prevalence of hypertension has been widely reported in various regions of the world [4-5]. It is ranked third as a cause of disability-adjusted life-years and is a leading risk factor for mortality[6]. By 2025, 1.56 billion people are expected to have hypertension [7]. In Malaysia, the prevalence of hypertension amongst adults aged 30 years and above has increased from 32.9% in 1996[8] to 40.5% in 2004[9] and to 42.6% in 2006 [10]. Elevated blood pressure tracks relatively well from youth to adulthood, making blood pressure in youth a useful predictor of essential hypertension in adulthood [11]. The objective of this study was to determine the prevalence of hypertension and factors associated among Malay secondary school students in Putrajaya.

MATERIALS AND METHODS

A cross sectional study was carried out in Putrajaya, Malaysia. The sampling frame consisted of a list of all the 12 secondary schools in Putrajaya. Three schools were selected using table of random numbers. All Malay students aged 13 years old to 17 years old from the three selected school students were included in the study. Trained interviewers administered a pre-tested validated questionnaire to obtain data on age, gender, family history of hypertension. A standardised format questionnaire was used to collect the data on age, gender family history of hypertension. The interviewer obtained verbal consent from the subjects before administrating the interview.

*Corresponding author: *rampal@medic.upm.edu.my*

Blood pressure measurements

Blood pressure was measured after the respondents had rested for at least 5 minutes using a standard mercury sphygmomanometer. The respondents were examined in a seated position with the arm placed at the heart level. Three blood pressure measurements were taken for each respondent. Systolic blood pressure [SBP] was defined as the average of three SBP readings and diastolic blood pressure [DBP] was defined as the average of three DBP readings. The average of the three values was used in the analysis. The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents was modified and used in this study to classify the respondents [12]. In this study respondents were categorized as normal if the average SBP or DBP levels was less than the 90th percentile and prehypertension if the average SBP or DBP levels was greater than or equal to the 90th percentile, but less than the 95th percentile. The respondents were categorized as hypertension if the average SBP and/or DBP that was greater than or equal to the 95th percentile for sex, age, and height.

Anthropometric measurements

Weight was recorded using the digital bathroom scale TANITA model HD-312 weighing machine on which the students were made to stand. This digital bathroom scale has scale marked in kilogram and measures weight to the nearest 0.2 kilogram. Two measurements were taken for both weight and height and the average of the two values were used in the analysis. After each respondent, the weighing machine was reset to zero. It was checked frequently by the use of a known weight. The students were requested to stand barefoot on the middle of the weighing machine, with the head looking straight in front, arms by the side and with only basic clothing. When the reading of the weighing machine was stable, the weight was recorded. Height was measured by using SECA Body meter Model 208 (made in Hamburg). The accuracy of this device is up to 0.05 centimeter. Height was measured by suspending the SECA bodymeter, two meters high from the floor against a straight wall, parallel to either doorframe or pillar. The student was then requested to stand barefoot under the center of the measuring tongue of the body meter without cap or songkok and then to lean against the wall with the back and head looking straight ahead so that an imaginary plane that would connect the eyes and ears were parallel to the floor. The student's heels were made to rest together against the wall or pillar, and the hands were loosely by the sides. The measuring tongue was lowered towards the head until it gently touched the head. Height measurement that appeared in the read-off area was then recorded.

Data Analysis

Statistical analysis was carried out using SPSS version 18. Categorical variables were presented as frequencies and percentages. The Pearson's chi-square test (x^2) test was used to determine the associations between categorical variables. Continuous variables were presented as means with their 95% confidence interval (CI) and standard deviation. Pearson correlation coefficient was performed to determine the correlation between two continuous variables. Independent sample t-test was used to compare the means of two independent continuous variables. The one-way Analysis of Variance (ANOVA) was used to test for differences among at least three groups. Post Hoc - Tukey test was used to perform multiple comparisons. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

A *p*-value of < 0.05 was considered as statistically significant.

RESULTS

Out of 1900 selected students aged 13-17 years old in the 3 selected schools, 1778 (93.6%) participated in the study. Non-respondents were those who were absent from class or those who did not give the consent to participate in the research. Table 1 shows the socio-demographic characteristics of respondents. Among the 1778 respondents, 899 (50.6%) of them were males. There was no significant difference in the mean age between males 14.7 (95% CI =14.7 - 14.8) years and 14.8 (95% CI =14.7-14.9) years respectively in the females (t= -0.67, df= 1776, p= 0.50). Out of the 1778, 40.4% had a family history of hypertension.

Blood pressure measurements

Table 1 and 2 show the mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) according to age and gender. The overall mean SBP and DBP for 1778 respondents was 108.9 and 63.2 mmHg respectively. Among the males, the mean SBP significantly increased with age except at 17 years, wherein there was a marginal decline in SBP (-0.3). For the females, the SBP also increased with age except at 14 years when there was a fall in SBP (- 1.0) mmHg. The DBP increased with age from 13 to 17 years in both sexes. The increase in the mean systolic

blood pressure with age was significant both in males (One way ANOVA T-test (F= 18.95, df= 4, p<0.001) and females (F= 4.54, df= 4, p= 0.001). Using Post Hoc - Tukey test to perform multiple comparisons between all the age groups for males showed that there was a significant difference in the mean systolic blood pressure levels between age group 13 and 15 (p = 0.001), 13 and 16 (p = 0.001), 13 and 17 (p = 0.001), 14 and 16 (p = 0.001), 14 and 17 (p = 0.001), 15 and 16 (p = 0.003), 15 and 17 (p = 0.005). However, there was no significant difference in the mean systolic blood pressure levels between age group 13 and 14 (p = 0.08), 14 and 15 (p = 0.43), 16 and 17 (p = 0.99). For females, significant difference in the mean systolic blood pressure was only found between age group 14 and 16 (p = 0.01), and between age group 14 and 17 (p = 0.01). The increase in the mean diastolic blood pressure with age was significant in males (One way ANOVA test F= 26.39, df= 4, p <0.001) but not in females (F= 2.11, df= 4, p=0.08). Using Post Hoc - Tukey test to perform multiple comparisons between all the age groups for males showed that there was a significant difference in the mean diastolic blood pressure levels between age group 13 and 14 (p = 0.007), 13 and 15 (p = 0.001), 13 and 16 (p = 0.001), 13 and 17 (p = 0.001), 14 and 15 (p = 0.002), 14 and 16 (p = 0.001), 14 and 17 (p = 0.001), 15 and 17 (p = 0.03). For females, there was no significant difference in the mean diastolic blood pressure between any age group (p > 0.05). The mean SBP was significantly higher in males 111.7 (95% CI = 110.8 – 112.5) mmHg as compared to 106 (95% CI = 105.6 – 112.2) mmHg in females (t= 9.5, df= 1776, p= 0.001). The difference in the mean diastolic blood pressure in males (63.5, 95% CI = 62.8 - 64.2 mmHg) was slightly higher as compared to 62.9, (95% CI = 62.3 – 63.6 mmHg) in females but the difference was not statistically significant (t= 1.2, df=1776, p= 0.23).

Table 1: Mean systolic blood pressure levels according to age and gender

Gender / Age (Years)	Number of respondents	Systolic blood pressure (mmHg)		
		Mean	95% CI	Std. Deviation
Male				
13	180	106.8	104.8-108.7	13.3
14	251	109.9	108.4-111.4	12.0
15	213	111.8	110.3-113.4	11.7
16	120	116.9	114.8-119.0	11.7
17	135	116.5	114.2-118.8	13.5
Total	899	111.7	110.8-112.5	12.9
Female				
13	168	105.1	103.3-107.0	12.1
14	204	104.3	102.7-105.8	11.2
15	251	105.5	104.0-106.9	11.6
16	152	108.4	106.4-110.4	12.6
17	104	108.9	106.5-111.3	12.4
Total	879	106.0	105.2-106.8	12.0
Both Gender				
13	348	106.0	104.6 – 107.3	12.7
14	455	107.4	106.3 – 108.5	12.0
15	464	108.4	107.3 – 109.5	12.1
16	272	112.2	110.6 – 113.7	12.9
17	239	113.2	111.5 – 115.0	13.6
Total	1778	108.9	108.3 – 109.5	12.8

Table 2: Mean diastolic blood pressure levels according to age and gender

Gender / Age (years)	Number of respondents	Diastolic blood pressure (mmHg)		
		Mean	95% CI	Std. Deviation
Male				
13	180	58.2	56.8-59.7	10.1
14	251	61.6	60.3-63.0	10.7
15	213	65.2	63.8-66.6	10.1
16	120	66.8	65.1-68.6	9.7
17	135	68.4	66.7-70.2	10.3
Total	899	63.5	62.8- 64.2	10.8
Female				
13	168	62.0	60.6-63.4	9.1
14	204	62.4	61.2-63.6	9.0
15	251	62.9	61.7-64.0	9.0
16	152	63.2	61.5-65.0	10.8
17	104	65.2	63.4-67.0	9.2
Total	879	62.9	62.3-63.6	9.4
Both Gender				
13	348	60.1	59.0 – 61.1	9.8
14	455	62.0	61.1 – 62.9	10.0
15	464	63.9	63.1 – 64.8	9.6
16	272	64.8	63.6 – 66.1	10.4
17	239	67.0	65.8 – 68.3	9.9
Total	1778	63.2	62.8 – 63.7	10.1

Prevalence of hypertension by age, gender, family history and nutritional status

Table 3 shows that the overall prevalence of pre-hypertension and hypertension was 11.1% and 11.6% respectively. The prevalence increased with age. From age 14 years to 16 years and there was a significant linear increase in trend in the prevalence of hypertension with age ($\chi 2$ for linear trend = 4.1, p =0.04). The data was than reanalysed by combining the pre-hypertension and hypertension group into one and compared to the normal group by age. The results showed that there was a significant association between prevalence of pre-hypertension/ hypertension and age ($\chi 2$=36, df= 4, p =0.001) and there was a linear increase in trend in the prevalence from age 14 to 17 years ($\chi 2$ for linear trend = 13.8, p =0.001). Among the male, the prevalence of pre-hypertension and hypertension was 16.2% and 12.9% respectively. The prevalence of pre-hypertension and hypertension in the female was 5.8% and 10.2% respectively. The results showed that there was a significant association between prevalence of hypertension and gender ($\chi 2$= 56.3, df= 2, p= 0.001).

Family History of Hypertension

Table 3 also shows that out of the 1778 respondents, 719 (40.4%) had family history of hypertension. The prevalence of hypertension was higher among those who had family history (12%) as compared to those who had no family history of hypertension (11.3%). However this difference was not statistically significant (p >0.05). There was no significant difference in the mean systolic blood pressure among respondents with family history and respondents without family history (t= -0.23, df= 1776, p= 0.81). The results also showed that there was no significant difference also in the mean diastolic blood pressure among respondents with family history and respondents without family history (t = -1.85, df= 1776, p= 0.06).

Table 3: Prevalence of hypertension according to age, gender, family history and nutritional status

Characteristic	Prevalence of Hypertension			
Gender /Age (years)	Normal	Pre-hypertension	Hypertension	Total
Male				
13	142(78.9%)	20(11.1%)	18(10.0%)	180
14	198(78.9%)	22(8.8%)	31(12.4%)	251
15	150(70.4%)	33(15.5%)	30(14.1%)	213
16	67(55.8%)	36(30.0%)	17(14.2%)	120
17	80(59.3%)	35(25.9%)	20(14.8%)	135
Total	637(70.9%)	146(16.2%)	116(12.9%)	899
Female				
13	143(85.1%)	7(4.2%)	18(10.7%)	168
14	181(88.7%)	9(4.4%)	14(6.9%)	204
15	211(84.1%)	18(7.2%)	22(8.8%)	251
16	121(79.6%)	8(5.3%)	23(15.1%)	152
17	82(78.8%)	9(8.7%)	13(12.5%)	104
Total	738(84.0%)	51(5.8%)	90(10.2%)	879
Both Gender				
13	285 (81.9%)	27(7.8%)	36(10.3%)	348
14	379(83.3%)	31(6.8%)	45(9.9%)	455
15	361(77.8%)	51(11.0%)	52(11.2%)	464
16	188(69.1%)	44(16.2%)	40(14.7%)	272
17	162(67.8%)	44(18.4%)	33(13.8%)	239
Total	1375(77.3%)	197(11.1%)	206(11.6%)	1778
Family History				
Yes	557 (77.5%)	76 (10.5%)	86(12%)	719
No	818 (77.3%)	121(11.4%)	120 (11.3%)	1059
Nutritional status				
Lean	172 (91.5%)	10 (5.3%)	6 (3.2%)	188
Normal	971 (83.8%)	113(9.7%)	75 (6.5%)	1159
Risk of Overweight	149 (66.8%)	37 (16.6%)	37 (16.6%)	223
Overweight	83 (39.9%)	37 (17.8%)	88 (42.3%)	208

Prevalence of overweight and association between BMI and blood pressure

The prevalence of 'at risk of overweight' and overweight was 12.5% and 11.7% respectively. There was a significant correlation between BMI and SBP ($r = 0.52$, $r^2 = 0.27$, $p= 0.001$) and DBP ($r = 0.38$, $r^2 = 0.15$, $p= 0.001$). The results also showed that the prevalence of hypertension was significantly higher in the children who were overweight and those 'at risk of overweight' as compared to those children who were normal or lean (Table 3).

