Neuropsychological Correlates of Hippocampal Volumes in First Episode Psychosis

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Introduction

There is increasing evidence that schizophrenia is a brain disorder and numerous neuroimaging studies show brain abnormalities in schizophrenia patients. Magnetic resonance imaging (MRI) studies have linked hippocampal abnormalities in the pathophysiology of psychosis (Bogerts et al, 1990; Bogerts et al, 1993). A meta-analysis of 18 structural MRI studies (Nelson et al, 1998)[1] reported a 4% reduction in bilateral hippocampal volumes in schizophrenia patients compared to normal controls.

As highlighted by several reviews (Moser et al, 1999; Strange et al, 1999), there are neuroanatomical and functional differences between anterior and posterior hippocampus projections. While the anterior hippocampus is predominantly connected to other limbic and striatal systems, the posterior hippocampus receives converging sensory input from posterior cortices. In fact, Suddath et al (1990) and Weinburger et al (1992) found abnormalities in anterior hippocampus in relation to other parts of the hippocampus between monozygotic twins discordant for schizophrenia.

However, as most of these studies involved chronic patients, their findings could be confounded by chronicity of the illness and long-term medication effects. Investigating patients with first-episode psychosis circumvents the problem and allows us to look at structural brain changes directly relevant to the disease process.

In attempting to understand how neuroanatomical abnormalities translate into cognitive performance, studies have investigated the neuropsychological profile of patients with first-episode psychosis (Censits et al, 1997; Bilder et al, 2000; Addington et al, 2003).[2] They found that patients showed deficits in domains such as verbal fluency, executive function, attention and memory.

Few have looked at neuropsychological performance and anterior versus posterior hippocampal volumes in first-episode patients. Studies by Bilder et al (1995)[3] and Szeszko et al (2002)[4] found an association between smaller anterior hippocampus and worse executive and motor functions. This study aims to look at neuropsychological correlates of hippocampal volumes in Asian patients with first-episode psychosis.

Materials and Methods

Consecutive patients accepted into the Early Psychosis Intervention Programme (EPIP) were approached for participation. Exclusion criteria were neurological problems, significant medical illness, alcohol or drug abuse and mental retardation. Diagnosis was made using the Structured Clinical Interview for DSM-IV diagnoses (SCID). Duration of psychosis (DUP) was determined from time of onset of first psychotic symptoms to time of definitive diagnoses as assessed from structured interviews with patients and relatives. Written informed consent was obtained and patients were scanned within 4 weeks of starting antipsychotics. Patient demographic and clinical details are provided in Table 1.

Table 1. Patients' Demographic and Clinical Characteristics

Variable	No. of patients	
Gender		
Female	5	(35.7%)
Male	9	(64.3%)
Race		
Chinese	12	(85.7%)
Indian	2	(14.3%)
Diagnosis		
Schizophrenia	10	(71.4%)
Schizoaffective disorder	1	(7.1%)
Schizophreniform disorder	1	(7.1%)
Bipolar disorder with psychotic features	1	(7.1%)
Brief psychotic disorder	1	(7.1%)
	Mean (SD)	
Age (y)	26.5	(6.6)
Duration of untreated psychosis (mo)	11.5	(11.6)
Years of schooling	11.6	(2.7)

MRI Data Acquisition and Image Analysis

Patients underwent imaging on a 1.5T MRI scanner (NVi, GE Medical Systems, Wisconsin, MI) with high resolution, fast gradient recalled (FGRE) 3D volumetric scans (TR/TE/TI/flip angle 6.4/1.5/400/20; matrix 256 x 256, FOV mm²) with coronal orientation, covering the whole brain for structural-anatomic detail.

Hippocampal volume measurements were performed using MRreg v.1.6.2 software by an experienced reader blinded to left-right anatomic status. Anterior-posterior hippocampal volume delineation was done using published procedure (Sullivan et al, 1995).[5]

Neurocognitive Battery

A neurocognitive battery was administered within 2 months when patients were deemed clinically stable by their clinicians. Tests used in the neurocognitive battery are shown in Table 2.

Results

Fourteen patients were recruited. Spearman's partial correlations examined the relationship between hippocampal volume and

Table 2. Tests Used in Neuropsychological Battery

Premorbid Cognitive Functioning
National Adult Reading Test (NART)
Current Cognitive Functioning
Raven's Progressive Matrices (RPM)
Attention
Continuous Performance Test (CPT)
Digit Span, Wechsler Adult Intelligence Scale – 3rd edition (WAIS – III)
Executive Functioning
Trail-making Test, Parts A and B
Wisconsin Card Sort Task (WCST)

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neuropsychological tests, controlling for age, gender and years of education.

Significant correlations were found for scores on Trails Part A and left anterior hippocampal volume ($r = 0.81$, $P < 0.05$), and Trails Part B and total anterior hippocampal volume ($r = 0.76$, $P < 0.05$), suggesting better performance with bigger hippocampus. No other significant correlations were found.

Discussion

Even upon first presentation, our first-episode patients had significant impairment on tasks of executive function. In particular, anterior hippocampal volume was significantly correlated with executive functioning, which is not explained by years of education. This is consistent with results obtained by Bilder et al (1995)[3] and Szeszko et al (2002).[4] The association of executive functioning deficits with reduced anterior hippocampus is also compatible with structural and functional-anatomic data relevant to schizophrenia.

Our study sampled first-episode psychosis patients with no long-term medication or a history of substance abuse. Further research using in vivo functional Magnetic Resonance Imaging (fMRI) techniques will shed more light on this structural-functional link.

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Special Poster Presentation

Nuclear Factor kappa B Transcription Profiling of Genes Protect Against Nitric Oxide-induced Neuronal Apoptosis

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EXTENDED ABSTRACTS

Introduction

Nitric oxide (NO) is a multifaceted molecule that is involved in a wide range of physiological and pathophysiological processes. It is also implicated in a variety of neurological disorders like stroke, seizure and Alzheimer's disease.

Nuclear Factor kappa B (NF-κB) is a transcription factor that contributes to synaptic transmission, neural development and is also involved in neurodegenerative diseases in the nervous system. In a previous report, we showed that NF-κB plays a protective role against nitric oxide-induced apoptosis in neuroblastoma cell line, SHEP-1.[1] However, the neuroprotective mechanism of NF-κB remains to be elucidated.

Neuronal cell death can also be modulated by other factors. Insulin factor-like growth factor (IGF-1) is a potent neurotrophic and anti-apoptotic factor which activates NF-κB. In this study, the role of IGF-1 in NF-kB -mediated neuroprotection of NO-induced apoptosis is investigated.

Material and Methods

Cell Culture

Human SHEP-1 neuroblastoma cells were maintained in Dulbecco's modified Eagle's medium containing 10% foetal calf serum, 100 units/mL penicillin, and 100 µg/mL streptomycin at 37°C in humidified 5% CO_2. To clarify the role of NF-κB in NO-induced apoptosis in SHEP-1, a stable transformed SHEP-1 neuroblastoma cells expressing I-κBαM, deviating from I-κBα that binds and inhibit NF-κB was created using LipofectAMINE and maintained with 500 µg/mL G418.

NF-κB p65 Activator Assay

SHEP-1 cells were treated with IGF-1 at the indicated time frames. Proteins were harvested and subjected to the reporter assay according to the protocol of TransAM NF-κB p65 activator assay (Active Motif). Protein concentration was determined by Bio-Rad protein assay.

Determination of Cell Viability

Neuronal cell viability was assessed by crystal violet staining where SHEP-1 and I-κBαM cells were grown in 96-well plates in triplicates and treated with SNP for up to 24 h.

cDNA Microarray

RNA of the I-κBαM and IGF-1 treated SHEP-1 were harvested and subjected to a human 12K cDNA microarray (BD Biosciences Clontech).

RT-PCR and PCR

Total RNA was harvested from IGF-1 treated cells using Trizol reagent. The reverse transcription and polymerase chain reaction amplification (RT-PCR) from total RNA was performed as described in Superscipt™ III first strand synthesis system for RT-PCR. PCR amplification was performed using the primers of biglycan, Matrix Gla protein and Tenascin C with actin as control.

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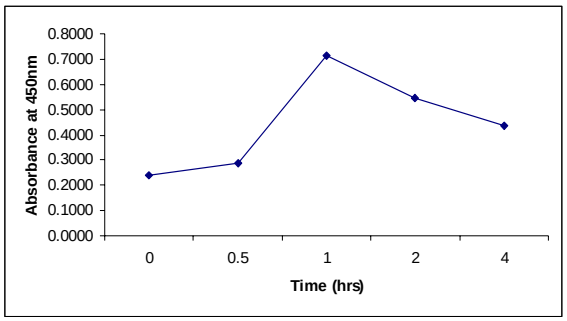

Fig. 1a.

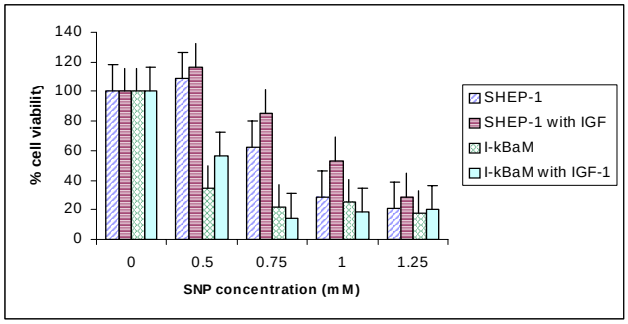

Fig. 1b.

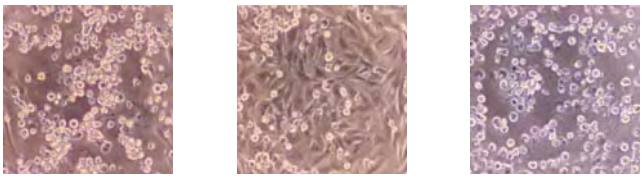

Fig. 1c.

Fig. 1. IGF-1 activates NF-κB and protects SHEP-1 cells against NO induced apoptosis. (a) IGF-1 treated SHEP-1 nuclear protein is subjected to NF-κB p65 activator assay where the absorbance is measured at wavelength of 405 nm with reference wavelength of 650 nm. (b) SHEP-1 and I-κBαM cells were exposed to the indicated SNP concentrations in the presence or absence of IGF-1 (200 ng/mL) for 24 hours. Cell viability was quantitated using crystal violet staining. (c) Photos of SHEP-1 cells treated with 0.75 mM NO (left), 0.75 mM NO and 200 ng/mL IGF-1 (middle) and I-κBαM cells treated with 0.75 mM NO and 200 ng/mL IGF-1 (magnification x400).

Increased gene expression

Anti-apoptosis
Biglycan
Glutathione peroxidase 3 (plasma)

Growth/Repair/Differentiation
Carbonic anhydrase IX
Serine (or cysteine) proteinase inhibitor, clade F
(alpha-2 antiplasmin, pigment epithelium derived factor), member 1
Colony stimulating factor 1 (macrophage)

Immune/Inflammation
Complement component 1, r subcomponent
Leukotriene b4 receptor (chemokine receptor-like 1)
Hypothetical protein FLJ10143

Signal transduction/Transcription
G protein-coupled receptor 38
RNA-binding protein gene with multiple splicing
G protein-coupled receptor 27

Metabolism
Transmembrane 7 superfamily member 2
Stearoyl-CoA desaturase (delta-9-desaturase)
Elastin microfibril interface located protein

Structural
EGF-containing fibulin-like extracellular matrix protein 1
Actinin, alpha 1
Actin related protein 2/3 complex, subunit 1A (41 kD)

Channels/Transporters
Solute carrier family 22 (organic cation transporter), member 4
Selenium binding protein 1
Potassium voltage-gated channel, Isk-related family, member 1

Hormone/Neurotransmmiters
Adenylate cyclase activating polypeptide 1 (pituitary)
Carboxypeptidase E
Prodynorphin

Electron transport
Oxidase (cytochrome c) assembly 1-like
Protease inhibitor 3, skin-derived (SKALP)
Hypothetical protein LOC51061

Other proteins
Matrix Gla protein
Glutamate-ammonia ligase (glutamine synthase)
Hypothetical protein PRO2121

Decreased gene expression

Signal transduction/Transcription
Arg/Abl-interacting protein ArgBP2

Metabolism
Adenosine deaminase

Hormones/Neurotransmmitor

Adrenomedullin
Channels/Transporters
Retinol binding protein 1, cellular
Glutamate receptor, ionotropic, kainate 3
Potassium inwardly-rectifying channel, subfamily J, inhibitor 1

Structural
Bicaudal D homolog 1 (Drosophila)
Other proteins
Nucleoredoxin 1
Protein expressed in thyroid
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2
Palmitoyl-protein thioesterase 2
U6 snRNA-associated Sm-like protein LSm7
Guanine nucleotide binding protein 11
Hypothetical protein HSN44A4A
CGI-63 protein
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, isoform 2
ATPase, H+ transporting, lysosomal 14kD, V1 subunit F
Stem cell growth factor; lymphocyte secreted C-type lectin
CGI-67 protein
Serine/threonine kinase 12
Tumor necrosis factor, alpha-induced protein 2

Fig. 2a.

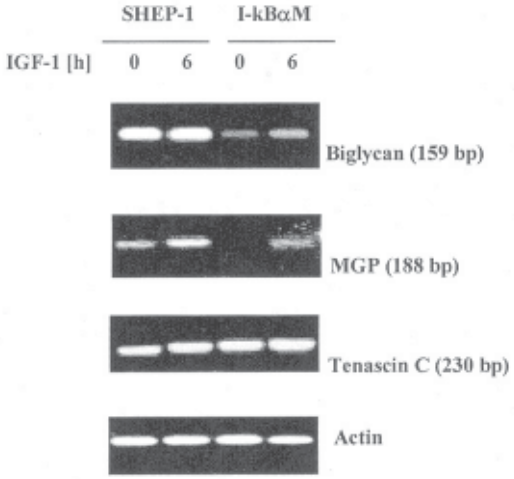

Fig. 2b.

Determination of Biglycan in Cell Culture

SHEP-1 and I-κBαM cells were treated with 200 ng/mL IGF-1 in serum-free DMEM medium. The medium was then collected, filtered and digested using 100 mU of chondroitinase ABCase for 16 h. Proteins were concentrated by incubating the medium with StrataClean™ resin, resuspended in 2 x SDS sample buffer and subjected to Western Blot analysis.

Western Blot Analysis

Biglycan protein was resolved by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane and probed with primary antibody against biglycan in PBS-T (provided by Dr KikuchiA, NCNP, Japan). After washing with PBS-T, the membrane was probed with horseradish peroxidase-conjugated antiserum to rabbit and developed by the enhanced chemi-luminescence's method.

Results

IGF-1 activates NF-κB in SHEP-1 cells

We have previously shown that IGF-1 induces the activation of NF-κB in SHEP-1 cell but not in I-κBαM cells using immunocytochemical staining of p65 subunit.[1] NF-κB reporter assay performed showed that NF-κB activity started increasing at 30 min, reached its peak at 1 h and decreased substantially after 2 h of IGF-1 treatments confirming NF-κB activation in SHEP-1 cells by IGF-1 (Fig. 1a).

Neuroprotective effects of IGF-1 against NO-induced apoptosis in an NF-κB-dependent manner

The effects of IGF-1 in NO-induced apoptosis were later examined. As seen in Figure 1b, I-κBαM cells were more sensitive to apoptosis induced by varying SNP concentrations as compared to control SHEP-1 cells. The administration of 200 ng/mL IGF-1 promoted the survival of NO-treated SHEP-1 in a dose dependent manner but not the case with I-κBαM cells (Fig. 1b and Fig. 1c). These results suggest that IGF-1 protects SHEP-1 cells against NO-induced apoptosis in an NF-κB dependent manner although the actual mechanism is still unclear.

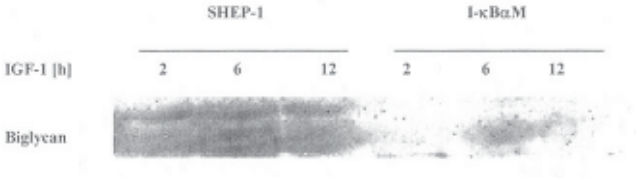

Fig. 2c.

Fig. 2. Analysis of cDNA microarray results. (a) RNA was extracted from 200ng/mL IGF-1 treated SHEP-1 cells treated with 200 ng/mL IGF-1 and I-κBαM cells and subjected to 12K human cDNA microarray analysis. Altered expression of genes that had more than 5 fold are divided into different categories. (b) The gene expression of biglycan, Matrix Gla protein and Tenascin C were evaluated using RT-PCR with actin as internal control. (c) Immunoblotting analysis of biglycan from IGF-1 treated SHEP-1 and I-κBαM cell culture medium at the indicated time frames.

cDNA Microarray Results

To elucidate the genes that are involved in the protective role of IGF-1 in NO-induced apoptosis in SHEP-1, cDNA of I-κBαM cells and IGF-1 treated SHEP-1 were harvested for cDNA microarray analysis. The results were analysed and only genes with more than 5-fold change in expression were listed and categorised in Figure 2a. As seen in Figure 2b, the administration of IGF-1 upregulated biglycan, Matrix Gla protein mRNA in SHEP-1 cells as compared to those in I-κBαM cells.

