SUPPLEMENT 6

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Health and Medical Research Fund

Research Dissemination Reports

醫療衞生研究基金

研究成果報告

Children's health
兒童健康

Reproductive health
生殖健康

Stroke / brain injury
中風/腦損傷

Supplement 6

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HONG KONG MEDICAL JOURNAL 香港醫學雜誌
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SUPPLEMENT 6

Health and Medical Research Fund

Research Dissemination Reports

STROKE / BRAIN INJURY

Editorial

Dissemination reports are concise informative reports of health-related research supported by the Health and Medical Research Fund (and its predecessor funds) administered by the Food and Health Bureau. In this edition, we present 13 dissemination reports of projects related to children's health, neurology, reproductive health, and stroke and brain injury. In particular, three projects are highlighted due to their potentially significant findings, impact on healthcare delivery and practice, and/or contribution to health policy formulation in Hong Kong.

Necrotising enterocolitis (NEC) is one of the most devastating complications of prematurity. Despite advances in neonatal management for preterm very-low-birth-weight infants, NEC-associated morbidities and mortality remain high. Ng et al[1] analysed gene expression microarray data of surgical tissues from infants with NEC and controls to identify potential biomarkers. These biomarkers were then validated in another case-control cohort. Performance of these biomarkers and existing biomarkers was compared. They found that two proteins, namely hepatocarcinoma-intestine-pancreas protein and intestinal bile acid binding proteins, are novel biomarkers for early diagnosis of NEC when used in combination, with reasonably high sensitivity of 85% and specificity of 91%. Use of these biomarkers in a risk stratification scheme could promptly help in the identification and management of preterm infants with NEC.

Over 13 million babies preterm (under 37 gestational weeks) are born each year globally. Currently, the best predictive markers for spontaneous preterm birth are shortened cervical length and elevated cervicovaginal foetal fibronectin, but their sensitivity at high specificity is only moderate. Chim et al[2] systematically profiled the transcriptome of maternal peripheral blood collected during preterm labour. They found that two RNA transcripts related to inflammation and defence response to bacteria were up-regulated in preterm labour, compared with control groups. In a validation cohort, preterm labour women test-positive for the two RNA transcripts were more likely to deliver sooner. Thus, maternal peripheral blood can be used as a relatively non-invasive way for predicting spontaneous preterm birth.

Dysexecutive syndrome (DES) is an impairment of executive functions such as goal setting, planning, action initiation and inhibition, social cognition, theory of mind, insight, and metacognition. DES comprises behavioural and cognitive domains, and behavioural DES (BDES) is a common condition among stroke survivors. Tang et al[3] performed magnetic resonance imaging on 369 patients with first-ever or recurrent acute ischaemic stroke and studied the clinical course of BDES. At 3 months after stroke, BDES was identified in 18.7% of stroke survivors. More severe anxiety symptoms, presence of current depression, and poor cognitive functioning predicted BDES at 3 months after stroke. BDES significantly improved at 38 months after stroke. No radiological variable was found to be associated with BDES.

We hope you will enjoy this selection of research dissemination reports. Electronic copies of these dissemination reports and the corresponding full reports can be downloaded individually from the Research Fund Secretariat website (https://rfs2.fhb.gov.hk/). Researchers interested in the funds administered by the Food and Health Bureau also may visit the website for detailed information about application procedures.

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References

1. Ng EW, Ng PC, Lam HS, et al. Gut barrier proteins in diagnosing necrotising enterocolitis in preterm infants: abridged secondary publication. Hong Kong Med J 2020;26(Suppl 6):S4-5.
2. Chim SS, Chan TF, Leung TY. Whole-transcriptome analysis of maternal blood for identification of RNA markers for predicting spontaneous preterm birth among preterm labour women: abridged secondary publication. Hong Kong Med J 2020;26(Suppl 6):S20-3.
3. Tang WK, Wong KS, Mok VC, Chu CW, Wang D, Wong A. Behavioural dysexecutive syndrome after stroke: abridged secondary publication. Hong Kong Med J 2020;26(Suppl 6):S30-3.

Gut barrier proteins in diagnosing necrotising enterocolitis in preterm infants: abridged secondary publication

EWY Ng, PC Ng, HS Lam, MMT Lam, HM Cheung, TPY Ma, KYY Chan, POR Wong, KT Leung, K Li, TCW Poon *

KEY MESSAGES

1. Our study provides the first evidence that hepatocarcinoma-intestine-pancreas (HIP) and intestinal bile acid binding protein (I-BABP) were specific, novel biomarkers for early diagnosis of necrotising enterocolitis (NEC).
2. Use of HIP followed by I-BABP significantly improved the diagnostic performance. A stepwise risk stratification scheme for preterm infants suspected of NEC is proposed.
3. This risk stratification scheme can facilitate neonatologists in identification and management of preterm infants with NEC.

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HMRF project number: 01120296

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Introduction

Necrotising enterocolitis (NEC) is one of the most devastating complications of prematurity. Despite advances in neonatal management for preterm very-low-birth-weight infants, NEC-associated morbidities and mortality remain high.[1] NEC often manifests in a fulminant manner with minimal antecedent signs and symptoms; it is important to recognise the initial bowel injury early so that neonatologists can promptly initiate treatment to minimise further damages to the bowel.

Acute-phase proteins, cell surface antigens, cytokines, and chemokines have been used to identify sepsis and NEC cases, but these mediators are unable to differentiate the two conditions.[2,3] Proteins originated from the bowel such as intestinal-fatty acid binding protein, liver-fatty acid binding protein, trefoil factor-3 (TFF3), and claudin-3 can be used as biomarkers for diagnosing NEC.[4] Nonetheless, these biomarkers are only useful in differentiating severe (surgical) cases from milder (medical) cases, and they are not clinically useful for detection of early bowel injury.[4] Such biomarkers are also unable to differentiate mild NEC cases from septicaemic or control patients.

Significant and extensive changes of gene expression are associated with multiple pathways involving inflammation, hypoxia and oxidative stress, cell adhesion and chemotaxis, extracellular matrix remodelling, angiogenesis, muscle contraction, and arginine metabolism.[5] These molecular responses correspond closely with the pathophysiology of NEC. We therefore hypothesised that novel tissue-specific or NEC-specific biomarkers could be discovered through searching for (1) gut-specific genes with mRNA levels dysregulated in NEC tissues, (2) genes that were up-regulated in accordance to the pathophysiologic mechanisms (eg, microbial infection and hypoxic and oxidative stress), and (3) proteins that shared the same protein family with known NEC biomarkers. We used a systemic bioinformatic approach to discover novel biomarkers through mining of the global gene expression data in diseased small bowel tissues collected from NEC infants. The objective was to discover novel biomarkers with good diagnostic value (sensitivity and specificity >85%) for diagnosing both medical (mild) and surgical (severe) NEC at the early phase.

Methods

This study comprised three phases: a discovery phase based on analysis of transcriptomes of NEC tissues to identify genes encoding for potential biomarker candidates, an exploratory phase to confirm level changes of selected markers, and a validation phase to examine the diagnostic value of plasma levels of the candidate proteins.

In the discovery phase, we analysed gene expression microarray data[5] of surgical small bowel tissues from infants with proven NEC (n=5), spontaneous intestinal perforation (n=5), and surgical control (n=4) to identify genes in which mRNA levels were altered in the NEC tissues in the same direction as those genes-encoding NEC

biomarkers. Among the gene list, only those that were predominantly expressed in the mucosa of human small intestine were retained by examining their baseline expression patterns in normal human tissues, and formed the disease site–specific list. This step aimed to filter out genes-encoding proteins that were not specific to the disease site of NEC. Using functional annotation analysis among the site-specific gene list, we retained genes in which expressions were likely to be up-regulated in response to microbial infection, hypoxia, and/or oxidative stress and genes that encoded proteins belonging to the same protein family of previously reported NEC biomarkers. Proteins in the final gene list were considered as potential NEC biomarker candidates.

In the exploratory phase, these proteins were examined in a case-control cohort consisted of 10 NEC patients, 20 non-NEC septicaemia patients, and 20 non-NEC non-septicaemia patients.

In the validation phase, plasma levels of the potential biomarker candidates and previously reported potential NEC biomarkers (intestinal-fatty acid binding protein, liver-fatty acid binding protein, TFF3, claudin-3, and cytosolic beta-glucosidase) were measured to determine their values in differentiating NEC cases (n=20) from septicaemic cases (n=40).

The Mann-Whitney *U* test was used for between-group comparisons. The Kruskal-Wallis test was used for comparisons of more than two groups, followed by Dunn's post-hoc tests. P values were adjusted by Bonferroni correction or Benjamini-Hochberg procedure for multiple comparisons where appropriate. The receiver operating characteristic curve, drawn by plotting sensitivity against 1-specificity at various cutoff values, was used to determine the diagnostic utilities of the biomarkers. All statistical analyses were performed using SPSS (Windows version 22; IBM Corp, Armonk [NY], US).

Results

Of 16 potential NEC biomarkers identified, 11 were quantified in NEC patients, non-NEC septicaemia patients, and non-NEC non-septicaemia patients in the exploratory phase.

In the validation phase, plasma levels of galectin-4, hepatocarcinoma-intestine-pancreas (HIP), intestinal bile acid binding protein (I-BABP), liver-fatty acid binding protein, and TFF3 were significantly higher in the NEC groups than both the non-NEC septicaemia group (P<0.011) and non-NEC non-septicaemia group (P<0.001). Galectin-4, I-BABP, HIP, and TFF3 had the highest diagnostic value in distinguishing NEC cases from non-NEC septicaemia cases and non-NEC non-septicaemia cases (area under the receiver operating characteristic curve was 0.84-0.91, all P<0.001).

Combination of the best two markers (HIP and I-BABP) was evaluated for improvement of their diagnostic performance. Using a risk-stratified approach by firstly using HIP to screen patients of high NEC risk and then using I-BABP to identify NEC cases among the high-risk group, the diagnostic performance of 85% sensitivity and 91% specificity was achieved.

Discussion

This study provides evidence that plasma HIP and I-BABP are tissue-specific novel biomarkers for early diagnosis of NEC. We formulated a stepwise risk stratification scheme by combining HIP and I-BABP to achieve diagnostic performance of 85% sensitivity and 91% specificity. These novel biomarkers were discovered using a comprehensive and systemic bioinformatic approach based on tissue expression profiles, dysregulatory pathways of intestinal injury, and pathophysiologic mechanisms of NEC, as well as through mining of the global gene expression data in diseased small bowel tissues collected from infants with NEC. Our new risk stratification scheme enables neonatologists to identify NEC cases during presentation of early and non-specific symptoms, and to facilitate decisions on timely application of treatment strategies to minimise gut damage.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120296). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

References

1. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med 2011;364:255-64.
2. Ng PC, Chan KY, Poon TC. Biomarkers for prediction and diagnosis of necrotizing enterocolitis. Clin Perinatol 2013;40:149-59.
3. Ng PC. Biomarkers of necrotising enterocolitis. Semin Fetal Neonatal Med 2014;19:33-8.
4. Ng EW, Poon TC, Lam HS, et al. Gut-associated biomarkers L-FABP, I-FABP, and TFF3 and LIT score for diagnosis of surgical necrotizing enterocolitis in preterm infants. Ann Surg 2013;258:1111-8.
5. Chan KY, Leung KT, Tam YH, et al. Genome-wide expression profiles of necrotizing enterocolitis versus spontaneous intestinal perforation in human intestinal tissues: dysregulation of functional pathways. Ann Surg 2014;260:1128-37.

Traffic-related air pollution and Hong Kong school children: abridged secondary publication

XQ Lao *, APS Lau, CHY Wong, TSI Yu

KEY MESSAGES

Generally, poorer respiratory health was associated with higher level of air pollution at school environment and higher total traffic count surrounding school.

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Introduction

Traffic exhaust is a principal source of ambient air pollution in urban areas. Gasoline- and diesel-powered vehicles emit various pollutants including carbon monoxide, nitrogen oxide, black carbon (BC), volatile organic compounds, and particulate matter (PM); all of which pose health risks. Traffic has been reported to be associated with respiratory symptoms, poor pulmonary function, wheezing, and asthma.[1,2] However, results have not always been consistent,[3] partly owing to inaccuracies in exposure assessments.

There is limited knowledge about air pollutants that are most likely to affect health.[4] Studies have focused on mixtures of air pollution, but different pollutant sources and components have different effects. A large proportion of pollution is attributable to exhaust fumes from diesel-powered vehicles, which have more serious health consequences than gasoline fumes. Optimal legislation and control strategies require accurate exposure assessment and adverse health effects characterisation of traffic-related air-borne pollutants.

Methods

This was a cross-sectional study. We chose 12 primary schools based on their nearby roads and traffic densities. The schools were grouped into three categories: (1) schools surrounded by main roads with a high traffic density, (2) schools surrounded by roads with mid-level or low traffic density, and (3) schools far away from main roads. We invited all years 2 to 6 students to participate in a health survey. Among approximately 2800 invited students, 2319 (1148 boys and 1171 girls) participated.

Height and weight were measured with light clothing and with no shoes on. The spirometry tests were conducted with Micro Medical SpiroUSB in accordance with the standardised criteria of the American Thoracic Society. The lung function parameters evaluated included the forced vital capacity, forced expiratory volume in the first second, peak expiratory flow, and maximum mid-expiratory flow (MMEF).

Information on non-infectious rhinitis completed by parents or guardians was retrieved. Non-infectious rhinitis was defined as affecting children who have had nasal symptoms (such as nasal blockage, sneezing, and running nose) and itching eye or lachrymation in the absence of common cold in the previous 12 months. Those who answered 'yes' were asked to state the months during which they had rhinitis. Only those children whose responses were consistent were deemed to have rhinitis.

Information on self-reported respiratory diseases was also retrieved. Parents were asked whether the children had been diagnosed with the following diseases by a doctor in the past 12 months: asthma, allergic rhinitis, sinusitis, bronchitis, bronchiolitis, pneumonia. Those answering 'yes' to any one of the diseases were asked to indicate the months in which their child was diagnosed. Only those children whose responses were consistent were deemed to have respiratory diseases.

Air quality assessments in the school environment and on the main roads surrounding each school were conducted. To address seasonal variations, the assessment was conducted twice (once in summer and once in winter), each for 4 to 5 days during school time (8:00 am to 3:00 pm), generally in front of the main buildings in the schools. One or two routes (main road) surrounding the schools were also assessed using the mobile real-time air monitoring platform. PM 2.5 was measured by TSI DustTrak Aerosol Monitor. Nitrogen dioxide

(NO2), ozone, and carbon monoxide were measured by gaseous analysers; BC was measured by the aethalometer.

Data analyses were carried out using SAS 9.3 (SAS Institute, Cary [NC], USA). All P values were derived from two-sided statistic tests, and those <0.05 were considered statistically significant. Mix linear regression and logistic regression analyses were used to assess the relationships between health outcomes and the school environment air pollution as well as the traffic counts.