DISCUSSION

In Malaysia, cardiovascular diseases have been the leading cause of death for the past 40 years [9]. The mortality, morbidity and disability attributable to non-communicable diseases is currently high and continues to grow. The prevalence of hypertension in adults is high; there is low level of awareness, low treatment rates and poor control

of hypertension [10]. Only 35.8% of respondents with hypertension were aware that they had hypertension and only 31.4% were currently being treated, and only 8.2% had their blood pressure under control [10]. The prevalence of risk factors for hypertension continues to show an increasing trend. The prevalence of obesity amongst Malaysians 18 years and above has increased from 4.4% in 1996 [8] to 12.3% in 2004 [13]. and 14.2% in 2006 [10]. In a study carried out by Rampal *et al.* in 2005 among 3,333 school children aged 13-17 years in the Klang district, showed that the prevalence of 'at risk of overweight' and 'overweight' was 11.4% and 8.2% respectively [14]. Hypertension is an established risk factor and contributes substantially to cardiovascular disease incidence and premature mortality [15]. In the next 10 years, China, India, and Britain will lose $558 billion, $237 billion, and $33 billion, respectively as a result of largely preventable heart disease, strokes, and diabetes [16-17]. Increased blood pressure levels during childhood strongly predict hypertension in young adulthood [18-19]. Among adolescents and young adults; elevated blood pressure is also associated with the presence of early atherosclerotic lesions[20]. In our current study, the prevalence of pre-hypertension and hypertension among the male was 16.2% and 12.9% respectively. The prevalence of prehypertension and hypertension in the female was 5.8% and 10.2% respectively. The overall prevalence of pre-hypertension and hypertension was 11.1% and 11.6% respectively, which is high. The prevalence of 'at risk of overweight' and overweight was 12.5% and 11.7% respectively. The prevalence of hypertension was significantly higher in the adolescents who were overweight or at risk of being overweight. The results also showed that there was a significant correlation between BMI and hypertension ($r = 0.52$, $r^2 = 0.27$, df=2, $p= 0.001$). Obesity, glucose intolerance, and hypertension in childhood were strongly associated with increased rates of premature death from endogenous causes in this population [21]. Even in USA blood pressure has increased over the past decade among children and adolescents. This increase is partially attributable to an increased prevalence of overweight [22-23]. In a study carried out among school children aged 8 years to 13 years in Fort Worth, Texas, Urrutia-Rojas *et al.* [24] reported an overall, the prevalence of HBP was 20.6 % and the likelihood of having hypertension was three times higher for overweight children in their study($p < 0.001$). In another study, school-based screening was performed in 5102 students aged 10 to 19 years in 8 Houston public schools from May 2002 to November 2002. The prevalence of elevated blood pressure on first screen was 19.4% [25]. Liang, Ya-Jun *et al.* [26] also reported an upward trend in blood pressure and hypertension in Chinese children and adolescents. The prevalence of pre-hypertension and hypertension increased dramatically from 1991 to 2004, with average relative increases of 6.38% and 8.13% in children and adolescents, respectively. Sharma *et al.* [26] reported that 5.9% schoolchildren aged 11-17 years in Shimla, India had hypertension and 12.3 % had prehypertension. In our study, both systolic and diastolic pressures showed a rising trend with age in both sexes. Similar results have also been reported in many other studies [28-29]. Multiple long-term cohort studies and randomized clinical trials have shown that the risks from raised blood pressure can be partially reversed [30-31]. Implications of this study are that the results show that the risk factors for cardiovascular diseases are already present in school children aged 13 to 17 years in Malaysia. These findings have been highlighted to the members of Ministry of Health staff. There is a need to develop, implement and evaluate effective national policies and sustainable practice for work on healthy eating and physical activity in schools. Second, early adolescents need better education on the need for healthy eating and physical activity in schools in a positive and sustainable way. A comprehensive approach involving behavioral modification intervention both in schools and in the community should be implemented. Involvement of the people at all levels especially those in policy-making and implementation is important. Interventions in schools to increase physical activity should ensure activities are challenging and fun and everyone should be able to participate. Dietary programmes should be integral to both the prevention and management program. There must also be a broader adherence to daily intake of fruit, vegetables, fish and fatty acid composition. School policy should address health-related issues relating to children who are hypertensive's, overweight or physically inactive and not only addressing factors in individual children's lifestyles but also those in the school environment, both physical and social. The Parent Teachers Association could be used as a vehicle for the headmasters, teachers, school health nurses, physicians/dentists and the canteen owners to work cooperatively with parents and the community to achieve the common goal of providing school children with the programs and environment necessary to promote health and improve learning. The parents, teachers and students need to be empowered and we must enable them to have greater control over efforts to improve their health. Third, capacity building and leadership of teachers, health care workers, community leaders and non-government organizations need to be enhanced. The Malaysian Health Promotion Board (MHPB) which is a statutory body established under an Act of Parliament of Malaysia Act 2006 can be approached to assist in capacity building [32]. Lastly, routine blood pressure measurements in school children should be carried out every year. School children with pre-hypertension and hypertension should be referred to the physician for management. This would help to improve the detection, prevention and treatment of hypertension. In conclusion, prevalence of hypertension and pre-hypertension is high among school children aged 13-17 years in Putrajaya. There is an urgent need for implementation of a comprehensive CVD prevention program and routine blood pressure measurements should be taken in school children to improve the detection, prevention and treatment of hypertension.

ETHICAL APPROVAL

Approval from the Faculty of Medicine and Health Science, University Putra Malaysia human research committee was received before commencement of the study. Permission was also obtained from the Ministry of Education, Malaysia. Informed consent was also obtained from the each respondent before data was collected.

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Prevalence of Hypertension and its Associated Factors Among University Staff

[1]L Rampal*, [2]AB Somayeh, [1]MS Salmiah, [1]I Faisal & [1]SY Sharifah Zainiyah

[1]Consultant Fellow, Department of Community Health, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

[2]Masters Student, Department of Community Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Introduction: In Malaysia, cardiovascular diseases (CVD) have been the leading cause of death for the past 40 years. Hypertension is the leading treatable risk factor for CVD mortality. **Objectives:** to determine the prevalence and factors associated with hypertension among University Putra Malaysia staff. **Methods:** A Cross sectional study design was used in this study. The sample was selected using table of random numbers. Two blood pressure measurements were taken from respondents aged 30 years and above. Data on socio-demographic variables and lifestyle-related risk factors were collected using a pre-tested structured questionnaire. Weight and height measurements were also taken. **Results:** Out of 517 respondents selected, 454 subjects agreed to participate, giving a response rate of 87.8%. The overall mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) for 454 respondents was 126.2 mmHg and 80.17 mmHg respectively. The mean SBP was significantly higher in males (129.68 mmHg) as compared to the females (122.65 mmHg). The mean SBP and DBP significantly increased with age in both males and females ($p<0.05$). There was a significant relationship between SBP and BMI ($r = 0.55$, $r^2 = 0.30$ $p <0.001$) and diastolic blood pressure and BMI ($r = 0.53$, $r^2 = 0.28$, $p <0.001$). The overall prevalence of hypertension was 34.4% and 33.9% had pre hypertension. Hypertension was significantly associated with age, gender, family history of hypertension, BMI and alcohol consumption. **Conclusions:** Prevalence of hypertension and pre-hypertension is high. There is an urgent need for implementation of a comprehensive CVD prevention program. Routine blood pressure measurements should be taken to improve the detection, prevention and treatment of hypertension.

Keywords: Hypertension, Prevalence, Risk Factors, University Staff,

INTRODUCTION

Cardiovascular disease (CVD) is responsible for 30% of all deaths worldwide[1]. CVD mortality is likely to continue to increase in developing countries, if no appropriate action is taken[2]. In Malaysia, CVD has been the leading cause of death for the past 40 years[3]. The burden of mortality, morbidity and disability attributable to CVD is currently high and continues to grow. The most important risk factors for cardiovascular diseases are hypertension, obesity, high blood cholesterol, cigarette smoking, diabetes, physical inactivity and stress. Hypertension is the leading treatable risk factor for CVD mortality as it has been widely reported in various regions of the world[4-6]. It is ranked third as a cause of disability-adjusted life-years and is a leading risk factor for mortality and 1.56 billion people are expected to have hypertension by 2025[7]. It causes more than seven million deaths every year worldwide[5, 6]. In Malaysia, the prevalence of hypertension amongst adults aged 30 years and above has increased from 32.9% in 1996[8] to 40.5% in 2004[3] and to 42.6% in 2006[9]. In Malaysia, it has been estimated that there are 4.8 million Malaysian residents who have hypertension[10]. From an economic perspective, the costs attributed to hypertension are substantial. It was estimated that about 10% of global healthcare expenditures went on suboptimal blood pressure in 2001[10]. Valid information regarding the number of individuals affected by hypertension is the starting point for public health policy makers to direct the efforts to make the population aware of their condition and have them treated. Screening for hypertension is straightforward and not only detects hypertension but also provides an opportunity for patient learning and treatment[11]. The objective of this study was to determine the prevalence of hypertension and factors associated among university staff.

MATERIAL AND METHODS

Study location/study design

This cross sectional study was carried out in Universiti Putra Malaysia (UPM) which is situated 22 km south of

*Corresponding author: *rampal@medic.upm.edu.my*

Kuala Lumpur, the capital of Malaysia and 12 km from Putrajaya, the new and ultra modern administrative seat of the Malaysian government. The university was established in 1931 and consists of 16 faculties and 9 institutes.

Study Population/ Sampling Frame/ Sample Size/Sampling Technique

The study population of this study was all Malaysian UPM staff aged ≥30 years. The estimated sample size was 517. The complete lists of all staff of both genders aged ≥30 years served as sampling frame. Simple random selection techniques using the table of random numbers were used to select the sample.

Instruments and procedures

A pre-tested validated questionnaire was used to obtain data on age, gender, ethnicity, education, family history of hypertension, self history of hypertension, smoking status, physical activity, alcohol consumption, awareness of hypertension and antihypertensive treatment.

Blood pressure measurements

Blood pressure was measured after the respondents had rested for at least 5 minutes using a standard mercury sphygmomanometer. The respondents were examined in a seated position with the arm placed at the heart level. Two blood pressure measurements were taken for each respondent. Systolic blood pressure [SBP] was defined as the average of the two SBP readings and diastolic blood pressure [DBP] was defined as the average of the two DBP readings. The average of the two values was used in the analysis. Respondents were classified as having normal blood pressure if they had a mean SBP <120mmHg, and mean DBP <80, prehypertension if they had a mean SBP 120 to 139 mmHg or mean DBP 80 to 89 mmHg. and hypertensive if they had a mean SBP ≥140 mmHg, and/or mean diastolic blood pressure (DBP) ≥90 mmHg and/or by self-reports of a medical diagnosis of hypertension and current treatment for hypertension with antihypertensive medication. Hypertension awareness was defined as a positive answer to the question 'Have you ever been told by a doctor that you have high blood pressure (hypertension)'.

Body Mass Index (BMI)

Weight was measured using a digital bathroom scale (TANITA Model HD 319), calibrated before use. Height was measured using a SECA Body Meter Model 206. Height was measured to the nearest 0.1 cm with the subject without shoes and weight was measured to the nearest 0.5 kg with the subject in light clothing. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Respondents were classified as obese if their BMI was 30 kg/m^2 or higher, in accordance with World Health Organization's recommendation[12].

Smoking status

Current cigarette smoking status was classified into three categories according to current, never smoker and ex-smokers (has smoked before but has not smoked in the past 1 month). The smokers were also classified as light smokers (less than 10 cigarettes/day), moderate smokers (10 – 20 cigarettes/day) and heavy smokers (>20 cigarettes/day).

Alcohol consumption

For alcohol consumption, persons were classified into three groups: never a drinker, former drinker and current drinker.

Statistical analysis

Statistical analysis was carried out using SPSS version 17. Categorical variables were presented as frequencies and percentages. The Pearson's chi-square test ($\chi 2$) test was used to determine the associations between categorical variables. Continuous variables were presented as means with their 95% confidence interval (CI) and standard deviation (SD). Pearson correlation coefficient was performed to determine the correlation between two continuous variables. Independent sample t-test was used to compare the means of two independent continuous variables. Multivariate analysis was performed using multiple logistic regressions. Result of logistic regression was expressed as odds ratio and 95% CI. A two-sided p value less than 0.05 was considered statistically significant.