IGF-1 Upregulates Biglycan in SHEP-1 Cells

Biglycan was shown to be nitric oxide-regulated and to promote the survival of mesangial cells.[2] We investigated the protein levels of biglycan in IGF-1 treated SHEP-1 and I-κBαM cells using immunoblot analysis. Biglycan was upregulated for the first few hours and reached its peak at 6 h post treatment of IGF-1. However, with prolonged exposure of up to 12 hours, Biglycan was shown to decrease substantially (Fig. 2c). Similar trend of results were observed in IGF-1 treated I-κBαM cells although the levels of biglycan was much reduced. These results suggest that biglycan might be one of the genes targeted by NF-κB in triggering off the NF-κB-mediated neuroprotection mechanism.

Conclusion

In this study, we have shown that IGF-1 is involved in the neuroprotection against NO-induced apoptosis in a NF-κB-dependent manner with altered expression of various genes like Matrix Gla protein and biglycan. This information obtained can potentially be used in therapeutic approaches in neurological disorders.

Acknowlegement

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Comparison of Body Mass Index and Subjective Global Assessment as Indices of Nutrition in Hospital Inpatients

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Introduction

There are many different indices of nutrition available for assessing nutritional status and diagnosing malnutrition. Some are easily performed, readily available and inexpensive, whereas, others are sophisticated, not available and expensive.[1] Anthropometric methods are commonly used to describe nutritional status as it provides an overview of the human body composition.[2] Weight, height and the derived body mass index (BMI) are easily available, inexpensive and practical indices. In adults, weight is usually stable and a change in weight suggests an abnormal process, nutritionally or otherwise.[1] Another practical method is Subjective Global Assessment (SGA). This is a clinical method for evaluating nutritional status that includes a patient's weight history, symptoms as well as physical parameters.[2] In this study we compared the use of the BMI and Subjective Global Assessment in patients admitted to an acute medical ward to evaluate their sensitivity in diagnosing malnutrition.

Materials and Methods

This was a prospective study conducted from 1 to 31 August 2003 in an acute admitting renal ward in a tertiary hospital in Singapore. In total, 55 patients who had both their BMI and Subjective Global Assessment evaluated by 2 dietitians were included in the study. Patients with oedema were excluded, as their dry weight was difficult to ascertain.

The mean age (± SD) of the subjects was 57.4 ± 14.5 years. Sixty-six per cent were diabetic and 58% had end-stage renal failure. Twenty-nine percent were males. Forty-five per cent were Chinese, 31% (Malay), 20% (Indian) and 4% (Others).

Body Mass Index: This was calculated using the formula: weight (kg)/height^2 (m). A BMI of <18.5 was considered underweight, 18.5 to 24.9 as normal weight and ≥25 as overweight. A BMI of <18.5 was considered indicative of malnutrition.

Subjective Global Assessment (SGA): A revised 7-point scale Subjective Global Assessment was used.[4] Subjective Global Assessment scores ranged from 1 to 7 with a score of 1 indicating severe malnutrition and 7 indicating normal nutrition. A Subjective Global Assessment of 6 or less was considered indicative of malnutrition.

All data were analysed using SPSS version 11.5. BMI and Subjective Global Assessment scores were cross-tabulated and the significance of the distribution was measured using the Chi-square test. *P* value of <0.05 was taken as statistically significant.

Results

The comparison of BMI and Subjective Global Assessment score data are shown in Table 1. Mean height (± SD) of the patients was 1.57 (± 0.08) metres and mean weight (± SD) was 60.62 (± 13.87) kg.

Three patients out of 55 (5%) had BMI <18.5. Of these, 2 out of 3 patients (67%) had Subjective Global Assessment <6. Twenty-six patients out of 55 (47%) had a Subjective Global Assessment <6. Of these, only 2 out of 26 patients (7.7%) had a BMI of <18.5. Only 2 out of 55 patients (4%) had a BMI <18.5 and a Subjective Global Assessment of <6. This distribution is statistically significant (*P* <0.05).

Table 1. Comparison of BMI[a] and SGA[b] Score in 55 Patients

SGA scoreBMI	<6	≥6	Total
<18.5	2	1	3
18.5 – 24.9	17	11	28
>25	7	17	24
Total	26	29	55

The value for *P* ≤0.05, Pearson's R.

Discussion

This study shows that Subjective Global Assessment usage may detect a higher incidence of malnutrition compared to the use of BMI.

BMI seems to be less sensitive in detecting malnutrition. This may be because it does not take into account a patient's weight and dietary history, symptoms like nausea, vomiting or diarrhoea, functional status relating to nutrition and physical parameters. In addition, although metabolic changes in relation to energy and protein deficiency can occur within hours or days of reducing nutrient intakes, anthropometric changes may take much longer.[3,4] In an acute admitting hospital this may be very prevalent. The changes in weight may not manifest. Therefore in this situation the Subjective Global Assessment may be a more sensitive indicator of malnutrition.

Nutritional status of patients is an important and modifiable factor that may influence disease processes and outcome.[2] Early detection and intervention is therefore important. An appropriate and sensitive method of assessment of malnutrition is therefore essential. This study shows that a method such as the Subjective Global Assessment which takes into account not only anthropometric measures like weight but also includes patient's symptoms and signs may be more appropriate.

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The Impact of Time-to-Balloon on Outcomes in Patients Undergoing Modern Primary Angioplasty for Acute Myocardial Infarction

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Introduction

Rapid time to treatment with thrombolytic therapy is associated with lower mortality in patients with acute ST-segment elevation myocardial infarction (STEMI).[1] However, the importance of time-to-primary percutaneous coronary intervention (PCI) in acute myocardial infarction (AMI) remains controversial. Cannon et al reported a delay in door-to-balloon time to be the major outcome predictor but not symptom-to-balloon time.[2] Other studies found symptom-to-balloon time to be more crucial.[3] Going by "open artery" theory, the more rapidly reperfusion is achieved with primary angioplasty, the better the outcome and conversely, delays in achieving reperfusion result in higher mortality.

Our study aimed to evaluate, in a single-centre cohort of patients with STEMI, the relationship between delay in symptom-to-treatment and door-to-treatment time on short- to medium-term clinical outcomes

Materials and Methods

Our study is a single-centre, prospective observational study conducted at NUH, Singapore. Acute STEMI was defined as a patient with a history suggestive of AMI accompanied by electrographic (ECG) evidence of ST-segment elevation of at least 0.1 mV in 2 or more ECG leads. There were no exclusion criteria. Between June 2001 and May 2003, a total of 208 consecutive, unselected patients with STEMI and had underwent primary PCI (without antecedent fibrinolytic therapy) were included.

Symptom-to-balloon time was defined as the interval between the time of patient's reported symptom(s) onset and time of first balloon inflation. Door-to-balloon time was the interval between the time of patient registration at emergency department and time of first balloon inflation.

Our hospital has fully computerised patient database systems that include EMDS (Emergency Database System), CPSS (Computerised Patient Support System) and catheterisation laboratory database (4D client, © 4D, Inc. 1995-2004). All data were acquired from the above databases.

Patients were divided into several pre-specified groups, first by time from symptom onset to first balloon inflation, and then by time of door to first balloon time. Baseline characteristics, mortality rate and major adverse cardiac event (MACE) rates were examined across these time categories. The primary endpoints of this study were mortality rate and MACE rate at 1 month and 6 months post-event. MACE was defined as death, myocardial infarction and repeat target vessel revascularisation.

All statistical analysis was performed using SPSS 11.5. Univariate analyses were conducted to identify the variables linked to mortality and MACE across the different time categories. Multivariate analysis was performed using logistic regression with adjusting for appropriate covariates. Statistical significance was assumed if $P < 0.05$.

Results

The demographic and clinical characteristics of 208 patients according to time-to-balloon are shown in Table 1. The ethnic composition of patients consisted of Chinese (65%), Indian (18%), Malay (15%) and others (2%). Patients were predominantly male and aged less than 70 years old. Patients with a longer ischaemic time were older, more often diabetic, and hypertensive. The majority of patients presented directly to NUH (86%), with transfers from TTSH (10%) and AH (4%) making up the rest. Two-thirds of the patients (65%) arrived at the hospital during office hours and weekdays.

Table 1. Baseline Variables by Symptom-to-Balloon Time

Baseline variable	Symptom-to-balloon time				
	<2 h (n = 7)	2-4 h (n = 100)	4-6 h (n = 48)	>6 h (n = 39)	*P* Value
% of total patients	3.6%	51.6%	24.7%	20.1%	N/A
Age <70 years	7 (100%)	87 (87%)	39 (81.3%)	34 (87.2%)	0.537
Age (y) (mean ± SD)	50.1 ± 8.3	54.3 ± 12.3	59.3 ± 11.5	57.5 ± 10.6	0.042
Men	7 (100%)	92 (92%)	41 (85.4%)	33 (84.6%)	0.391
Diabetes mellitus	0 (0%)	19 (19%)	13 (27.1%)	13 (33.3%)	0.124
Hypertension	3 (42.9%)	49 (49%)	28 (58.3%)	24 (61.5%)	0.454
Current smoker	3 (42.9%)	51 (51%)	23 (47.9%)	20 (51.3%)	0.927
Prior CABG	0 (0%)	1 (1%)	0 (0%)	0 (0%)	1.000
Prior MI	2 (28.6%)	7 (7%)	3 (6.3%)	5 (12.8%)	0.159
Anterior Wall Infarction	4 (57.1%)	60 (60%)	28 (58.3%)	19 (48.7%)	0.407

CABG: coronary artery bypass grafting; MI: myocardial infarct
Note: 14 (6.7%) missing data

Cardiogenic shock was presented in 16 (7.7%) of patients. The culprit vessel resulting in acute STEMI consists of left anterior descending artery (57%), right coronary artery (34%) and circumflex artery (9%). Adjuvant therapeutics administered includes coronary stenting (97%), glycoprotein IIb/IIIa inhibitors (47%), thrombectomy device (40%) and distal protection device (10%).

The median symptom to reperfusion and door-to-balloon times were 3 hours 55 minutes and 110 minutes respectively. Only 3.6% of patients achieved time to reperfusion in less than 2 hours after symptom onset. 35% of patients achieved door-to-balloon times of <90 minutes.

Clinical Outcomes by Time-to-balloon

Mortality at 1 month and 6 months was 8.7% and 10.7% respectively, whereas MACE at 1 month and 6 months were 8.9% and 14.1% respectively. The mortality at 1 month was reduced to 4.8% if cardiogenic shock patients were excluded.

Mortality and MACE rate both consistently showed an escalating trend with longer symptom-to-balloon and door-to-balloon time. (Fig.2) However, all outcomes did not reach statistical significance. Longer symptom-to-balloon time was a significant predictor of MACE event at 1 month (OR 1.441, p 0.011, CI 1.088-1.908) and 6 months (OR 1.184, p 0.048, CI 1.001-1.400), but not mortality after adjusting for differences in baseline variables by multivariate analysis. Door-to-balloon time, however, did not demonstrate any significant effect on outcomes.

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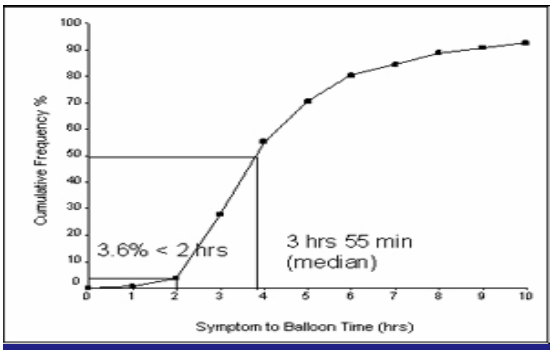
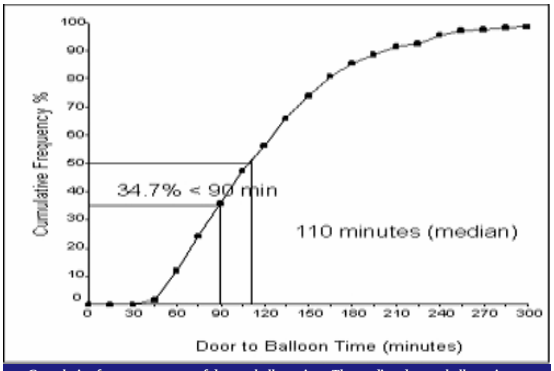
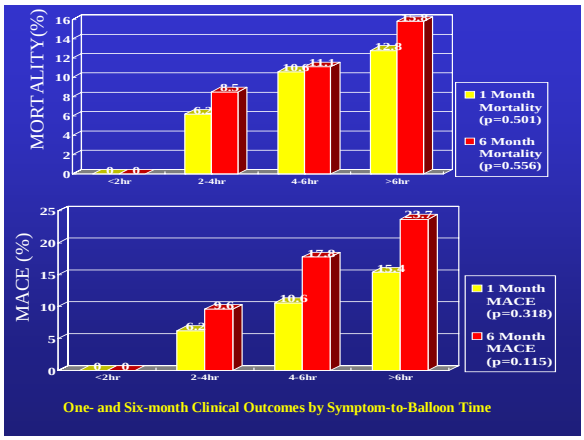


Figure 1. Cumulative frequency curves of Symptom to Balloon time. The median reperfusion time was 3 hours and 55 minutes, and 3.6% of patients achieved reperfusion in <2 hours.



Cumulative frequency curves of door to balloon time. The median door to balloon time was 110 minutes and 35% of patients achieved door to ballooon times of <90 minutes



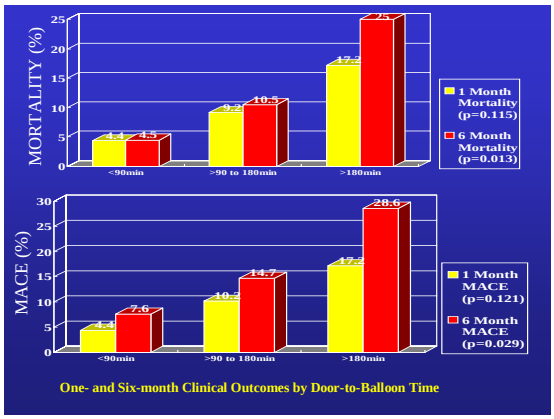


Fig. 2. One- and six-month clinical outcome by symptom-to-balloon and door-to-balloon time.

Discussion

The main finding of the present study is that among patients with STEMI undergoing modern mechanical reperfusion, delay in time from symptom onset to balloon is an important predictor of poor outcome. We are one of the first centres to report on this important issue in an Asian population.

The association between increased duration of coronary vessel occlusion and degree of myocardial necrosis has been well-characterised in animal models.[4] Therefore, late reperfusion is expected to result in poor flow, less myocardial salvage and thus suboptimal cardiovascular outcomes, even after optimal mechanical reperfusion. However, Zijlstra et al[5] reported that mortality increased linearly with time delay only in patients treated with thrombolysis, whereas it was relatively stable in patients treated by primary angioplasty. Nevertheless, evidence is gradually mounting that time to reperfusion is just as important in primary angioplasty, as it is in thrombolytic therapy. In our cohort of 208 patients with STEMI undergoing primary angioplasty, our findings support the prognostic role of early restoration of myocardial perfusion.

The fact that only 3.6% of patients achieved a symptom-to-balloon time of less than 2 hours is alarming. Merely 35% of our patients achieved a door-to-balloon time of less than 90 minutes in accordance to the recommendation of American College of Cardiology/ American Heart Association (ACC/AHA) guidelines for the management of AMI.[6] These findings imply that there are many opportunities for improvement in our current myocardial infarction management pathway.

Our finding that two-thirds of patients presented during office hours (Monday to Friday: 0800 h to 1700 h; Saturday: 0800 h to 1230 h) suggests the possibility that delay in presentation may have occurred as a consequence of inaccessibility to medical facilities during after hours and on weekends. This factor could contribute to a considerable delay in the recognition of AMI.

Despite consistent increase in mortality and MACE with longer delay for symptom-to-balloon or door-to-balloon time (Fig. 2), statistical significance was not reached. These findings are most likely explained by the limitation of small sample size with low rate of mortality. Nonetheless, symptom-to-balloon time has been positively associated with MACE at both 1 month and 6 months.

Educating public and healthcare providers plays a paramount role in minimising delay at both times. Other emerging strategies include the administration of pharmacological agents to facilitate the opening of occluded arteries in transition to PCI ("facilitated" PCI).

Conclusion

Improving public awareness and accessibility of health services to patients with STEMI is essential in reducing poor outcomes. Our data suggest that physicians, hospitals, and healthcare systems should work together to reduce symptom-to-balloon time.

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Special Poster Presentation

Signal-averaged Electrocardiograms of Children with Ventricular Septal Defects Before and After Surgical Repair

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EXTENDED ABSTRACTS

Introduction

Ventricular septal defect (VSD) is one of the most commonly encountered congenital heart defects in infants and children. The incidence of isolated VSD is about 2 per 1000 live births. While medical management is often the choice of treatment, surgical closure of the VSD may be indicated in some patients during the course of their disease. Operative mortality is often less than 5%.[1] Nevertheless, the incidence of ventricular dysrhythmias has been observed to increase with duration of postoperative follow-up.[2] The underlying mechanism(s) of such dysrhythmias have not been fully established.

The objective of this study was to assess the changes in signal-averaged ECG (SAECG) and the occurrence of late potentials (LP) in paediatric patients with ventricular septal defects, before and after corrective surgery. The hypothesis is that the presence of a VSD and/or surgical scarring of the myocardium may induce conduction disturbances that result in late potentials. These late potentials are high frequency, low amplitude potentials seen at the terminal portion of QRS and early ST segment of the ECG. Their presence may predispose an individual to ventricular dysrhythmias.[3]

Materials and Methods

Subjects

Paediatric patients with isolated VSD, either before (n = 148, 85 males, 63 females) or after (n = 66, 43 males, 23 females) corrective surgery were studied. Their ages ranged from 1 day to 17 years.