Results

The mean age of participants was 9.5±0.81 (range, 7-14) years. 49.5% were boys. The mean body mass index was 17.6±2.8 kg/m^2. Baseline characteristics were similar across the three school categories (Table 1).

Generally, higher levels of air pollution were associated (not necessarily significantly) with lower lung function parameters, except for the pollutant ozone (Table 2). PM 2.5 was significantly associated with a lower forced expiratory volume in the first second and a lower peak expiratory flow, after adjusting for potential confounders. PM 2.5, BC, NO2, and total traffic count were significantly associated with lower MMEF level after adjusting for potential confounders.

PM 2.5, NO2, and total traffic count were significantly associated with the occurrence of rhinitis. PM 2.5 was also significantly associated with self-reported respiratory diseases (Table 3).

Discussion

We investigated the respiratory health effects of air pollutants (PM 2.5, BC, NO2, and ozone) in the school environment and the traffic counts surrounding the schools. Generally, a higher level of air pollution was associated with poorer respiratory health in students, consistent with a previous study.[5] Specifically, a higher level of PM 2.5 was associated with lower forced expiratory volume in the first second, peak expiratory flow, and MMEF, and a higher prevalence of rhinitis and self-reported respiratory diseases; a higher level of BC was associated with a lower MMEF; a higher level of NO2 was associated with a higher prevalence of rhinitis; and a higher traffic count was associated with a lower MMEF and a higher prevalence of rhinitis.

MMEF was the most sensitive parameter. A lower MMEF level was associated with levels of PM 2.5, BC, and NO2 as well as total traffic count after adjusting for potential confounders. MMEF is an important indicator of a small airway function. Our study suggests that air pollution mainly affects the small airway function.

PM 2.5 was associated with health outcomes. This implies that PM 2.5 may contain various toxic chemicals. The total traffic count (but not the diesel vehicle count) was positively associated with a lower MMEF and a higher prevalence of rhinitis. This may be due to the poor correlations between traffic volume surrounding the school and pollutant concentrations in the school environment.

Our findings have important public health implications. Our results suggest urgency to develop strategies on air pollution mitigation in the school environment to protect children's health. Higher traffic volume surrounding schools was associated with poor respiratory health in primary schoolchildren. This finding suggests that urban planning for school location should consider the effects of traffic exhaust on school environment.

TABLE 1. Baseline characteristics of participants*

Characteristic	Overall	Schools surrounded by main roads with a high traffic density	Schools surrounded by roads with mid-level or low traffic density	Schools far away from main roads
Age, y	9.5±0.81	9.6±0.85	9.42±0.89	9.64±0.88
Male	49.5	49.1	50.1	48.9
Height, cm	135.9±7.6	136.6±8.2	134.9±7.9	136.1±8.1
Weight, kg	32.8±8.1	33.4±8.7	31.9±8.5	33.1±8.3
Body mass index, kg/m^2	17.6±2.8	17.7±3.1	17.3±3.0	17.6±3.5
Forced vital capacity, mL	1977±353	2002±355	1938±358	1991±356
Forced expiratory volume in the first second, mL	1734±299	1751±302	1694±306	1757±310
Maximum mid-expiratory flow, mL/s	1984±453	2012±477	1950±463	1991±563
Peak expiratory flow, mL/s	3949±686	4013±733	3859±736	3974±563
Rhinitis	38.4	37.8	40.5	39.1
Self-reported diseases	35.4	38.2	33.6	36.2

* Data are presented as mean ± standard deviation or % of participants

TABLE 2. Association between lung function and air pollutants / traffic counts in the primary school children

Lung function parameter	Unadjusted model		Adjusted model	
	Effect estimate (95% confidence interval)*	P value	Effect estimate (95% confidence interval)*	P value
Forced expiratory volume in the first second				
Particulate matter 2.5	-15.4 (-40.2 to 10.5)	0.31	-32.6 (-56.5 to -2.1)	0.033
Black carbon	-15.3 (-31.6 to 5.7)	0.140	-20.8 (-38.1 to 8.5)	0.12
Nitrogen dioxide	-4.1 (-19.4 to 12.3)	0.58	-7.3 (-18.9 to 11.7)	0.62
Ozone	7.8 (-14.9 to 30.6)	0.48	9.7 (-12.8 to 29.6)	0.49
Total traffic count	-10.3 (-36.1 to 12.8)	0.45	-15.2 (-40.2 to 3.6)	0.48
No. of trucks and buses	-13.7 (-28.6 to 21.4)	0.69	-16.6 (-29.4 to 7.3)	0.55
Forced vital capacity				
Particulate matter 2.5	-12.6 (-36.8 to 20.5)	0.68	-15.1 (-36.5 to 19.5)	0.54
Black carbon	-8.6 (-40.1 to 17.4)	0.42	-10.3 (-38.7 to 21.1)	0.47
Nitrogen dioxide	-4.8 (-28.2 to 19.7)	0.66	-6.1 (-28.1 to 19.9)	0.63
Ozone	4.3 (-29.4 to 36.1)	0.75	5. 8 (-26.5 to 32.3)	0.56
Total traffic count	-9.5 (-38.2 to 16.9)	0.38	-13.2 (-49.2 to 8.6)	0.48
No. of trucks and buses	-10.8 (-59.7 to 18.2)	0.68	-15.3 (-60.2 to 14.3)	0.52
Peak expiratory flow				
Particulate matter 2.5	-60.5 (-113.6 to 8.6)	0.05	-51. 1 (-109.2 to -2.3)	0.05
Black carbon	-63.9 (-118.6 to 13.9)	0.07	-49.2 (-99.8 to 10.6)	0.11
Nitrogen dioxide	-14.5 (-59.3 to 31.9)	0.61	-13.7 (-56.1 to 29.2)	0.56
Ozone	37.5 (-31.3 to 101.6)	0.33	39.7 (-22.8 to 121.8)	0.32
Total traffic count	-46.3 (-84.1 to 23.5)	0.36	-48.2 (-86.2 to 23.5)	0.40
No. of trucks and buses	-49.6 (-91.5 to 32.5)	0.51	-51.1 (-96.0 to 29.3)	0.58
Maximum mid-expiratory flow				
Particulate matter 2.5	-56.4 (-89.1 to -27.6)	0.01	-61.8 (-92.7 to -32.3)	0.01
Black carbon	-49.5 (-97.5 to -11.7)	0.01	-53.7 (-106.1 to -11.5)	0.01
Nitrogen dioxide	-16.0 (-54.3 to 21.5)	0.16	-17.8 (-74.2 to 37.8)	0.25
Ozone	40.2 (-13.6 to 98.6)	0.33	45.6 (-21.3 to 96.8)	0.29
Total traffic count	-22.6 (-58.3 to -9.8)	0.01	-26.3 (-60.1 to -11.8)	0.01
No. of trucks and buses	-25.7 (-59.1 to 6.8)	0.17	-26.4 (-64.2 to 11.9)	0.21

* Effect estimates were calculated as changes in per interquartile increase in air pollutant concentration or the traffic counts

TABLE 3. Association between rhinitis/self-reported disease and air pollutants / traffic counts in primary school children

Air pollutant	Adjusted odds ratio (95% confidence interval)	
	Rhinitis	Self-reported disease
Particulate matter 2.5	1.87 (1.14-2.54)	1.72 (1.11-2.38)
Black carbon	1.36 (0.93-2.13)	1.28 (0.89-2.09)
Nitrogen dioxide	1.21 (1.01-1.98)	1.26 (0.87-2.08)
Ozone	0.98 (0.93-1.81)	0.99 (0.87-1.98)
Total traffic count	1.48 (1.03-2.16)	1.56 (0.98-2.06)
No. of trucks and buses	1.31 (0.84-2.31)	1.24 (0.94-2.26)

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This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#11121031). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

References

1. Gehring U, Wijga AH, Hoek G, et al. Exposure to air pollution and development of asthma and rhinoconjunctivitis throughout childhood and adolescence: a population-based birth cohort study. Lancet Respir Med 2015;3:933-42.
2. McConnell R, Islam T, Shankardass K, et al. Childhood incident asthma and traffic-related air pollution at home and school. Environ Health Perspect 2010;118:1021-6.
3. Schultz ES, Litonjua AA, Melen E. Effects of long-term exposure to traffic-related air pollution on lung function in children. Curr Allergy Asthma Rep 2017;17:41.
4. Lippmann M, Chen LC. Health effects of concentrated ambient air particulate matter (CAPs) and its components. Crit Rev Toxicol 2009;39:865-913.
5. Chen Z, Salam MT, Eckel SP, Breton CV, Gilliland FD. Chronic effects of air pollution on respiratory health in Southern California children: findings from the Southern California Children's Health Study. J Thorac Dis 2015;7:46-58.

Secular trends of blood pressure in children and adolescents in Hong Kong: abridged secondary publication

MK Kwok *, YK Tu, IOL Wong, SL Lin, CM Schooling

KEY MESSAGES

1. In children and adolescents in Hong Kong, a U-shaped trend in blood pressure and an increasing trend in body mass index were observed until recent years.
3. The recent levelling off in body mass index could be related to school-based health promotion campaign.
4. Such discordant trends suggest identifying unknown determinants of blood pressure besides body mass index is warranted.

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Introduction

Secular trends in blood pressure (BP) and body mass index (BMI) in children and adolescents foreshadow trends in cardiovascular disease in adults. They are useful sentinels for population cardiovascular health. Changes in BP do not mirror changes in BMI, as factors determining trends in BP and BMI may be less evident in different settings. Understanding the concomitant trends in BP and BMI and delineating their potential driving forces would provide aetiological insights for prevention. Specifically, the discordant trends in children and adolescents, which could not be explained by adult lifestyle risk factors or medication, suggest the importance of identifying factors in the early life course.[1] Age-period-cohort modelling decomposes temporal trends into the relative contribution of age, calendar period, and year of birth. This makes it possible to distinguish the impact of early life cohort-specific factors (cohort effect) and the impact of contemporaneous population-wide factors (period effect) from the physiological changes of BP and BMI with increasing age (age effect). We delineated the relative contribution of age, period, and birth cohort to the trends in BP in children and adolescents aged 9 to 18 years from 1999 to 2014 and BMI for those aged 6 to 18 years from 1996 to 2014 in Hong Kong.

Methods

The study was approved by the Institutional Review Board of The University of Hong Kong / Hospital Authority Hong Kong West Cluster. The Student Health Service of the Department of Health provides free annual health assessments for all primary and secondary school students in Hong Kong. The Student Health Service was introduced in 1995/96 for primary school students, and was extended to secondary school students in 1996/97, but was suspended for secondary school students in year 2 or above in 2009/10 because of the Human Swine Influenza Vaccination Programme.

BP for primary 5 (age 10-11 years) onwards was measured twice a year using an automated oscillometric device. If initial BP was over the 90th percentile for sex, age, and height local reference, re-checking would be performed. Weight and height for primary 1 (age 6-7 years) onwards was measured yearly using a digital scale and stadiometer, respectively. Coverage was incomplete in the early years; trends of BP from 1999 and BMI from 1996 were considered. We randomly selected one time point per participant so that there is no correlation between multiple measurements for the same participant.

We examined the overall secular trends in age-, sex- and height-standardised BP from 1999 to 2014 and age- and sex-standardised BMI from 1996 to 2014. We decomposed the trends of BP and BMI into the effects of three components, namely age, period, and birth cohort. Given age, period, and cohort are linearly dependent (ie, non-identifiability problem), age-period-cohort partial least squares regression imposes constraints that are exactly the inherent mathematical relations within the data of the age, period, and cohort variables (ie, age + cohort = period),[2] with no additional arbitrary constraints. We examined the curvilinear relationship of age-period-cohort on BP or BMI by including age, period, and cohort as categorical variables. We plotted the resultant regression

coefficients with 95% confidence intervals. Statistical analyses were performed using Stata version 12.1 (Stata Corp, College station, Texas, USA) and R version 3.0.1 with the command plsr (R Development Core Team, Vienna, Austria).

Results

BP data of 402 040 students aged 9 to 18 years from 1999 to 2014 and BMI data of 1 898 816 students aged 6 to 18 years from 1996 to 2014 were included. Overall, systolic and diastolic BP trends declined from 1999 and bottomed in 2003-2005 but increased afterwards (Fig. 1). Systolic and diastolic BP was higher in earlier cohorts until about 1984. BMI trend gradually increased from 1996 before stabilised around 2009-2010 (Fig. 2). Those born in earlier years until about 1984 had higher BMI, whereas those born in 2003-2004 had lower BMI.

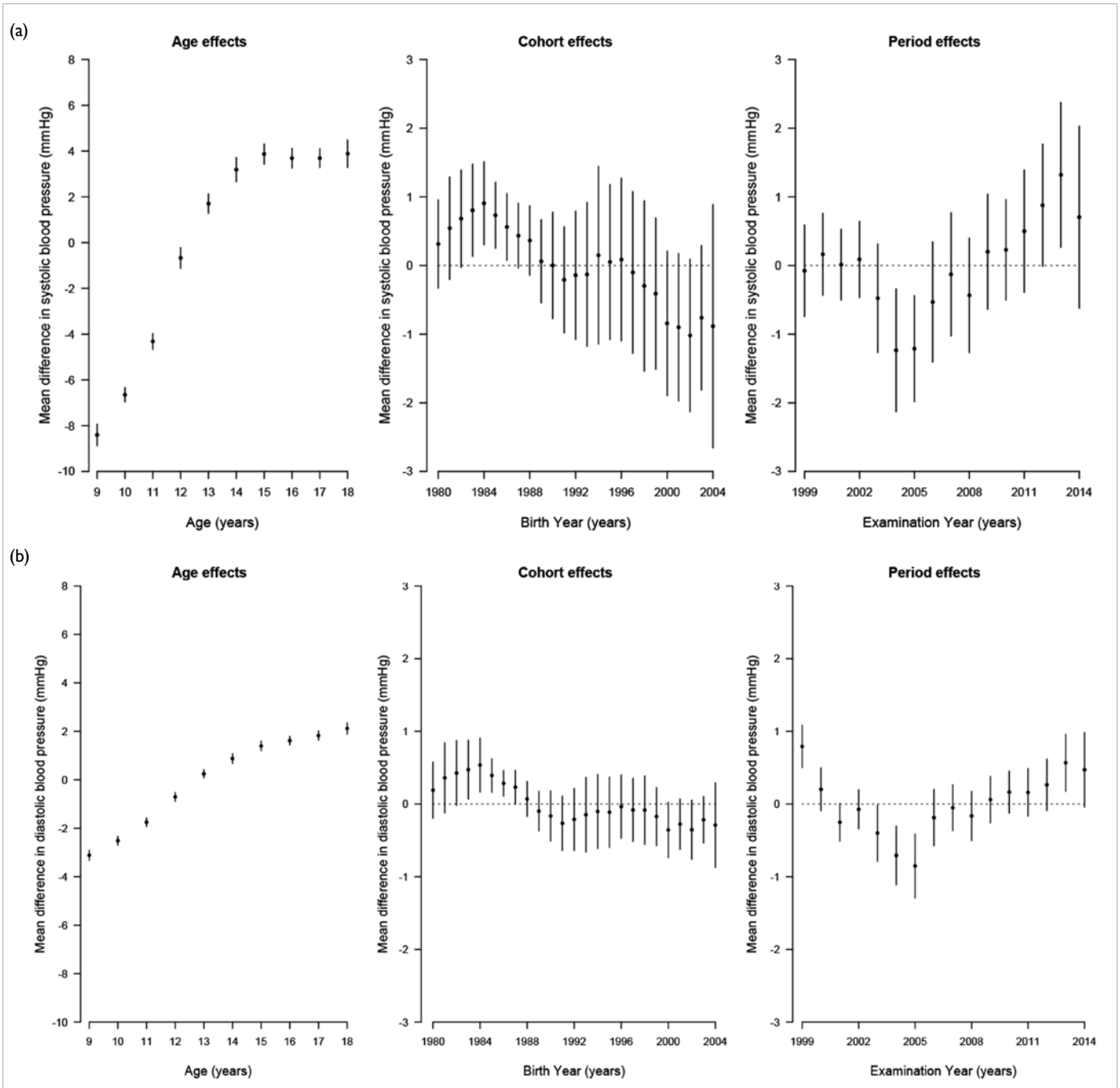

FIG 1. Parameter estimates of age, cohort, and period effects on (a) systolic and (b) diastolic blood pressure among children and adolescents aged 9 to 18 years from 1999 to 2014 (being born from 1980-1981 to 2004-2005) using partial least squares regression with two components and categorical variables