Ethical Approval

Approval from the Faculty of Medicine and Health Science, University Putra Malaysia human research committee was

received before commencement of the study. Informed consent was also obtained from the each respondent before data was collected.

RESULTS

Table 1 shows the socio-demographic characteristics of the respondents. Out of the 517 subjects selected, 454 agreed to participate giving a response rate of 87.8%. The mean age of the respondents was 42.86 years (95% CI 41.97-43.74). The result shows that out of the 454 respondents 50.9% were males. The males had significantly ($p<0.001$) higher mean age (45.53 ± SD 10.13 years) as compared to the females (40.09 ± SD 8.20 years). The majority (86.3%) were Malays, 84.8% of the respondents were married, and 51.5% of the respondents reported that they had a positive family history of hypertension. Table 2 shows the life-style factors and body mass index of the respondents. The result indicates that 31.1% of the respondents were overweight and 11.9% were obese, 10.1% were current smokers, 93% had never taken alcohol and 55.3% had been sufficiently active.

Table 1: Socio-demographic characteristics of respondents

Factor	Frequency	Percentage
Age (yrs)		
30-39	205	45.2
40-49	127	28.0
50-59	100	22.0
60 and above	22	4.8
Gender		
Male	231	50.9
Female	223	49.1
Ethnicity		
Malay	392	86.3
Chinese	40	8.8
Indian	22	4.9
Marital Status		
Single	59	13.0
Married	385	84.8
Divorced/Widowed	10	2.2
Level of Education		
Primary	7	1.5
Secondary	113	24.9
Tertiary	334	73.6
Position at work		
Academic	244	53.7
Non academic	210	46.3
Monthly Family Income		
<3000	99	21.8
3000-4999	129	28.4
5000-6999	74	16.3
7000 and above	152	33.5
Family History of Hypertension		
Yes	234	51.5
No	176	38.8
Don't Know	44	9.7

Table 2: Life-style factors and body mass index of the respondents

Characteristics	Frequency	Percentage
Smoking status		
Never smoker	368	81.1
Former smoker	40	8.8
Current smoker	46	10.1
No of cigarettes/day		
<10 (Light)	20	43.5
10-20 (Moderate)	25	54.3
More than 20 (Heavy)	1	2.2
Body Mass Index (kg/m2)		
Underweight (< 18.5)	44	9.7
Normal (18.5-24.99)	215	47.3
Overweight (25-29.99)	141	31.1
Obese (30 and above)	54	11.9
Alcohol consumption		
Never a drinker	422	93.0
Former drinker	17	3.7
Current drinker	15	3.3
Currently drinking status		
Light	12	80.0
Moderate	1	6.7
Heavy	2	13.3
Physical activity		
Inactive	126	27.8
Insufficiently active	77	16.9
Sufficiently active	251	55.3

Blood pressure measurements

Systolic blood pressure

Table 3 shows the overall mean SBP by age and gender. The overall mean SBP for 454 respondents was 126.2 mmHg (95%CI 124.99, 127.46). The mean SBP was significantly (p = 0.001) higher in males (129.68 mmHg) as compared to the females (122.65 mmHg). The mean SBP significantly increased with age in both males and females. The increase in the mean SBP with age was significant both in males (One way ANOVA T-test (F= 32.17, p=0.001) and females (F= 53.08, p= 0.001). Using Post Hoc - Tukey test to perform multiple comparisons between all the age groups for males showed that there was a significant difference in the mean SBP levels between age groups 30-39 and 40-49 (p = 0.001), 30-39 and 50 -59 (p = 0.001), 30-39 and 60 and above (p = 0.001). Significant difference in the mean SBP levels were also noted between age groups 40-49 and 50 -59 (p = 0.001), 40-49 and 60 and above (p = 0.05). However there was no difference in the mean SBP levels between age groups 50 -59 and 60 and above. For females, there was

a significant difference in the mean SBP levels between age groups 30-39 and 40-49 (p = 0.001), 30-39 and 50 -59 (p = 0.001), 30-39 and 60 and above (p = 0.01). Significant difference in the mean SBP levels were also noted between age groups 40-49 and 50 -59 (p = 0.001). However there was no difference in the mean SBP levels between age groups 40-49 and 60 and above and 50-59 and 60 and above.

Table 3: Mean systolic blood pressure levels by age and gender

Gender / Age (Years)	Number of respondents	Systolic blood pressure (mmHg)		
		Mean	95% CI	Std. Deviation
Male				
30-39	79	121.26	119.00-123.52	10.09
40-49	69	130.33	127.72-132.93	10.85
50-59	63	137.15	134.45-139.84	10.69
60 and above	20	137.15	133.64-140.65	7.49
Total	231	129.68	128.09-131.26	12.22
Female				
30-39	126	115.36	113.62-117.10	9.87
40-49	58	128.37	125.40-131.33	11.26
50-59	37	137.60	133.93-141.28	11.02
60 and above	2	139.00	137.35-240.64	11.31
Total	223	122.65	120.85-124.45	13.64
Both Gender				
30-39	205	117.64	116.21-119.06	10.34
40-49	127	129.43	127.49-131.37	11.04
50-59	100	137.32	135.18-139.45	10.76
60 and above	22	137.31	133.96-140.67	7.56
Total	454	126.22	124.99-127.46	13.39

Diastolic blood pressure

Table 4 shows the overall mean DBP by age and gender. The overall mean DBP for 454 respondents was 80.17 mmHg (95%CI 79.32, 81.03). The mean DBP was significantly (p = 0.001) higher in males (82.64 mmHg) as compared to the females (77.62 mmHg). The mean DBP significantly increased with age in both males and females. The increase in the mean DBP with age was significant both in males (One way ANOVA T-test (F= 23.75, p =0.001) and females (F= 39.01, p= 0.001). Using Post Hoc - Tukey test to perform multiple comparisons between all the age groups for males showed that there was a significant difference in the mean DBP levels between age group 30-39 and 40-49 (p = 0.002), 30-39 and 50 -59 (p = 0.001), 30-39 and 60 and above (p = 0.003). Significant difference in the mean DBP levels were also noted between age groups 40-49 and 50 -59 (p = 0.001). However there was no difference in the mean DBP levels between age groups 40-49 and 60 and above and between age groups 50 -59 and 60 and above (p >0.05). For females, there was a significant difference in the DBP levels between age groups 30-39 and 40-49 (p = 0.001), 30-39 and 50 -59 (p = 0.001) and 40-49 and 50 -59 (p = 0.011). However there was no difference in the mean DBP levels between age groups 30-39 and 60, 40-49 and 60 and above and 50 -59 and 60 and above.

Table 4: Mean diastolic blood pressure levels by age and gender

Gender / Age (years)	Number of respondents	Diastolic blood pressure (mmHg)		
		Mean	95% CI	Std. Deviation
Male				
30-39	79	77.80	76.24-79.36	6.97
40-49	69	82.35	80.40-84.30	8.13
50-59	63	88.46	86.50-90.41	7.75
60 and above	20	84.42	81.22-87.62	6.84
Total	231	82.64	81.52-83.75	8.58
Female				
30-39	126	73.00	71.72-74.28	7.25
40-49	58	81.69	79.52-83.86	8.25
50-59	37	86.66	84.24-89.07	7.24
60 and above	2	83.50	62.62-229.62	16.26
Total	223	77.62	76.39-78.85	9.33
Both Gender				
30-39	205	74.85	73.82-75.88	7.50
40-49	127	82.05	80.62-83.48	8.16
50-59	100	87.79	86.29-89.29	7.58
60 and above	22	84.34	81.05-87.62	7.41
Total	454	80.17	79.32-81.03	9.29

Prevalence of hypertension

Table 5 shows prevalence of hypertension by age and gender. The overall prevalence of hypertension and prehypertension amongst the 454 staff aged 30 years and above was 34.4% and 33.9% respectively. The prevalence of hypertension and prehypertension amongst the 231 males was 45.5% and 33.3% respectively. For the 223 females, the prevalence of hypertension and prehypertension was 22.9% and 34.5% respectively. Table 6 shows prevalence of awareness, treatment and control of hypertension by gender. Among those 156 respondents classified as hypertensive, 100 (64.1%) were aware they had hypertension. However, out of these 100 respondents who were aware they had hypertension, 86 (86%) respondents stated that they were taking antihypertensive medication. Out of these 86 respondents who stated that they were being treated only 39 (45.3%) had their hypertension under control. The result shows that out of the 156 respondents who had hypertension, there were only 39 (25%) had their blood pressure under control. Although the blood pressure under control was low in both sexes, females (31.4%) had a higher proportion as compared to males (21.9%). Table 7 shows prevalence of hypertension and factors associated. Bivariate analysis showed that prevalence of hypertension was significantly associated with age, gender, marital status, level of education, family income, family history of hypertension, physical inactivity. Results of the Logistic model (Table 8) showed that prevalence of hypertension was significantly associated with age, gender, family history of hypertension, BMI and alcohol consumption (Nagelkerke $R^2 = 0.59$; Hosmer and Lemeshow Test, $p = 0.09$; the overall accuracy of this model to predict the subjects having hypertension is 83.2%; area under ROC curve = 0.90 (95%CI: 0.87 – 0.93); there is no multicollinearity and interaction between variables). Obese individuals (BMI $\geq$ 30) were eleven times more likely to have hypertension than individuals with a normal BMI (OR 11.37, 95% CI 4.36–29.62). Individuals with a family history of hypertension were five times as likely to have hypertension than those without a family history of hypertension (OR 5.25, 95% CI 2.80 - 9.85). Individuals who consume alcohol were seven times as likely to have hypertension than those without a family history of hypertension (OR 7.14, 95% CI 1.75 - 29.16).

Table 5: Prevalence of hypertension by age and gender

Gender /Age (years)	Prevalence of Hypertension			
	Normal	Pre-hypertension	Hypertension	Total
Male				
30-39	37 (46.8%)	32 (40.5%)	10 (12.7%)	79
40-49	9 (13.1%)	33 (47.8%)	27 (39.1%)	69
50-59	3 (4.8%)	9 (14.3%)	51 (80.9%)	63
60 and above	0 (0.0%)	3 (15.0%)	17 (85.0%)	20
Total	49 (21.2%)	77 (33.3%)	105 (45.5%)	231
Female				
30-39	82 (65.1%)	36 (28.5%)	8 (6.4%)	126
40-49	10 (17.3%)	31 (53.4%)	17 (29.3%)	58
50-59	3 (8.1%)	10 (27.1%)	24 (64.8%)	37
60 and above	0 (0.0%)	0 (0.0%)	2 (100.0%)	2
Total	95 (42.6%)	77 (34.5%)	51 (22.9%)	223
Both Gender				
30-39	119 (58.0%)	68 (33.2%)	18 (8.8%)	205
40-49	19 (15.0%)	64 (50.3%)	44 (34.7%)	127
50-59	6 (6.0%)	19 (19.0%)	75 (75.0%)	100
60 and above	0 (0.0%)	3 (13.6%)	19 (86.4%)	22
Total	144 (31.7%)	154 (33.9%)	156 (34.4%)	454

Table 6: Prevalence of Awareness, Treatment and Control of Hypertension by Gender

Status	Male	Female	Both sexes
	Frequency (%)	Frequency (%)	Frequency (%)
Hypertensive			
Aware	66 (62.9)	34 (66.7)	100 (64.1)
Aware and Treated	53 (80.3)	33 (97.1)	86 (86.0)
Treated and Controlled	23 (43.4)	16 (48.5)	39 (45.3)
Hypertensive			
Overall Control	23 (21.9)	16 (31.4)	39 (25.0)

Table 7: Prevalence of hypertension and factors associated

Variables	With Hypertension		Without Hypertension		Total	P value
	Number	%	Number	%		
Age (yrs)						
30-39	18	8.8	187	91.2	205	<0.001*
40-49	44	34.6	83	65.4	127	
50-59	75	75.0	25	25.0	100	
60 and above	19	86.4	3	13.6	22	
Gender						
Male	105	45.5	126	54.5	231	<0.001*
Female	51	22.9	172	77.1	223	
Ethnicity						
Malay	129	32.9	263	67.1	392	0.177
Chinese	19	47.5	21	52.5	40	
Indians	8	36.4	14	63.6	22	
Marital Status						
Single	8	13.6	51	86.4	59	<0.001*
Married	144	37.4	241	62.6	385	
Divorced/Widowed	4	40.0	6	60.0	10	
Level of Education						
Primary/Secondary	51	42.5	69	57.5	120	0.029*
Tertiary	105	31.4	229	68.6	334	
Family Income (RM)						
Low (<3000)	23	23.2	76	76.8	99	<0.001*
Medium (3000-5999)	45	27.0	122	73.0	167	
High (6000 and above)	88	46.2	100	53.1	188	
Family History						
Yes	112	47.9	122	52.1	234	<0.001*
No	38	21.6	138	78.4	176	
Don't Know	6	13.6	38	86.4	44	
Physical Inactivity						
Yes	57	45.2	69	54.8	126	0.003*
No	99	30.1	229	69.9	328	