Age- and gender-matched healthy controls were similarly studied. The controls had no medical or cardiovascular disease by clinical examination and echocardiography.

All patients and controls had their SAECG measured. Relevant clinical variables including age, gender, height, weight, body surface area (BSA) and body mass index (BMI) were also measured.

Measurement of SAECG

SAECG parameters included 3 variables i.e., duration of filtered QRS (TQRS), duration of high frequency, low amplitude signals in the terminal portion of QRS complex that are <40μV (HFLA) and root mean square voltage of the last 40 ms of the filtered QRS (RMS_{40}). These were measured using a set of high-resolution ECG equipment (MAC-15, Marquette Electronics, Milwaukee, Wisconsin, USA) and the Frank orthogonal XYZ lead system. Each subject was measured at rest and in a supine position. Surface ECG signals were processed for analysis of high-frequency, low-amplitude signals. Signal averaging reduced noise and improved the signal-to-noise ratio. A minimum of 200 cardiac cycles to a maximum of 600 cycles was averaged for each subject. The process of averaging was terminated when noise level was <0.3 μV or up to a maximum of 600 cardiac cycles, whichever occurred earlier. Signal-averaged QRS complexes were filtered with a spectral filter having a high-pass frequency of 40 Hz and a low-pass cut-off at 250 Hz. Determination of TQRS, HFLA and RMS_{40} was done using the computer algorithm.

Age- and gender-specific criteria for defining LP was previously derived using a local population of healthy normal Chinese infants and children.[4] LP was defined as present when an individual had 2 or more abnormal SAECG parameters present as defined by the age- and gender-specific criteria.

Statistical Analysis

Independent-samples T-tests were used to compare differences in SAECG and clinical variables between different groups.

Results

The median age of patients with preoperative and postoperative VSD was 7.2 months (range, 1 day to 15.6 years) and 40.4 months (range, 2.4 months to 17.4 years) respectively. 15.5% of preoperative and 28.6% of postoperative VSD children had right bundle branch block (RBBB). Among the postoperative VSD children, 19.7% had LP, in contrast to 2.7% of preoperative VSD children (P <0.001) and 0.6% of controls.

SAECG and clinical variables of preoperative children were not different from that of controls but their BMI was significantly lower (14.9 ± 2.0 kg/m^2 versus 16.0 ± 2.3 kg/m^2, P <0.0001).

Postoperative VSD children had significantly prolonged TQRS and HFLA and lower RMS_{40} when compared to age- and gender-matched controls (Table 1) while their weight, height and BMI were not significantly different. While postoperative VSD children were significantly older, they had significantly prolonged TQRS and HFLA and lower RMS_{40} when compared to preoperative VSD

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Table 1. Comparison of Clinical Data, TQRS, HFLA and RMS_{40} of Postoperative VSD Children and Controls

	Postoperative (n = 66)	Control (n = 66)
Height (cm)		
Mean ± SD	104.8 ± 33.0	106.7 ± 33.2
95% CI	96.7, 112.9	98.5, 114.9
Range	59.0-171.0	55.0-178.0
Weight (kg)		
Mean ± SD	20.3 ± 16.1	21.5 ± 15.3
95% CI	16.3, 24.3	17.7, 25.2
Range	5.0-67.9	5.5-72.0
BSA (m^2)		
Mean ± SD	0.75 ± 0.41	0.78 ± 0.40
95% CI	0.65, 0.85	0.68, 0.88
Range	0.27-1.75	0.27-1.86
BMI (kg/m^2)		
Mean ± SD	15.9 ± 3.0	16.8 ± 2.2
95% CI	15.2, 16.7	16.2, 17.3
Range	11.9-28.3	13.0-24.1
TQRS (ms)		
Mean ± SD	121.3 ± 22.8*	00.8 ± 8.1
95% CI	115.7, 126.9	198.8, 102.8
Range	86.0-175.0	82.0-127.0
HFLA (ms)		
Mean ± SD	24.4 ± 9.5*	21.4 ± 5.7
95% CI	22.1, 26.7	20.0, 22.8
Range	12.0-58.0	11.0-37.0
RMS_{40} (μV)		
Mean ± SD	63.9 ± 46.4*	89.7 ± 54.0
95% CI	52.5, 75.3	76.5, 103.0
Range	12.0-217.0	28.0-263.0

* Postoperative versus Control, P <0.05

BSA: Body Surface Area
BMI: Body Mass Index
TQRS: Duration of filtered QRS
HFLA: Duration of high frequency, low amplitude signals in the terminal portion of QRS complex that are <40μV
RMS_{40}: Root mean square voltage of the last 40 ms of the filtered QRS

children (Table 2).

Discussion

The prevalence of LP in both the controls and preoperative VSD children are low, although the VSD children had slightly higher prevalence of LP (2.7%). Whether the presence of the structural cardiac defect, or the size and position of the VSD has any influence on the conducting pathways in the ventricular septum is not determined.

The prevalence of LP in VSD children after surgical correction of VSD is significantly higher (19.7%). Almost all these children had right ventriculotomy done during surgical repair of their VSD.

The presence of LP indicates slow, irregular or delayed propagation of electrical impulses in abnormal myocardium. As a consequence of such delayed activation or depolarisation of the myocardium high-frequency, low-amplitude micropotentials are seen at the terminal portion of the QRS complex and early ST segment of the ECG, thus giving rise to LP.[3] It may be postulated that in patients after surgical correction of VSD, myocardial scarring and fibrosis may be present at the site of surgical incision. Such fibrotic tissue can give rise to barriers to impulse conduction, thus lengthening the excitation pathway. The higher prevalence of RBBB in our postoperative VSD children adds support to this postulation.

Various studies have indicated the prognostic and predictive value of LP for ventricular dysrhythmias in patients with myocardial infarction, sudden cardiac death and cardiomyopathy.[5,6] The findings of this study imply that children after surgical correction of VSD and who had LP may be predisposed to ventricular dysrhythmias. The presence of myocardial scarring may be a contributory factor to an increased risk of ventricular dysrhythmias.

Table 2. Comparison of Clinical data, TQRS, HFLA and RMS_{40} of Preoperative and Postoperative VSD Children

	Preoperative (n = 148)	Postoperative (n = 66)
Age (years)		
Mean ± SD	2.5 ± 3.5	5.4 ± 5.0*
95% CI	1.9, 3.0	4.2, 6.7
Range	0.003-15.630	0.197-17.425
Height (cm)		
Mean ± SD	80.1 ± 31.0	104.8 ± 33.0*
95% CI	75.0, 85.1	96.7, 112.9
Range	46.5-163.0	59.0-171.0
Weight (kg)		
Mean ± SD	11.4 ± 10.3	20.3 ± 16.1*
95% CI	9.7, 13.0	16.3, 24.3
Range	2.3-72.8	5.0-67.9
BSA (m^2)		
Mean ± SD	0.48 ± 0.31	0.75 ± 0.41*
95% CI	0.43, 0.53	0.65, 0.85
Range	0.17-1.78	0.27-1.75
BMI (kg/m^2)		
Mean ± SD	14.9 ± 2.0	15.9 ± 3.0*
95% CI	14.6, 15.2	15.2, 16.7
Range	10.3-27.4	11.9-28.3
TQRS (ms)		
Mean ± SD	97.1 ± 11.5	121.3 ± 22.8*
95% CI	95.2, 99.0	115.7, 126.9
Range	70.0-148.0	86.0-175.0
HFLA (ms)		
Mean ± SD	20.0 ± 5.6	24.4 ± 9.5*
95% CI	19.0, 20.9	22.1, 26.7
Range	10.0-46.0	12.0-58.0
RMS_{40} (μV)		
Mean ± SD	137.7 ± 80.6	63.9 ± 46.4*
95% CI	124.6, 150.8	52.5, 75.3
Range	12.0-425.0	12.0-217.0

* Postoperative versus Preoperative, P <0.05

BSA: Body Surface Area
BMI: Body Mass Index
TQRS: Duration of filtered QRS
HFLA: Duration of high frequency, low amplitude signals in the terminal portion of QRS complex that are <40μV
RMS_{40}: Root mean square voltage of the last 40 ms of the filtered QRS

In summary, it is noted that there is a higher prevalence of children have RBBB and LP after the surgical repair of VSD, as compared to preoperative VSD children. The presence of LP may predispose these children to ventricular dysrhythmias.

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Causes of Isolated Prolonged Activated Partial Thromboplastin Time (APTT) in an Acute General Hospital: A Guide to Fresh Frozen Plasma (FFP) Usage

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Introduction

Activated partial thromboplastin time (APTT) is a commonly requested coagulation test to assess the intrinsic pathway of coagulation. Fresh frozen plasma (FFP) has been used to correct prolonged APTT, often without further investigations. In our previous study, we found that about one-third of FFP requests were for inappropriate indications and a significant number of these requests were for the correction of prolonged coagulation tests, often without establishing the causes of the coagulation abnormalities.[1] We decided to investigate the causes of isolated prolonged APTT in an acute general hospital to further rationalise FFP usage.

Materials and Methods

Consecutive patients with prolonged APTT (>43 s) and normal prothrombin time (PT <15 s) in Tan Tock Seng Hospital between February 2002 and January 2004 were enrolled in the study after informed consent. Patients with prolonged TT were excluded from the study to exclude cases resulting from heparin contamination. The schema for investigation is shown in Figure 1.

APTT, PT and TT were performed using Actin FSL (Dade Behring, Marburg, Germany), STA $CaCL_2$ 0.025M, STA Neoplastine CI Plus, and STA Thrombin 2 (Diagnostica Stago, France) reagents respectively.

Lupus anticoagulant (LA) detection was based on the prolongation of lupus anticoagulant sensitive APTT (Diagnostica Stago, France), and correction by the addition of phospholipids. Two different assays were used: a dilute Russell's Viper Venom time-based assay with calculated ratio using reagent without and with phospholipids. A ratio of more than 1.2 was considered positive (Dade Behring, Germany). This was confirmed with a second test using hexagonal phase phosphatidyle-ethanolamine as the source of phospholipid. A difference in APTT of more than 8 s with and without HPE was considered positive (Staclot LA Kit, Diagnostica Stago, France).

Coagulant factor VIII (FVIII), IX, XI, and XII assays were APTT-based assays using factor-deficient plasma (Diagnostica Stago, France). Factor VIII inhibitor was quantified by mixing the test plasma with control plasma containing a known amount of FVIII. The level of inhibitor present was then calculated by comparing the residual FVIII activity of a patient-control mixture and a buffer-control mixture and expressed in Bethesda Units. Plasma Von Willebrand factor antigen (vWF:Ag) was quantified by immuno-turbidimetric method (STA Liatest vWF, Diagnostica Stago, France).

All the above tests were performed using automated machine STA Compact except for Staclot LA, which was performed using ST Art (both by Diagnostica Stago, France).

The causes of prolonged APTT were assigned according to the following criteria. If LA was positive, the cause was LA except when the test for specific FVIII inhibitor was positive, which would mean that the cause was FVIII inhibitor. Factor deficiency was diagnosed when factor VIII, IX, XI and XIII levels were less than 41%, 42%, 56% and 44% respectively, based on the sensitivity of our APTT reagent. Probable von Willebrand disease (VWD) was assigned if vWF:Ag level was less than 50% based on previously established laboratory reference range. Cause was classified as unknown if above criteria were not met.

Results

Two hundred and four samples were analysed, with 27 excluded due to prolonged TT, leaving 177 for the final analysis. The median age of the cohort was 52 years old (range, 13 to 104). The female and male ratio was 1:1.27. Racial distribution was as follows: 133 Chinese, 20 Malays, 16 Indians and 8 belonging to other races, hence representative of the general hospital population of Singapore.

The most common causes were LA and unknown (53.1% and 31.6% respectively) and factor deficiencies were rare (Fig. 1). Not all cases positive for LA had non-correctable APTT. Amongst the 56 with unknown causes, 40 had correctable APTT whilst 16 had non-correctable APTT after 50:50 plasma correction. APTT was considered correctable if it shortened to within 5 s of control APTT or it was shortened by more than 50% from the original APTT compared to control. In this group, APTT prolongation is usually mild and less than 1.5 times of the mean APTT.

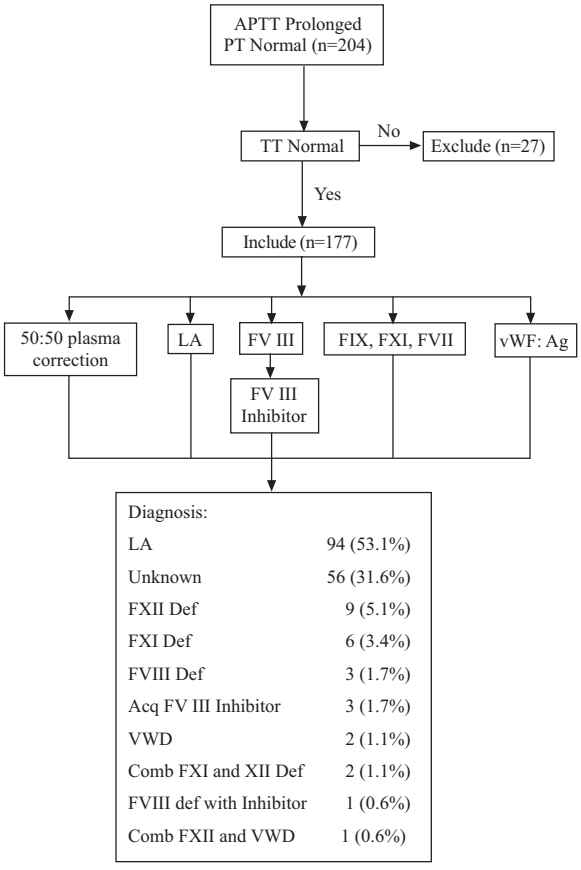

Fig. 1. Study schema and causes of isolated prolonged APTT detected.

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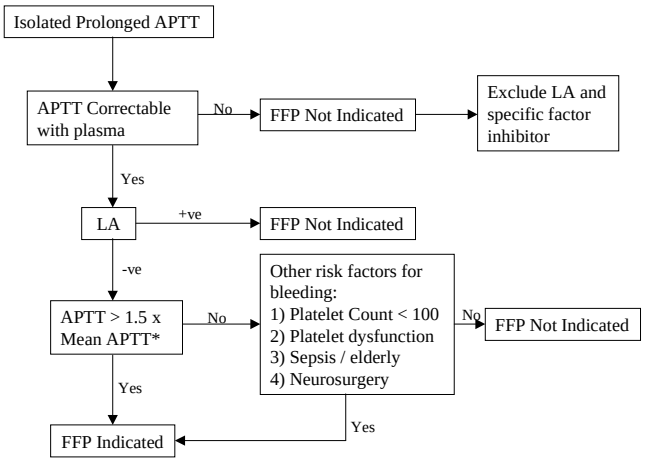

Fig. 2. Investigation algorithm for isolated prolonged APTT and decision for FFP usage.

Discussion

In our study, the most common cause of isolated prolonged APTT is LA, which predisposes patients to thrombosis, and FFP should not be used in these patients. The second most common group is those without detectable causes. The APTT is usually only mildly prolonged in this group and possible causes include inappropriate blood draw technique, deficiency of contact factors that are clinically insignificant or very weak LA where FFP is not indicated. Amongst the rest, only patients with Factor VIII, IX and XI deficiency may benefit from FFP treatment and these constitute a very small number. Even then, haemophilia A and B should preferably be treated with specific factor concentrates rather than FFP. This is also true for VWD. Factor XII deficiency is more often associated with thrombosis than with haemorrhage.[2] FVIII inhibitors require specific treatment and not FFP. Usually only factor XI level below its haemostatic level of 30% is associated with bleeding.[3]

In our cohort, this corresponds to APTT of more than 51 s, which is 1.5 times our laboratory's mean APTT. Most guidelines state that FFP transfusion is indicated in bleeding patients or preoperatively only if APTT is more than 1.5 times the mean APTT.[4,5] The basis may be that only APTT level above this level is associated with significantly low levels of coagulation factors. However, these general guidelines may not apply to patients who may have additional risk for excessive bleeding like thrombocytopenia.

Based on our findings, we have devised an algorithm for investigating isolated prolonged APTT and for the ordering of FFP to correct the coagulation defect (Fig. 2). Using this scheme, patients with FVIII inhibitor may sometimes be inappropriately given FFP but FVIII inhibitors usually arise in known haemophiliacs, who will be monitored for inhibitor development. They can also be acquired. These patients usually present with extensive bruising and bleeding. In this situation, factor assays will usually be done in the setting of prolonged APTT (usually non-correctable with plasma). Patients with FVIII inhibitor should be recognised and managed accordingly to prevent uncontrollable bleeding.

Conclusion

Isolated prolonged APTT is usually due to causes that do not lead to increased bleeding. An investigational algorithm may help in determining the cause of the coagulation defect as well as in rationalising FFP use.

Acknowledgement

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Special Poster Presentation

MARS Liver Dialysis in Children with Acute Liver Failure

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Introduction

Molecular Adsorbents Recirculating System (MARS™) has been shown to be an effective mode of liver dialysis in adults with acute liver failure (ALF) and acute-on-chronic liver failure. The MARS™ liver dialysis system is an albumin-filled circuit, which allows for the removal of both water-soluble and protein bound toxins such as bilirubin, bile acids, aromatic amino acids and mercaptans from blood. Clinical studies over the last decade have demonstrated that the removal of these toxins is accompanied by a lightening of the degree of hepatic encephalopathy, as well as improvement in cardiovascular and renal functions.[1-3]

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However, there is still currently, limited information on the safety or effectiveness of MARS™ in children.