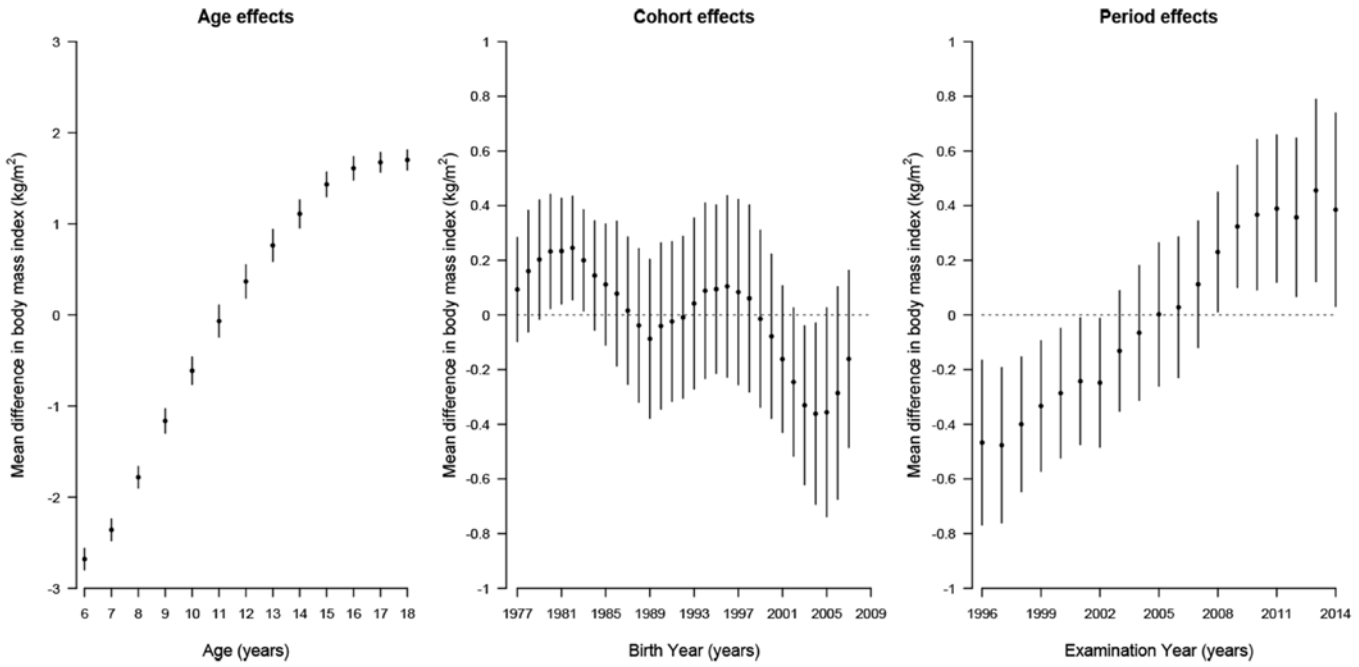

FIG 2. Parameter estimates of age, cohort, and period effects on body mass index among children and adolescents aged 6 to 18 years from 1996 to 2014 (being born from 1977-1978 to 2007-2008) using partial least squares regression with two components and categorical variables

Discussion

In Hong Kong, divergent secular trends in BP and BMI in children and adolescents were evident, with a U-shaped trend in BP but an increasing trend in BMI until levelling off in recent years. In addition to the physiological age effect, both period and cohort effects contributed to trends in BP and BMI.

Changes in BP do not necessarily occur with similar changes in BMI. Higher BMI is a major risk factor of BP. The modest BP downward trend before 2005 despite increasing BMI would have been more salient if BMI had not increased. Nonetheless, considering the trend in BP is not fully explained by the trend in BMI, and adjustment of BMI only accounts for a small proportion of trends in BP in elsewhere,[3] other factors may have contributed to changes in BP.

The positive period effect for BMI until recent years is consistent with westernisation of diet and lifestyle in a more obesogenic environment. As such, the recent levelling off in the BMI trend in Hong Kong may indicate introduction of effective interventions. In 2006, school-based health promotion campaign-EatSmart@school.hk-targeted primary schools with healthier lunch and snacks and modestly improved students' diet.[4]

The negative period effect for BP from about 1999 to 2003-2005 followed by a positive period effect suggests that contextually specific population-wide factors could be involved. In Hong Kong, high salt intake, low fruit and vegetables consumption, and physical inactivity was unlikely to have changed in similar U-shaped patterns as the trend in BP.[5] Moreover, in a South Korea study, changes in sodium or potassium intake, physical activity, smoking, stress, family size, and household income did not explain the declining trend in BP.

The cohort effects for BP are more synchronised with those for BMI. There may be common factors driving both BP and BMI.

Several limitations are noted. First, attending health assessment is voluntary. Differential selection by child health status or parental attributes or family socioeconomic position over time could bias the results, which is unlikely given health assessment at the Student Health Service is free and accessible to all public or private school students. Second, using a single BP measurement at a single visit may slightly overestimate average BP, but would not affect changes over time. Third, random measurement error is possible, but our large sample size minimises potential under- or over-estimation. Finally, the study is descriptive. We can only speculate about the aetiologies of the observed changes in BP and BMI. Nonetheless, these help generate hypotheses for further testing in different settings.

In Hong Kong, contemporaneous population-wide factors and cohort-specific factors may have contributed to the divergent trends in BP and BMI. Dual actions in tackling rising BMI and identifying

other determinants of BP are imperative for improving future population cardiovascular health, as well as focusing on the poorer profile in boys.

Acknowledgements

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Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#11121371). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

Disclosure

The results of this research have been previously published in:

1. Kwok MK, Tu YK, Kawachi I, Schooling CM. Age-period-cohort analysis of trends in blood pressure and body mass index in children and adolescents in Hong Kong. J Epidemiol Community Health 2017;71:1161-8.

2. Kwok MK, Wong IOL, Schooling CM. Age-period-cohort projection of trends in blood pressure and body mass index in children and adolescents in Hong Kong. BMC Pediatr 2020;20:43.

References

1. Lawlor DA, Smith GD. Early life determinants of adult blood pressure. Curr Opin Nephrol Hypertens 2005;14:259-64.
2. Tu YK, Kramer N, Lee WC. Addressing the identification problem in age-period-cohort analysis: a tutorial on the use of partial least squares and principal components analysis. Epidemiology 2012;23:583-93.
3. Khang YH, Lynch JW. Exploring determinants of secular decreases in childhood blood pressure and hypertension. Circulation 2011;124:397-405.
4. Department of Health, Hong Kong SAR. Assessment of dietary pattern in primary schools 2012 executive summary 2012. Available from: http://school.eatsmart.gov.hk/files/pdf/dietary_assessment_2012_executive_summary.pdf.
5. Woo J, Chan R, Li L, Luk W. A survey of infant and young child feeding in Hong Kong: diet and nutrient intake. Hong Kong: Department of Medicine and Therapeutics and Centre for Nutritional Studies, The Chinese University of Hong Kong and Family Health Service, Department of Health, Hong Kong SAR Government; 2012.

Burden of fatal and nonfatal injury in Hong Kong: abridged secondary publication

CB Chow *, P Ip, SM McGhee, SHM Tsui, CW Kam, HFP Ho, EWY Chan, HSW Wong, IWS Chiu

KEY MESSAGES

1. This study aimed to quantify injury burden in Hong Kong in terms of disability weights and disability-adjusted life-years (DALYs).
2. Disability weights in Hong Kong were lower than those in foreign countries, especially those of lifelong effects.
3. Between 2001 and 2012 in Hong Kong, a mean of 85 151 DALYs were lost due to injury annually, equivalent to 1192 DALYs per 100 000 population.
4. The study design could only capture injury mortality and injury-related inpatient cases. Owing to a lack of routine injury data at accident and emergency departments, the injury burden may be underestimated.
5. Healthcare professionals and policymakers can reference the results of this study to make informed decisions on local and territory-wide injury prevention strategies.

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Introduction

Injury is one of the leading causes of mortality and morbidity, and is also a common health problem in Hong Kong, especially unintentional injury.[1] It is the leading cause of adolescent death and disability in Hong Kong,[2] accounting for 3.1 deaths per 100 000 person-years among those aged 0 to 4 years and 3.7 deaths per 100 000 person-years among those aged 15 to 24 years.[3] Quantifying injury burden is essential for priority setting and allocation of limited resources.

Disability-adjusted life-years (DALYs), devised from the Global Burden of Disease Study,[4] is used to quantify burden of disease and injuries in a standardised manner to enable valid comparison between diseases and countries. It summarises mortality, morbidity, and disability of a disease into one single score. By generating DALYs from injuries, healthcare professionals and policymakers can objectively assess the injury burden in Hong Kong and make informed decisions on injury prevention strategies.

Methods

DALYs was calculated using disability weights (DW), years lost due to premature mortality (YLL), and years lived with disability (YLD). DW was estimated in patients who were injured and admitted to the accident and emergency departments of three trauma centres (Queen Elizabeth Hospital, Queen Mary Hospital, and Tuen Mun Hospital).

Health-related quality of life was assessed using the Short Form-12 version 2. Pre-injury health-related quality of life was assessed retrospectively by asking subjects to recall their health state 1 week prior to injury. Follow-up telephone interviews were made at 1 and 4 months after injury or until the patient resumed normal health status, whichever earlier. All subjects were contacted at 12 months after injury. Scores from Short Form-12 version 2 were used to derive the Short Form-6D single utility index. The index from those who were recovered by 12 months was used to compute the population mean score. The annualised DW was estimated by averaging the DWs at all post-injury follow-ups. Acute and lifelong DWs were calculated.

In the event of insufficient case recruitment in particular injury types, a linear regression analysis was used to impute the DWs of these injuries using age, sex, and length of hospital stay from the results of recruited subjects.

YLL was calculated using age- and sex-specific injury mortality from 2001 to 2012 from Centre for Health Protection, with ICD-10 causes of death. Life expectancies for each age group and sex were extracted from the 2011 life table by the Census and Statistics Department.

Short- and long-term YLDs were mapped into EUROCOST injury categories. The short-term YLDs was computed by multiplying the annualised DW with the corresponding number of the injuries.

The long-term YLDs was computed by multiplying the lifelong DW with the corresponding number of injuries, the proportion of lifelong consequences, and the remaining life expectancy of the patient.

Results

Of 1728 (90%) subjects contacted, 775 (45%) were still affected by the injury at 1 month after injury. Of whom, 337 (47%) were still affected by the injury at 4 months after injury. At 12 months after injury, the response rate was 81%, with 201 (13%) subjects still affected by the injury, which was considered as having lifelong consequences. The Short Form-6D single utility index troughed at 1 month after injury and increased steadily from 4 to 12 months, with men reporting higher scores than women at all time points.

Acute and lifelong DWs and the proportion of lifelong consequences of each EUROCOST injury type are shown in Table 1. Lifelong DW was lower than acute DW, as not all injury cases have lifelong effects on patient's quality of life.

From 2001 to 2012, injury accounted for 1 021 815 DALYs, which was equivalent to 85 151 DALYs annually or 1192 DALYs per 100 000 population (Table 2). Men contributed more DALYs

TABLE 1. Acute and lifelong disability weights and proportion of lifelong consequences of EUROCOST injury types.

EUROCOST injury types	Acute disability weight	Lifelong disability weight	Proportion of lifelong consequences
Concussion	0.044	0.005	6.7%
Other skull-brain injury	0.079	0.001	6.3%
Open wound head	0.010	0.000	0.0%
Eye injury	0.025	0.004	2.5%
Fracture of facial bones	0.151	0.026	21.1%
Open wound face	0.043	0.000	8.4%
Fracture/dislocation/strain/sprain of vertebrae/spine	0.056	0.002	11.0%
Whiplash, neck sprain, distortion of cervical spine	0.136	0.016	32.0%
Spinal cord injury	0.654	0.394	100.0%
Internal organ injury	0.216	0.055	59.1%
Fracture of rib/sternum	0.092	0.000	6.1%
Fracture of clavicle/scapula	0.141	0.021	12.6%
Fracture of upper arm	0.183	0.002	25.1%
Fracture of elbow/forearm	0.174	0.016	12.5%
Fracture of wrist	0.138	0.002	10.5%
Fracture hand/fingers	0.115	0.032	22.4%
Dislocation/strain/sprain of shoulder/elbow	0.075	0.014	13.6%
Dislocation/strain/sprain of wrist/hand/fingers	0.083	0.013	14.5%
Injury of upper extremity nerves	0.142	0.022	34.6%
Complex soft tissue injury of upper extremity	0.123	0.003	17.5%
Fracture of pelvis	0.041	0.001	8.9%
Fracture of hip	0.150	0.077	57.4%
Fracture of femur shaft	0.286	0.080	72.8%
Fracture of knee/lower leg	0.222	0.029	31.6%
Fracture of ankle	0.168	0.028	18.1%
Fracture of foot/toes	0.141	0.020	12.7%
Dislocation/strain/sprain of knee	0.069	0.006	13.1%
Dislocation/strain/sprain of ankle/foot	0.065	0.000	10.0%
Dislocation/strain/sprain of hip	0.060	0.018	21.1%
Injury of lower extremity nerves	0.230	0.060	100.0%
Complex soft tissue injury of lower extremities	0.198	0.001	0.0%
Superficial injury, including contusions	0.037	0.005	8.3%
Open wounds	0.030	0.002	5.2%
Burns	0.030	0.016	4.9%
Poisoning	0.025	0.000	21.1%
Foreign body	0.000	0.000	0.0%
Other injury	0.047	0.008	11.9%