*significant at $p < 0.05$

Table 8: Logistic regression analysis of the factors associated with hypertension

Variables	β	SE	OR	95% CI	*P* value
Gender					
Female			1		
Male	0.63	0.37	1.878	1.03 – 3.42	<0.039*
Age Group					
30 - < 40			1		
40 - < 50	1.71	0.37	5.55	2.68 -11.50	< 0.001*
50 - < 60	3.09	0.42	21.87	9.53 - 50.20	< 0.001*
60 and above	4.12	0.89	61.37	10.68 - 352.75	< 0.001*
Family History					
No			1		
Yes	1.70	0.32	5.25	2.80 - 9.85	< 0 .001*
Alcohol Consumption					
Never			1		
Former Drinker	0.80	0.77	2.23	0.50 – 10.00	0.290
Current Drinker	1.97	0.72	7.14	1.75 - 29.16	0.006*
BMI					
Normal/underweight			1		
Overweight	1.26	0.49	3.54	1.91 - 6.55	< 0.001*
Obese	2.43	0.47	11.37	4.362 - 29.62	< 0.001*

*significant at $p < 0.05$

Note: Nagelkerke $R^2 = 0.59$; Hosmer and Lemeshow Test, $p = 0.09$; the overall accuracy of this model to predict the subjects having hypertension is 83.2%; area under ROC curve = 0.90 (95%CI: 0.87 – 0.93); there is no multi-co linearity and interaction between variables.

DISCUSSION

The prevalence of hypertension amongst UPM staff aged 30 years and above in our study was 34.4% which is lower than the prevalence of 40.5% reported by Rampal *et al*.[3] and 42.6% reported by the third National Health Morbidity Survey[9]. The lower prevalence of hypertension recorded in this study could be due to the fact that university staff are among the highly educated part of the society and are exposed to more information than the general population. Thus, they are more likely to be concerned about their health by choosing a healthier lifestyle. In two surveys conducted among university populations in Nigeria, the prevalence of hypertension was 33% and 21%[13, 14]. Individuals with prehypertension have a two-fold risk of developing clinical hypertension compared with normotensive individuals[15]. Prehypertension is not categorized as a disease. However, by identifying these individuals with prehypertension both the patients and clinicians are alerted to this risk and encouraged to intervene and prevent or delay the disease from developing. Individuals with diabetes or kidney disease and also prehypertension should be considered for appropriate drug therapy if lifestyle modifications fails to bring down the blood pressure to 130/80 mmHg or less[16]. In addition to prevalence of hypertension of 34.4%, the overall prevalence of prehypertension in this study was high (33.9%). Lee *et al*.[17] recorded a prevalence of 28.5% and 18.7% for hypertension and pre-hypertension, respectively, among Singaporean population. Prospective studies strongly suggest that SBP rather than DBP is a better predictor of CVD risk especially in adults aged 55 years and above in whom most deaths from CVD occur[4]. In our study, the overall mean SBP in males was 129.68 mm Hg which is higher than the age-standardized mean SBP worldwide for males was 128.1 mm Hg[18]. For the females, in our study, the overall mean SBP was 122.65 mmHg which is lower than the age-standardized mean SBP worldwide (124.4 mm Hg) for females[18]. The overall mean SBP is higher than those from higher income countries such as the age-standardized mean SBP amongst females in Australasia (117.6 mm Hg), South Korea (116.9 mm Hg), North America (118.4 mm Hg) and those from Asia pacific (120.5 mm Hg)[18].

A key predictor of blood pressure in many populations is age. In our study, the mean SBP and DBP significantly increased with age in both males and females and prevalence of hypertension significantly increased with age in both sexes. Rampal *et al*.[3] reported similar results in their national study involving 16,440 subjects3. Numerous other studies have also reported that the prevalence of hypertension significantly increased with age [2, 16, 19, 20]. In our study, the prevalence of hypertension was higher in males compared to females for those aged less than 60 years. For those aged 60 years or more, the prevalence of hypertension was higher among the females. In a national study carried out by Rampal *et al.* in 2004[3], the prevalence of hypertension also increased with age in both sexes. The prevalence of hypertension was higher in males compared with females for those aged less than 50 years. For those aged 50 years or more, the prevalence of hypertension was higher among females. In a systematic review of data worldwide, at younger age men are more often affected by hypertension than women, whereas in older people hypertension was higher in women than in men[21]. It has been suggested that female sex hormones may contribute to the gender difference in blood pressure regulation[22]. However, the effect of oestrogen on blood pressure is still controversial[23]. In our study, hypertension was significantly associated with family history of hypertension. This result is consistent with several other studies[24, 25]. Our study also showed that hypertension was significantly associated with obesity. The prevalence of obesity amongst Malaysians 18 years and above has increased from 4.4% in 1996[8] to 12.3% in 2004[26] and 14.2% in 2006[9]. The rising problem of obesity is a cause of concern. Implications of this study are that the results show that prevalence of hypertension and pre-hypertension is high among the University staff. Only 25% have their blood pressure under control. Routine blood pressure measurements should be taken to improve the detection, prevention and treatment of hypertension. In conclusion, prevalence of hypertension and pre-hypertension is high. There is an urgent need for implementation of a comprehensive CVD prevention program.

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Nutritional Status and the Use of Protease Inhibitors Among Hiv-infected Children in Klang Valley, Malaysia

[1]MT Mohd. Nasir*, [1]J Yeo, [1]MSL Huang, [2]MT Koh, [3]R Kamarul Azhar & [4]GL Khor

[1]Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia
[2]Department of Paediatrics, Faculty of Medicine, University Malaya Medical Centre
[3]Department of Paediatrics, Paediatric Institute, Kuala Lumpur Hospital
[4]Department of Nutrition and Dietetics, School of Pharmacy and Health, Faculty of Medicine and Health, International Medical University

ABSTRACT

This study determined the association between nutritional status and the use of protease inhibitors (PI) containing regimen among HIV-infected children receiving treatment at the referral centres in Klang Valley. A total of 95 children currently on antiretroviral (ARV) therapy, aged one to eighteen years, were recruited using purposive sampling. Demographic data, anthropometric measurements, medical history, were collected using a structured questionnaire. Serum micronutrients levels and lipid profile were also examined using blood samples. Mean age was 8.8±3.9 years and 44.2% were on PI. Age ($\chi 2 = 10.351$, $p = .006$), weight-for-age ($\chi 2 = 6.567$, $p = .010$), serum selenium ($\chi 2 = 4.225$, $p = .040$) and HDL-C ($\chi 2 = 7.539$, $p = .006$) were significantly associated with the use of PI. Fewer children on PI were deficient in selenium as compared to those not on PI. On the contrary, more children on PI were underweight and had low HDL-C. The use of PI was found to have both positive and negative effects with better selenium level but poorer HDL-C level and weight status.

Keywords: HIV, protease inhibitors, nutritional status, HIV-infected children, Malaysia

INTRODUCTION

Human Immunodeficiency Virus (HIV) remains of the biggest threat to global health and people living with HIV including children vulnerable to various health-related issues.[1] One of the most affected outcomes of HIV would be the nutritional status of these children. HIV-infected children are prone to malnutrition due to inadequate intake of nutrients, nutrient loss due to malabsorption, metabolic alterations and drug-nutrient interactions.[2, 3] A state of malnutrition would render these children more prone to infections and further deterioration of their overall health specifically their nutritional status.[4] Micronutrients such as selenium, zinc, vitamin A and E are found to be most affected among HIV-infected patients.[5, 6, 7]

The advancement of antiretroviral (ARV) therapy has brought about a dramatic increase in life expectancy of people living with HIV and AIDS.[8] However, its use was not without adverse effects in some patients. Nausea, diarrhoea, fatredistribution, pancreatitis, lactic acidosis, lipid abnormalities, and hyperglycaemia were among the common effects associated with the use of ARV therapy.[8, 9] Specifically, the incorporation of protease inhibitors (PI) into the ARV regimen has been implicated as a possible cause of HIV-associated lipodystrophy not only among adults but also in children.[10]

PI are highly potent antiretroviral agents that act by selectively blocking HIV-1 protease, an enzymenecessary for HIV-1 replication in the later stages ofvirus production.[11] It is usually used in combination with other antiretroviral drugs to maximise the reduction in viral load and increase in CD4+ cell count.[12] In Malaysia, recommendations for first line therapy for children and adolescents are adapted from the US Department of Health and Human Services Guidelines for the Use of Antiretroviral Agents.[13] Some of the recommended choices of regimen to be used include PI as part of the first line therapy and thus, it is not surprising to note that most of these children may be exposed to these medications.

Current literatures pointed to the need for more data on the use of PI and its association with the nutritional status of these children. A longitudinal study conducted by Miller *et al.*,[14] among 67 HIV-infected children found that PI therapy has a positive effect on several growth parameters, including weight, weight/height, and muscle mass. On the contrary, another longitudinal study among 25 Italian HIV-infected children noted that PI treatment did not seem to have significantly influenced their body weight. However, the author highlighted that combined therapy including PI may result in an improved height trend among the children.[15]

*Corresponding author: *nasir.jpsk@gmail.com*

Furthermore, Bitnun *et al.,*[10] noted that studies comparing PI and PI-naive group in terms of lipid profiles and other micronutrients were relatively few. A recent study by Chantry *et al.,*[16] found that the use of PI was associated with the worsening of lipid profiles. Its effect was even worse when combined with a non-nucleoside reverse transcriptase.

This study aimed to describe the nutritional status of HIV-infected children as well as to determine the differences in nutritional status between the children who were taking PI as part of the ARV regimen and the children who do not. This would further enhance and extend our understanding on the effect of PI on the nutritional status of the children.

METHODS

This study was carried out at the Paediatric Institute of the Kuala Lumpur Hospital and the Paediatric Department of the Universiti Malaya Medical Centre (UMMC) involving children receiving outpatient care for HIV infection. The number of HIV-infected children being treated at the Paediatric Institute totalled 111 and 10 were being treated at the UMMC. The inclusion criteria for the study include: a) HIV-infected children aged 1 to 18 years; and b) HIV-infected children currently treated with ARV medications. The exclusion criteria include: a) HIV-infected children who were yet to start HAART treatment; b) HIV-infected children who were hospitalised due to infections; and c) HIV-infected children who refused to participate or were absent from their appointment. This study utilised a non-probability sampling method whereby both the hospitals were selected as they are the centres of referral for HIV cases among children below the age of 19 years. All children who met the selection criteria were included. Prior consent was obtained from parents/guardians before the child could be included in the study. The response rate of this study was 79.3%.

Ethical approval for the study was obtained from the research ethics committees of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia; University Malaya Medical Centre; and the Ministry of Health, Malaysia (Research ID: NMRR-08-1233-2501).

All parents/guardians of the children were interviewed using structured questionnaires which include questions on socio-demographic variables (age, sex, ethnicity, and living arrangement), and antiretroviral medications history (duration on treatment, and types of medications prescribed). The medical records of the HIV-infected children were used to validate the information provided by their parents/guardians.

Weight and height were measured using the Tanita digital scale model 314 and SECA wall stadiometer model 206 respectively. Duplicate measurements of each parameter were done and the means of the height and weight were then used for the calculation of Body Mass Index (calculated as weight in kilograms divided by height in meters squared). Body Mass Index-for-age (BMI-for-age), height-for-age (Ht-for-age) and weight-for-age (wt-for-age) were expressed as z-scores to adjust for age and sex. WHO Anthroplus[17]software was used for the calculation of the z-scores. To determine the growth status of the children, the World Health Organization (WHO) Growth Standards 2006[18] and WHO Growth Reference 2007[17] were then used. Weight-for-age computations were only up to the age of 10.0 years due to the limitation of the WHO reference used.

The children were also required to provide non-fasting blood samples for the assessment of serum level of zinc, selenium, vitamin E, vitamin A and lipids. In this study, non-fasting blood samples were used because the children were required to take the prescribed regimen of drugs on time after meals. Therefore, it was deemed unethical to require fasting blood samples from these children.