We report our experience of MARS™ in 4 children with acute liver failure.

Materials and Methods

This is a retrospective chart review of children with acute liver failure who received MARS™ liver dialysis over the period 2001 to 2004 at the Children's Medical Institute, National University Hospital. Aetiologies of liver failure were: acute Wilson's Disease (2) acute Hepatitis B1 and acute liver failure of unknown aetiology (1) (Table 1). The weight of the children ranged from 18.6 kg to 55 kg. All the patients had been listed for liver transplant having met the King's College criteria for transplantation,[4] and were being managed in the Paediatric Intensive Care Unit. All 4 patients were in grade 3 to 4 encephalopathy, and were mechanically ventilated and sedated. One patient was on intravenous inotropic support. Neurological status was assessed clinically. These included pupil size, reactivity and equality, blood pressure, plantar tone and reflexes. Liver function was monitored before and after each MARS™ session.

There was no modification of the circuit for these paediatric patients. Standard Baxter BM 25 and Gambro AK 100 renal dialysis machines with paediatric blood circuits were used, coupled with the MARS™ albumin circuit and monitor. No anticoagulation was employed.

Table 1. Demographic of Patients

Patient	Age (y)	Sex	Aetiology	Maximum PT (sec)
Pt.1	5	Male	Acute Wilson's disease	53.3
Pt.2	11	Female	Unknown, non A to E hepatitis	94.6
Pt.3	15	Male	Acute hepatitis B	81.4
Pt.4	6	Male	Acute Wilson's disease	67.1

PT: prothrombin time; sec: seconds

Results and Outcomes

A total of 9 sessions of liver dialysis were carried out were carried out between the 4 patients with an average of 2 sessions each, each lasting an average of 6 hours. All MARS™ dialysis sessions were uneventful and well-tolerated.

Three patients had normal Computer Tomography scans of the brain prior to MARS™ dialysis. This was mainly to exclude intracranial haemorrhage prior to liver transplant. Two out of these 3 patients had intravenous mannitol. All patients' pupils remained active throughout the dialysis sessions.

All 4 children had total bilirubin levels ranging from 282 mmol/L to 1247 mmol/L (mean = 615). There was significant fall in both conjugated bilirubin as well as total bilirubin. Reduction in conjugated bilirubin ranged from 21 mmol/L to 658 mmol/L with a mean of 176 mmol/L (SD = 231.54). The mean fall in total serum bilirubin was 160 mmol/L, with the average drop ranging from 4 mmol/L to 609 mmol/L (SD = 227.28) (Fig. 1). However, there were no significant changes in unconjugated bilirubin. In fact, there was some increase in the post-MARS™ results.

Maximum prothrombin time (PT) ranged from 53.3 to 94.6 seconds (mean = 74.1), a reflection of the severity of liver disease, and PT results did not change significantly with MARS™ treatments.

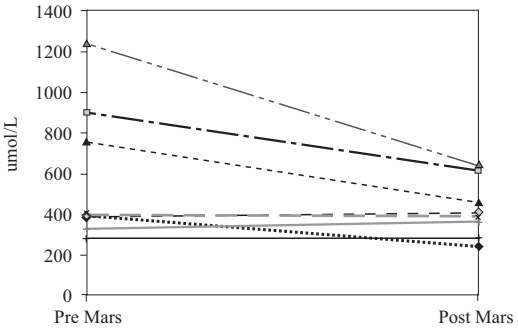

Fig. 1. Reduction in total bilirubin pre and post MARS™.

Two of the patients, 1 with Wilson's Disease, and the other with non A to E hepatitis received living related liver donors 3 days after the commencement of MARS™. Both received left lateral graft segments, the boy from his mother, and the girl from her father. The other 2 patients died 2 days after the commencement of MARS™ from liver failure, complicated by multi-organ failure. One had no compatible living related liver donor and the other deteriorated while a potential donor was being worked up.

Discussion

Despite new developments in paediatric intensive care management, the mortality of children with ALF remains high (50% to 90%, depending on the aetiology of disease).[5] Mortality is usually related to complications such as cerebral oedema, sepsis and multiple organ failure. Urgent orthotopic or living related liver transplantation is currently the only effective treatment for these patients who are unlikely to recover spontaneously. However, liver transplantation is not always possible because of the shortage of organs or incompatible living related liver donors.

Artificial liver-assist devices such as MARS™ have the potential to prolong survival of patients with ALF; acting as a temporary support for the patient's liver function until the native liver recovers or as a bridging device until a liver graft becomes available.

Our experience has shown that MARS™ can be safely performed in children, and the efficacy in decreasing total serum bilirubin is similar to what is described in adult populations. In our local context, liver dialysis in children with acute liver failure may be useful in buying time for urgent assessment of a living donor.

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The Influence of Breast Feeding Compared to Formula Feeding on Infant Adiposity

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Introduction

Adult health may be influenced by foetal and early childhood nutrition. We postulate that breast milk or breastfeeding may have a beneficial effect on the growth pattern and body composition of infants, which may result in a lower incidence of obesity and its attendant complications later on in life.

Materials and Methods

We compared growth, skin-fold measurements and fat mass in breastfed (BF, n = 63) versus formula-fed (FF, n = 37) full-term infants, from birth until 12 months of age. The BF group was exclusively breastfed up to 2 months of age, while the FF group was predominantly or exclusively formula-fed. Anthropometry, skin-fold measurements at 4 different sites and percentage fat mass as determined by the Deuterium Oxide dilution technique for measuring total body water were performed at 3, 6 and 12 months of age.

Deuterium Oxide Dilution Technique

Deuterium is a stable non-radioactive isotope of hydrogen. It is accurate and non-invasive, similar to total body potassium (TBK) or total body electric conductivity (TOBEC), but unlike Dual-energy X-ray Absorptiometry (DXA). Total body water was calculated as 0.95 X dose/saliva concentration. The body fat percentage was then calculated from total body water using age-specific hydration factors of the fat-free mass and subtracting total body water from body weight. A pre-dose saliva sample (1 mL to 2 mL) was collected in a small plastic test tube about 2 hours before the next feeding. A weighed (accurate to 0.01 g) tracer dose of 0.2 g/kg (1 mL to 2 mL on average) deuterium oxide was fed orally to the infant and the tracer allowed to distribute evenly in the body water pool for 2 hours. A second saliva sample (1 mL to 2 mL) was collected after 2 hours. Saliva samples were stored in firmly closed test tubes at -20°C until analysis. After thawing and centrifuging at 2000 G, for 20 minutes, supernatants were pair-wise analysed using Fourier Transformed Infrared Spectroscopy (FTIR) and compared against weighed standards of deuterium oxide in water and 2 quality-control samples (low and high concentrations) after every 10 samples.

Results

All 100 babies were full-term at birth [(BF) 39.3 ± 0.9 weeks versus (FF) 38.8 ± 1.0 weeks]. Maternal characteristics such as ethnicity, age and body mass index, and the infants' sex ratio, weight, length, head circumference, body mass index and mid-arm circumference at birth were similar between the 2 groups. Solids were first introduced at 5.2 ± 1.1 (BF) versus 4.7 ± 0.9 (FF) months (P = 0.026). There were no significant differences between the 2 groups with regards to anthropometry, mid-arm circumference (MAC), skin-fold measurements, body mass index (BMI) or percentage fat mass (% Fat) at 3,6 or 12 months as summarised (mean ± SD, P >0.05) (Table 1).

Conclusion

There was a trend towards breastfed infants being leaner when compared to formula fed infants before and even after solids were introduced, although skin-fold measurements and body mass index showed the converse. If early nutrition has an effect on obesity later on in life, then exclusive breastfeeding for at least 2 months should be strongly advocated.

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Table 1

Age (mo)	Diet	MAC (cm)	Triceps (mm)	Bicep (mm)	Subscapular (mm)	Suprailiac (mm)	BMI	% Fat
3	BF	14.9 ± 1.9	9.1 ± 1.8	6.4 ± 1.3	8.4 ± 1.4	8.4 ± 2.1	17.0 ± 1.7	22.0 ± 6.5
	FF	14.2 ± 0.9	9.1 ± 1.7	6.9 ± 1.9	8.2 ± 1.5	8.1 ± 1.9	16.7 ± 1.9	22.7 ± 5.8
6	BF	15.1 ± 1.0	10.3 ± 2.2	6.1 ± 1.5	8.5 ± 1.9	7.3 ± 1.8	16.9 ± 1.7	22.8 ± 7.4
	FF	14.7 ± 1.1	9.1 ± 1.8	6.0 ± 1.4	8.6 ± 2.4	7.3 ± 1.6	16.8 ± 1.4	22.2 ± 5.2
12	BF	15.0 ± 0.9	8.6 ± 1.6	5.8 ± 0.9	8.4 ± 1.7	6.5 ± 1.2	16.5 ± 1.5	18.4 ± 2.7
	FF	15.0 ± 1.1	8.1 ± 1.3	5.8 ± 1.9	7.8 ± 1.9	6.8 ± 2.0	16.5 ± 1.1	19.1 ± 2.9

BF: breast fed; BMI: body mass index; FF: formula fed; MAC: mid-arm circumference

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Use of Complementary and Alternative Medicine in Paediatric Oncology Patients in Singapore

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Introduction

Complementary and alternative medicine (CAM) has been gaining acceptance worldwide.[1,2] Although usage is thought to be widespread among paediatric cancer patients, local studies have not been done.

In our study, we aim to: (1) determine the prevalence of CAM usage before and after cancer diagnosis, (2) assess factors associated with CAM usage, (3) describe the reasons for usage or non-usage of CAM therapies, (4) estimate the costs incurred, and (5) assess parental perceptions towards CAM.

The results were anticipated to provide a profile of those parents likely to choose CAM therapies for their child, and then assist them to make their decision in an informed way.

Materials and Methods

Our study population comprised 73 paediatric cancer patients <15 years old (42 male children and 31 female children) from the Children's Cancer Centre of KK Women's and Children's Hospital, which treats two-thirds of paediatric oncology patients in Singapore. The primary caregiver completed an interviewer-administered questionnaire.

A pilot study, with standardisation of inquiry by interviewers and revision of the questionnaire, was done. The study proper was performed in May 2002. A subsequent telephone survey reached 59 of the original 73 caregivers (25 CAM users for cancer, and 34 non-users). This second phase focused on questions relating to spirituality as a treatment modality, benefits of CAM use, and overall satisfaction with the CAM therapy used, which were not sufficiently addressed in the original questionnaire.

Statistical Analysis

Data collected were analysed using SPSS v11.0. Descriptive and univariate analyses were performed to determine the factors associated with CAM usage. Bivariate analysis, including Pearson Chi-Square test was performed for categorical variables to determine if differences between each group were statistically significant.

Results

The demographics of the population interviewed were comparable to the Singapore Cancer Registry in terms of gender and race. Seventy-four per cent of the children were undergoing active treatment (either as inpatients or as day patients in the day therapy unit), with the remaining 26% on follow-up. 97.3% received chemotherapy, 15.0% received radiotherapy, and 13.7% received surgery. One received a bone marrow transplant.

Patterns of CAM Use

Forty-nine caregivers (67.1%) reported having used at least 1 CAM therapy since diagnosis of the child's cancer. The therapies most commonly instituted were changes in diet (55.1%), health supplements (44.9%), herbal tea (36.7%) and bird's nest (36.7%) ingestion (Fig. 1). Many respondents reported increased intake of fruits, vegetables or large amounts of fruit juice, and avoidance of meat. One patient relied on "mushroom water" (drinking only water boiled with mushrooms). None adopted organic, macrobiotic or other unorthodox diets. Excluding dietary modification, 60.3% used at least 1 form of CAM. About 20 different kinds of health supplements were used, including cactus juice, noni juice, wheatgrass, plant extracts, pure wild honey, growth factors and enzymes. Herbal teas (like herbal soups, chrysanthemum tea and barley drink), regarded as "cooling" in traditional Chinese medical practice, were believed to decrease the "heatiness" of the body induced by chemotherapy. Only 6 patients (8.2%) consulted a traditional Chinese physician.

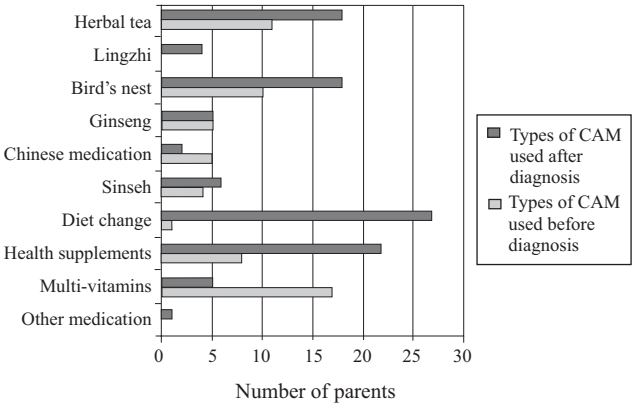

Fig. 1. Types of CAM used.

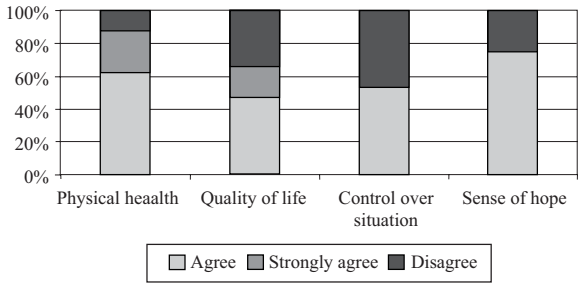

Fig. 2. Benifits of CAM used.

Phase II of the survey found that 25.4% of the 59 telephone respondents had used some form of spirituality, such as formal prayer (8 patients), laying on of hands (3), seeking help from a bomoh (2) or temple medium (1).

Predictors of CAM Use

Twenty-six of the 49 CAM users had used 1 or more forms of CAM since birth, the most popular being herbal tea, bird's nest and multivitamins.

Prior CAM usage (relative risk 1.93), Chinese race (P <0.001),

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Buddhism/Taoism religion (P <0.001) and dissatisfaction with conventional treatment were positive predictors. However, users and non-users did not differ significantly by household income, parental education, cancer type or conventional treatment modality used. Severe disease symptoms, treatment side effects or presence of cancer relapse were not associated with use.

Reasons and Expectations for CAM Use

Before diagnosis, the main reason for CAM use was to improve general health. Subsequently, most (85.7%) used it as an adjunct to conventional cancer treatments, with only 20.4% of respondents anticipating curative or anti-cancer effects. Other reasons cited were: to strengthen the immune system (63.3%) and to control chemotherapy side effects (25.0%).

Sources of Information

Most relied on more than 1 source, the most common being friends (51%), followed by other patients, then parent's own knowledge base. Eight per cent were recommended products by staff of health food stores.

Doctor-Patient Relationship

Although a majority (61.2%) felt it was important for the doctor to be aware of their CAM usage, only 44.9% informed the physician. Many parents felt that CAM remedies are derived from natural sources and are thus non-toxic. Some believed that the doctor would be unsupportive and discourage usage. On a positive note, 41.7% of non-CAM users followed advice from an oncologist and 87.5% of respondents were themselves wary of adverse interactions with chemotherapy.

93.9% of CAM users would continue such treatment even after completion of conventional therapy, with 65.3% recommending it to other parents.

Expenditure on CAM

Only 1 prior CAM user discontinued use after diagnosis. Overall, parents spent an average of $197.90 per month. Prior CAM users spent an average of $226 per month on their child, up from $73.30. Despite the substantial costs incurred, many caregivers deemed it as money well spent.

Satisfaction with CAM and Conventional Treatment

The majority of CAM users (63.6%) agreed, with 24.2% strongly agreeing, that CAM improved their child's physical health and well-being (Fig. 2). Most (64.7%) felt their child's quality of life improved. CAM conferred psychological benefits of hope (32%) and a sense of control (50%). Overall satisfaction with both CAM (76.5% satisfied, 17.6% very satisfied) and conventional treatment (61% satisfied, 27.1% very satisfied) was high. Sixty per cent felt CAM was more easily obtainable and had fewer side effects than conventional treatment. However, CAM was not deemed to be safer, cheaper or more effective.

Discussion

The local prevalence rate of 67.1% is comparable to that previously reported in the literature.[3] In Malaysia, Arrifin et al[4] found that one-third of parents sought aid from traditional healers, with 13% delaying conventional treatment. A Taiwanese study by Yeh CH et al[5] found a CAM usage prevalence rate of 73%.

However, factors including parental education and poor prognosis were previously found to be predictive of use but we could not confirm this. The most common newly initiated therapies such as herbal teas or bird's nest are similar to those found by the Taiwanese study, probably because many of our patients are Chinese. The spectrum of CAM appears to be relatively narrow since therapies such as bioelectromagnetics, crystals and homeopathy were not popular locally. Parents within this small community may mutually influence each other's choice of CAM.

Our study highlighted the significant costs involved, which may constitute an unnecessary financial burden. Another point of concern is that 55.1% of patients were reluctant to disclose CAM usage to their doctors. The oncologist should initiate pre-emptive discussion in a non-judgmental manner. This may avoid disrupting the doctor-patient relationship and hopefully encourage compliance with conventional treatment.