TABLE 2. Disability-adjusted life-years (DALYs), years lost due to premature mortality (YLL), years lived with disability (YLD) [acute and lifelong], and YLL:YLD ratio stratified by sex, age, and year

	DALYs	YLL	YLD (acute)	YLD (lifelong)	YLL:YLD ratio
Sex					
Female	396 971	253 390	64 009	79 573	1.76
Male	624 844	459 626	64 663	100 555	2.78
Age, y					
0-14	40 957	23 967	6 994	9 995	1.41
15-59	734 971	579 912	54 945	100 114	3.74
≥60	245 887	109 136	66 732	70 019	0.80
Year					
2001	91 562	65 080	10 745	15 737	2.46
2002	93 451	67 891	10 452	15 108	2.66
2003	89 874	66 469	9 440	13 966	2.84
2004	95 215	71 127	9 771	14 316	2.95
2005	91 617	68 438	9 495	13 684	2.95
2006	83 991	60 714	9 524	13 753	2.61
2007	78 471	55 874	9 560	13 037	2.47
2008	77 016	53 347	10 186	13 483	2.25
2009	84 216	57 504	11 309	15 403	2.15
2010	85 802	56 069	12 441	17 292	1.89
2011	74 592	44 772	12 712	17 108	1.50
2012	76 008	45 731	13 037	17 240	1.51

TABLE 3. Total disability-adjusted life-years (DALYs) and DALYs per 100 000 population by district between 2001-2012*

District	Population in 2006	Total DALYs	DALYs per 100 000
Hong Kong Island	1 268 112	173 229	1138
Central & Western	250 064	32 654	1088
Wan Chai	155 196	20 250	1087
Eastern	587 690	79 947	1134
Southern	275 162	40 379	1223
Kowloon	2 019 533	319 874	1320
Yau Tsim Mong	280 548	49 687	1476
Sham Shui Po	365 540	61 159	1394
Kowloon City	362 501	46 893	1078
Wong Tai Sin	423 521	73 914	1454
Kwun Tong	587 423	88 222	1252
New Territories	3 573 635	488 529	1139
Kwai Tsing	523 300	77 932	1241
Tsuen Wan	288 728	33 530	968
Tuen Mun	502 035	77 028	1279
Yuen Long	534 192	78 354	1222
North	280 730	42 932	1274
Tai Po	293 542	42 006	1192
Sha Tin	607 544	76 525	1050
Sai Kung	406 442	44 752	918
Islands	137 122	15 470	940
Total	**6 861 280**	**981 633**	**1192**

* Cases contributing to 40 182 DALYs did not specify residential district

than women (61.2% vs 38.8%). Generally, the YLL:YLD ratio was >1, which indicated that injury morbidity contributed less DALYs than mortality. DALYs by residential district are shown in Table 3, with Yau Tsim Mong ranking highest in terms of DALYs per 100 000 population.

Discussion

The DWs for most injury types were significantly lower in our study than in foreign studies, in particular annualised DWs. The DWs, especially lifelong DWs, in our study were generally lower than the confidence intervals reported in a meta-analysis of six developed countries injury cohort studies.[5] Although the linear regression analysis showed that it was statistically reliable to compute DWs of under-represented injury types from length of hospital stay data collected from recruited cases, lower DWs may indicate that the relationship between computed values in the prediction may not be linear.

Our study has limitations in estimating injury burden in Hong Kong holistically, owing to a lack of routine injury data from accident and emergency departments. Thus, our study reflects injury burden only from injury mortality and injury-related inpatient cases. DALYs in our study was only 34% to 41% of that in overseas studies (UK 2005: 2924 DALYs per 100 000 population; global 2013: 3459 DALYs per 100 000 population). This may indicate that the injury burden may be severely underestimated using only inpatient and mortality injury data. To accurately estimate the injury burden in Hong Kong, a comprehensive injury surveillance system inside and outside healthcare system should be implemented to collect routine injury data.

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References

1. Surveillance and Epidemiology Branch CfHP. Injury Survey 2008. Department of Health, Hong Kong SAR; 2010.
2. Child Health Survey 2005-2006. Department of Health, Hong Kong SAR; 2009.
3. Department of Health annual report 2001-2002. In: Department of Health, Hong Kong SAR; 2003.
4. Christopher JL, Murray ADL. The global burden of disease: a comprehensive assessment of mortality and disability from disease, injuries and risk factors in 1990 and projected to 2020. 1996.
5. Gabbe BJ, Lyons RA, Simpson PM, et al. Disability weights based on patient-reported data from a multinational injury cohort. Bull World Health Organ 2016;94:806-16C.

Psychometric evaluation of the Chinese version of the Fatigue Scale for Children: abridged secondary publication

JOK Chung *, WHC Li, GCF Chan, SY Chiu, KY Ho

KEY MESSAGES

1. The Chinese version of the Fatigue Scale for Children (FS-C) is a reliable and valid tool in assessing cancer-related fatigue among Hong Kong Chinese children who have survived cancer.
2. The Chinese version of FS-C is crucial for guiding interventions to alleviate fatigue in children who have survived cancer.
3. Confirmatory factor analysis confirmed that there are three factors underlying the Chinese version of FS-C.

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Introduction

Cancer-related fatigue is the most common adverse effect reported by children who have survived cancer.[1] A valid and reliable instrument to measure patients' and survivors' fatigue level is crucial. The Fatigue Scale for Children (FS-C)[2] has not been translated into Chinese or used in Hong Kong children. Concepts or items in the original scale may be inapplicable to Hong Kong children. The FS-C was initially developed to assess the level of cancer-related fatigue in children during cancer treatment and thus may be inappropriate for survivors who have completed treatment. Confirmatory factor analysis was not used to test the hypothesised configuration of the factor structure or measurement model, owing to the small sample size. It is unknown whether the factor structure of the Chinese version of FS-C was congruent with the findings in previous exploratory factor analysis. Evaluation on both linguistic and cultural equivalence is required prior to the use of the Chinese version of FS-C for childhood cancer survivors in Hong Kong.

Methods

This cross-sectional study was approved by the Institutional Review Board of The University of Hong Kong / Hospital Authority Hong Kong West Cluster. Written informed consent was obtained from the parents. Children were asked to put their names on a special assent form and were told that their participation was voluntary. This study was conducted in the outpatient clinic of a public acute-care hospital in Hong Kong. Inclusion criteria were cancer survivors who had completed treatment at least 6 months previously, age of 7 to 12 years, and ability to speak Cantonese and read Chinese. Children with recurrence or second malignancies or those with cognitive or learning problems were excluded.

Subjects were asked to respond to the Chinese version of FS-C, the Center for Epidemiologic Studies Depression Scale for Children, and the Pediatric Quality of Life Inventory.

A panel of experts was asked to rate the semantic equivalence of each item of the Chinese version of the FS-C using a four-point scale (1=not equivalent, 4=most equivalent). Any item with a rating of 1 or 2 by >20% of panel members was amended accordingly. Similarly, the content equivalence of each item was rated using a four-point scale (1=not relevant, 4=very relevant).

For construct validity, the known-group technique was used with a one-way between-group analysis of variance to compare the levels of fatigue among 50 childhood cancer survivors, 50 cancer children receiving treatment, and 50 healthy counterparts.

Convergent validity was assessed using the Pearson correlation coefficient to explore the correlation between scores on the Chinese versions of the FS-C and Center for Epidemiologic Studies Depression Scale for Children. Discriminant validity was determined by examining the correlation between scores of the Chinese version of the FS-C and Pediatric Quality of Life Inventory.

Confirmatory factor analysis was conducted using LISREL (version 8.8 for Windows; Scientific Software International, Lincolnwood [IL], USA) to enable more precise testing of the configuration of the factor structures of the Chinese version of the FS-C and to examine whether the proposed factor

TABLE. Levels of fatigue among cancer survivors, cancer children receiving treatment, and healthy counterparts

	Cancer survivors (n=50)	Cancer children receiving treatment (n=50)	Healthy counterparts (n=50)
Level of fatigue	26.4±9.7	31.1±7.6	23.0±5.6
	Cancer survivors vs cancer children receiving treatment	Cancer survivors vs healthy counterparts	Cancer children receiving treatment vs healthy counterparts
Mean difference	-4.7, P=0.01	3.4, P=0.03	8.1, P<0.00

structures (three-factor model) fitted the data adequately. The generally weighted least-squares method with asymptotic covariance matrixes was used to estimate the parameters. The χ^2 / degrees of freedom ratio, root mean square error of approximation, comparative fit index, and goodness-of-fit index were then calculated to examine the overall fit of the data model with the scale.

Internal consistency was assessed by calculating Cronbach's alpha. 20% of the survivors were randomly selected to be tested again through telephone after 2 weeks. The test-retest reliability was estimated by calculating the intraclass correlation coefficient.

Results

54.5% of the survivors were male. 46.5% had received chemotherapy and 36.5% had received mixed methods. 26.5% had completed treatment for 6 to 12 months. Demographics of the childhood cancer survivors, cancer children receiving treatment, and healthy counterparts were comparable.

The semantic equivalence for items of the Chinese version of FS-C ranged from 83% to 100%, indicating high meaning equivalence to the original version. The content validity index (CVI) of the scale was 0.80, with CVI of each item ranging from 0.17 (item 8) to 1. All items except item 8 were relevant to the concept of fatigue in Hong Kong Chinese children cancer survivors. After removal of item 8, the CVI of the scale increased to 0.85, with CVI of each item ranging from 0.83 to 1.

For construct validity, the mean score of the Chinese version of FS-C in cancer survivors was significantly lower than that in cancer children receiving treatment but significantly higher than that in healthy counterparts (26.4 vs 31.1 vs 23.0, Table). This indicated good known-group validity.

There were a strong positive correlation (r=0.51, P<0.01) between the Chinese version of FS-C and Center for Epidemiologic Studies Depression Scale for Children, and a strong negative correlation (r= -0.52, P<0.01) between the Chinese version of FS-C and Pediatric Quality of Life Inventory.

In the three-factor model (13-item), the factor loadings ranged from 0.38 to 0.95, with positive correlations between parameters. The residuals ranged from 0.14 to 0.37. This reflected that the measurement errors were small. The χ^2 / degrees of freedom ratio was 1.15; root mean square error of approximation was 0.03; comparative fix index was 0.96; and goodness-of-fit index was 0.95. The results demonstrated that the three-factor model adequately fit the data collected using the Chinese version of FS-C.

The intraclass correlation coefficient at 2-week interval was 0.92. The internal consistency (Cronbach's alpha) of the Chinese version of FS-C (14 items) was 0.91. The corrected item-total correlations ranged from 0.13 (item 8) to 0.73. All items except item 8 correlated with the total score of the scale. After removal of item 8, the Cronbach's alpha of the Chinese version of FS-C (13 items) was 0.92.

Discussion

Confucianism emphasises achieving harmony in body and daily lives. Therefore, Hong Kong children are more likely to stay calm when facing adversity like cancers and tend not to show negative emotions including madness to others.[3] The CVI for item 8 was particularly low, indicating irrelevance with the concept of cancer-related fatigue among Hong Kong childhood cancer survivors. Therefore, item 8 was removed from the scale.

The construct validity of the Chinese version of FS-C was good, indicating that it is a valid instrument to identify different groups of children with different levels of fatigue.

Convergent validity and discriminant validity of the Chinese version of FS-C were good. Scores of the Chinese versions of FS-C were strongly and positively correlated with the score of the Center for Epidemiologic Studies Depression Scale for Children. This indicated that children with higher levels of fatigue are more likely to report depression.[4] Scores of the Chinese version of the FS-C have a strong negative correlation with the score of the Pediatric Quality of Life Inventory. This indicates the decrease in quality of life by cancer-related fatigue among childhood cancer survivors.[5]

Confirmatory factor analysis revealed that the good-fit indices supported the three-factor model.

The congruence could be explained by the adverse effects of cancer-related fatigue, which severely deteriorates ability to perform daily activities.[5] The deterioration was so severe that cultural discrepancies between Hong Kong and the West only slightly affect survivors' perceptions and experience of fatigue, resulting in the presentation of similar symptoms.

The Chinese version of FS-C should be used by healthcare professionals and the community to assess and monitor the levels of fatigue in Hong Kong childhood cancer survivors, in combination with other validated scales that assess depressive symptoms and quality of life, so that early screening and interventions can be implemented.

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Disclosure

The results of this research have been previously published in:

1. Ho KY, Li WH, Lam KW, Chiu SY, Chan CF. The psychometric properties of the Chinese version of the fatigue scale for children. Cancer Nurs 2016;39:341-8.

References

1. Chung OK, Li HC, Chiu SY, Ho KY, Lopez V. The impact of cancer and its treatment on physical activity levels and behavior in Hong Kong Chinese childhood cancer survivors. Cancer Nurs 2014;37:E43-E51.
2. Tomlinson D, Hinds PS, Ethier MC, Ness KK, Zupanec S, Sung L. Psychometric properties of instruments used to measure fatigue in children and adolescents with cancer: a systematic review. J Pain Symptom Manage 2013;45:83-91.
3. Li HC, Chung OK, Ho KY, Chiu SY, Lopez V. Coping strategies used by children hospitalized with cancer: an exploratory study. Psychooncology 2011;20:969-76.
4. Hockenberry MJ, Hooke MC, Gregurich M, McCarthy K, Sambuco G, Krull K. Symptom clusters in children and adolescents receiving cisplatin, doxorubicin, or ifosfamide. Oncol Nurs Forum 2010;37:E16-E27.
5. Zebrack BJ, Chesler MA. Quality of life in childhood cancer survivors. Psychooncology 2002;11:132-41.

Whole-transcriptome analysis of maternal blood for identification of RNA markers for predicting spontaneous preterm birth among preterm labour women: abridged secondary publication

SSC Chim *, TF Chan, TY Leung

KEY MESSAGES

1. Accurate prediction of spontaneous preterm birth (sPTB) before 37 weeks among women presenting with preterm labour may facilitate better patient care.
2. RNA-seq facilitates systematic search of markers in the transcriptome of maternal peripheral blood, which is relatively non-invasive, compared with amniotic fluid, chorionic villi, or foetal membrane.
3. We identified 68 differentially expressed transcripts between those preterm labour women ending in sPTB and those ending in term birth. Preterm labour women tested positive for two transcripts deliver significantly sooner than those tested negative.
4. The up-regulated transcripts in sPTB were over-represented with gene-ontology terms in inflammation and defence response to bacteria and other organisms.
5. Maternal peripheral blood with sPTB-associated transcripts are potentially useful for predicting and studying sPTB.

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Introduction

Globally, over 13 million babies were born preterm (birth before 37 gestational weeks) each year. It is estimated that 10 neonates die per minute worldwide as a result of preterm birth. Only a certain proportion of women presenting with preterm labour (PTL) eventually end in spontaneous preterm birth (sPTB). Currently, the best predictive markers for sPTB are shortened cervical length and elevated cervicovaginal foetal fibronectin, but their sensitivity at high specificity is only moderate. Thus, new markers for sPTB are needed.