Fifteen millimetres (ml) of blood samples were drawn by trained nurses from the hospitals. Serum Zn and Se levels were measured using inductively coupled plasma-mass spectrometry (ICP-MS). High-performance liquid chromatography (HPLC) with UV detector was used to measure the serum levels of vitamin A and E. Serum triglycerides, total cholesterol (TC), and high density lipoprotein cholesterol (HDL-C) levels were measured byenzymatic methods. Low-density lipoprotein cholesterol (LDL-C) concentrations were derived by calculation using the Friedewald formula.[19] These analyses were conducted by the Gribbles Pathology (M) Sdn. Bhd. according to laboratory-based protocols.

The cut-off reference values used for vitamins and minerals examined in this study were based on the laboratory values recommended by WHO 2001 report.[20] Cut-offs for lipids are based onthe National Cholesterol Education Program (NCEP)[21] classifications for children and adolescents.

PASW Statistics version 18.0 was used for data management and statistical analysis. The data was analysed descriptively. Chi-square test was used to determine the association between nutritional status and the use of PI. The critical level of $\alpha = 0.05$ was used for statistical significance.

RESULTS

A total of 95 children participated in this study. The age of these children ranged from 15 months to 17 years old with a mean of 8.8 ± 3.9 years. Slightly more respondents were male (56.8%) as compared to female. By ethnicity, majority (53.7%) were Malays, followed by Chinese (32.6%) and other ethnic groups made up the remaining 13.7%. While 64.2% lived with families, 8.4% were adopted and 27.4 % were living in shelter homes around Klang Valley.

A majority (61.5%) of the children were diagnosed with HIV or AIDS during the first two years of life and the mean age (in months) of first diagnosis was 28.3 ± 33.6 months. Overall, the mean number of months since diagnosis was 105.5 ± 46.3 months. The average number of months the children had been on ARV was 67.9 ± 38.3 months. Only 4.2% had been on the regimen for less than a year. A total of 42 children (44.2%) were taking PI as part of the regimen. None of the children was on monotherapy. The mean percentage of CD4% was 24.9 ± 8.8 ranging from as low as 2% to as high as 45%. In terms of CD4+ absolute count, the mean was 884.1 ± 510.8 cells/mm3 and ranged between 34 and 2444 cell/mm3.

Table 1: Demographic background and nutritional status of the children and the association with the use of PI containing regimen (n=96 unless otherwise stated)

Characteristics	Intake of Protease Inhibitors (PI)			
	PI	Non-PI	Total	p-value
Demographic background				
Age				0.006*
6.0	8a (23.5b)	26 (76.5)	34 (35.8)	
7.0 to 10.0	19 (61.3)	12 (38.7)	31 (32.6)	
11.0	15 (50.0)	15 (50.0)	30 (31.6)	
Sex				1.000
Male	24 (44.4)	30 (55.6)	54 (56.8)	
Female	18 (43.9)	23 (56.1)	41 (43.2)	
Ethnicity				-
Malay	21 (41.2)	30 (58.8)	51 (53.7)	
Chinese	17 (54.8)	14 (45.2)	31 (32.6)	
Indian	3 (60.0)	2 (40.0)	5 (5.3)	
Sarawak Bumiputera	0 (0.0)	2 (100.0)	2 (2.1)	
Sabah Bumiputera	1 (100.0)	0 (0.0)	1 (1.0)	
Myanmar (Refugees)	0 (0.0)	5 (100.0)	5 (5.3)	
Anthropometric measurements				
BMI-for-age				0.274
Overweight/Obese	11 (33.3)	22 (66.7)	33 (34.7)	
Normal	27 (50.9)	26 (49.1)	53 (55.8)	
Thinness	4 (44.4)	5 (55.6)	9 (9.5)	
Mean Z-score	-0.31 ± 0.15			
Wt-for-age (n=59)				0.010*
Normal/Too heavy	15 (30.6)	34 (69.4)	49 (83.1)	
Underweight	8 (80.0)	2 (20.0)	10 (16.9)	
Mean Z-score	-1.19 ± 0.15			
Ht-for-age				0.135
Normal	23 (37.7)	38 (62.3)	61 (64.2)	
Stunted	19 (55.9)	15 (44.1)	34 (35.8)	
Mean Z-score	-1.54 ± 0.15			

Continuation

Table 1. Demographic background and nutritional status of the children and the association with the use of PI containing regimen (n=96 unless otherwise stated)

Characteristics	Intake of Protease Inhibitors (PI)			
	PI	Non-PI	Total	p-value
Biochemical Indicators				
Selenium (µmol/L)				0.040*
Adequate (0.9)	40(48.2)	43 (51.8)	83 (87.4)	
Deficient (<0.9)	2 (16.7)	10 (83.3)	12 (12.6)	
Zinc (µmol/L)				1.000
Adequate (9.0)	39 (43.8)	50 (56.2)	89 (93.7)	
Deficient (<9.0)	3 (50.0)	3 (50.0)	6 (6.3)	
Vitamin A (µmol/L)				0.148
Adequate (1.05)	19 (36.5)	33 (63.5)	52 (54.7)	
Deficient (<1.05)	23 (53.5)	20 (46.5)	43 (45.3)	
Vitamin E (µmol/L)				0.795
Adequate (25.0)	32 (45.7)	38 (54.3)	70 (73.7)	
Deficient (<25.0)	10 (40.0)	16 (60.0)	25 (26.3)	
Lipid Profile				
Total cholesterol (mg/dL)				0.088
Normal (<170)	10 (29.4)	24 (70.6)	34 (35.8)	
Borderline (170-199)	16 (50.0)	16 (50.0)	32 (33.7)	
High (200)	16 (55.2)	13 (44.8)	29 (30.5)	
HDL-C (mg/dL)				0.006*
Normal (>40)	33 (38.8)	52 (61.3)	85 (83.3)	
Deficient (40)	9 (75.0)	1 (25.0)	10 (16.7)	
LDL-C (mg/dL) (n=93)				0.386
Normal (<110)	24 (43.6)	31 (56.4)	55 (59.2)	
Borderline (110-129)	9 (34.6)	17 (65.4)	26 (27.9)	
High (130)	7 (58.3)	5 (41.7)	12 (12.9)	

* Statistically significant at = 0.05
a = Frequency b = Row %

Table 1 reveals that the intake of PI were associated with age [$\chi 2 = 10.351$, p = .006], weight-for-age [$\chi 2 = 6.567$, p = .010], selenium [$\chi 2 = 4.225$, p = .040], and HDL-C [$\chi 2 = 7.539$, p = .006] classifications.

Significantly higher percentages of children from the younger age groups of below 6 years (76.5% vs. 23.5%) were not on PI as compared to children from the older age groups. However, as the age increases, more children were treated with PI containing regimen as seen in those aged between 7 to 10 years. Half of the children in their teenage years were on PI containing regimen. On the contrary, no significant association was found between the sexes in terms of treatment approach. Weight-for-age classifications were found to be significantly associated with the use of PI. Significantly more underweight children were on PI as compared to the non-PI. No significant association were found between BMI-for-age classifications and PI groups. Similarly, height-for-age was not associated with the intake of PI

although more children in the PI group were found to be stunted.

Selenium deficiency was more common (83.3%) among children not on PI containing regimen as compared to those on PI. No significant association were noted for serum zinc, vitamin A and vitamin E levels with the use of PI containing regimen. In terms of lipid profile, 75.0% of the children on PI were found to have a low level of HDL-C. Total cholesterol and LDL-C levels were found to have no significant association with the use of PI.

DISCUSSION

It was found that there were more underweight children in the PI group as opposed to the non-PI group. It is unsure whether the poor weight status was due to the use of PI or due to changes in regimen to incorporate PI as part of the subsequent treatment approach given the poor weight status. Lack of data on any change of regimen among the children made it impossible to distinguish whether these children were treated with PI containing regimen as a result of a combination of poor weight status as well as poor immunological and virological factors. It is also worth noting that patients who were found to be losing weight were also more likely to be placed on PI as recommended by WHO treatment guidelines in combination with other factors.[9] However, finding from other studies showed sustained weight gain among those who were treated with PI both in adults[22] and children.[15, 23] A prospective longitudinal study which followed a group of children for a median of 2.4 years even found that PI therapy has a positive effect on muscle mass and not just on weight, height and weight-for-height of the children.[14]

Micronutrient deficiencies are common in HIV, both in the early as well as the late stages of the progression of the infection.[5, 24] The biochemical markers examined in this study showed deficiency in several micronutrients. Deficiency in serum level of vitamin A was more commonly seen among HIV-infected children as compared with other micronutrients. Low status of micronutrients seen in early asymptomatic HIV is mainly due to reduced absorption as a result of structural and functional changes in intestinal tract characterized by villous atrophy and crypt hyperplasia,[25] increased utilization and loss of micronutrients when diarrhoea and other co-infections become more frequent.[26] As such, dietary intake becomes increasingly important and its level of requirement may eventually be higher than that of non-infected children.[7] The treatment approach using PI containing regimen was found to have positive effect on serum selenium level with less children experiencing deficiency as compared to those not on PI. Previous studies have found that serum selenium levels were improved on antiretroviral treatment generally.[6, 7] On the contrary, a 3-year observational study by Rousseau *et al.,*[27] among 44 patients found that serum selenium level were not significantly different between those on antiretroviral treatment and those who do not.

The use of PI as part of the ARV treatment has been associated with dyslipidemia which include elevated levels of total cholesterol, triglycerides, LDL-C and decreased level of HDL-C.[15, 28, 29, 30, 31, 32] In this study, only HDL-C levels were associated with the use of PI containing regimen. HDL-C was lower among children who were on PI. Similarly in a study by Rose *et al.*,[33] it was found that those treated with PI containing regimen for 3 to 6 years period were less likely to have high HDL-C level. However, contradicting findings on the effect of PI on HDL-C levels had been shown in another study by Bitnun *et al.*,[10] which found that children who were on PI containing regimen had significantly higher total cholesterol, LDL-C and triglycerides levels but no effect on HDL-C level.

There are several limitations in this study. First, due to the nature of the cross sectional study design, the temporal relation between the variables remained unclear. Thus, it is not possible to distinguish whether poor weight status of the children preceded the use of protease inhibitors. Secondly, lack of data on the use of supplementation limits the interpretation in terms of micronutrients adequacy. Nevertheless, vitamin A deficiency remained one of the most affected micronutrients given the high percentage of inadequate level among the children. It is also worth noting that the lipid profile from this study can only provide an insight for screening purposes and no diagnostic conclusion can be drawn for clinical purposes.

With more children being treated with PI containing regimen, there is a need to ascertain its effect on micronutrient levels. Cohort studies looking at micronutrient levels and the duration of the use of PI containing regimen and other antiretroviral drug groups would be insightful. Furthermore, comprehensive nutritional assessments which include anthropometric, biochemical, clinical and dietary assessment should be an on-going evaluation for these children as the use of PI may have possible effects on the lipid profile and weight status of the children. Intervention trials focusing on the effectiveness of nutrition counselling among children on ARV therapy may be beneficial to help the children and their parents/guardians move towards improvements in nutritional status and overall growth.

CONCLUSION

In conclusion, children receiving PI containing regimen were found to have better selenium levels as compared to the non-PI group. On the contrary, the use of PI therapy among HIV-infected children was found to be associated with poorer HDL-C level and weight status. Our findings suggest that routine monitoring of serum lipids in HIV-infected children particularly those receiving PI containing regimen may be beneficial. Early detection of dyslipidemia and poor growth may warrant for nutritional interventionto be incorporated in an attempt to provide a more holistic approach

in treating HIV-infected children. Dietary counselling and regular exercise may be helpful in the management of dyslipidemia particularly among children receiving PI containing regimen.

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Evaluation of Two Cell Culture Media in Culturing Rat Full Term Amniotic Fluid Cells

[1, 2]N. Ferdaos, [1, 2]T. Karuppiah, [1, 2]R. Rosli, [2]M. N. Yazid & [1, 2]N. Nordin*

[1]Stem Cell Research Laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia
[2]Clinical Genetics Unit, Department of Obstetrics & Gynaecology,
Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

ABSTRACT

Introduction: Amniotic fluid (AF) consists of heterogenous population of cells with high diagnostic and therapeutic values. The study of rat amniotic fluid cells is very limited, despite the extensive use of this animal model in biomedical research. Primary culture of rat AF cells, especially from full term pregnancies has not been well established. Here we attempt to determine the suitable medium in culturing rat AF cells that would enhance the cell viability, growth rate and heterogeneity. **Methods:** The cell viability, growth rate and heterogeneity of rat AF cells were compared upon culturing the primary cells in two different media; Amniomax or RPMI. Cell viability study was carried out using trypan blue staining, while the growth rate was monitored based on the time required to passage the cells (population doubling time in hour). The heterogeneity of cells was examined based on the morphology of the cells. Statistical analysis was performed using t-test. **Results:** Amniomax was observed to provide a better culture condition in culturing rat AF cells as the cells are more viable, grow faster and more heterogenous as compared to the cells grown in RPMI. **Conclusion:** Amniomax is a more suitable medium for high quality and viability of full term rat AF cell culture, as compared to RPMI. Thus, warranting propagation of more rat AF cells for biomedical research.