Finally, though only 3 patients experienced ill effects with CAM, natural products are not necessarily safe or harmless. Of 260 traditional Chinese medicines investigated by Ko,[6] 32% contained undeclared pharmaceuticals or heavy metals. Dietary changes and nutritional supplementation may affect tumour growth and drug bioavailability. Even antioxidants (vitamins C/E) can reduce chemotherapy effectiveness.

Conclusion

In conclusion, patients report physical and psychological benefits when they use CAM. Having a child with cancer is highly stressful for families. For parents disillusioned with the harsh treatments that may be necessary, CAM presents a supposedly non-toxic and holistic substitute. It is the argument to "leave no stone unturned"—to do everything possible—that may be hardest to refute.

It is foreseeable that CAM will have a growing impact on every facet of the healthcare system. Future research is thus important to clarify distinctions between potentially harmful alternative "cancer cures" and potentially beneficial complementary therapies employed as adjuncts to cancer treatment.

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Psychiatric Morbidity Among Emergency Department Doctors and Nurses after the SARS Outbreak

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Introduction

During the outbreak of Severe Acute Respiratory Syndrome (SARS) from 13 March to 31 May 2003, the study emergency department (ED) was designated as Singapore's national screening centre for SARS and was closed to all other patients. Overnight, the healthcare workers (HCW) of the study ED became the nation's front-liners in the battle against SARS, doing work and facing risks that were different from those of HCW in the rest of the hospital and in the ED of the other 5 public hospitals. To help HCW cope with the unusual stressors e.g., high rate of transmission among HCW, stigmatisation, taking care of sick colleagues, the study hospital and ED introduced psychosocial interventions.

Objectives

This study aimed to (1) determine the psychiatric morbidity among ED doctors and nurses 6 months after the outbreak, and (2) examine the coping strategies adopted by these doctors and nurses.

Materials and Methods

A self-administered questionnaire survey of ED doctors and nurses was conducted 6 months after the outbreak in November 2003. Inclusion criteria were all ED doctors and nurses who had patient contact at any time during the 80 days of the outbreak. The questionnaire was anonymous and participation was voluntary. Data collected included demographics and responses to these validated instruments (a) Impact of Event Scale (IES),[1] (b) General Health Questionnaire 28 (GHQ 28),[2] (c) General Health Questionnaire 12 (GHQ 12)[2] to measure psychiatric morbidity, and (d) Coping Orientation to Problems Experienced (COPE)[3] to assess coping strategies. The hospital ethics committee approved this study.

The IES is a 15-item questionnaire. Based on the subject's response, each item was scored 0, 1, 3 or 5 giving a maximum score of 75, with higher scores reflecting higher impact. For this study, IES total score $\geq$26 was the chosen threshold for psychiatric morbidity. The GHQ 28 had 28 items and GHQ 12 had 12 items. Based on the subject's response, each item was scored 0 or 1, with a maximum score of 28 for GHQ 28 and 12 for GHQ 12. Psychiatric morbidity was defined as a score of $\geq$5 on GHQ 28 or $\geq$4 on GHQ 12.

COPE is a multidimensional inventory with 60 items, of which sets of 4 are grouped to form 15 conceptually distinct coping strategies. The 15 scales are in turn categorised as: (a) problem-focused coping, (b) emotion-focused coping, and (c) less-useful coping.[3] Problem-focused and emotion-focused responses were considered more adaptive than less-useful coping.

Results

Thirty-eight out of 41 (92.7%) doctors and 58 out of 83 (69.9%) nurses responded. There was no difference between the doctors' mean age of 31.7 years (95% Confidence Interval [CI], 30.1-33) and that for the nurses at 32.1 years (95% CI, 29.7-34.6). The gender difference between doctors and nurses was highly significant (P <0.0001), with 83.3% of doctors being men and 80.3% of nurses being women (Table 1). On IES, 17.8% of respondents scored $\geq$26, while on GHQ 28, 18.8% scored $\geq$5, indicative of psychiatric morbidity. Doctors reported significantly less (P = 0.03) impact, scoring 12.2 (95% CI, 8.6-15.8) on IES compared to nurses scoring 17.6 (95% CI, 13.4-21.7). The GHQ 28 was significantly correlated with IES total score (P = 0.04).

Respondents reported a preference for problem-focused and emotion-focused i.e., adaptive coping measures and almost never used alcohol or drug to cope (Table 2). There was no difference between doctors and nurses in their use of adaptive or less-useful coping strategies. Compared to nurses, a significant number of doctors chose humour (P <0.0001) as a coping response. Compared to other ethnic groups, a significant number of Filipino HCW turned to religion (P <0.0001) as a coping response. On the GHQ 28, those with psychiatric morbidity scored significantly higher (P = 0.002) in less-useful coping compared to those without psychiatric morbidity. There was no correlation between age and the number of years in current profession with the above scores.

Discussion

In June 2002, 9 months before the SARS outbreak, the mental health of the study ED HCW was assessed with GHQ 28, which showed that 29.4% of 17 doctors and 33.3% of 24 nurses had minor psychiatric disorder. Six months after SARS, the rates of psychiatric morbidity were surprisingly low among the study ED doctors and nurses, compared to the pre-SARS baseline and 2 other SARS-related studies.[4,5] The authors speculate that the SARS outbreak was such a life-defining event for the respondents that their perceptions of stress changed drastically.

Table 1. Demographic Characteristics of Emergency Department Doctors and Nurses

Characteristic	Doctors (n = 38)	Nurses (n = 58)	*P* value
Response rate	92.7%	69.9%	<0.0001
Male	65.8%	8.6%	<0.0001
Mean age (y) (95% Confidence interval, CI)	31.6 (30.1-33)	32.1 (29.7-34.6)	0.69
Mean no. of years in current profession (95% CI)	7 (5.6-8.3)	10 (7.5-12.4)	0.03
Marital status			0.03
Single	44.7%	63.8%	
Married	55.3%	31%	
Divorced/separated	0	5.2%	
Nationality			0.21
Singapore	70.3%	67.2%	
Philippines	16.2%	19%	
Malaysia	10.8%	5.2%	
China, India	2.7%	8.6%	
Religion			0.52
Christianity	60.5%	44.8%	
Buddhism	13.2%	20.7%	
No religion	13.2%	13.8%	
Islam	5.3%	15.5%	
Hinduism, other	7.8%	5.2%	

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Table 2. Median Scores in Coping Responses

	[a]Problem-focused coping	[b]Emotion-focused coping	[c]Less-useful coping	Humour	Alcohol/ Drug use
Professional groups					
Doctors (n = 38)	52	50	23	10	6
Nurses (n = 58)	49.5	49	23	6	4
P value	0.1	0.75	0.69	<0.0001	0.74
Ethnic groups					
Chinese (n = 64)	47.5	47	22	8	4
Filipino (n = 17)	52	55	25	8	4
Malay, Indian (n = 13)	50	52	21	7	4
P value	0.3	0.02	0.39	0.8	0.97
Psychiatric morbidity					
IES <26 (n = 76)	48	47	22	8	4
IES ≥26 (n = 17)	55	61	25	7	4
P value	0.001	<0.001	0.02	0.19	0.62
GHQ 28<5 (n = 77)	50	48.5	22	8	4
GHQ 28 ≥5 (n = 18)	52.5	51.5	29	8.5	4
P value	0.17	0.09	0.002	0.6	0.17

[a] Problem-focused coping: sum of active coping, planning, seeking instrumental support, suppression of competing activities and restraint coping
[b] Emotion-focused coping: sum of seeking emotional support, positive reinterpretation, acceptance, denial and turning to religion
[c] Less-useful coping: sum of focus on and venting of emotion, mental disengagement and behavioural disengagement

Coping responses are influenced by 2 sets of factors: environmental factors and personal-internal characteristics. Many of the measures adopted by the hospital during the outbreak influenced the ED environment: (1) Intra-hospital communications e.g., daily emails from the CEO, daily updates by head of department, (2) communications with the community and public relations management e.g., meetings with and pronouncement of support from political, community and business leaders and (3) welfare and psychological interventions e.g., food and welfare gift packs donated by well-wishers, display of thousands of tokens of appreciation and encouragement from the community. Certain conditions unique to the ED created opportunities for HCW to bond and cope better than expected: (a) all ED HCW report to the same head of department, (b) there was already a strong sense of identity as the ED team before the outbreak, (c) the ED HCW were relatively young and hence more receptive to changes, (d) the higher level of disaster awareness and preparedness helped with the crisis mode of operation during the outbreak and (e) the ED senior medical and nursing team personally sponsored in cash or kind departmental celebrations and activities to encourage and enable HCW to relax and have fun despite the seriousness of the outbreak. All these measures influenced the coping responses of ED HCW.

Conclusions

With a supportive hospital and departmental environment, respondents chose adaptive coping in response to the outbreak and reported low psychiatric morbidity. The trend was for nurses to report higher stress levels compared to the doctors, reaching statistical significance on IES but not on the GHQ. Doctors chose humour while Filipino chose religion as their preferred coping responses. Psychosocial interventions to help HCW need to take into account these preferences.

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Special Poster Presentation

Impact of Reversibility of No-reflow Phenomenon on 30-day Mortality Following Percutaneous Revascularisation for Acute Myocardial Infarction – Insights from a 1328-Patient Registry

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Introduction

Occurrence of no-reflow during percutaneous coronary intervention (PCI) is associated with a high incidence of death and adverse events.[1-3] The risk of no-reflow is particularly high in myocardial infarction (MI) interventions. Various vasodilators including verapamil, adenosine and nitroprusside have been used to treat no-reflow phenomenon. While most patients respond to these medications with restoration of normal antegrade coronary flow, some are refractory to the treatment and the final flow remains impaired. The impact of reversibility of the no-reflow on clinical outcomes is currently unclear. In this study, we sought to determine whether reversal of the no-reflow is associated with improved survival compared with refractory no-reflow.

Materials and Methods

The PCI registry at the Singapore National University Hospital was used to gather patient data for this analysis. Patients who underwent PCI for MI at our institution from January 2000 to June 2004 were recruited for analysis. The diagnosis of MI was based on chest pain lasting ≥30 minutes, ST segment elevation ≥2 mm in at least

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2 contiguous ECG leads and a greater than 3-fold increase in serum creatine kinase (CK) levels.

All procedures were performed according to standard techniques, and the interventional strategy was left to the discretion of individual operator. All patients were on life-long aspirin administration and received a loading dose of clopidogrel (300 mg). All patients who received coronary stents received clopidogrel maintenance (75 mg/day) for >1 month.

No-reflow during the procedure was defined as Thrombolysis In Myocardial Infarction (TIMI) flow <3 in the absence of flow-limiting stenosis or thrombus or dissection that occurred after initial restoration of flow. All patients with no-reflow received intracoronary nitroglycerin in repeated 100 µg to 200 µg boluses at the time no-reflow was diagnosed. In addition, various drug therapies including adenosine, verapamil and nitroprusside or a combination of these drugs were administered at the discretion of the operator, according to the recommendation from the Society of Cardiac Angiography and Interventions.[4]

Our analysis stratified patients according to the occurrence and persistence of no-reflow during PCI: (1) Adequate reflow: without no-reflow occurrence; (2) Reversible no-reflow: no-reflow occurred, but final TIMI 3 flow restored after intracoronary medications; (3) Refractory no-reflow: no-reflow occurred and persisted, final TIMI flow <3 despite medications. Thirty-day mortality rates were determined for each group.

Results

A total of 1328 patients who underwent PCI for MI were analysed. Coronary stenting was performed in 92% of the patients. Among the study patients, no-reflow (including reversible and refractory) occurred in 135 patients (10.2%). Of the 135 patients with no-reflow, intracoronary verapamil, adenosine, nitroprusside or a combination of drugs was used to treat the no-reflow in 70.0%, 17.7%, 3.5% and 8.8% of cases respectively. Intracoronary medications successfully restored final TIMI 3 in 108 patients (80%, reversible no-reflow). The remaining 27 patients (20%) have final TIMI <3 (refractory no-reflow). The baseline demographic characteristics and angiographic details of the 3 groups are shown in the Table. After controlling for other factors, patients with higher baseline haemoglobin (OR 1.67; 95% CI, 1.06-2.62, $P = 0.0026$) and platelet (OR 1.01; 95% CI, 1.00-1.02, $P = 0.044$) levels were more likely to have refractory no-reflow.

The 30-day mortality of the adequate reflow, reversible no-reflow and refractory no-reflow groups are shown in Figure 1. We observed a higher mortality in the refractory no-reflow group. In comparison with patients with adequate reflow, occurrence of no-reflow (including reversible and refractory) had a higher 30-day mortality (4.1% versus 9.8%, OR 2.5; 95% CI, 1.26-5.04, $P = 0.007$). Among the patients with no-reflow occurred, reversible no-reflow was associated with a significantly lower 30-day mortality (3.3% versus 36.4%, OR 16.57; 95% CI, 3.92-70.08, $P < 0.001$) compared with the refractory no-reflow.

Discussion

The major finding of the present study is that in comparison with refractory no-reflow, reversible no-reflow is associated with a lower 30-day mortality.

No-reflow after PCI has been shown to be associated with worsened short- and long-term outcomes. In accordance to the previous reports, our study shows that the occurrence of no-reflow is associated with a >2-fold higher mortality. Although intracoronary medications can improve coronary flow, the effect on mortality is not clear. We found that reversible no-reflow is associated with a 10-fold lower 30-day mortality compared with refractory no-reflow.

Recently, Resnic et al reported that despite improvement in antegrade flow, administration of verapamil or nitroprusside was not associated with improved in-hospital outcomes in patients with no-reflow.[3] While our data show that reversible no-reflow is associated with better 30-day outcomes, these data are not conflicting. Although

Table 1. Baseline and Lesion Characteristics of the Study Patients

Characteristics	Good flow (n = 1193)	Reversible no-reflow (n = 108)	Refractory no-reflow (n = 27)
Age (y)	55.68 (11.38)	55.06 (11.19)	58.08 (14.24)
Female sex	207 (17.4%)	19 (17.6%)	10 (37.0%)
Ethnicity			
Chinese	804 (67.4%)	68 (63.0%)	19 (70.4%)
Malay	181 (15.2%)	16 (14.8%)	4 (14.8%)
Indian	181 (15.2%)	21 (19.4%)	3 (11.1%)
Others	27 (2.3%)	3 (2.8%)	1 (3.7%)
Risk factors			
Hypertension	658 (55.2%)	53 (49.1%)	14 (51.9%)
Diabetes mellitus	346 (29.0%)	35 (32.4%)	9 (33.3%)
Hyperlipidaemia	669 (56.1%)	54 (50.0%)	12 (44.4%)
Family history	52 (4.4%)	1 (0.9%)	0 (0%)
Smoker	539 (45.2%)	38 (35.2%)	10 (37.0%)
Medical history			
Stroke	46 (3.9%)	4 (3.7%)	3 (11.1%)
Peripheral vascular disease	10 (0.8%)	0 (0%)	1 (3.7%)
COPD	1 (0.1%)	0 (0%)	0 (0%)
Renal failure	10 (0.8%)	0 (0%)	0 (0%)
Previous PCI	64 (5.4%)	6 (5.6%)	1 (3.7%)
Previous CABG	18 (1.5%)	1 (0.9%)	0 (0%)
Left ventricular function			
≥50%	518 (63.2%)	60 (69.0%)	8 (53.3%)
35% to 49%	251 (30.6%)	24 (27.6%)	6 (40.0%)
<35	51 (6.2%)	3 (3.4%)	1 (6.7%)
Baseline haemoglobin (g/dL)	13.86 (1.84)	13.91 (1.82)	13.78 (3.32)
Baseline platelet ($x10^9$/L)	260.91 (76.04)	251.30 (67.78)	284.33 (94.14)
Baseline creatinine (µmol/L)	99.21 (71.27)	93.90 (23.85)	109.48 (54.47)
Multi-vessel disease	214 (18.0%)	26 (24.5%)	3 (11.1%)
Glycoprotein IIb/IIIa inhibitors	226 (18.9%)	39 (36.1%)	12 (44.4%)
Target vessel			
LAD*	617 (51.7%)	64 (59.3%)	17 (63.0%)
LCx†	145 (12.2%)	9 (8.3%)	1 (3.7%)
RCA‡	417 (35.0%)	34 (31.5%)	9 (33.3%)
SVG§	8 (0.7%)	1 (0.9%)	0 (0%)
Left main	6 (0.5%)	0 (0%)	0 (0%)
AHA/ACC lesion type			
A/B1	247 (20.7%)	19 (17.6%)	2 (7.4%)
B2/C	945 (79.3%)	89 (82.4%)	25 (92.6%)
Eccentric lesion	897 (84.1%)	78 (85.7%)	20 (90.9%)
Thrombus-laden lesion	491 (41.2%)	59 (54.6%)	19 (70.4%)
Ostial lesion	37 (3.4%)	4 (3.8%)	1 (4.0%)
Irregular contour	380 (35.6%)	51 (50.0%)	11 (44.0%)
Angulated lesion	95 (8.9%)	5 (5.0%)	4 (16.0%)
Calcified lesion	77 (92.8%)	6 (5.8%)	0 (0%)
Bifurcation	170 (15.8%)	22 (21.0%)	4 (16.0%)
Tortuous lesion	46 (4.3%)	1 (1.0%)	1 (4.2%)
Baseline TIMI flow			
0-2	571 (48.5%)	65 (60.2%)	21 (77.8%)
3	607 (51.5%)	43 (39.8%)	6 (22.2%)
Final TIMI flow			
0-2	59 (5.0%)	0 (0%)	27 (100%)
3	1115 (95.0%)	108 (100%)	0 (0%)
Peak creatine kinase (U/L)	2223 (3225)	3052 (3028)	3337 (2791)
Peak CKMB (µg/L)	158 (190)	235 (189)	231 (187)

Values are expressed as number (relative percentage) or mean values ± SD
* left anterior descending artery; †left circumflex artery; ‡right coronary artery; § saphenous vein graft

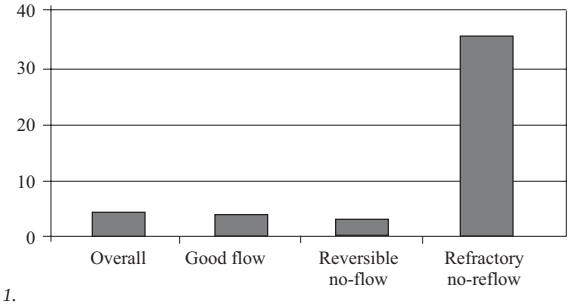

Fig. 1.

administration of vasodilators may not improve the clinical outcomes, it helps to separate the patients with better prognosis (reversible no-reflow) from those with worse prognosis (refractory no-reflow).