Discovering sPTB-associated markers is challenging, because it is unethical to obtain human placental or foetal gestational membranes, or myometrium before term from healthy pregnancies as control for comparison. We propose to use markers from maternal peripheral blood, which can be readily obtained before term.

Using strand-specific massively parallel cDNA sequencing (RNA-seq), we have systematically profiled the transcriptomes of maternal peripheral blood during the presentation of PTL. We compared the transcriptomes between sPTB and term birth (TB). We hypothesised that the blood concentration of certain RNA transcripts differed between PTL women ending in sPTB and those ending in TB. We also hypothesised that the maternal blood concentration of such RNA transcripts during PTL can be used to detect the imminent sPTB.

Methods

This case-control study was performed in two phases. With ethics approval from the institutional review board and informed consent from participants, blood samples were obtained from women during the PTL presentation. We followed up these pregnancies for delivery outcomes.

Inclusion criteria were women with uterine contractions more than once every 10 minutes before 34 weeks, an intact membrane, singleton pregnancy, and a Chinese or Korean ethnicity. Women were excluded if pregnancy was complicated with preterm prelabour rupture of membrane, multiple gestation, preeclampsia, foetal growth restriction, macrosomia, foetal distress, antepartum haemorrhage, foetal chromosomal or structural abnormalities, a history of uterine abnormality or cervical surgery, or indicated preterm births before 37 weeks (induction of labour, elective or emergency term caesarean deliveries) where delivery is iatrogenic, usually because of medical complications. Gestational age was established based on menstrual date confirmed by ultrasonographic examination prior to 20 weeks gestation.

We compared those women ending in sPTB

(<34 weeks) and those ending in TB (≥37 weeks). RNA-seq was performed on 20 women ending in sPTB (n=10) or TB (n=10). We then validated the differential expression of the 10 most-promising transcripts identified by RNA-seq using a different method on a different set of blood samples.

Results

A total of 129 PTL women were recruited. Upon quality check of the RNA using the Bioanalyzer (Agilent, Pico RNAchip), eight samples with an unacceptable low RNA integrity number were removed from analysis. Finally, 20 RNA samples of high quality were used for RNA-seq and the remaining 119 RNA samples were used for validation of the initial RNA-seq findings.

RNA-seq enabled summarisation of RNA levels at both gene levels and transcript levels or even exon levels. As one gene contains multiple transcripts and exons, multiple identifiers were generated to uniquely identify each transcript and exon in the whole genome. Each exon was uniquely identified by a 7-digit number preceded by 'e'. To account for technical variation in data (eg, different sequencing depth per sequencing library and intra- and inter-run variation), we normalised data using the established method[1] before differential expression testing.[2] After adjustment for multiple testing using the False Discovery Rate method,[3] we identified 68 differentially expressed RNA transcripts with ≥2-fold change (57 up-regulated and 11 down-regulated) between the sPTB and the TB groups (adjusted P<0.05, Fig. 1). Of these, 10 transcripts were selected for the validation study.

The validation set involved 119 RNA samples independent from the RNA-seq samples. To account for the difference in the varying amount of total RNA input, we divided the RNA level of the target transcript in a sample by the level of an empirically chosen reference transcript. We then $\log_2$-transformed that ratio and expressed it as the normalised RNA levels (Fig. 2). The normalised RNA levels in maternal peripheral blood of PTL women were significantly different between the sPTB and the TB groups in all 10 selected transcripts (Fig. 2), concordant with the RNA-seq data, suggesting that our RNA-seq dataset is valid.

The interquartile ranges of the sPTB and the TB groups did not overlap in four transcripts. The optimal cutoff in predicting sPTB was determined using the receiver operating characteristic (ROC) curve analysis. The area under the ROC curve ranged from 0.746 to 0.908. We designated the transcripts with the two greatest areas as the *PUT1* and *PUT2* mRNA, where *PUT* represents preterm up-regulated transcripts. Based on these optimal cutoffs, we achieved 92.6% (95% confidence interval (CI)=84.6%-97.2%) sensitivity and 69.6% (95% CI=57.3%-80.1%) specificity for the *PUT1* mRNA and 63.0% (95% CI=51.5%-73.4%) sensitivity and 85.5% (95% CI=75.0%-92.8%) specificity for the *PUT2* mRNA in predicting sPTB among PTL women.

To characterise the relationship of the blood levels of *PUT1* and *PUT2* mRNA transcripts and the timing of sPTB, we compared the Kaplan-Meier curve of women tested positive for these transcripts across the duration between blood sampling and delivery, with that of women tested negative. The median duration between blood taking and delivery was 5.09 days in the *PUT1*-positive women and 58.1 days in the *PUT1*-negative women (log-rank test, χ^2=43.32, degree of freedom=1, P<0.0001, hazard ratio=5.10 [95% CI=3.14-8.29], Fig. 3). Similarly, *PUT2*-positive women delivered sooner after blood sampling compared with *PUT2*-negative women (5.09 days vs 56.2 days, log-rank test, χ^2=44.66, degree of freedom=1, P<0.0001, hazard ratio=5.67 [95% CI=3.41-9.44], Fig. 3).

Discussion

PTL women with the *PUT1* and *PUT2* mRNA in their blood appear to deliver sooner. To explore whether our lists of up-regulated and down-regulated transcripts in sPTB bear any biological significance, we analysed the gene-ontology

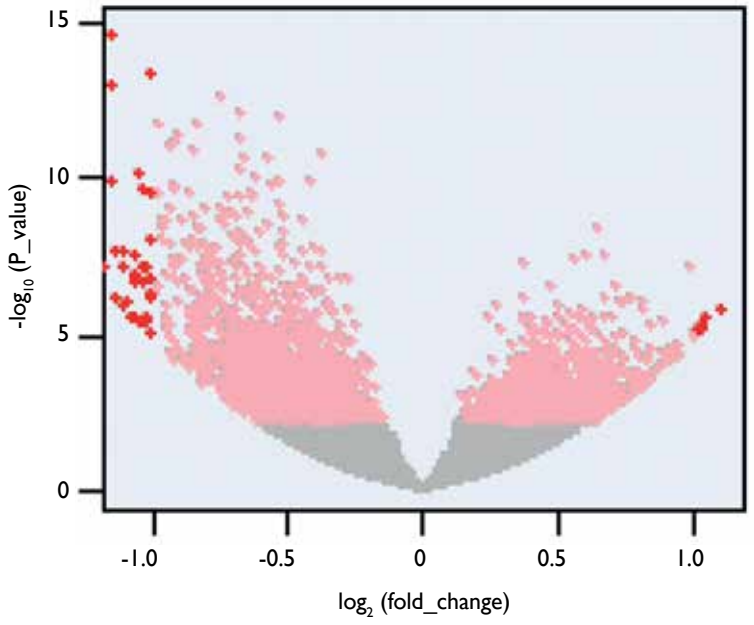

FIG 1. Volcano plot of the DESeq2 differential gene expression analysis of the RNA-seq experiment. Significantly changed transcripts between the spontaneous preterm birth (sPTB) and the term birth (TB) groups are highlighted in pink. Significantly up-regulated and down-regulated transcripts in sPTB with absolute fold-change of >2 are highlighted in red. These transcripts in red are used for validation in a different set of preterm labour women.

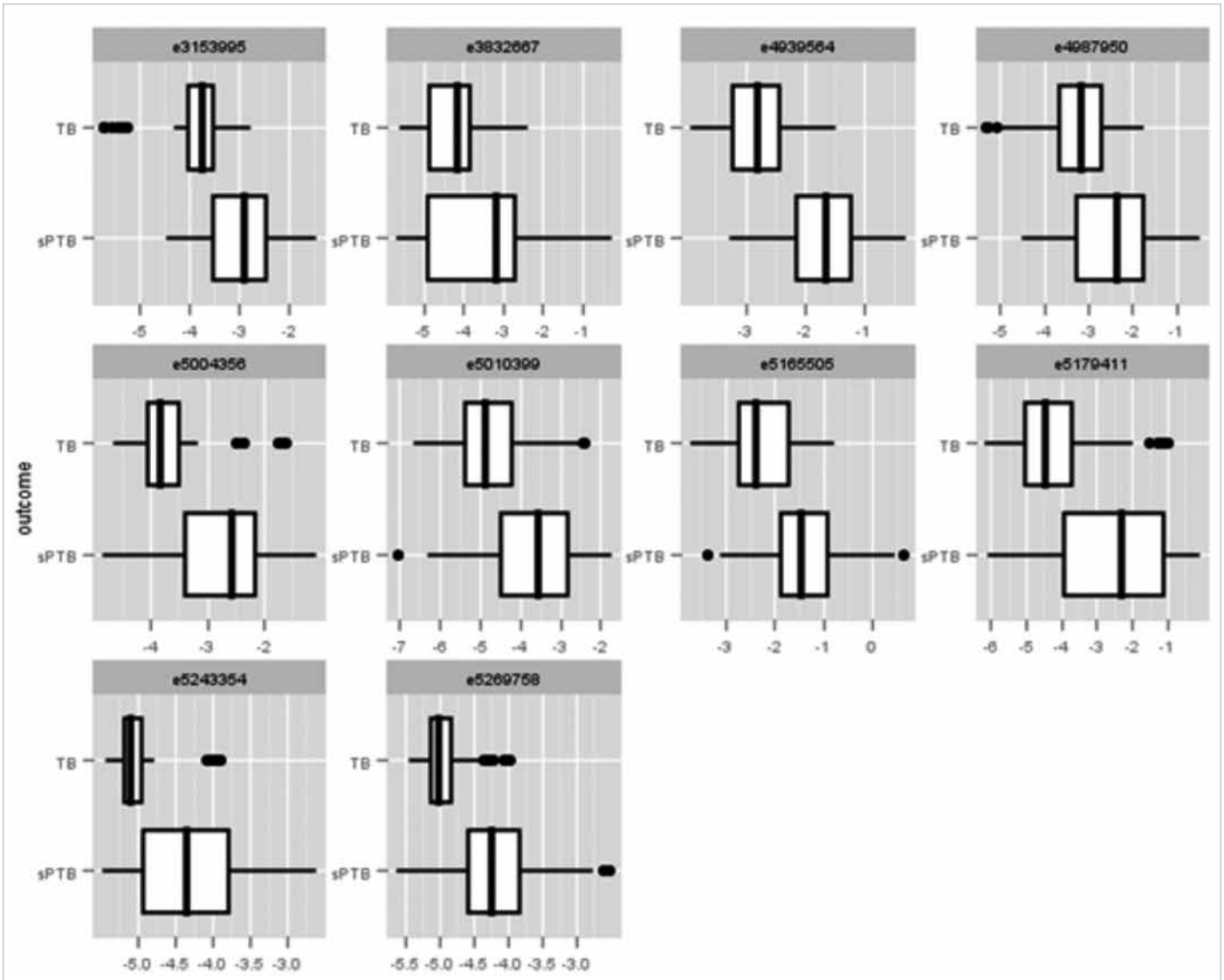

FIG 2. Box-plots of the normalised RNA levels (in log_2-scale) in the validation cohort of the 10 most promising transcripts identified using RNA-seq. Blood RNA levels are sampled in women with preterm labour and plotted according to eventual delivery outcome, either spontaneous preterm birth (sPTB) before 37 weeks or term birth (TB) on or after 37 weeks. Each assay is named by its exon ID of the transcript and normalised to one or more reference transcripts. The RNA transcripts with the greatest area under the receiver operating characteristic curve are the *PUT1* and *PUT2* mRNA.

terms annotating transcripts using the tools at the PANTHER[4] website and the gene-ontology terms in molecular function, biological process, and cellular components. Altogether, there are 329 up- and 239 down-regulated transcripts with ≥1.5-fold change in the sPTB group, compared with the TB group. Statistical over-representation tests showed that the sPTB-upregulated list comprised genes annotated more frequently with gene-ontology–biological process terms in acute inflammatory response (9.2-fold over-represented), positive regulation of defence response (4.0-fold), response to bacterium (3.6-fold), defence response to other organism (3.5-fold), innate immune response (3.1-fold), among other terms not associated with the immune system. In contrast, the sPTB-downregulated list comprised genes annotated more frequently with gene-ontology–biological process terms in complement activation (classical pathway) [16.6-fold], among a few other terms. For a more intuitive comparison, we expanded those gene-ontology–biological process terms related to the immune systems. The terms associated with innate immune response was over-represented among the sPTB-upregulated transcripts (3.1-fold), whereas those associated with humoral immune response mediated by circulating immunoglobulin (15.2-fold) was over-represented among the list of sPTB-downregulated transcripts. Apparently, there is a big difference in the immune system in the PTL women ending in sPTB, compared with those

ending in TB. Intriguingly, over-representation of terms associated with response to bacteria, defence response to other organism, and inflammation in the sPTB group are consistent with the literature on the potential pathogenesis of sPTB.

Our study is limited by a small sample size and recruitment of only East Asian women. Further studies are warranted to determine whether the predictive performance of the identified transcripts can be generalised to other ethnic groups.

Conclusion

PTL women tested positive for *PUT1* and *PUT2* in maternal peripheral whole blood are more likely to deliver sooner, compared with those tested negative. There is an over-representation of genes in inflammation and immune response against bacteria and other organisms in the list of up-regulated transcripts in PTL women ending in sPTB, compared with those ending in TB. Accurate identification of women at high risk for sPTB may facilitate appropriate management, including timely transfer to a hospital with neonatal intensive care ward and timely administration of antenatal corticosteroid to mature the foetal lungs.

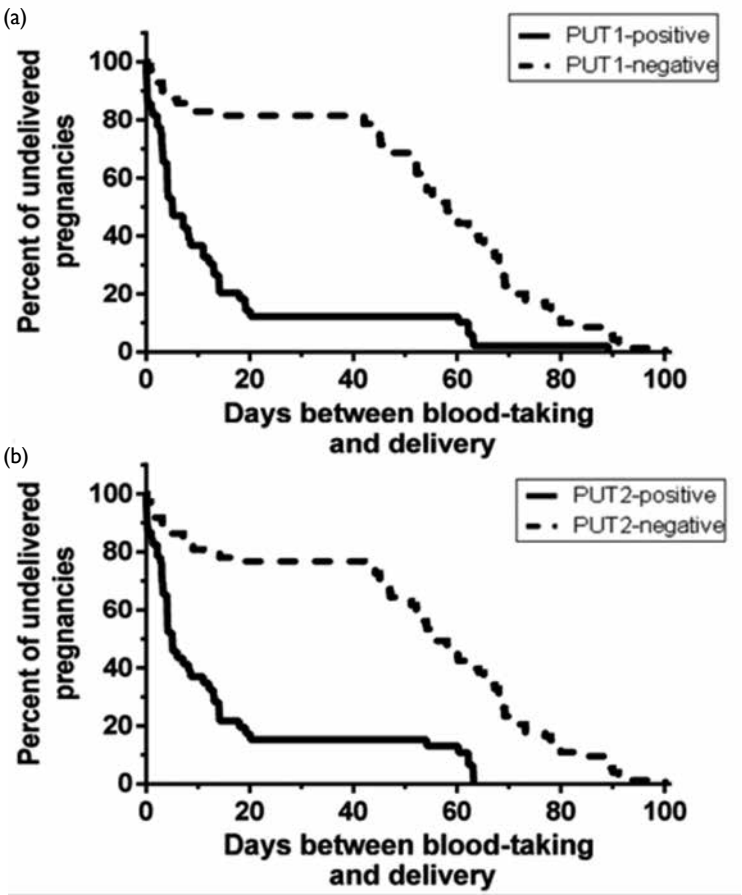

FIG 3. Percentage of undelivered pregnancies across gestational age in preterm labour women tested positive or negative for the *PUT1* and *PUT2* mRNA transcripts in maternal peripheral blood. (a) The median duration between blood taking and delivery is 5.09 days in the *PUT1*-positive women and 58.1 days in the *PUT1*-negative women (log-rank test, χ^2=43.32, degree of freedom=1, P<0.0001, hazard ratio=5.10 [95% CI=3.14-8.29]). (b) Similarly, *PUT2*-positive women delivered sooner than *PUT2*-negative women (5.09 days vs 56.2 days, log-rank test, χ^2=44.66, degree of freedom=1, P<0.0001, hazard ratio=5.67 [95% CI=3.41-9.44]).