Keywords: Full term rat amniotic fluid, cell culture, Amniomax, RPMI, amniotic fluid cells

INTRODUCTION

Amniotic fluid (AF) has been widely used in clinical setting for prenatal diagnosis, for more than 50 years, due to the presence of fetal cells in the fluid [2]. The fluid consists of a highly heterogenous population of differentiated and undifferentiated cells [1, 4, 16, 17]. The heterogeneity of AF cells might be explained by the direct contact of the fluid with the fetus [10] and the origin of the cells, which is from the three primary germ layers of amnion, fetal and embryonic tissues [1, 4, 7, 16, 17].

Recently, the use of AF has not been limited to only prenatal diagnosis, but they have been found to reside high potential stem cells, thus increases its therapeutic values. Exploring the potential values of these cells is indeed essential. AF can be retrieved from either mid- or full-term pregnancies, but most of stem cells findings from AF were established from mid-term pregnancies, even though the procedure to collect AF from this term is invasive (amniocentesis), with 1% risk of miscarriage and maternal/fetal complications [19, 21]. Minimizing these risks to the patient is important, thus full-term AF could be used as alternative, as they are usually discarded after birth and the procedure is much safer [6, 20, 21].

Despite the extensive use of rat animal model in biomedical research [5, 9, 10, 14], the culture of rat AF cells has not been well established, especially the AF cells harvested from full term pregnancies. Previous studies using human full term AF cells have demonstrated that the cells were difficult to grow and required long time for cell attachment [6]. Full term AF has been shown to contain less viable cells [4] as the fluid generally consists of excess cellular debris [12]. Therefore, establishment of appropriate conditions in culturing full term rat AF cells is indeed essential.

One of the important aspects in studies using rat AF is the culturing of the primary AF cells. Cell culture media is a factor that could affect AF cell culture [18] and even though various types of medium have been used in culturing AF cells [3-4], the best medium to culture AF cells from rat samples is still unknown.

Commercial and well-known medium to culture human AF cells, Amniomax has reported to be successful in

*Corresponding author: *shariza@medic.upm.edu.my*

propagating the bovine AF cells [3], hence providing an alternative option to be considered for culturing rat AF cells. Another common, cost-effective cell culture medium for various types of cell lines, RPMI could also be considered, as it has been used to culture murine AF cells in previous study [13]. Therefore, this study aims to evaluate the effects of these two different media on the growth rate, cell viability and heterogeneity of the rat AF cells as an effort to establish an optimal culture condition for the propagation and survival of rat full term AF cells. The established culture condition will certainly be useful in promoting future studies using AF cells. Thus, allowing the potential therapeutic values of AF cells to be explored.

MATERIALS AND METHODS

Sample collection

Time-mated pregnant Sprague Dawley rats from full-term pregnancies (day 20; n=8) were collected from animal center in University Malaya (UM) and sacrificed by excessive carbon dioxide (25-30L/min, 1000psi). Amnion sacs were removed from the uterus and washed with cold PBS (1x). Subsequently, amnion sacs were removed from the uterus by cesarean section and washed with PBS before placed in a petri dish. Then, AF from each sac were drawn and pooled under sterile condition. All rats received humane care and observed by a veterinarian in UM. Animal sampling procedures were approved by Animal Care and Use Committee (ACUC) of UPM.

Culturing and maintainence of AF cells

Prior to culturing, AF samples were centrifuged at 160xg for 10 minutes to pellet the cells. The cells were then resuspended in fresh medium by gentle pipetting and distributed into 6 well plates before incubated at 37°C in a humidified 5% CO2 incubator. The cells were subcultured after they reached 80-90% confluency by washing the cells with 1x PBS twice and trypsinized with 0.25% trypsin in 1mM EDTA (Gibco-Invitrogen). The trypsin was deactivated with medium containing serum and population doubling time (PDT) of the cells were recorded according to the formula: PDT = duration of culture x log2/log (inoculum cell number/cells harvested) [21].

Media preparation

Amniomax (Gibco-Invitrogen) complete medium was prepared by adding the supplements (containing serum and antibiotics) into the basal medium. RPMI 1640 with L-glutamine (Gibco-Invitrogen) was supplemented with 7.5% sodium bicarbonate (Sigma), 1% penicillin-streptomycin (Gibco- Invitrogen) and 20% fetal bovine serum (Gibco-Invitrogen).

Cell viability study & statistical analysis

Trypsinization and preparation of single cell suspension was performed prior to trypan blue staining. 10µl of the cell suspension was mixed with 10µl of trypan blue dye (Sigma) and the number of viable cells was determined using haemocytometer. The unstained cells were identified as viable cells whereas blue stained cells were identified as dead cells, as the dye was only absorbed by damaged cells. The experiments were performed in triplicate and for statistical analyses, t-test was conducted and P values <0.05 were considered statistically significant.

RESULTS

Two different media (Amniomax and RPMI) were used to evaluate the growth and maintenance of full term rat amniotic fluid (AF) primary cell culture based on cell viability, growth rate and heterogeneity. To compare the effects of Amniomax and RPMI, cells from the same batch of AF specimens were cultured in both medium upon collection for a culture period of 14 days, and in the same culture conditions.

VIABILITY OF AF CELLS

Both Amniomax and RPMI were observed to provide short attachment time of the primary rat AF cells to culture flask. The cells in both media were found to attach within 48 hours. However, the quality of the AF cells cultured in the respective medium was significantly different and this was assessed through cell viability study. From the cell viability study performed on the first and second passages of the cells, Amniomax was observed to significantly maintain more viable cells compared to RPMI. It was discovered that the number of viable cells grown in RMPI was reduced more than 70% (Figure 1), which may indicate that RPMI is not supporting the growth of AF cells or may suggest that RPMI is selective towards certain type of cells in AF.

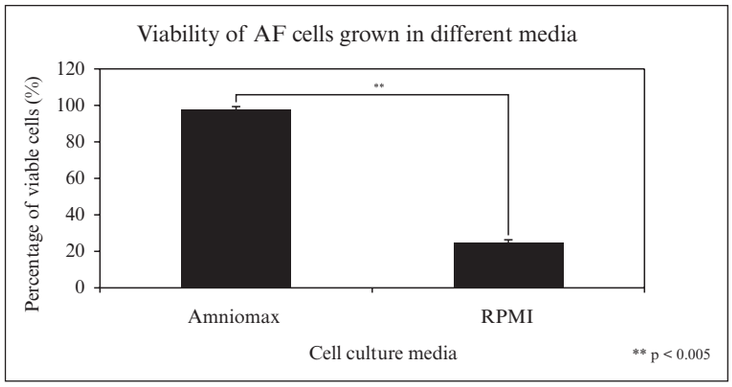

Figure 1. Comparison of AF cells viability grown in two different media, Amniomax and RPMI. Amniomax medium shows higher cell viability compared to RPMI medium (P2 & P3). Error bar is presented as mean ± S.E.M of 8 independent experiments conducted in triplicate, n=24

GROWTH RATE OF AF CELLS

The growth rate of AF cells in both media was also monitored. The cells were found to exhibit different growth behaviour. Cells grown in RPMI were observed to take longer time to reach confluency compared to cells cultured in Amniomax. The population doubling time for cells in RPMI was 125 hours compared to 40 hours for cells in Amniomax. These results highly suggest that Amniomax is providing a better culture condition in promoting the growth rate of AF cells compared to RPMI.

HETEROGENEITY OF AF CELLS

More heterogenous population of AF cells was observed when AF cells were grown in Amniomax compared to in RPMI (Figure 2). AF cells cultured in Amniomax exhibit at least four different morphology of cells; amniotic fluid cells (AF)-type (Figure 2A), epithelial–like (Figure 2B), fibroblast–like (Figure 2C), and neuronal-like cells (Figure 2D). Even though cells in RPMI took longer time, different types of cells was also observed in this medium. AF-type (Figure 2E), epithelial–like (Figure 2F) and fibroblast-like cells (Figure 2G) were observed being grown in RPMI. However, unlike in Amniomax, neuronal-like cells were not observed in RPMI. These observations suggest that Amniomax may be less selective compared to RPMI, thus promoting the propagation of more heterogeneous population of full term AF.

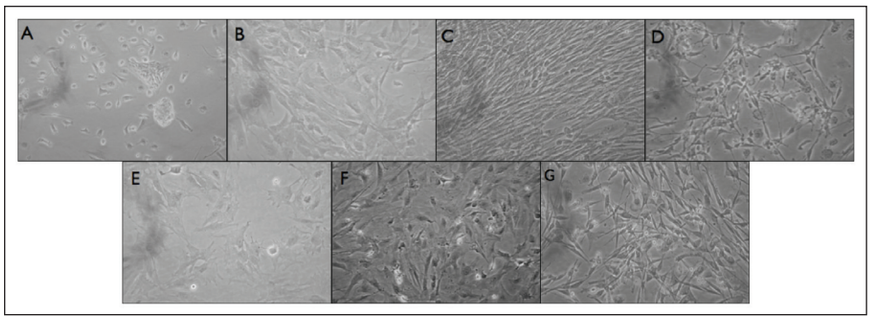

Figure 2. Heterogenous population of rat AF cells cultured in different media. More heterogeneous morphology of cells was observed when the cells were cultured in Amniomax; AF-type (A), epithelial–like (B), fibroblast–like (C), neuronal-like cells (D) compared to RPMI; AF-type (E), epithelial–like (F) and fibroblast-like cells (G). The magnification is 10X

DISCUSSION

The present study aims to establish a culture condition that would support the propagation of AF cells harvested from full term pregnant rats. AF cells harvested at this stage of pregnancy has been claimed to contain less number of viable cells [4], therefore culturing of the cells requires a good culture condition. One important aspect in establishing this

condition is to find the best suitable medium that would promote the cell attachment and enhance the viability, growth rate and heterogeneity of these cells. In this study, two types of media that have been used in culturing AF cells were evaluated. Amniomax, a specific medium to culture human AF cells, that has been previously reported to culture AF cells from bovine origin [3], and RPMI, a common medium that has been used to culture murine AF cells [13]. The ability of these two media in propagating and maintaining the rat AF cells was evaluated.

Both media managed to provide an environment that supported cell attachment. However, Amniomax showed better condition to culture rat AF cells as it was observed to support more viable cells than RPMI. The significance of the cell viability study was supported with the growth rate results, where AF cells cultured in Amniomax has lower PDT, indicating a faster growth rate.

The overall assessment of the cell culture success was observed by cell morphology, where more types of cells were observed in Amniomax, supporting the heterogeneous population of AF cells. At least four types of cells were observed in Amniomax compared to three in RPMI. Therefore, based on the cell viability, growth rate and heterogeneity, we discovered that rat AF cell culture quality is better with Amniomax medium as compared to RPMI (Figure 1, 2). Previous study on bovine AF cells supports our finding in which the AF cells grow very poorly in RPMI, but very well in Amniomax[3]. This may due to the variable contents of the medium, where Amniomax may have more additional supplements consisting growth factors and hormones specifically for the growth of rat AF cells [3], thus better in culturing AF cells, than RPMI [3, 15]. We suppose the basic basal components of RPMI may not be sufficient for rat AF cells, even though the difference of the constituents between these two media were unknown, as the composition of the Amniomax media is unavailable.

Overall, in our hands, Amniomax medium was found to be significantly better in culturing rat AF cells compared to RPMI. In conclusion, although Amniomax is a specific medium to culture human AF cells, it is also recommended for culturing the full term rat AF cells. This is the first finding to establish a culture condition that is not only managed to propagate the rat AF cells harvested from full-term pregnancies, but also enhanced the growth rate, viability and heterogeneity of the cells. The results will certainly be useful in promoting future studies using AF cells, especially full term AF cells. In that, it gives us the opportunity to have sufficient number of cells for isolation of stem cells residing in the fluid.