The 30-day mortality of patient with reversible no-reflow was found to be similar to those with adequate reflow in our study. In contrast, the PAMI investigators found that reversible no-reflow during PCI for MI was associated with a higher mortality compared with those without no-reflow.[5] There are several fundamental differences in the 2 studies that may explain the different findings. Only stable patients that fulfill the stringent criteria were recruited in the PAMI trial while our registry has essentially no exclusion criteria and patients treated are those of 'real world' ones. No-reflow was defined as TIMI flow 0-1 in the PAMI study and 0-2 in our registry. The number of patients in the reversible no-reflow group in the PAMI was small (n = 16).

There are limitations in our study. We did not collect data on the myocardial blush scores (MBG) and TIMI frame count. These assessments are often time-consuming and require sophisticated equipment and trained personnel for their accuracy and reproducibility. In contrast, epicardial TIMI flow can be reliably determined with visual inspection of angiograms by most interventional cardiologists during the procedure. Thus, unlike the previously mentioned techniques, identifying patients by visual angiography as having no-reflow during PCI allows for easy detection of a reversible versus refractory no-reflow.

In conclusion, our results suggest that the occurrence of no-reflow was associated with increased 30-day mortality. Among those with no-reflow occurred, reversible no-reflow was associated with a lower 30-day mortality compared with the refractory counterpart.

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Special Poster Presentation

Cardiovascular Abnormalities in Children on Long-term Dialysis: Analysis of Risk Factors

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Introduction

Cardiovascular disease is a major cause of mortality in adults with end-stage renal failure, accounting for 50% of all deaths.[1] Cardiovascular disease is also a significant cause of morbidity and mortality in children with end-stage renal failure on long-term dialysis. It accounts for 30% to 40% of all deaths in this group of patients.[2]

Left ventricular hypertrophy has been shown to be an independent risk factor for mortality. A report in 1992 stated that 30% of children on continuous ambulatory peritoneal dialysis and 22% of transplant children had left ventricular hypertrophy on echocardiographic examination. Another study in 2000 reported that 75% of their paediatric study group had left ventricular hypertrophy. In our local paediatric population, 34.5% of those with end-stage renal disease have severe left ventricular hypertrophy defined as LV mass index (LVMI) >51g/m^2.

This study examined potential risk factors that may affect cardiovascular status as measured on 2-dimensional echocardiography by left ventricular mass index (LVMI g/m^2) and fractional shortening (FS%).

Materials and Methods

Data from 40 children with end-stage renal failure managed at our institution were retrospectively reviewed. Their mean age was 15.95 ± 6.33 years (range, 3.72-25.92 years) and they had undergone dialysis, mainly peritoneal dialysis, for 4.1 ± 2.9 years (range, 0.3-13.5 years). There were 15 males and 25 females. They had reached end-stage renal failure due to a variety of causes, including systemic lupus erythromatosis, cystic dysplastic kidneys and reflux nephropathy.

The patients were evaluated for any change in their cardiovascular status based on the change in left ventricular mass index (ΔLVMI) and

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fractional shortening (ΔFS). These parameters were calculated based on echocardiographic measurements taken at the initiation of dialysis and at the most recent examination. We then analysed possible risk factors for cardiovascular disease, including age, race, sex, duration of dialysis (DUR), mean body mass index, mean systolic and diastolic blood pressures, mean haemoglobin, mean serum calcium (Ca), mean phosphate (Pi), mean intact parathyroid hormone and mean ferritin. Multivariate linear regression analysis was performed with either DLVMI or ΔFS as the dependent variables.

Results

The mean LVMI at baseline was 49.1 ± 17.1g/m^2 while the mean FS was 33.2 ± 5.5%. At the end of the follow-up period, the LVMI had improved to 47.8 ± 18.7 g/m^2 while the FS improved to 35.0 ± 6.6%. The change in left ventricular mass index (ΔLVMI) over this duration ranged from -38.9 to 45.8 g/m^2, (mean, -1.33 ± 19.10 g/m^2) whereas the change in ejection fraction (ΔFS) ranged from -10.7% to 23.8% (mean, 1.86 ± 6.61%). Multivariate linear regression analysis showed that only serum phosphate (P <0.001) was a significant predictor of ΔLVMI, whereas the serum calcium (P = 0.004) and dialysis duration (P = 0.005) were independent predictors of ΔFS. The linear regression equation for predicted DLVMI (g/m^2) = 26.52*Pi - 51.47, while ΔFS (%) = 12.52*Ca + 0.89*DUR - 32.4.

Discussion

Cardiovascular disease accounts for almost 50% of all deaths in dialysis patients. The incidence of cardiovascular death is much higher in dialysis patients compared to the normal population.

It has been increasingly recognised that abnormal calcium and phosphate metabolism play a part in the cardiovascular mortality and morbidity seen in patients with end-stage renal failure. Many studies have shown the association between elevated serum phosphate and cardiac deaths in patients on haemodialysis.[3] An elevated phosphate or calcium-phosphate product is also an independent risk factor for all-cause mortality.[4]

Much attention has been placed on the association between calcium and phosphate metabolism and calcification of cardiac vessels and cardiac valves.[5]

The prevalence and extent of vascular calcifications are strong predictors of cardiovascular and all cause mortality in haemodialysis patients.

There have been very few reports on the impact of calcium and phosphate metabolism on cardiac function as measured by left ventricular function. Rostand et al found a higher myocardial calcium content in patients on haemodialysis versus controls. The increased myocardial calcium was positively associated with an increase in the calcium-phosphate product and inversely associated with left ventricular ejection fraction.[6] Similarly, in our study, we found that increasing serum calcium and phosphate influences the left ventricular structure, as measured by the left ventricular mass index, as well as left ventricular function, as measured by fractional shortening.

Surprisingly, the association between anaemia and left ventricular hypertrophy was not significant although it is now widely accepted that anaemia is an important factor in the development of left ventricular hypertrophy.

Our finding that there was a significant association between phosphate and the change in LVMI, and calcium and the change in fractional shortening, suggests that abnormal calcium and phosphate metabolism may affect left ventricular function. It also implies that good control of phosphate and calcium may help improve left ventricular function in children with renal failure on long-term dialysis.

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EXTENDED ABSTRACTS

Special Poster Presentation

Umbilical Cord Blood Stem Cell from Unrelated Donors is a Feasible Alternate Stem Cell Source for Transplant in Patients with Genetic Diseases

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Introduction

Blood/marrow transplantation (BMT) using unaffected related matched sibling donors cures approximately 20% of patients with genetic blood and immunodeficiency disorders. The majority of patients, however, lack such donors. Transplantation using matched unrelated adult blood/marrow stem cell donors is an alternative but is constrained by a limited racially appropriate donor pool, time disadvantage in the search process, high costs, and unacceptable risks of graft-versus-host disease (GVHD) when using un-manipulated stem cells, and rejection as well as infective risks when using manipulated stem cells.

To justify unrelated donor BMT, a relatively high-risk and high-

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cost procedure in patients with non-malignant diseases where safer, albeit non-curative supportive therapies are available, BMT needs to be safe and effective. To this end, focus has been placed on reduced intensity conditioning (RIC) to minimise regimen-related toxicity and the use of stem cells that are associated with lower risks of GVHD, such as umbilical cord blood (UCB) stem cells.

As the rejection risks with this strategy may be increased, novel regimens are required to improve the chance of success. In the last decade, there have been few anecdotal reports of successful unrelated UCB transplantation for patients with thalassaemia major (TM), fanconi anaemia (FA) and immunodeficiency disorders using conventional conditioning but none for patients with hyper-immunoglobulin M (hyper-IgM) syndrome. We report our experience with unrelated UCB transplantation for a small series of patients with genetic diseases such as these, who underwent both conventional as well as RIC regimens.

Materials and Methods

Over the last 6 years, 5 patients with genetic diseases [1 FA with myelodysplastic syndrome (MDS), 2 TM, 1 severe combined immunodeficiency (SCID), 1 hyper-IgM syndrome] received unrelated UCB transplantation at our institution. Except the 2 TM patients, the other 3 patients required emergent transplants due to deteriorating underlying conditions.

All patients were male with a median age of 5.5 (range, 0.5 to 17.0) years at transplant. All except 1 (patient with TM) received up to 2 antigens mismatched UCB grafts. UCB units were obtained from local (3 units) and overseas (3 units) banks. All except the 2 patients with immunodeficiency disorders (SCID and hyper-IgM syndrome) received conventional myeloablative conditioning.

The RIC regimens consisted of a combination of fludarabine with either intravenous cyclophosphamide or busulphan. The 17-year-old patient with hyper-IgM syndrome received RIC with dual UCB units' transplantation. All patients received anti-thymocyte globulin (equine or rabbit) during conditioning. GVHD prophylaxis was with cyclosporine A and short-course methotrexate or mycophenolate mofetil. The median body weight of the patients at the time of transplant was 19.2 (range, 4.8 to 60) kg. The median total nucleated cell dose was 5.9 (range, 5.5 to 10.1) x 10^7/kg.

Results

Sixty per cent of the patients demonstrated myeloid engraftment at a median of 17 (range, 14 to 39) days. All engrafted patients achieved red blood cell and platelet engraftment within the first 100 days after transplant. At a median follow-up of 27 (range, 1.7 to 39.0) months, 80% of patients were surviving disease-free with documented donor chimerism in 3 of 4 patients. As of last follow-up, 2 of 3 patients had documented stable mixed chimerism while 1 (patient with TM) was 100% donor chimera by molecular studies.

There was 1 primary graft rejection with autologous reconstitution (patient with TM) and 1 early death secondary to infection (patient with FA and MDS). There was no regimen-related mortality. Only 1 patient (patient with TM) had grade 1 acute GVHD of the skin and none of the long-term survivors had chronic GVHD.

Conclusion

The results of unrelated UCB transplantation in this small series of patients with genetic diseases are encouraging. The conditioning regimen evolved over time with a focus on reducing the intensity of the conditioning regimen in later patients without compromising engraftment rates. The use of a novel strategy combining RIC and multiple unit UCB units to enhance cell dose without increasing risks of cross-rejection or GVHD is attractive, requires further study and holds a promise of safe and effective transplants for patients with genetic diseases.

Special Poster Presentation

Double Deletions of Glutathione S-transferase Genes (GSTM1 and GSTT1) Reduce the Risk of Early Relapse in Childhood B-lineage Acute Lymphoblastic Leukaemia (ALL)

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Introduction

The treatment of childhood acute lymphoblastic leukaemia (ALL), the most common form of childhood cancer, is one of the great success stories of medicine as locally >75% of children with ALL are long-term survivors. This has been achieved through increased intensity in therapy and improved scheduling of the same successful chemotherapeutic drugs in vogue for the last 20 years. However, despite improved chemotherapeutic regimens, 25% of patients relapse, and drug resistance remains the underlying cause.

Inherited differences in the metabolism and disposition of drugs due to the polymorphism in the genes that encode drug-metabolising enzymes, transporters, or targets can profoundly influence the efficacy and toxicity of therapy, affecting therapeutic response.[1] Glutathione S-transferase (GST) is an interesting candidate gene to study as it is involved in the breakdown of many classes of chemotherapy drugs. Specifically, crucial anticancer drugs, like prednisolone, dexamethasone, cyclophosphamide and anthracyclines are substrates of GST.

GST conjugates glutathione to active cytotoxic drugs, rendering them inactive. There are several subfamilies of isoenzymes including GSTM, GSTT and GSTP. Homozygous deletions in the GSTM1 and GSTT1 gene produce null genotypes, resulting in no activities. These null genotypes will result in lower degradation of active drugs and hence increased efficacy or unfortunately increased toxicity. Similarly,

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polymorphisms in the GSTP1 gene have also resulted in lower enzyme activity.[2]

The impact of GST polymorphisms on outcome of childhood ALL has been studied in Western populations with disparate results. Specifically, the European BFM study group found that both GSTT1 and GSTM1 null genotypes reduce the risk of relapse but the St Jude and CCG investigators found no effect of GST genotypes on treatment outcome in childhood leukaemia.[5,6]

In our study, we explore the impact of specific GST genotypes on early relapse (≤30 months), and relapse outcome in B-lineage ALL. We enrolled 98 B-lineage childhood ALL consisting of 41 relapsed patients [early relapse = 20 (≤30 months), late relapse = 21 (>30 months)] and 57 patients who have achieved complete clinical remission (CCR) for at least 30 months. The patients were recruited from 2 centres, National University Hospital, Singapore, and University of Malaya Medical Centre, Malaysia.

Materials and Methods

Patients were treated in consecutive clinical trials: NUH-ALL and HK-ALL at the National University Hospital, Singapore, and UH-ALL studies at the University of Malaya Medical Centre, Malaysia. Criteria for risk-stratification were based on initial total white blood cell count (TWBC), cytogenetics and age. Patients with poor prognostic features: TWBC $\geq 100 \times 10^9$/L, cytogenetics: t(9;22), t(4;11), hypodiploid, and age <2 years and >10 years were considered as high risk. Treatment for the standard risk group in the ALL studies was almost similar. Patient exclusion criteria in the analysis includes 1) T-lineage ALL, 2) infant leukaemia, 3) induction failure, 4) toxic death, and 5) non-compliance. There were 63 males and 35 females, and the median age was 4.2 years.

DNA and Genotyping

DNA was extracted from BM aspirates using Trizol (Invitrogen) methodology. Genotyping for GSTT1, GSTM1nd GSTP1 polymorphisms were carried out by RFLP and PCR.[3] Only GSTP1 codon105 polymorphism was analysed.

Statistical Analysis

Genotype frequencies in relapse and CCR were assessed with the Chi-square test. Survival estimates were based on Kaplan-Meier analysis and the Breslow statistic was used to compare survival distributions for the different composite genotypes. Relapse-free survival estimates were defined as time from date of diagnosis to relapse or CCR.

Results

The different GST genotypes detected in the 2 groups of patients are shown in Table 1. The effect of the different GST genotypes on relapse-free survival is shown in Figure 1. Among the 3 subfamilies of GST studied, M1 null has the highest protective value on patients from early recurrence of the disease (early relapse = 30% versus non-early relapse = 56%, $P = 0.046$). Double null GST genotype showed a significant protective value on early relapse as compared to both GSTM1 and GSTT1 present ($P = 0.0214$, Breslow). Although there was a trend towards improvement, double null GST genotype when compared to single null genotype on early relapse was not statistically significant.

No significant association of GSTP1 genotype on early relapse was observed in our study ($P = 0.115$).

Discussion

Patients vary widely in their responses to drug therapy, and functional polymorphisms in genes encoding enzymes, which affect drug efficacy, may underline these inter-individual differences. Efforts

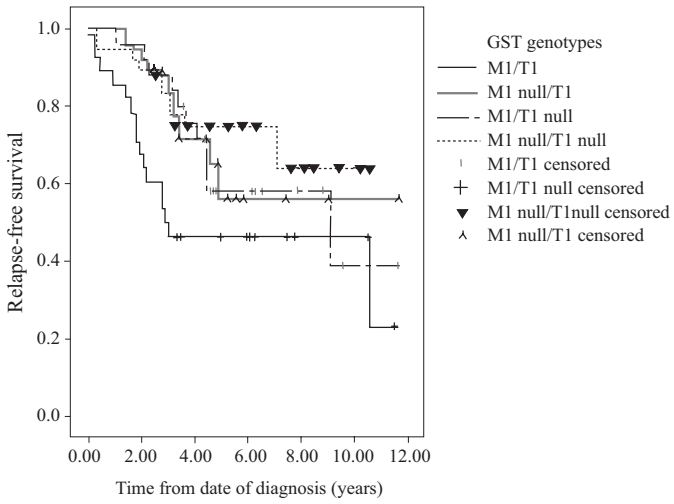

Fig. 1. The effect of the 4 different GST genotypes on relapse-free survival.

Table 1. Frequencies of the different GST genotypes in the 2 cohorts of patients: early relapse and non-early relapse. Note that the percentages may not add to 100% as some patients have deletion of more than 1 gene.