Acknowledgements

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Funding

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References

1. Risso D, Ngai J, Speed TP, Dudoit S. Normalization of RNA-seq data using factor analysis of control genes or samples. Nat Biotechnol 2014;32:896-902.
2. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550.
3. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol 1995;57:289-300.
4. Mi H, Muruganujan A, Casagrande JT, Thomas PD. Large-scale gene function analysis with the PANTHER classification system. Nat Protoc 2013;8:1551-66.

Maternal and obstetric factors of hepatitis B immunisation failure in Hong Kong: a multicentre prospective study: abridged secondary publication

KW Cheung *, MTY Seto, ASY Kan, D Wong, TKO Kou, PL So, WL Lau, RMS Wong, CP Lee, EHY Ng

KEY MESSAGES

Viral load of 8 $\log_{10}$ copies/mL at 28 to 30 weeks of gestation could be the optimal hepatitis B virus DNA cutoff to predict immunoprophylaxis failure. Starting antiviral treatment at 30 weeks could reduce the viral load and hence the immunoprophylaxis failure rate.

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Introduction

Hepatitis B virus (HBV) infection remains the most common form of chronic hepatitis worldwide. The risk of vertical transmission leading to chronic infection is dramatically reduced by administering hepatitis B immunoglobulin to newborns at birth together with a complete course of HBV vaccination.[1] A high maternal HBV DNA level during pregnancy is the strongest risk factor leading to immunoprophylaxis failure.[2] The optimal HBV DNA level to identify pregnancies associated with subsequent immunoprophylaxis failure remains unclear, owing to the retrospective nature[3] and heterogeneity of the studied population,[4] and different or unknown timing of HBV DNA quantification.[3-5] We aimed to evaluate the risk of immunoprophylaxis failure in relation to the maternal HBV DNA level at 28 to 30 weeks of gestation.

Methods

This prospective multicentre study was conducted from January 2014 to December 2016 at five hospitals in Hong Kong. Pregnant women were tested for hepatitis B surface antigen (HBsAg) during their first antenatal visit. Women with a positive HBsAg status were recruited. Women receiving antiviral treatment during pregnancy were excluded. All women provided written informed consent and were enrolled under protocols approved by the institutional review board of each hospital.

Maternal hepatitis B e antigen (HBeAg) was tested once upon recruitment, and the HBV DNA was quantified at 28 to 30 weeks using the COBAS TaqMan HBV Monitor Test coupled with the COBAS Ampliprep extraction system (both Roche Diagnostics, Branchburg, NJ), with a lower limit of detection of 100 copies/mL (~17.2 IU/mL) and upper limit of 990 000 000 copies/mL (~170 103 092 IU/mL) (1 IU=5.82 copies). All newborns received both 10 μg HBV vaccines (Engerix-B, GlaxoSmithKline, Belgium) and 110 IU hepatitis B immunoglobulin (HyperHEP B, Grifols [CA], USA) intramuscularly at a different site within 12 hours of birth, followed by hepatitis B vaccine at the same dosage at 1 and 6 months of life. HBsAg of infants was examined at age 9 to 12 months. Immunoprophylaxis failure of infants was defined as HBsAg positive status at age 9 to 12 months.

The sample size was calculated based on comparing the proportions of immunoprophylaxis failure in infants between high and low maternal pre-delivery HBV DNA levels. The proportions were assumed to be 2.5% and 0.01% for the groups of high and low maternal pre-delivery HBV DNA levels, respectively. A total of 624 subjects were required to have a power of 80% and a type I error of 5%. The Student's *t* test or Wilcoxon rank sum test was used to compare quantitative variables, and the Chi-square test or Fisher's exact test was used to compare qualitative variables. A P value of <0.05 was considered statistically significant. Data

were analysed with SAS software (version 9.2, SAS Institute. Cary [NC], USA).

Results and discussion

Data from 641 women and 654 infants (13 pairs of twins) were available for final analysis. All infants received hepatitis B immunoglobulin within 12 hours of birth and completed the whole course of hepatitis B vaccine on schedule. Of the women, 155 (24.2%) were HBeAg positive. There were seven (1.1%) cases of immunoprophylaxis failure; all born to women with positive HBeAg status and HBV DNA of >8 $\log_{10}$ copies/mL (>17 000 000 IU/mL). The risk of immunoprophylaxis failure with HBV DNA level of <8, 8-8.99, >9 $\log_{10}$ copies/mL were 0%, 8.6%, and 3.1%, respectively (Table). Significant predictors of immunoprophylaxis failure at 28 to 30 weeks were positive HBeAg (4.5% vs 0%, P<0.0001) and HBV DNA of ≥8 $\log_{10}$ copies/mL (≥17 000 000 IU/mL) [5.8% vs 0%, P<0.0001].

TABLE. Immunoprophylaxis failure rate in different hepatitis B virus (HBV) DNA levels and hepatitis B e antigen (HBeAg) statuses

HBV DNA at 28-30 weeks, $\log_{10}$ copies/mL	No. of patients	Immunoprophylaxis failure rate	
		No. (%)	95% confidence interval
<6 (~171 821 IU/mL)	474	0	0-0.78
6-6.99	24	0	0-14.25
7-7.99	22	0	0-15.44
≥8 (~17 000 000 IU/mL)	121	7 (5.79)	2.36-11.56
8-8.99	58	5 (8.62)	2.86-18.98
≥9 (~170 000 000 IU/mL)	63	2 (3.17)	0.39-11.00
HBeAg status			
Negative	486	0	0-0.76
Positive	155	7 (4.52)	1.83-9.08

Acknowledgements

We thank Chi Tao Ng, Clinical Trials Centre, The University of Hong Kong, for the statistical analysis.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#11121661). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

Disclosure

The results of this research have been previously published in:

1. Cheung KW, Seto MTY, Kan ASY, et al. Immunoprophylaxis failure of infants born to hepatitis B carrier mothers following routine vaccination. Clin Gastroenterol Hepatol 2018;16(1):144-145.

References

1. Lee C, Gong Y, Brok J, Boxall EH, Gluud C. Effect of hepatitis B immunisation in newborn infants of mothers positive for hepatitis B surface antigen: systematic review and meta-analysis. BMJ 2006;332:328-36.
2. Cheung KW, Seto MT, Wong SF. Towards complete eradication of hepatitis B infection from perinatal transmission: review of the mechanisms of in utero infection and the use of antiviral treatment during pregnancy. Eur J Obstet Gynecol Reprod Biol 2013;169:17-23.
3. Zou H, Chen Y, Duan Z, Zhang H, Pan C. Virologic factors associated with failure to passive-active immunoprophylaxis in infants born to HBsAg-positive mothers. J Viral Hepat 2012;19:e18-25.
4. Wen WH, Chang MH, Zhao LL, et al. Mother-to-infant transmission of hepatitis B virus infection: significance of maternal viral load and strategies for intervention. J Hepatol 2013;59:24-30.
5. Wiseman E, Fraser MA, Holden S, et al. Perinatal transmission of hepatitis B virus: an Australian experience. Med J Aust 2009;190:489-92.

Three-dimensional versus two-dimensional ultrasound-guided embryo transfer: a randomised control study (abridged secondary publication)

TC Li *, S Saravelos, WS Kong

KEY MESSAGES

1. There was no significant difference in the live birth rate after two- or three-dimensional ultrasound-guided embryo transfer.
2. Although three-dimensional ultrasonography is a newer tool for embryo transfer, it should not be recommended as a strategy to improve clinical outcomes.

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Introduction

The use of ultrasonography (US) in gynaecology enables diagnosing pathologies of the uterus, tubes, and ovaries. In reproductive medicine, it can be used to monitor endometrial thickness, follicular status and growth, and to guide retrieval of oocytes from the ovaries and transfer of fertilised embryos into the uterus.[1] These are critical procedures of artificial reproductive technology (ART). Three-dimensional (3D) US images can be acquired and analysed live or retrospectively. Its scanning procedure does not differ from the routine two-dimensional (2D) US, and acquisition of a 3D volume requires only a few seconds. As a result, 3D US is considered to be the most accurate non-invasive modality to diagnose uterine anomalies.[2,3] Furthermore, 3D US allows accurate volume calculations of structures such as the endometrium and follicles. Coupled with advanced software for automated measurements, 3D US may improve accuracy, reduce inter-observer variability, and increase efficiency of ART.[4] Therefore, we conducted a randomised controlled trial to compare 3D US with 2D US in guiding embryo transfer in terms of pregnancy outcomes.

Methods

This was a single-blind, single-centre prospective randomised controlled trial. The study was approved by the Institutional Review Board (reference number CREC 2014.650) and was registered online at Clinicaltrials.gov (registration number NCT02413697). All patients were fully counselled and completed a written informed consent prior to participation.

Consecutive women undergoing US-guided embryo transfer in our unit were included. Those aged 42 years or older or women whose endometrial cavity could not be visualised adequately were excluded.

On the morning of the embryo transfer procedure, women were randomised into the 3D or the 2D US guidance group in a 1:1 ratio using a computer-generated list and sealed opaque envelopes prepared by one of the research nurses. All patients were blinded to their allocation. All US examinations were performed by a single experienced operator using a General Electric Voluson Expert series US machine (E8 or V730) with a 3D/4D RAB6-D trans-abdominal probe (GE Medical Systems Kretztechnik, Austria). The technique and settings were kept standardised throughout the trial.

The primary outcome measure was the live birth rate. The secondary outcome measures included implantation rate, clinical pregnancy rate, ongoing pregnancy rate, multiple pregnancy rate, early miscarriage rate, and ectopic pregnancy rate.

Results

Of 546 women assessed for eligibility, 481 were recruited and 474 completed the study (Fig.). The 3D and 2D US groups were comparable in terms of baseline characteristics, except for the median level of oestradiol on the day of trigger (9216 pmol/L vs 10654 pmol/L, P<0.02, Table 1). However, this variable was not found to be predictive of any of the outcome measures. There was no significant difference between the two groups in terms of the live birth rate (32.1% vs 32.5%, P=0.92, Table 2), positive human chorionic gonadotropin

rate, implantation rate, biochemical pregnancy rate, clinical pregnancy rate, early miscarriage rate, ongoing pregnancy rate, ectopic pregnancy rate, or multiple pregnancy rate.

Discussion

To the best of our knowledge, this is the first randomised controlled trial to compare the use of 3D versus 2D US guidance during embryo transfer. There was no significant difference between the two groups; this contradicts the postulated benefits reported in the literature. In our experience, the echogenic tip embryo transfer catheter allows 2D US to provide a clear appreciation of its location within the uterine cavity in most cases, even without the benefit of the 3D coronal plane. In addition, in cases where the embryo transfer catheter was found along the lateral wall of the cavity on 3D US, it was not always possible to correct this as the catheter could only be adjusted in an 'in-out' motion and not a 'left-right' motion. Although 3D US may theoretically allow for a more accurate transfer of the embryo, whether the position of transfer translates into improved clinical outcomes remains unclear, as the embryo may migrate within the uterine cavity after transfer.[5]

One limitation of the present study is that it included an unselected population of women undergoing ART. However, US guidance during embryo transfer has traditionally concerned unselected populations of women undergoing ART, as reflected in the methodology of previous studies comparing 2D US guidance versus clinical touch embryo transfer. Another limitation is the degree of heterogeneity in the characteristics of the recruited women. Nonetheless, randomisation along with serial logistic regression and subgroup analyses were applied to control for this. In addition, there were limited prospective data recorded regarding the exact timing and difficulty of each transfer, which may have been informative. A single experienced operator performed both 2D and 3D US in this trial; it remains unknown whether the results would be similar with different operators or those early on in their learning curve.

Conclusion

There was no significant difference in the pregnancy rate after 3D or 2D US-guided embryo transfer. Although 3D US is a newer tool for embryo transfer, it should not be recommended as a strategy to improve clinical outcomes.

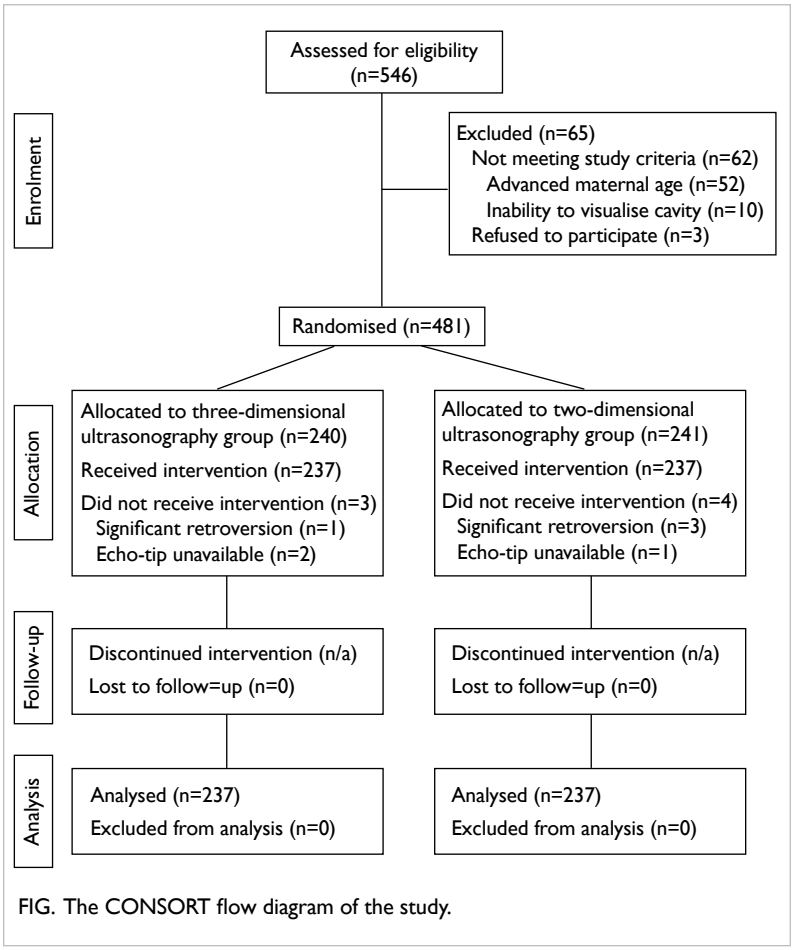

FIG. The CONSORT flow diagram of the study.