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INVITED REVIEW

Plant-Derived Antimalarial Agents: From Crude Extracts To Isolated Bioactive Compounds

[1]A Wan Omar* & [2]I Patimah

[1]Departament of Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor

[2]Departament of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor

ABSTRACT

Despite decades of intense research, malaria remains a deadly disease of the developing worlds. Drug-resistance to limited available antimalarials, in part, has contributed to the persistence of this infectious disease. Likewise, the use of antimalarials such as artemisinin, though effective in global malaria control programs, is hampered by high cost and limited supply. Therefore, identification of an antimalarial drug that is easy to isolate and produce, inexpensive, and demonstrates little toxicity across a diverse population represents the ideal agent needed for global malaria control programs and eradication of this deadly disease. This review discusses several antimalarial compounds containing unique structural composition that have been isolated and characterized from plant sources. These compounds have exhibited promising antimalarial activities in vitro and in vivo. However, limitations such as toxicity, low bioavailability and/or poor solubility have probably restricted the scope of use for several plant products in humans. Nevertheless, plants provide novel leads, which can be developed into safe drugs by synthetic strategies as exemplified by artemether and quinoline class of antimalarials. Therefore, plant bioactive compounds described herein provide useful alternatives, which could be modulated to obtain antimalarials active against not only drug-sensitive, but also drug-resistant and multi-drug resistant strains of *Plasmodium*. In this direction, semi synthetic approaches to newer and modified antimalarials have provided useful insights into their applicability in antimalarial drug discovery.

Keywords: Malaria; plant products; *Plasmodium*; antiplasmodial activity

INTRODUCTION

Malaria remains one of the most important infectious diseases in the developing world affecting millions of people causing up to 3 million death annually [1, 2]. This vector-borne infectious disease affects the productivity of individuals, families and the whole society, since it causes more loss of energy, more debilitation, more loss of work capacity and more economic damage than any other human parasitic diseases [3]. Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development. Malaria kills over a million people each year, with as many as 300-500 millions people being infected, with extremely high fatality rates among young children below 5 years of age. It is widespread in tropical and subtropical regions, including parts of the Americas, Asia and Africa. A total of 109 countries were endemic for malaria in 2008, 45 within the WHO African region [4]. By the year 2000, the WHO Global Malaria Control Strategy aimed to reduce malaria mortality by at least 20%, compared with 1995, whereby the aim of control was merely in at least 75% of the affected countries.

The human malaria, transmitted by female *Anopheles* mosquitoes has four *Plasmodium* species as its aetiological agents, namely *P. falciparum, P. vivax, P. ovale* and *P. malariae*. The most widespread and severe disease is caused by *P. falciparum*, which transiently infects the liver before invading red blood cells of the mammalian host. Clinical manifestations occur at the erythrocytic stage and can include fever, chills, prostration and anaemia, as well as delirium, metabolic acidosis, cerebral malaria and multi-organ system failure, which may be followed by coma and death [5, 6, 7].

*Corresponding author: *wanomar@medic.upm.edu.my*

A traditional antimalarial compound, Quinine (an aminoquinoline alkaloid) was isolated from the bark of *Cinchona* species (Rubiaceae) in 1820 by Pelletier and Caventou. It is one of the oldest and most important antimalarial drugs and is still used today. For almost three centuries, this alkaloid was the sole active principle effective against *Plamodium falciparum*, and it has been considered that this compound was responsible, after the Second World War, for the development of synthetic antimalarial drugs belonging to the classes of 4- and 8-aminoquinolines, such as chloroquine and primaquine), among others. Until recently, chloroquine) was the only drug used for the treatment of malaria [8, 9]. The appearance of drug-resistance *P. falciparum* strains since 1960, in particular to chloroquine, has made the treatment of malaria increasingly problematic in virtually all malarious regions of the world [10].

Currently the most promising active compounds are derived from Chinese medicinal plant, "Qinghasu" and especially artemisinin, one of the derived compound, has potential as an alternative to chloroquine. There is no single drug that is effective for treating multi-drug resistant malaria. Drugs combination which include artemisinin derivatives such as artesunate, or mixtures with older drugs such as the atovaquone – proguanil combination Malarone® [2, 10] provide effective combination therapy. Unfortunately first reports on drug resistance to artemisinin-derivatives [11] and to drug combination therapies [12] have appeared. So, in the absence of a functional, safe and widely available malaria vaccine, continuous efforts toward the development of new antimalarial drugs should be an urgent priority.

Natural products have been playing a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases [13]. Undoubtedly, the vast majority of the existing antimalarial chemotherapeutic agents are based on natural products, and this fact anticipates that new leads may certainly emerge from the tropical plant sources, since biological chemodiversity continues to be an important source of molecular templates in the search for antimalarial drugs [14 - 16].

In Malaysia, chloroquine resistant case was first reported in 1963 [17, 18]. Subsequently, several chloqoquine resistant cases have been reported in Sabah, West Malaysia [19]. In addition, other drug resistant cases were also detected. For example, the combination of sulfonamides-pyrimethamine [20, 21] and sulfadoxine-pyrimethamine resistant [22, 23]. The study reported by Lokman *et al* (1996) revealed a widespread resistance of Falciparum malaria to both chloroquine and sulfadoxine-pyrimethamine in endemic areas of Peninsular Malaysia [24].

Traditional and modern medicine share a common resource. They both utilize either plants, animals, micro-organisms or minerals. Malaysia, a developing country, is rich in natural resources. More than 20,000 species of angiosperms and 600 species of ferns in Malaysia, 1,082 species (15%) and 76 species (13%), respectively, are reported to have medicinal properties. A big portion of plants have long been in use by the Malay community because of their claim to be of medicinal value in the treatment of a variety of infections including malaria.

WHO estimates 80% of the world population uses herbal crude extracts for treatment of various ailments other than for culinary purposes. In addition the majority of the rural poplation in poor countries have limited access to formal health services and the distribution of health personnels and medical supplies are largely concentrated in the urban areas. Medicinal plants, whether prepared as crude extracts or in semi-purified forms are widely used in the treatment of a variety of disorders.

CRUDE PLANT EXTRACTS WITH ANTIPLASMODIAL ACTIVITY

Although many crude plant extracts often show only modest activity against the parasites in vitro or against malaria in mice, suggesting that the species in question probably have only a limited effect in man and that cure of the disease is unlikely. However, this may not necessarily mean that medicines made from these species are of no value, since partially effective treatments might be beneficial in those cases that the course of the disease is shortened by reducing anaemia and lowering the risk of death or serious illness from other anaemia-related diseases. Moreover, benefits may also include the alleviation of symptoms such as pain and fever and immunomodulation leading to increased immunity [25]. Finally, it is important to stress that plant extracts could also be effective against the parasite on hepatic stage [26].

In one study [27], crude extracts of ten Malaysian medicinal plants were screened for their antiplasmodial activity. These plants were selected based on their traditional claims for treatment of ailments or to relieve fever (Table 1). The 10 plants species selected in this study were: *Tinospora crispa, Xylocarpus granatum, Jasminium sambac, Andrographis paniculata, Physalis minima, Carica papaya, Ocimum sanctum, Nigella sativa, Ardisia crenata, Cinnamomum inners*. The 7-day suppressive test was employed to determine the parasitemia suppression of the plant extracts against *P. berghei*.

Out of 10 plants used in this study, 4 plants, *T. crispa, X. granatum, J. sambac and A. paniculata* showed very good in vitro antiplasmodial activities to *P. falciparum*. Their percentage inhibition were more than 50% inhibition (Table 2) to parasite growth at the 0.03 ug/ml extract concentrations. *T. crispa* showed the maximum at 90 % inhibition, followed

by *X. granatum* at 80 % and *J. sambac* and *A. paniculata* both at 70 %. These four plants showed minimal cytotoxicity on MDBK cells were *T. crispa, X. granatum, J. sambac* and *A. paniculata*. Their respective percent growth inhibition at 64 ug/ml concentration were 15.64 ± 1.19, 19.63 ± 0.62, 14.87 ± 0.47 and 18.89 ± 0.31 (Table 2).

Table 1. The local names of plants, parts used, method of consumption and their traditional

Plant	Local name	Plant part used and method of consumption
Tinospora crispa	Patawali	The stem is boiled and made into a drink to treat fever
Xylocarpus granatum	Nyireh	The stem is boiled and drunk for the treatment of fever, dysentery and filariasis.
Jasminum sambac	Melati or Melur	To treat a fever, drink water in which the flowers have been boiled
Andrographis paniculata	Hempedu bumi or Pokok Cerita	Water in which the leaves have been soaked drunk to treat a fever.
Physalis minima	Letup-letup	Water boiled with the plant is drunk to protect one against worm infestation and fever
Carica papaya	Papaya	Young leaves are pounded and mixed with water to create a drink
Ocimum sanctum	Selasih	Water that has been soaked with the seeds is drunk for coughs and to treat fever.
Nigella sativa	Jintan hitam	Water boiled with crushed seeds is drunk to cure rheumatism, fever and to improve general well-being.
Ardisia crenata	Mata ayam	To treat fever and diarrhea, the root is pounded till fine and eaten
Cinnamomum iners	Teja lawang	The root is boiled and water drunk for treating rheumatism and fever.

Table 2. *In vitro* inhibition of *P. falciparum* of methanol plant extracts and cytotoxicity to MDBK cells

Species	Plant part	Antiplasmodial activity (% of growth inhibittion or IC_{50})*	Cytotoxicity to MDBK cells (IC_{50})**
Tinospora crispa	Stem	>90	15.64 ± 1.19
Xylocarpus granatum	Barks	>80	19.63 ± 0.62
Jasminum sambac	Flowers	>70	14. 87 ± 0.47
Andrographis paniculata	Leaves	>70	18.89 ± 0.31
Physalis minima	Whole plant	34.68 ± 5.31	Not active
Carica papaya	Leaves	16.80 ± 1.29	Not active
Ocimum sanctum	Whole plant	14.20 ± 0.49	Not active
Nigella sativa	Seeds	18.03 ± 0.69	Not active
Ardisia crenata	Roots	9.95 ± 1.44	Not active
Cinnamomum iners	Roots	12.73 ± 3.04	Not active

* Results are recorded as the % of growth inhibition (or IC_{50}). The results for *T. crispa, X. granatum, J. sambac* and *A. paniculata* were given in percent of growth inhibition because the growth were beyond the 50 % level at the last concentration tested that is 0.03 ug/ml. All test samples were performed in triplicates and reported as mean ± SD. Not active because there was no growth inhibition.

**Cytotoxicity to MDBK cells. All tests were performed in triplicates and reported as mean ± SD

In mice infected with *P. berghei*, the group which received treatment with *T. crispa* showed the highest inhibitory effect (parasitemia suppression) on parasite growth *in vivo* (Table 3). At the end of day 5 all infected mice in the control groups died. Mean parasitemia suppression in the *P. berghei*-infected mice ranged from 1.14 ± 0.22 on day 1 to 50.73 ± 1.32 on day 6 (Table 3). By day 7 all mice died. Mice that survived until the sixth day were those treated with *T. crispa* (n=3) and *X. granatum* (n=2). The parasitemia suppression ranged from 50.73 ± 1.32 and 49.78 ± 2.30 for *T. crispa* and *X*. granatum respectively. Generally parasitemia suppression gradually increase and this probably extend the days of survival of treated mice to the infection. The mean percentage parasitemia per 1000 erythrocytes in the two control groups were between 1.67 ± 0.42 on day 1 to 40.68 ± 1.02 by day 4. There was a gradual increase in parasitemia which reached maximum of 40.68 ± 1.02. All control mice died by day 5.

Table 3. *In vivo* percentage parasitemia suppression* of *Plasmodium berghei* in mice treated with plant extracts and mean parasitemia in the control

No. of days	% parasitemia suppression in treated mice				**Control (% parasitemia per 1000 RBCs)
	T. crispa	*X. granatum*	*J. sambac*	*A. paniculata*	
Day 1	1.74±0.13	1.14±0.22	1.72±0.9	1.35±0.42	1.67±0.42
Day 2	1.26±0.25	2.89±1.05	2.94±0.71	3.17±0.69	6.94±1.10
Day 3	13.83±1.64	18.01±3.94	17.65±1.42	18.51±1.03	17.68±3.45
Day 4	32.11±1.78	34.28±5.90	26.44±5.19	28.38±2.26	40.68±1.02
Day 5	42.18±2.10	41.69±0.92	31.21±1.03	37.50±3.30	All died
Day 6***	50.73±1.32	49.78±2.30	All died	All died	
Day 7	All died	All died			

* Mice were divided into two experimental groups consisting of 8 mice per group and parasitemia suppression reported as mean ± SD each day for every plant extract

** Control received distilled water. Percentage of parasitemia was counted based on infected erythrocytes calculated per 1000 erythrocytes. Mice were divided into two control groups consisting of 8 mice per group and parasitemia levels reported as mean ± SD each day for every plant extract.