GST genotypes	Early ≤30months (n = 20)	Non-early relapse CCR >30 months (n = 78)
M1 and T1 present	11 (55%)	18 (23%)
Single null (M1 null *or* T1 null)	6 (30%)	38 (49%)
Double null (M1 null *and* T1 null)	3 (15%)	22 (28%)
M1 null	6 (30%)	44 (56%)
T1 null	6 (30%)	38 (49%)
P1 present	11 (55%)	53 (68%)
P1 homozygous	0 (0%)	6 (8%)
P1 heterozygous	9 (45%)	19 (24%)

have been made to gain closer insight into the role of these genes on both disease susceptibility and drug metabolism, and risk of relapse in childhood ALL.[1,2]

We observed a significant impact of double null genotype (GSTM1 null/GSTT1 null) on early relapse and treatment outcome ($P = 0.0214$, Breslow) compared to GSTM1 and GSTT1 present. Double null GSTM1 and GSTT1 genotypes conferred a 40% reduction in risk of early relapse as compared to the presence of both GSTM1 and GSTT1 (Table 1). GSTM1 null has the highest protective value on patients from early relapse as compared to GSTT1 null, and GSTP1 polymorphism ($P = 0.046$). The impact of double null genotype (GSTM1 null/GSTT1 null) is greater than single null genotype (GSTM1 null or GSTT1 null) on early relapse and treatment outcome (Fig. 1). This may be explained in part by the role of GST in the detoxification pathway.

We studied children with B-lineage ALL, and patients who were non-compliant, failed to achieve remission in induction, had infant leukaemia and were below 1 year of age were excluded in the analysis. The excluded patients have other factors that are likely to impact on the outcome, like failure to take medications and very resistant disease that confounds the outcome despite the GST. We are currently prospectively collecting both toxicity and outcome data in our Singapore-Malaysia ALL trial and analysing this in the context of the patient's pharmacogenetic status. This study provides the basis for a larger prospective study. We hope that in the near future, individualised therapy based on a patient's pharmacogenetic make-

up can be tailored to improve cure rates, and this may warrant a high throughput screening to genotype multiple genes on a single platform.

Acknowledgement

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Special Poster Presentation

Evaluation of Particulate Respirators During the SARS Outbreak

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Introduction

Particulate respirators are designed to protect and minimise healthcare workers' (HCWs) exposure to airborne pathogens. To be effective, they should fit different facial sizes and be properly fit-tested to prevent leakage. Prior to SARS, the 2 makes that were in use in Tan Tock Seng Hospital (TTSH) were 3M and Kimberly Clark. According to the TTSH Infection Control Unit's fit test records, 65.8% of HCWs were fitted with the 3M 1860S (small size), while 26.5% were fitted with 3M 1860 (regular size). With the outbreak of SARS in Singapore, there was a drastic increase in demand for high-filtration masks and alternative makes of masks were sourced and evaluated. This presented a unique opportunity to test several different makes of masks for facial fit and leakage.

Materials and Methods

A qualitative fit test was used to assess the adequacy of respirator fit by observing HCW's response to the test agent Bitrex. Bitrex is a bitter solution containing water, sodium chloride and denatonium benzoate manufactured by 3M. This solution is used in many consumer products as an aversive agent against ingestion, hence it is safe if swallowed. Sensitivity test and fit test were conducted using the 3M qualitative fit test apparatus. Three staff who wore "small" size and 3 staff who wore "normal" size (masks based on tests with conventional brand) participated in the evaluation. Staff had been advised not to eat, drink or chew sweets for at least 15 minutes before the test.

A sensitivity test was first done to ensure that the staff could detect the bitter taste of the solution at very low levels. The sensitivity test solution is a dilute version of the fit test solution. The staff were required to put on the hood and collar to contain the aerosol and instructed to breathe through his/her mouth while the sensitivity test aerosol was injected into the hood using a nebulizer. All the staff were able to detect the bitter taste of the solution.

The staff were given a few minutes to rinse their mouths with water to clear the taste. They were then instructed to don a respirator and fit testing was repeated, this time with an undiluted fit test solution. After the initial injection of aerosol, the staff were asked to perform normal breathing, to turn their heads side-to-side and to nod their heads up and down. The staff were asked to count from one to ten as they performed the action. This was to detect any leakage when they talked. After that, the staff were instructed to take deep breaths. The test was terminated at any time the bitter taste of the aerosol was detected by the staff, as this would indicate leakage of the mask.

Staff who failed the first time were given a few minutes to rest

Table 1. Outcome of Mask Evaluation

Particulate respirators			
Brand	Model	Size	Number of staff who passed fit testing
1	A1	S	0
		M/L	3
	A2	S	0
2	B1	S	0
	B2	S	0
		M/L	0
3	C	F	0
4	D	F	0
5	E	F	0
6	F	F	0
7	G	F	0
8	H1	F	6
	H2	F	0
9	I	F	0
10	J	F	0
11	K	F	0
12	L	F	0
13	M	F	1
14	N	F	6
15	O1	F	0
	O2	F	0
16	P	F	1
17	Q	S	0
		M/L	0

S: small; M: medium; L: large; F: free size

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and the fit test was repeated after redonning and readjusting the respirator. A second failure indicated that a different size or model respirator was needed. The test was successful and adequate respirator fit demonstrated if the entire test was completed without the staff detecting the bitter taste of the aerosolised solution. The process of fit test was repeated for the other makes of respirators.

Results

Twenty-one models of masks from 17 brands were evaluated (4 brands had 2 different models). Of these 21 models, 3 models of masks came in 2 sizes (small and medium/large), while 2 other models were available only in the small size. The other 16 models were "free" sizes (Table 1). None of the HCWs who usually wear the "small" size could fit the 5 small-sized masks. Only 2 makes of the 16 free-sized masks could fit this group. The rest of the masks were either too big, or the nose metal piece too rigid to be moulded for proper fit.

Conclusion

The majority of the particulate respirators tested were not suitable for use in our sample of HCWs in TTSH. These results reinforce the need to evaluate different makes of masks, and are important for deciding which makes of masks to order should there be new outbreaks of airborne diseases.

Special Poster Presentation

Laparoscopic Radical Nephrectomy: Oncologic Outcome in 100 Cases

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Introduction

Until recently, open radical nephrectomy has been the standard of care for localised renal cell carcinoma. In 1990, Clayman and colleagues performed the first laparoscopic nephrectomy on a tumour-bearing kidney. This procedure has since become widely accepted in many centres throughout the world, and is fast becoming the new standard of care for localised renal cell carcinoma.

Materials and Methods

We retrospectively analysed 100 cases of clinical stage T1-T2 renal cell carcinoma treated by laparoscopic radical nephrectomy between October 1998 and July 2003 in a single institution (Westmead Hospital, New South Wales, Australia).

Preoperative imaging, operation reports, histopathology reports and follow-up information were studied.

Results

Fifty-nine male and 41 female patients with a median age of 61 years (range, 23 to 85) were included. The mean size of tumours was 4.6 cm (range, 2.0 to 10.0). Operating time ranged from 60 to 255 minutes, with a mean of 120 minutes.

There were 5 open conversions for the following reasons: obesity, dense peritoneal adhesions, tumour thrombus, hepatic vein injury and colonic injury.

Intraoperative complications included inferior vena cava injury (n = 1), splenic laceration (n = 1), hepatic vein injury (n = 1), colonic injury (n = 1). There was no perioperative mortality. Postoperative morbidity included respiratory complication (n = 3), ileus (n = 2), pulmonary embolism (n = 1) and wound cellulites (n = 1).

Pathological stage distribution was as follows – T1a: 53 patients; T1b: 27 patients; T2: 9 patients; T3a: 6 patients; T3b: 5 patients. Histological cell type was as follows – conventional clear cell: 79 patients; papillary: 16 patients; chromophobe: 2 patients; collecting duct: 3 patients.

The mean follow-up duration was 24 months (range, 2 to 59). Five-year cancer-specific survival by Kaplan-Meier analysis was 91.6 % overall for all stages (T1a 96%, T1b 95.5%, T2 88.9%, T3a 80%, p3b 60%).

Discussion

In our experience with these 100 cases, we found laparoscopic radical nephrectomy to be technically feasible, and safe. Oncologic efficacy is at least equivalent to open radical nephrectomy. The 5-year cancer-specific survival of 91.6% overall compares favourably with recent published series (91% to 98%).

The advantages of faster postoperative recovery, decreased analgesic requirements, and shorter hospital stay have been shown in many published series.

We believe laparoscopic radical nephrectomy to be the new standard of care for localised renal cell carcinoma not amenable to nephron-sparing surgery.

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Aquaporin-4 is Correlated with Peri-tumoural Oedema in Meningiomas

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Introduction

Meningiomas are the most common non-glial primary intracranial tumours and they make up 15% to 20% of all primary brain tumours. They arise from specialised meningothelial cells mainly in arachnoid granulations or even develop from any meningeal structure or from ectopic cells rests of meningeal derivation. Significant cause of morbidity and death in meningiomas is contributed by tumour growth and compression of vital brain structures as well as peri-lesional brain oedema. Approximately 60% of meningiomas are associated with peri-lesional brain oedema. Several aspects such as age and gender of patient, size, location and grade of tumour have been evaluated in order to understand the pathophysiological mechanisms of oedema. However, at present the causative factors remain unclear.[1]

Brain oedema is classified by Igor Klatzo into 2 main types: vasogenic and cytotoxic.[2] Vasogenic oedema involves the breakdown of blood-brain barrier (BBB) and extravasation of blood plasma into the brain parenchyma. Cytotoxic oedema is characterised by cellular swelling in the absence of any measurable breakdown of BBB.[3] Central nervous system (CNS) neoplasm-induced oedema is an example of vasogenic oedema. Vasogenic oedema fluid is extracellular and accumulates primarily in white matter. Tight junctions in microvascular endothelia open in vasogenic oedema.[4] Tight junctions are well-developed intercellular structures that form an impermeable seal between adjacent endothelial cells of blood vessels in the CNS. Tight junction proteins e.g., occludin, line the cytoplasmic face of intact tight junctions[5] and establish the BBB.

Little is known about the regulation of water transport across the BBB, but it is well-recognised that the aquaporin family of water channel proteins is the major pathway by which water rapidly crosses cell membranes.[6] The aquaporins are small (~30kDa) hydrophobic proteins that assemble in membranes as tetramers. Each monomer, consisting of 6 membrane-spanning tilted a-helical domains with cytoplasmically oriented amino and carboxy-termini, contains a distinct water pore.[7,8] At least 10 aquaporins have been cloned. Aquaporin 4 (AQP4) is the predominant water channel in the brain.[9] Within the brain, it is also expressed at the blood-brain and brain-cerebrospinal fluid interfaces.[10] At the cellular level, AQP4 is expressed abundantly in a highly polarised distribution in ependymal cells and astroglial membranes facing capillaries and forming the glia limitans.[11] Recent studies have reported that AQP4 negatively influences the outcome from brain oedema[12] and suggested that AQP4 contributes to the development of brain oedema.[13,14]

In this study, we investigate the role of tight junction proteins as well as AQP4 expression and its relationship to peri-tumoural oedema in human meningiomas.

Materials and Methods

After institutional ethics review and approval as well as informed consent, we studied 17 meningioma specimens after surgical resection. The specimens were predominantly skull base meningiomas excised by the senior author (IN). Ten of the tumours had severe peri-lesional oedema on MRI scans (T2 weighted images) while the other 7 tumours had no demonstrable oedema. Review of the MRIs was performed by a member of the team blinded to the laboratory analysis (DNSL). Meningiomas were snap frozen in liquid nitrogen and stored at -80°C till analysis. An additional piece was placed in 4% paraformaldehye for frozen section. A structurally normal cerebral cortex was obtained from a patient undergoing temporal resection to access a mesial temporal lobe lesion. All laboratory analysis was performed blinded to MRI data (TWL).

Immunohistochemistry

Immunoreactive occludin and AQP4 were detected by the labeled streptavidin-biotin method. Several contiguous 10 μm sections were mounted on poly-L-Lysine-treated slides, One section was stained with haematoxylin and eosin. The remaining sections were extensively rinsed in PBS and endogenous peroxidases were quenched in 3% hydrogen peroxide for 5 minutes. After being washed in PBS, slides were incubated with primary antibody at room temperature for 2 hours with a 1:100 dilution of mouse anti-human occludin monoclonal antibody (Zymed Laboratories, CA, USA) or a 1:100 dilution of goat anti-human AQP4 polyclonal antibody (Santa Cruz Biotech Inc., CA, USA). Following 3 rinses with PBS, slides were then incubated with linking antibody (Dako) for 15 minutes, followed by 15 minutes with streptavidin-horseradish-peroxidase and then incubated for 8 minutes with the chromogen 3,3'-diaminobenzidine tetrahydrochloride (DAB; Dako). After, each incubation sample was rinsed 3 times with distilled water. Samples were counterstained with haematoxylin for 1 minute and nuclei blued in water. Slides were then dehydrated and mounted.

Western Blot analysis

Specimens were homogenised in triton lysis buffer containing 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% triton and 5 mM EDTA with protease inhibitor cocktail (Sigma-Aldrich, USA). Protein concentrations were determined by Bradford assay using bovine serum albumin as the standard control. 75 mg of protein was electrophoresed on a 12% SDS-PAGE gel and then electroblotted onto nitrocellulose membrane. The membrane was then blocked with 5% dried milk in Tris buffered saline with 1% Tween 20 and probed with 1:100 dilution of mouse anti-human occludin monoclonal antibody or 1:50 dilution of goat anti-human AQP4 polyclonal antibody. The membrane was then washed and incubated with horseradish-peroxidase(HRP)-conjugated goat anti-rabbit (Santa Cruz Biotech Inc., CA, USA) or rabbit anti-goat antibody (Chemicon International, CA, USA). Protein expression was detected with an enhanced chemiluminescence detection system (Pierce Biotechnology Inc., IL, USA). Bands were visualised on film and densitometry was used to estimate the relative amount of proteins.

Statistical Analysis

All statistics were performed with the SPSS software (Version 12; SPSS, Inc., Chicago, Illinois). Categorical variables were analysed using the Chi-square test. For means, a Student's *t*-test was used.

Results

Immunohistochemical staining

Alterations in BBB integrity via immunohistochemical staining of

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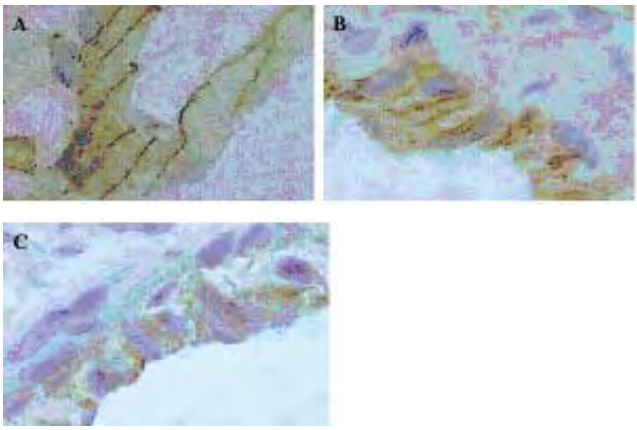

Fig. 1a. Immunohistochemical staining of the tight junction protein occludin in A. normal brain, B. oedematous meningioma and C. non-oedematous meningioma. Both groups of meningioma showed diffuse and fragmented staining of occludin.

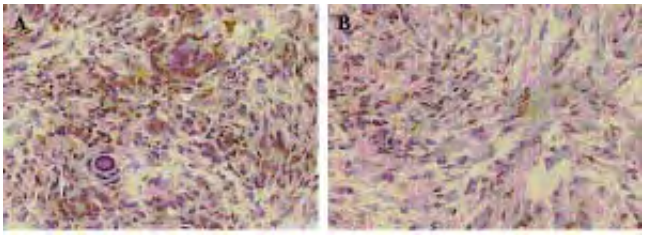

Fig. 1b. Immunohistochemical staining of AQP4 in A. oedematous meningioma and B. non-oedematous meningioma. AQP4 was upregulated in both groups of meningioma.

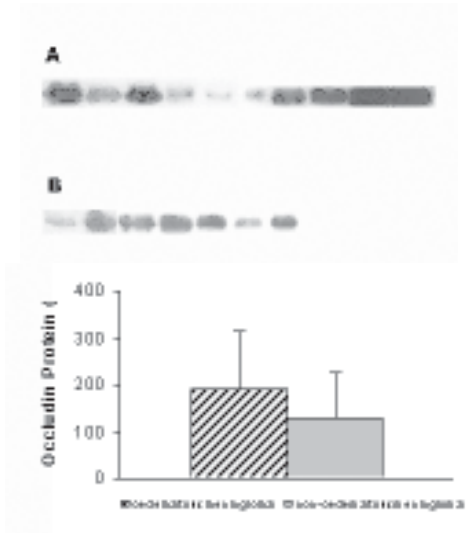

Fig. 2a. Expression of occludin protein (~59.1kDa) in (A) oedematous meningiomas and (B) non-oedematous meningiomas.

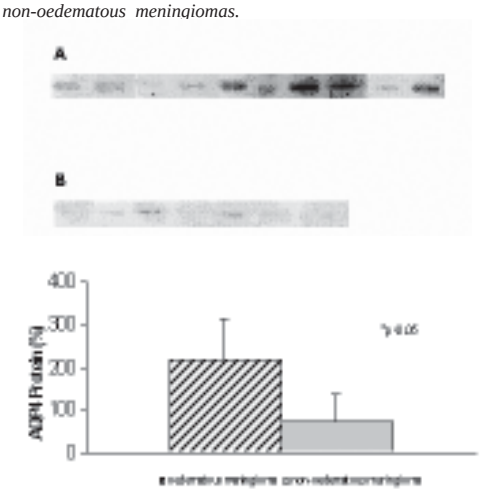

Fig. 2b. Expression of AQP4 protein (~34.8kDa) in (A) oedematous meningiomas and (B) non-oedematous meningiomas.

the tight junction protein occludin in the meingiomas showed significant disruption of BBB. It is demonstrated by fragmentation, absence or diffuse cytoplasmic localisation of DAB (brown staining) as compared to normal brain. However the degree of impairment of the BBB as observed under light microscopy was not significantly different between the meningiomas with marked oedema and no demonstrable oedema (Fig. 1a). Meningioma immunostained for AQP4 was not just restricted to the perimicrovessel region as observed in normal brain[11,15,16], but was upregulated throughout the specimens in both groups (Fig. 1b).