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Funding

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Disclosure

The results of this research have been previously published in:

1. Saravelos SH, Kong GW, Chung JP, et al. A prospective randomized controlled trial of 3D versus 2D ultrasound-guided embryo transfer in women undergoing ART treatment. Hum Reprod 2016;31:2255-60.

2. Saravelos SH, Jayaprakasan K, Ojha K, Li TC. Assessment of the uterus with three-dimensional ultrasound in women undergoing ART. Hum Reprod Update 2017;23:188-210.

TABLE 1. Baseline characteristics of the three-dimensional and two-dimensional ultrasound-guided embryo transfer groups

Parameter	Three-dimensional group (n=237)*	Two-dimensional group (n=237)*
Age, y	36 (34-38)	36 (33-38)
Body mass index, kg/m^2	22 (20-24)	22 (20-24)
Infertility duration, y	4 (2-7)	4 (2-7)
Type of infertility		
Primary	113 (47.7)	120 (50.6)
Secondary	124 (52.3)	117 (49.4)
Cause of infertility†		
Ovulatory	30 (12.7)	45 (19.0)
Tuberperitoneal	99 (41.8)	100 (42.2)
Male	96 (40.5)	101 (42.6)
Other/unexplained	48 (20.3)	40 (16.9)
Treatment protocol		
Agonist	140 (59.1)	139 (58.6)
Antagonist	97 (40.9)	98 (41.4)
Baseline follicle-stimulating hormone, IU/L	7.1 (6.2-8.3)	7.2 (6.2-8.4)
Baseline luteinising hormone, IU/L	3.6 (2.1-5.4)	3.5 (1.8-5.2)
Oestradiol on trigger day, pmol/L	9216 (6583-12832)	10654 (7133-15213)
Duration of stimulation, days	10 (10-12)	11 (10-12)
Total oocyte retrieved	9 (6-13)	10 (6-14)
Mature oocytes retrieved	7 (5-11)	8 (6-11)
Oocyte fertilised	6 (4-9)	6 (4-9)
Type of embryo transfer		
Fresh	118 (49.8)	119 (50.2)
Frozen	119 (50.2)	118 (49.8)
Stage of embryo		
Day 3	93 (39.2)	86 (36.3)
Day 5	144 (60.8)	151 (63.7)
Good quality	96 (40.5)	105 (44.3)
No. of embryo transferred		
1	158 (66.7)	161 (67.9)
2	79 (33.3)	76 (32.1)
Endometrial thickness, mm	11.1 (9.4-13.1)	10.7 (9.1-12.6)
Use of tenaculum	6 (2.5)	6 (2.5)

* Data are presented as median (interquartile range) or No. (%) of cases
† Some patients presented with more than one cause of infertility

TABLE 2. Pregnancy outcomes in the three-dimensional and two-dimensional ultrasound-guided embryo transfer groups

Parameter	Three-dimensional group (n=237)*	Two-dimensional group (n=237)*	P value	Rate ratio (95% confidence interval)
Positive human chorionic gonadotropin (hCG) rate	120 (50.6)	124 (52.3)	0.71	0.97 (0.81-1.15)
hCG day 14, IU/L	158 (76-262)	158 (74-243)	0.32	
hCG day 21, IU/L	2626 (757-4578)	2786 (1391-4595)	0.60	
Biochemical pregnancy rate	17 (7.2)	19 (8.0)	0.73	0.90 (0.48-1.68)
Implantation rate	37.1%±3%	38.0%±3%	0.84	
Clinical pregnancy rate	103 (43.5)	105 (44.3)	0.85	0.98 (0.80-1.20)
Ectopic pregnancy rate	3/103 (2.9)	3/105 (2.9)	0.98	1.02 (0.21-4.94)
Early miscarriage rate	16/100 (16.0)	14/102 (13.7)	0.65	1.17 (0.60-2.26)
Multiple pregnancy rate				
Twins	5/100 (5.0)	7/102 (6.9)	0.57	0.72 (0.24-2.20)
Triplets	1/100 (1.0)	1/102 (1.0)	0.99	1.02 (0.07-16.08)
Ongoing pregnancy rate	84 (35.4)	88 (37.1)	0.70	0.96 (0.75-1.21)
Live birth rate	76 (32.1)	77 (32.5)	0.92	0.99 (0.76-1.28)

* Data are presented as No. (%) of cases, median (interquartile range), or mean±standard error

References

1. Alcazar JL, Iturra A, Sedda F, et al. Three-dimensional volume off-line analysis as compared to real-time ultrasound for assessing adnexal masses. Eur J Obstet Gynecol Reprod Biol 2012;161:92-5.
2. Bermejo C, Martinez Ten P, Cantarero R, et al. Three-dimensional ultrasound in the diagnosis of Mullerian duct anomalies and concordance with magnetic resonance imaging. Ultrasound Obstet Gynecol 2010;35:593-601.
3. Saravelos SH, Cocksedge KA, Li TC. Prevalence and diagnosis of congenital uterine anomalies in women with reproductive failure: a critical appraisal. Hum Reprod Update 2008;14:415-29.
4. Raine-Fenning N, Jayaprakasan K, Deb S, et al. Automated follicle tracking improves measurement reliability in patients undergoing ovarian stimulation. Reprod Biomed Online 2009;18:658-63.
5. Saravelos SH, Wong AW, Chan CP, et al. Assessment of the embryo flash position and migration with 3D ultrasound within 60 min of embryo transfer. Hum Reprod 2016;31:591-6.

Behavioural dysexecutive syndrome after stroke: abridged secondary publication

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KEY MESSAGES

1. Behavioural dysexecutive syndrome (BDES) was common among stroke survivors. Its prevalence at 3 months post-stroke was 18.7%.
2. More severe anxiety symptoms, presence of current depression, and poor cognitive functioning predicted BDES at 3 months post-stroke.
3. No radiological variable was found to be associated with BDES.
4. BDES was related to poor executive function that involves conceptualisation, category fluency, motor programming, sensitivity to interference, inhibitory control, environmental autonomy, and semantic memory.
5. All patients with BDES at the 3-month follow-up were found to be remitted at the 40-month follow-up assessment. No patients from the non-BDES group developed BDES at the 38-month follow-up.
6. All stroke patients showed significant improvement in BDES symptoms at 38 months post-stroke.
7. BDES imposes a psychological burden on stroke patients and should not be neglected. Early identification and multidisciplinary intervention for BDES is essential.

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Introduction

Dysexecutive syndrome (DES) is an impairment of executive functions.[1] Executive functions involve goal setting, planning, action initiation and inhibition, social cognition, theory of mind, insight, and metacognition.[2,3] DES comprises behavioural and cognitive domains,[4] and behavioural and cognitive DES can present separately.[5,6] Behavioural DES (BDES) is a common condition following stroke, with a prevalence of 42.1% to 44.2%.[5,6]

To the best of our knowledge, there are few structural brain imaging studies of BDES or behavioural symptoms in stroke. These studies identified associations between BDES/behavioural symptoms and infarcts in the right hemisphere,[7] anterior capsule,[8] thalamus,[8] insular,[9] and white matter hyperintensities.[10] However, these studies were limited by small sample size,[7-10] biased sampling (from clinical trial samples),[10] inclusion of subjects with current psychiatric diagnoses,[7] lack of standardised assessment of BDES,[7-10] executive function,[7,9,10] or detailed radiological examination.[7,9]

The primary objective of this study was to evaluate the prevalence and predictors of BDES and its clinical, neuropsychological, and magnetic resonance imaging (MRI) correlates in a cohort of Hong Kong stroke survivors. The secondary objective was to determine the clinical course of BDES.

Methods

A total of 4581 patients with first-ever or recurrent acute ischaemic stroke were admitted to the Acute Stroke Unit of Prince of Wales Hospital between September 2013 and September 2017. Of whom, 1447 received MRI, and 384 of whom participated in the initial 3-month assessment.

The inclusion criteria were Chinese ethnicity, Cantonese as the primary language, age of ≥18 years, right-handedness, acute first ischaemic stroke occurring within 7 days before admission, and informed consent given. The exclusion criteria were a history of epilepsy, head injury, hydrocephalus, intracranial tumour, Parkinson disease, dementia, or other non-stroke neurological disease; a history or current diagnosis of depression, bipolar disorder, schizophrenia or alcohol/substance abuse/dependence; aphasia, defined as a score of ≥2 on the best language item of the National Institutes of Health Stroke Scale (NIHSS)[11], or auditory impairment; dementia, defined as a Mini-Mental State Examination (MMSE)[12] score of <20; contraindications for MRI such as a pacemaker in situ, physical frailty or severe claustrophobia; physical frailty; and recurrence of stroke prior to the 3-month assessment. Duplicate subjects and those who failed to complete the assessment were excluded from the analysis. Finally, 369 stroke patients and

237 healthy controls were included.

At 3 months after the onset of the index stroke, a trained research assistant blind to the stroke patients' radiological data administered the Chinese version of the Dysexecutive Questionnaire, which contains 20 items that assess the affective, motivational, behavioural, and cognitive symptoms of BDES using a 5-point Likert scale ranging from 'never' to 'very often', with higher scores indicating more severe BDES. A cutoff of 20 indicates the presence of BDES.

The demographic and clinical data of stroke patients were collected by a research nurse. The same nurse also assessed patients' stroke severity using the NIHSS and degree of disability in daily activities using the modified Rankin Scale within 2 days of admission. The research assistant administered an executive cognitive function battery (the Chinese version of the Frontal Assessment Battery, Colour Trails Test, Arrow Test, and Category Verbal Fluency Test) 3 months after stroke, and the MMSE, Barthel Index, the anxiety subscale of the Hospital Anxiety Depression Scale, 15-item Geriatric Depression Scale at the baseline and follow-up assessments. Healthy controls attended one assessment interview where the same research assistant administered the Dysexecutive Questionnaire, Geriatric Depression Scale, MMSE, and the executive cognitive function battery.

MRI was performed using a 3.0-T MRI system (Philips Achieva 3.0T, X Series, Quasar Dual MRI System) within 7 days of the index admission. An experienced neuroradiologist blind to the subjects' psychiatric diagnoses and BDES status assessed the MRI images. The number, volumes, and locations of acute infarcts and the number of old infarcts were evaluated. The number of lacunae, the number and location of cerebral microbleeds, and the Fazekas white matter hyperintensity score were recorded. Voxel-based lesion-symptom mapping was performed.

To examine the determinants of BDES, we compared demographic and clinical variables between the stroke and healthy control groups and between the BDES and non-BDES groups using the *t*-test, χ^2 test, Fisher's exact test, or Mann-Whitney *U* test, as appropriate. MRI variables in the BDES and non-BDES groups were also compared. Bonferroni adjustment was applied to multiple statistical comparisons of MRI variables. Multivariate logistic regression (forward Wald mode) was performed to determine the predictors of the presence of BDES at 3 months after the index stroke. A paired-sample *t*-test was applied to investigate differences in the stroke patients' clinical characteristics between the first and follow-up assessments. Analyses of covariance adjusted for age and education level were conducted to compare the performance of all groups on the cognitive battery. Two types of voxel-based lesion-symptom mapping analysis were conducted.

Results

The final sample (n=369) consisted of 236 (64%) men. The mean age of the sample was 66.5±9.8 years and the mean years of education was 7.7±4.4 years. The mean NIHSS score on admission was 4.0±3.9.

Of the 369 patients, 69 (18.7%) were diagnosed with BDES. In the multivariate logistic regression, predictors of the presence of BDES at 3 months post-stroke were the anxiety subscale score of the Hospital Anxiety Depression Scale (odds ratio [OR]=1.184, 95% confidence interval [CI]=1.083-1.295, P<0.001), presence of current depression (OR=4.055, 95% CI=2.060-7.983, P<0.001), and MMSE score (OR=0.805, 95% CI=0.705-0.906, P<0.001). After adjusting for multiple testing, no significant differences in any MRI characteristics were observed between the BDES and non-BDES groups. Similarly, the voxel-based lesion-symptom mapping analysis yielded no significant results.

The mean score of the Chinese version of the Frontal Assessment Battery was significantly lower in the BDES group than in the non-BDES group (10.1±2.5 vs 12.8±2.4, P=0.001). The BDES group made significantly more errors and nearly missed responses and required more prompts during the Colour Trails Test, compared with the non-BDES group (errors: 4.9±4.9 vs 0.6±1.7, P<0.001; nearly-missed responses: 6.2±7.3 vs 0.6±2.5, P<0.001; prompts: 7.2±7.4 vs 1.1±2.8, P<0.001). The time required to complete trail 2 in the Colour Trails Test was significantly longer in the BDES group than in the non-BDES group (215.0±80.4 vs 162.5±76.8 seconds, P=0.040).

In the Category Verbal Fluency Test, the BDES group made significantly more intrusion responses (1.4±1.8 vs 0.4±1.0, P<0.001) and fewer total correct responses (33.0±11.3 vs 41.3±10.6, P=0.025) than the non-BDES group. In the Arrow Test, the BDES group had a significantly longer mean response time than the non-BDES group (47.5±27.1 vs 24.6±10.5 seconds, P<0.001). The BDES group also had a significantly higher mean interference score than the non-BDES group (104.6±101.0 vs 24.8±36.2, P<0.001).

Of the 69 patients with BDES at the 3-month follow-up, 44 (63.8%) attended a second follow-up at a mean of 40 (range, 9-52) months after the index stroke. At the second follow-up, no patients presented with BDES, and the mean Dysexecutive Questionnaire score significantly decreased from 25.8±5.7 at 3 months to 2.2±3.2 (P<0.001). The 174 (58%) patients without BDES at the 3-month follow-up attended a second follow-up at a mean of 37.6 (range, 13-55) months after the index stroke. At the second follow-up, no patient had developed BDES,

and the mean Dysexecutive Questionnaire score significantly decreased from 8.1±6.1 at 3 months to 2.0±2.1 (P<0.001). Participants in both groups showed significant decreases in modified Rankin Scale score and Geriatric Depression Scale score and an increase in Barthel Index score (all P<0.001).

Discussion

BDES was identified in 18.7% of the stroke patients 3 months after the index stroke. Predictors of BDES at the 3-month follow up in patients with ischaemic stroke were a higher level of anxiety symptoms, the presence of current depression, and poor global cognitive functioning. BDES correlated with poor executive function (cognitive deficits) related to conceptualisation, category verbal fluency (information generation), motor programming, sensitivity to interference, inhibitory control, environmental autonomy, and semantic memory. Our results showed that BDES does not have a late onset and runs an acute course. Previous studies found that different behavioural and neuropsychiatric symptoms were associated with specific cognitive domains.