*** Day 6 : T. crispa: 3 survived and 2 survived with J. sambac

Nor Rain *et al.* in 2007[28] similarly studied antiplasmodial activities of plant extracts using the pLDH assay to *Plasmodium falciparum* D10 strain (sensitive strain) while the cytotoxic activities were carried out towards Madin-Darby bovine kidney (MDBK) cells using MTT assay. The concentration of extracts used for both screening assays were from the highest concentration 64 µg/ml, two fold dilution to the lowest concentration 0.03 µg/ml. *Goniothalamus macrophyllus* (stem extract) showed more than 60% growth inhibition while *Goniothalamus scortechinii* root and stem extract showed a 90% and more than 80% growth inhibition at the last concetration tested, 0.03 µg/ml. The *G. scortechini* (leaves extract) showed an IC50 (50 % growth inhibition) at 8.53 µg/ml, *Ardisia crispa* (leaves extract) demonstrated an IC50 at 5.90 ± 0.14 µg/ml while Croton argyratus (leaves extract) showed a percentage inhibition of more than 60% at the tested concentration. *Blumea balsamifera* root and stem showed an IC50 at 26.25 ± 2.47 µg/ml and 7.75 ± 0.35 µg/ml respectively. *Agathis borneensis* (leaves extract) demonstrated a 50% growth inhibition at 11.00 ± 1.41 µg/ml.

Riduan *et al.* 2006[29] studied the enhancement of antimalarial properties combination of goniothalamin with chloroquine. Percentage of parasite growth on treated infected mice were determined based on 4 Day Test. Oral treatment with 1 mg/kg BW of chloroquine on experimental mice suppressed 70% and 76.7% of both *Plasmodium yoelii* and *Plasmodium berghei*, respectively. The infection of *P. berghei* in mice was inhibited less than 50% by goniothalamin individual treatment at all doses in this study. About 27.8% and 18.5% inhibition of infection were observed in *P. yoelii* infected mice treated with 30 mg/kg and 60 mg/kg of goniothalamin respectively and the suppression exceed more than 50% at higher doses (90 and 120 mg/kg). Combination of 1 mg/kg chloroquine with either 30 mg/kg or 60 mg/kg of goniothalamin decreased the parasitemia of *P. yoelii* infected mice more than 90% and prolong the survival up to 100% after treatment. Similar treatment to *P. berghei* infected mice only shows about 60% reduction of parasitemia. The study findings showed that antimalarial property of goniothalamin was enhanced by combination with chloroquine at lower dose of each drug.

ISOLATED PLANT ANTIPLASMODIAL BIOACTIVE COMPOUNDS

Several classes of bioactive compounds isolated from plants possess antimalarial activity. However, the most important and diverse biopotency has been observed in alkaloids, quassinoids and sesquiterpene lactones. The chemical structures of some traditional antimalarials are shown in Figure 1.

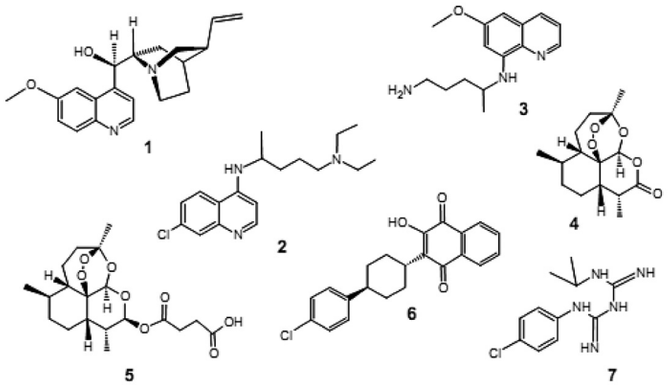

Figure 1. Chemical structures of some traditional antimalarial drugs. 1) Quinine (1), chloroquine(2), primaquine (3), artemisinin (4), artesunate (5), atovaquone (6) and proguanil (7)

Quinine, an aminoquinoline alkaloid isolated from the bark of Cinchona species (Rubiaceae) in 1820 by Pelletier and Caventou, is one of the oldest and most important antimalarial drugs and is still used today. For almost three centuries, this alkaloid was the sole active principle effective against *Plamodium falciparum*, and it has been considered responsible, after the Second World War, for the development of synthetic antimalarial drugs belonging to the classes of 4- and 8-aminoquinolines, such as chloroquine and primaquine, among others. Until recently, chloroquine was the only drug used for the treatment of malaria [30].

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The appearance of drug-resistance *P. falciparum* strains since 1960, in particular to chloroquine, has made the treatment of malaria increasingly problematic in virtually all malarious regions of the world. Several researchers have dedicated efforts to the development of new active compounds, especially from artemisinin, as an alternative to chloroquine. Currently no single drug is effective for treating multi-drug resistant malaria, and effective combination therapy includes artemisinin derivatives such as artesunate, or mixtures with older drugs such as the atovaquone–proguanil combination [31] provide effective combination therapy. Unfortunately first reports on drug resistance to artemisinin-derivatives and to drug combination therapies have appeared. So, in the absence of a functional, safe and widely available malaria vaccine, research to develop alternative therapies is greatly needed. To this end, plants used in traditional medicines may offer a promising source of compounds with antimalarial activity. Indeed, plant products and their derivatives have traditionally been a common source of drugs.

SECONDARY PHYTOCHEMICALS

Several classes of the secondary phytochemicals possess antimalarial activity. However, the most important and diverse biopotency has been observed in alkaloids, quassinoids and sesquiterpene lactones. The chemical structures of some traditional antimalarials are shown in Figure 1.

Alkaloids are the physiologically-active nitrogenous bases derived from many biogenetic precursors. A new bisbenzylisoquinoline alkaloid named as 2-N-methyltelobine together with twelve known alkaloids [32, 33, 34] of the same group were isolated from *Stephania erecta* (Menispermaceae). All the alkaloids inhibited the growth of cultured chloroquine-resistant and sensitive strains of *P. falciparum* [35, 36, 37, 38, 39].

A bisbenzylisoquinoline alkaloid dehatrine [40] isolated from the wood of *Beilschmiedia madang* (Lauraceae), exhibited potent inhibitory activity (IC50 0.017 mM) against the proliferation of malaria pathogen *P. falciparum*, which was comparable to quinine. Cryptolepine is an indolisoquinoline antimalarial alkaloid with IC50 value approximately

half that of chloroquine. In view of this high degree of in vitro activity, it was surprising that the isolated alkaloid proved to be inactive in mouse against the *P. berghei* model [41, 42, 43]. It was shown that the alkaloid might interact with DNA, and it appeared that two nitrogen atoms N and N-CH3 of cryptolepine interact with adenine–thymine base pair. There is also a possibility of formation of p–p charge transfer complex between purine–pyrimidine bases and cryptolepine [44, 45, 46].

Another interesting antimalarial compound tubulosine was found active in vitro against both sensitive and resistant strains of *P. falciparum* [47, 48, 49]. The indol moiety in tubulosine enhances the affinity for protozoan receptor, when compared with psychotrine and cephaeline [50, 51]. The relative in vitro inactivity of in comparison with can be explained by its double bond in ring C, which enhances the coplanar conformation and electron environment [52].

Quassinoids.

The quassinoids are heavily oxygenated lactones with majority of C20 basic skeleton named as picrasane. A wide spectrum of biological properties was reported for this class of compounds, of which antineoplastic and antimalarial have equal and parallel importance [53] The quassinoids brusatol, bruceantin and brucein A, B and C differ only in the nature of the ester moiety [54] Its IC_{50} values were similar for chloroquine-resistant and sensitive strains, suggesting that quassinoids may act upon malarial parasites by means of a fundamentally different mechanism from that of chloroquine. Cedronin possesses some of the structural requirements for cytotoxic activities. The results also suggested cedronin exhibits lower selective toxicity against Plasmodium than against mammalian cells [55] .

Sesquiterpenes

The discovery of Qinghaosu (artemisinin), a novel sesquiterpene lactone endoperoxide antimalarial constituent from the Chinese plant 'Qinghao' (*Artemisia annua*), prompted the investigation of some other naturally occurring peroxides for their schizonticidal activity. Artemisinin is a new class of antimalarials, where the endoperoxide moiety plays an important role. The definitive mode of action of this series of drugs is still not known. After being opened in the *Plasmodium* it liberates singlet oxygen and forms a free radical, both being strong cytotoxins. In vitro-testing using the inhibition of radio-labelled hypoxanthine uptake as an index of drug effect on parasite growth suggests that artemisinin causes a marked diminution of nucleic acid synthesis. The drug effect on this process is, however, rather slow; well-defined concentration response curves being generated only after a 6–8 h incubation period. Dihydroartemisinin is over 200 times more effective than artemisinin in reducing 3H-hypoxanthine uptake. The inhibitory action of artemisinin on the incorporation of 3H-leucine into the parasite protein is much more rapid than that of hypoxanthine, which has led some researchers to hypothesize that protein synthesis may be one of the prime targets of drug action. Unlike chloroquine, artemisinin does not directly cause malaria parasite haemozoin to clump, but it does inhibit clumping caused by subsequent exposure to chloroquine. It has also been reported that one of the mechanisms of action is due to its inhibition of cytochrome oxidase, which occurs at the plasma, the nuclear and the food vacuole-limiting membranes as well as in the mitochondria of the trophozoites of *P. berghei*. It was demonstrated in neurolenin B, that a, b -unsaturated keto function is one of the structural requirements for high in vitro antiplasmodial activity. Additionally, a free OH function at C-8 increases and at C-9 decreases the activity. The two endoperoxides: nardoperoxide and isonardoperoxide isolated from the roots of *Nardostachys chinensis*, showed strongest antimalarial effects. It is noteworthy that activity and selectivity of isonardoperoxide was comparable to those of quinine, a clinically used drug. Nardoperoxide and isonar doperoxide seem to be the promising lead compounds for antimalarial drugs [56, 57].

Triterpenoids

Triterpenoids isolated from different medicinal plants exhibit antimalarial property, for example, Gedunin activity of which is about three times higher than chloroquine, but twenty-times lower than quinine. Comparison of activities of gedunin and dihydrogedunin suggested that the reduction of the double bond in a ,b -unsaturated keto function lead to a decrease of antimalarial activity and increase in toxicity [58].

Flavonoids and xanthones

The antimalarial activity from these classes of compounds has not been described earlier, although it constitutes one of the most characteristic classes of compounds in higher plants. Flavonoids isolated from *Artemisia annua* were not found active against *P. falciparum*, but demonstrated a marked and selective potentiating effect on the antiplasmodial

activity of artemisinin [59, 60].

Quinones

Chemically, quinones are compounds with a 1,4-diketo-cyclohexa-2,5-dienoid or a 1,2-diketocyclohexa-3,5-dienoid moiety [61] The structure of many naturally-occurring quinones is based on the benzoquinone, naphthoquinone or anthraquinone ring system. Napththoquinones are rather promising as blood schizonticides, since they are highly active against *P. falciparum* in vitro [62] Roots of Nepenthes thorelii yielded plumbagin and 2-methylnaphthazarin both of which were evaluated against *P. falciparum*. The quinone structure was regarded essential for the activity of naphthoquinones like plumbagin[63, 64].

Miscellaneous compounds and Essential Oil

Various compounds with different chemical structures possessing antimalarial activity have been isolated from plants. The active constituents isolated from *Piptadenia pervillei, Moronobea coccinea, Holostylis reniformis* were phenolic compounds, benzophenones and aryltetralone lignans respectively. All these compounds were shown to possess significant antimalarial activity due to the presence of a, b - unsaturated carbonyl moiety [65, 66, 67]. The a, b -unsaturated carbonyl moiety was suspected to undergo a Michael reaction with nucleophilic sites in the parasite DNA molecule, thereby inhibiting the growth of *P. falciparum*.

The essential oil from the leaves and stem of *Tetradenia riparia* was tested. Moderate antimalarial activity was recorded against two strains of *P. falciparum*. Essential oils of *Artemisia vulgaris, Eucalyptus globulus, Myrtus communis, Juniperus communis, Lavandula angustifolia, Origanum vulgare, Rosmaricus officinalis* and *Salvia officinalis* were tested against two strains of *P. falciparum*, FcB1-Columbia and a Nigerian chloroquine resistant strain. Concentrations ranging from 150 mg/ml to 1 mg/ml inhibited 50% of the parasite growth in vitro which is obtained after 24 to 72 h of contact between the oil and parasite culture. The best results were obtained with *M. communis* and *R. officinalis* oils, which inhibited P. falciparum at a concentration ranging from 150 to 270 mg/ml [68].

CONCLUSION

Malaria is still the most destructive and dangerous parasitic infection in many tropical and subtropical countries. The burden of this disease is getting worse, mainly due to the increasing resistance of Plasmodium falciparum against the widely available antimalarial drugs. There is an urgent need for new, more affordable and accessible antimalarial agents possessing original modes of action. Plant products have played a dominant role in the discovery of leads for the development of drugs to treat human diseases, and this fact anticipates that new antimalarial leads may certainly emerge from tropical plant sources. The recently developed new isolation and characterization techniques together with development of new pharmacological testing undoubtedly shall further facilitate discovery of newer derivatives with improved properties. The search for additional antimalarials from higher plants must continue to fight the disease.

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