Protein Expression

Western blot analysis revealed that occludin protein level was not significantly different between the oedematous and non-oedematous meningiomas ($P = 0.28$) (Fig. 2a). However in AQP4 protein level, oedematous meningiomas had a significantly increased level of AQP4 expression compared with non-oedematous meningiomas (217.2 ± 94.8% versus 74.4 ± 63.1%, $P < 0.05$) (Fig. 2b).

Discussion

Cerebral oedema may result from a loss in structural integrity or from abnormalities of water homeostasis at the cellular level. As such we studied 2 areas; the integrity of a trans- membrane protein important in maintaining the structural integrity of the tight junctions and the expression of AQP4, a water channel recently implicated in cellular water homeostasis. In this study, we showed that AQP4 expression is upregulated in meningiomas and there is a significant correlation between AQP4 expression and the presence of oedema. Increased AQP4 expression suggests enhancement of oedema formation. The role of AQP4 in brain water homeostasis is still sparse but extrapolating the data from its function in the kidney, it functions most likely by transporting water from the extracellular space into glial cells, protecting surrounding neurons from further osmotic stress.[3] If the increase of AQP4 expression results in oedema formation and not oedema fluid clearance, its upregulation may be a maladaptive response, as is the case for upregulation of AQP2 in the kidney, in some fluid-retaining states.[4] However it is shown in studies that AQP4-knockout mice showed reduced oedema formation after focal cerebral ischaemia and water intoxication.[4,12,17] These studies provide evidence that AQP4 may be important in the formation of brain oedema.

The integrity of the BBB as observed in the immunohistochemical staining of occludin may have led to fluid leakage. However, the degree of disruption of occludin and its protein expression level were not significantly different between the oedematous and non-oedematous meningioma. Therefore BBB disruption and oedema formation may not be directly associated with tight junction pathology. However it has been shown by Vizuete et al that after neurological insult, striatal AQP4 mRNA was induced and it correlated with extravasation of Evans blue dye as a marker of BBB disruption and not with neuronal degeneration. Disruption of BBB could then have induced AQP4 expression to re-establish the brain osmotic equilibrium.[18]

This finding may be important as novel therapeutic targeting of aquaporin channels may limit oedema formation, which may greatly

ameliorate symptoms in non-operable cases where the symptoms may be due largely to oedema, greatly reduces the risk of surgery and aids in minimising the possibility of a stormy postoperative period from exacerbation of cerebral oedema.

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Special Poster Presentation

Estrogen Receptor Alpha Gene *Pvu*II Polymorphism And Polycystic Ovary Syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common pathological causes of female infertility, affecting 5% to 10% of women in the reproductive age group. It is highly prevalent within families, suggesting a genetic basis. Several candidate genes have been studied as predisposing genetic factors contributing to PCOS, but neither of them has been widely accepted as a major cause for this syndrome.[1] PCOS is characterised by anovulation, excessive ovarian androgen production, and, consequently, infertility.

Estrogen receptors, ERα and ERβ, mediate the physiological functions of estrogen. Estrogen not only acts as a key intraovarian modulator of ovarian activity but also exerts auto and paracrine roles on the follicle oocyte unit. It stimulates antral and pre-antral follicular growth.[2] In PCOS, the ovary is constantly exposed to estrogen and the multiple atretic and cystic follicles could result due to defective follicle protective action of estrogen.[3]

Several ERα gene polymorphisms have been identified, however *Pvu*II polymorphism in particular has been found to be associated with a number of clinical conditions. *Pvu*II polymorphism in ERα has been found to have a close association with breast cancer and spontaneous miscarriage.[2] Recently, an association between *Pvu*II polymorphism and outcome of ovarian stimulation in patients undergoing IVF has been reported.[2] In the present study an association of ERα gene *Pvu*II polymorphism with PCOS was determined.

Materials and Methods

Study Subjects

One hundred and thirty four Singaporean Chinese female subjects having infertility due to PCOS were recruited in this study. Their ages ranged from 14 to 39 years (25.6 ± 6.71 years; mean ± SD. One hundred normal ovulatory Chinese women ranging between 18 and 44 years (32.7 ± 4.56 years; mean ± SD) with regular menstrual cycles (intervals between 23 and 39 days) were used as control subjects.

Investigation Protocols

Investigations included ovulation tests, hormone measurements, and abdominal ultrasonography and laparoscopy to confirm the polycystic nature of the ovaries. Plasma levels of E_2, FSH, LH and T were analysed by their specific radioimmunoassay using reagents provided by the WHO (WHO, Method Manual, 1981).

Determination of ERα PvuII Polymorphism

Genomic DNA was extracted from peripheral leucocytes by a standard procedure. DNA amplification was carried out using specific primers, ERpvuF:

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5'-CTGCCACCCTATCTGTATCTTTTCCTATTCACC-3'

ERpvuR:

5'-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3'

as previously described.[2] RFLP analysis was performed using *Pvu*II as previously described.[2]

Statistical Analysis

The gene counting method determined gene frequencies. Statistical tests of significance and Chi-square and Fisher's exact test (2-sided) were carried out using SPSS for windows, version 12.0. One-way analysis of variance (ANOVA) was used for the statistical analysis of all serum values to achieve homogeneity of variance test. A P value <0.05 was considered statistically significant.

Results

The frequency of *Pvu*II polymorphism was significantly higher in the patients (0.53) than controls (0.47) (P = 0.008, χ^2 = 9.676) (Table 1). The occurrence of homozygous *Pvu*II polymorphism was also significantly higher in the patients (26.1%) than controls (11.0%) (P = 0.005) (Table 1). However, the occurrence of heterozygous polymorphism in patients was similar to that in controls.

Serum levels of E_2 was significantly higher in the homozygous group (355 pmol/L) than no polymorphism group (224 pmol/L) (P = 0.034), heterozygous group (238 pmol/L) (P = 0.020), and heterozygous and no polymorphism group taken together (233 pmol/L) (P = 0.001).

Serum levels of T and LH did not show any significant difference between the three genotypes ($0.436 \leq P \leq 0.999$). Serum level of FSH however, was significantly higher in the homozygous group (3.50 IU/L) than 2 other groups (2.57 IU/L) (P = 0.017) (Table 2).

Discussion

Various endocrine factors may contribute to the phenomenon of arrested follicular development, which is the hallmark of anovulatory infertility in PCOS. In PCOS, an increased recruitment of follicles or a decreased natural atresia occurs during stages of early folliculogenesis and development of early tertiary follicles. On the other hand atresia of tertiary follicles is accelerated compared with that in normal women and further follicle maturation is impeded.

Estrogen offers a folliculoprotective effect and plays an important role in recruiting or preventing atresia of follicles. In hypophysectomised rats, estrogen alone promotes preantral follicular development in the absence of endogenous gonadotropins.[4] Androgens when given systemically or produced locally through LH stimulation induce follicular atresia and administration of FSH prevents this effect.[5] Thus it is possible that estrogen alone could be responsible for increasing recruitment or preventing atresia of primary, secondary and preantral follicles in women with PCOS.

ERα receptors have been demonstrated in ovarian epithelial cells, granulosa cells and theca cells of the ovarian follicles,[2] indicating importance of the receptor in the development of follicles. From a clinical perspective it is possible that a defect in the E-ERα binding system resulting from any mutation or polymorphism of the receptor protein would affect the normal function of the hormone.

In our study the incidence of *Pvu*II polymorphism was higher in the patients than controls. The occurrence of homozygous *Pvu*II polymorphism was also higher in the patients than controls. However, homozygosity in none of the groups showed any significance to patients with particular symptoms like acne, hirsutism or obesity. In our study, no statistical significance in serum levels of LH or T was seen. Serum levels of FSH were significantly higher in the homozygous group but were within the normal range. Nevertheless, serum levels of E_2 were found to be significantly higher in the homozygous group than no polymorphism group and heterozygous group. This could be attributed to the mechanism of estrogen resistance.

The *Pvu*II polymorphism, though it does not result in amino acid change of the receptor protein, may co-segregate with mutations or regulatory sequence variations in the ER gene, which in turn may affect the ER expression or function.[6] Furthermore, it has been reported that genes containing SNPs can cause different structural folds of mRNA, resulting in different biological functions that interact with other cellular components.

Conclusion

Therefore genetic variability of estrogen receptor gene through its estrogen resistance and interaction with environmental factors, and a small number of major causative genes including those involved in the paracrine and autocrine modifications of follicular growth, may contribute to the development of PCOS in some Singapore Chinese women.

Table 1. Incidence of *Pvu*II Genotypes in PCOS Patients and Controls

Subjects	Genotypes			Frequency of P	P value
	pp	Pp	PP	q	
Patients (134)	26	73	35	0.53	-
Controls (100)	17	72	11	0.47	0.008

P: *Pvu*II positive; p: *Pvu*II negative; pp: no polymorphism: PP: homozygotes; Pp: heterozygotes

Table 2. Hormonal Values of PCOS Patients in each Genotype

Variable	No polymorphism pp (n = 26)	Heterozygous Pp (n = 73)	Homozygous PP (n = 35)	Non-homozygous pp +Pp (n = 99)
E_2* (pmol/L) (normal: 161-562)	224 ± 91	238 ± 106	355 ± 234[a]	233 ± 101
T[t] (nmol/L) (normal: 3.1-11.4)	8.39 ± 3.10	8.51 ± 3.61	8.77 ± 3.48	8.51 ± 3.74
LH* (IU/L) (normal: 2.0-10.5)	1.61 ± 9.68	9.4 ± 8.48	11.95 ± 8.53	10.05 ± 8.85
FSH* (IU/L) (normal: 1.6-6.1)	3.20 ± 1.58	2.31 ± 1.45	3.50 ± 2.50 [b]	2.57 ± 1.53

P: *Pvu*II positive; p: *Pvu*II negative; pp: no polymorphism: PP: homozygotes; Pp: heterozygotes

Note: Values are given as means ± SD in SI units.

[a] Significantly higher than pp group (P = 0.034), Pp group (P = 0.020) and these 2 groups taken together (P = 0.001)

[b] Significantly higher than the other 2 groups taken together (P = 0.017)

* Measured at day 4

[t] Measured at day 21

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Ultrarapid Prenatal Detection of Down Syndrome Using Real-time Multiplex Polymerase Chain Reaction (PCR) in Amniotic Fluid

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Introduction

Down syndrome (DS) or trisomy 21, affects 1 in 600 newborns. DS is the most common chromosomal aneuploidy causing severe mental retardation. In prenatal diagnosis, amniocentesis is the most common procedure for obtaining amniocytes for chromosomal analysis. Though reliable and accurate, 7 days to 21 days of culture for metaphase analysis are required before results are released. This extended period of waiting causes parental anxiety. Abnormal results are important in clinical decision-making, but normal results allay the anxiety associated with the longer wait for the full karyotype.

Alternative methods include analysis of uncultured amniocytes by fluorescence in situ hybridisation (FISH)[1] or by genomic DNA amplification and quantification.[2] In these tests, only common aneuploidies involving chromosomes 13, 18, 21, X and Y are analysed. FISH relies on visual counting of fluorescence signals within target foetal cells and is therefore labour-intensive, time-consuming and costly. The use of quantitative fluorescent-polymerase chain reaction (QF-PCR),[3] to amplify and quantitate short tandem repeats reduces cost and time. However, a minimum of 6 hours is still required for QF-PCR.

Real-time PCR combines the amplification and quantitation steps in real-time. We hypothesise that by making use of real-time multiplex PCR, reporting time can be further reduced. In this study, we were successful in the detection of DS within 3 hours of amniocentesis with 100% accuracy.

Materials and Methods

Sample Collection and DNA Isolation

Sample collection for research was approved by the Institutional Review Board. Two millilitres of amniotic fluid, surplus to requirement for cell culture for conventional karyotyping were obtained after written informed consent from 85 mothers undergoing amniocentesis between 14^{+4} to 35^{+2} gestational weeks. The amniotic fluid was centrifuged to obtain the amniocytes pellet for resuspension in PBS. DNA was isolated from 200 µL of the amniocytes/PBS suspension using the QIAamp DNA Blood Mini Kit according to the manufacturer's recommendations (Qiagen GmbH, Germany).

Relative Quantitation of APP Against β-globin Gene Expression to Detect DS Using Real-time Multiplex PCR

Real-time multiplex PCR analysis was performed with the use of a PE Applied Biosystems 7000 Sequence Detector. For the detection of DS, amyloid gene (*APP*) was used as the target gene as it is located on chromosome 21. Endogenous reference gene used was β-*globin*. To examine the coamplification of both target and endogenous reference genes, it is essential that the probe of each gene was labelled with a different fluorescence dye to enable differentiation. In this study, *APP* probe was labeled with 6-FAM while β-*globin* probe is labeled with VIC. The sequence of *APP* and β-*globin* primers and probe combinations had been described.[4,5] Commercial male and female genomic DNA were used as endogenous controls for the coamplification of *APP* and β-*globin* in all runs.

Qualitative Measurement of APP/β-globin Real-time Multiplex PCR Efficiencies

Commercial male genomic DNA was serially diluted 5-fold and set as the standard. The standard curve is required to measure the efficiencies of *APP*/β-*globin* coamplification. Each sample and standard was run in triplicates with both sample and standards running in parallel.

Results

Qualitative Analysis and Relative Quantitation of APP Gene Expression

In all cases (100%), DS diagnostic results in this blinded study were concordant with their respective karyotypes. Both *APP* and β-*globin* amplified in all endogenous controls (100%). To calculate the mean δC_T (β-*globin-APP*) values, the difference between C_T of β-*globin* and *APP* in each well of the triplicate was averaged. This gives the mean δC_T (β-*globin-APP*) for relative quantitation of *APP* gene expression against β-*globin*. The δC_T (β-*globin-APP*) values were low (mean ± SD = -1.41 ± 0.17) in 82 pregnancies and high (mean ± SD = -0.75 ± 0.08) in 3 pregnancies (Table 1). With conventional karyotypes, these 3 cases with high δC_T (β-*globin-APP*) were confirmed to be DS while the remaining 82 were excluded of DS. The difference in the δC_T (β-*globin-APP*) values between normal and DS cases is significant (P = 0.003, Mann-Whitney rank-sum test) (Fig. 1).

Sensitivities of APP/β-globin Real-time Multiplex PCR Assay

Sensitivities of both *APP* and β-*globin* amplifications in this *APP*/β-*globin* real-time multiplex PCR assay are high. All standards

Table 1. Difference in the δC_T (*β-globin-APP*) between Normal and DS Amniotic Fluid Samples

	δC_T (*β-globin-APP*)	
	Normal	*DS*
Mean	-1.406	-0.753
Median	-1.40	-0.75
SD	±0.17	±0.08

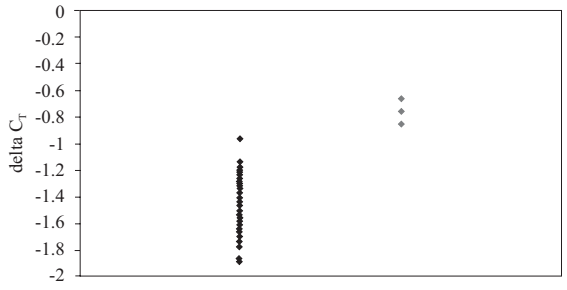

*Fig. 1. Distinct differences in delta C_T (*β-globin-APP*) between normal and DS amniotic fluid samples.*

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including the lowest dilution of 15 GE/mL (GE: genome equivalents) were amplified in both *APP* and β-*globin* coamplification.

Standard Curves

Regression coefficients for all runs were at -0.99 (ideal -1.00). For *APP* standard curves, the mean ± SD slope was -3.68 ± 0.14 with the mean ± SD intercept of 39.95 ± 0.77. For β-*globin* standard curves, the mean ± SD slope was -3.46 ± 0.08 with the mean ± SD intercept of 37.56 ± 0.45.

Discussion

Real-time multiplex PCR amplifies both the target and endogenous reference genes in the same tube. Such coamplification minimises well-to-well variation and increases sample throughput. By using the mean δC_T (β-*globin-APP*), we were able to compare the gene expression level of *APP* and β-*globin*. This is known as relative quantitation of gene expression. We were accurate in detecting 3 DS out of 85 amniotic fluid samples.

Ideally, specific indication of the normality of the foetus should be available on the same day as the diagnostic invasive procedure. This would be possible if analysis results of uncultured amniocytes after invasive testing were ready within hours of the procedure. In our study, the time taken for DNA isolation, amplification and analysis was within 3 hours of amniocentesis. We thus achieved our aim of ultrarapid diagnosis of DS.

We have demonstrated that real-time multiplex PCR of *APP* with β-*globin* as the endogenous reference in amniotic fluid samples is a rapid and reliable alternative technique to QF-PCR and FISH in prenatal diagnosis. This technique can be used to include other common foetal chromosomal aneuploidies like trisomy 18 (Edward's syndrome) and trisomy 13 (Patau's syndrome).

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