The prevalence of BDES in the current study (18.7%) is lower than that in previous studies (42.1%–44.2%).[5,6] The inconsistency may be attributable to the methodologies used. Different inclusion criteria for participants' level of impairment, global cognitive functioning, and the status of the stroke survivors were used, and the assessment time and measurement of BDES were also incompatible.

Our results were consistent with previous findings on mood symptoms[13-16] and impaired global cognitive functioning,[10,17,18] as correlates of individual neuropsychiatric symptoms. The literature associates DES with disruptions of the fronto-subcortical circuits.[8,19,20] However, our study did not find any association between lesion location and BDES.

Our results demonstrated that all stroke patients who presented with BDES at 3 months post-index event had recovered from BDES at 40 months. Furthermore, no delayed onset of BDES was observed at the 38-month follow up. The prevalence of BDES decreased from 18.7% at 3 months to none in the follow-up assessment. Previous longitudinal studies showed recovery of behavioural[7] and neuropsychiatric[21] symptoms in some patients 12 to 15 months after the index stroke. There are mixed findings on the late onset of neuropsychiatric symptoms after stroke, for instance, 17.4% of participants remained symptom-free for 1 year, whereas 10.8% had delayed onset of fatigue at 6-month follow-up.[22]

The main limitation of this study is the potential for selection bias. A relatively small proportion of the original cohort of ischaemic stroke patients was examined, and this may limit the generalisability of our findings. Although the present study investigated the course of BDES over 38 months, the change between the first and second assessment remained unclear. Furthermore, patients with previous stroke were included, and pre-existing infarcts may have contributed to the development of BDES.

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Disclosure

The results of this research have been previously published in:

1. Tang WK, Lau CG, Liang Y, et al. Prevalence and clinical correlates of poststroke behavioral dysexecutive syndrome. J Am Heart Assoc 2019;8:e013448. doi:10.1161/JAHA.119.013448

References

1. Godefroy O. Frontal syndrome and disorders of executive functions. J Neurol 2003;250:1-6.
2. Aboulafia-Brakha T, Christe B, Martory MD, Annoni JM. Theory of mind tasks and executive functions: a systematic review of group studies in neurology. J Neuropsychol 2011;5:39-55.
3. Lezak MD. The problem of assessing executive functions. Int J Psychol 1982;17:281-97.
4. Hanna-Pladdy B. Dysexecutive syndromes in neurologic disease. J Neurol Phys Ther 2007;31:119-27.
5. Godefroy O, Azouvi P, Robert P, et al. Dysexecutive syndrome: diagnostic criteria and validation study. Ann Neurol 2010;68:855-64.
6. Roussel M, Martinaud O, Hénon H, et al. The behavioral and cognitive executive disorders of stroke: the GREFEX study. PLoS One 2016;11:e0147602.
7. Castellanos-Pinedo F, Hernández-Pérez JM, Zurdo M, et al. Influence of premorbid psychopathology and lesion location on affective and behavioral disorders after ischemic stroke. J Neuropsychiatry Clin Neurosci 2011;23:340-7.
8. Lanna ME, Alves CE, Sudo FK, et al. Cognitive disconnective syndrome by single strategic strokes in vascular dementia. J Neurol Sci 2012;322:176-83.
9. Manes F, Paradiso S, Robinson RG. Neuropsychiatric effects of insular stroke. J Nerv Ment Dis 1999;187:707-12.
10. Mok VC, Wong A, Wong K, et al. Executive dysfunction and left frontal white matter hyperintensities are correlated with neuropsychiatric symptoms in stroke patients with confluent white matter hyperintensities. Dement Geriatr Cogn Disord 2010;30:254-60.
11. Brott T, Adams HP Jr, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989;20:864-70.
12. Chiu H, Lee H, Chung W, Kwong P. Reliability and validity of the Cantonese version of the mini-mental state

examination: a preliminary study. Hong Kong J Psychiatry 1994;4:25-8.
13. Scheffer M, Monteiro JK, de Almeida RMM. Frontal stroke: problem solving, decision making, impulsiveness, and depressive symptoms in men and women. Psychol Neurosci 2011;4:267-78.
14. Mutai H, Furukawa T, Houri A, Suzuki A, Hanihara T. Factors associated with multidimensional aspect of post-stroke fatigue in acute stroke period. Asian J Psychiatr 2017;26:1-5.
15. Duncan F, Lewis SJ, Greig CA, et al. Exploratory longitudinal cohort study of associations of fatigue after stroke. Stroke 2015;46:1052-8.
16. Galligan NG, Hevey D, Coen RF, Harbison JA. Clarifying the associations between anxiety, depression and fatigue following stroke. J Health Psychol 2016;21:2863-71.
17. Brodaty H, Sachdev PS, Withall A, Altendorf A, Valenzuela MJ, Lorentz L. Frequency and clinical, neuropsychological and neuroimaging correlates of apathy following stroke: the Sydney Stroke Study. Psychol Med 2005;35:1707-16.
18. Onoda K, Kuroda Y, Yamamoto Y, et al. Post-stroke apathy and hypoperfusion in basal ganglia: SPECT study. Cerebrovasc Dis 2011;31:6-11.
19. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology 1994;44:2308-14.
20. Cummings JL. Frontal-subcortical circuits and human behavior. Arch Neurol 1993;50:873-80.
21. Withall A, Brodaty H, Altendorf A, Sachdev PS. A longitudinal study examining the independence of apathy and depression after stroke: the Sydney Stroke Study. Int Psychogeriatr 2011;23:264-73.
22. Schepers VP, Visser-Meily AM, Ketelaar M, Lindeman E. Poststroke fatigue: course and its relation to personal and stroke-related factors. Arch Phys Med Rehabil 2006;87:184-8.

Bilateral movement computer games to improve motor function of upper limb and quality of life in patients with sub-acute stroke: a randomised controlled trial: abridged secondary publication

SSL Lam *, SSM Ng, CWK Lai, J Woo

KEY MESSAGES

1. Sixteen sessions of bilateral movement computer games training is superior to video-directed conventional training in promoting the recovery of motor control and functional use of a paretic upper limb after a stroke.
2. Bilateral movement computer games training is a useful complement to conventional upper limb rehabilitation for patients with subacute stroke.

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Introduction

In stroke survivors, a paretic upper limb greatly affects their functional independence and quality of life. Neuro-rehabilitation programmes should consist of repetitive, high-intensity, task-specific sensorimotor training in order to promote motor and functional recovery effectively.

Bilateral movement therapy using the non-paretic limb can enhance functional recovery of the paretic limb via the facilitative coupling effects.[1] Bilateral movement training promotes neural plasticity and facilitates control of the paretic limb's movement.[1] Virtual reality refers to interactive simulations with a computer-generated scenario that appears similar to the real world. Virtual reality is a promising rehabilitation intervention for improving upper limb motor control and ability in the activities of daily living after a stroke.[2] It provides a platform for highly repetitive bilateral movement training that is considered engaging, motivating, self-assisted, and task-orientated.

In a study of computer games with a movement-based controller, intervention significantly improved the upper limb motor performance of chronic stroke survivors.[3] However, the study was neither randomised nor controlled; only the immediate effects were studied; and the sample size was small. We therefore tested the hypothesis that those practising bilateral movement computer games training (BMCT) would experience greater improvement in motor control and functional performance than those with conventional training. The objective of this study is to investigate whether the BMCT is more effective than the video-directed conventional training (VDCT), in addition to conventional physiotherapy, in promoting the recovery of motor control and functional use of the paretic upper limb in activities of daily living and quality of life in patients with sub-acute stroke.

Methods

This study was a stratified, single-blinded, controlled clinical trial. It was approved by the Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee (reference: CRE-2012.343-T). Informed consent was obtained from each patient.

All patients referred to the geriatric day hospital at Shatin Hospital for stroke rehabilitation were screened by the physiotherapist-in-charge. Subjects were included if they were aged 45 to 85 years, diagnosed with stroke 1 week to 6 months previously, and scored ≤7 out of 10 on the Abbreviated Mental Test. Subjects were excluded if they had receptive dysphasia or any medical, cardiovascular, or orthopaedic condition that would hinder their proper assessment and treatment. Subjects were assigned to either the BMCT or VDCT group using stratified blocked randomisation, in addition to standard physiotherapy training. Interventions were delivered by the two physiotherapists. Assessments and data entry were performed by a research assistant who was blinded to the treatment group.

There were 16 sessions (twice a week for 8 weeks). Each session involved 1.5 hours of conventional physiotherapy and 1.5 hours of multidisciplinary occupational therapy as well as the corresponding intervention. The physiotherapy

TABLE 1. Details of conventional physiotherapy, bilateral movement computer games training, and video-directed conventional training

Conventional physiotherapy	Bilateral movement computer games training	Video-directed conventional training
Upper arm and hand function exercises (30 minutes)	3 different computer games (30 minutes)	Exercise in response to a video (30 minutes)
1. Passive stretching and weight bearing exercise (5 minutes) 2. Assisted or active mobilising exercise (5 minutes) 3. Progressive resisted exercise (10 minutes) 4. Task-orientated exercise (10 minutes)	1. Hitting single stationary targets which required movement in all directions and increasing reaction speed (10 minutes) 2. Hitting multiple moving targets or interacting with multiple moving targets which required directional control, strategy, and timing 3. Interacting with various stationary and moving targets which required strength, endurance, and timing.	Video prescribed the exercise movements of the upper limb that the subject had performed during conventional physiotherapy training (upper arm and hand function exercises)

session involved 60 minutes of lower limb strengthening, balance, and functional training, and 30 minutes of upper arm and hand function training (Table 1).

The BMCT subjects played the video game for 30 minutes. They were instructed to hold the game controller with the paretic hand, and the other end of the controller connected with a handlebar was held by the non-paretic hand. For subjects with severe upper limb motor impairment, the paretic hand was strapped to the game controller with a bandage. The subjects moved the paretic arm in a bilateral, nearly symmetrical and self-assistive pattern. Each subject played three different games for 10 minutes each during each session. A patient care assistant monitored the subject's practice under supervision of a physiotherapist. The games were organised in increasing levels of complexity requiring greater physical movement, skill, and concentration. Progression to more difficult games was directed by the case physiotherapist based on whether the subject could move the paretic arm easily while completing the challenges and whether the subject felt fatigued after each game. The programme was progressed by: (1) moving up the game levels to increase the numbers of targets and moving speed; (2) decreasing mouse sensitivity to increase fine movement control; and (3) adding weight to the handlebar. Training data and progress of each subject were presented in graphs, heat-maps, and scoreboards.

Those in the VDCT group continued to exercise for 30 minutes in response to a video that prescribed the exercise movements of the upper limb that the subject had performed during conventional physiotherapy training (upper arm and hand function training). Participation in video-directed exercise was monitored by the patient care assistant.

The primary outcome was the Fugl-Meyer Assessment - Upper Extremity score. Secondary outcomes were the Action Research Arm Test score, grip strength on the affected and non-affected side as measured with a digital dynamometer, and health-related quality of life measured with the 36-item Short-form Health Survey (SF-36). Subjects were assessed by a blinded assessor before treatment, after 8 sessions, after 16 sessions, and at 4 weeks after treatment.

TABLE 2. Patient characteristics of two groups

Characteristic	Bilateral movement computer games training (n=47)*	Video-directed conventional training (n=46)*
Sex		
Male	27 (57.4)	28 (60.9)
Female	20 (42.6)	18 (39.1)
Age, y	65.1±10.2	66.0±9.0
Body height, cm	162.5±7.6	163.2±8.5
Body weight, kg	58.5±8.1	59.9±9.9
Body mass index, kg/m^2	22.1±2.5	22.4±2.7
Post stroke days	57.6±24.7	63.4±39.6
≤8 weeks	29 (61.7)	25 (54.35)
>8 weeks	18 (38.3)	21 (45.65)
Type of stroke		
Infarct	38 (80.9)	38 (82.6)
Haemorrhage	9 (19.1)	8 (17.4)
Hemiplegic side		
Right	21 (44.7)	25 (54.3)
Left	26 (55.3)	21 (45.7)
No. of stroke	1.1±0.4	1.2±0.5
Abbreviated Mental Test	9.5±0.9	9.4±1.1

* Data are presented as mean±standard deviation or No. (%) of participants

The results of a pilot study predicted an effect size of 0.64. The alpha level was set at 0.05 and the design was based on a power of 80%. Assuming the possible drop-out rate of 10%, the sample size required was estimated to be 88 subjects.

Analysis of covariance adjusted with the

TABLE 3. Univariate analysis of changes in outcome measures from baseline to after 16 sessions of treatment

Outcome measure	Mean change (95% confidence interval)		P value (ANCOVA)
	Bilateral movement computer games training	Video-directed conventional training	
Fugl-Meyer Assessment - Upper Extremity	14.84 (12.42-17.26)	6.54 (5.05-8.02)	<0.001
Action Research Arm Test	13.64 (9.65-17.63)	6.61 (3.88-9.33)	0.006
Grip strength (affected)	4.89 (3.21-6.57)	1.72 (0.78-2.67)	0.002
Grip strength (non-affected)	1.38 (0.27-2.49)	1.04 (-0.00-2.08)	0.639
Physical Component Summary	3.85 (1.82-5.88)	3.23 (1.48-4.97)	0.701
Mental Component Summary	4.75 (1.78-7.72)	2.85 (0.01-5.68)	0.455

baseline measurements was used to investigate the significance of any observed differences between groups in changes of scores. The significance level was set at P≤0.05. Analyses were carried out using the SAS software (version 9.4). All subjects were in the intention-to-treat population. Missing values for dropouts were not replaced, and it was assumed that no changes occurred in any outcome measure. All P values were corrected using Bonferroni adjustment to maintain the overall type I error at 5%.

Results

Of 93 subjects, 47 (50.5%) were allocated to the BMCT group. 10 (10.8%) subjects dropped out, owing to hospital re-admission (secondary to re-stroke or other medical problems) or lost to follow-up. The mean days post-stroke at the beginning of the experiment was 57.6±24.7 days in the BMCT group and 63.4±39.6 days in the VDCT group (Table 2).

The mean change in the Fugl-Meyer Assessment - Upper Extremity score, Action Research Arm Test score, and grip strength (affected) from baseline was significantly greater in the BMCT group than in the VDCT group after 16 sessions (all P<0.05, Table 3).

Discussion

Reorganisation of the brain generally occurs in the first 6 months after stroke, determined by the patient's motivation, learning activity, family support, and the quality and intensity of rehabilitation therapies.[2] The BMCT resulted in significantly greater improvements in the mean Fugl-Meyer Assessment - Upper Extremity score, Action Research Arm Test score, and grip strength (affected) than VDCT, regardless of time. BMCT involved exercising the paretic upper limb with support and assistance from the non-paretic limb in a bilateral pattern of movement. The coupling effect of both upper limbs was assumed to facilitate the functional recovery of the paretic limb. It allows the undamaged brain hemisphere to enhance activation of the damaged hemisphere through inter-hemispheric connections.[1] Moreover, the excitation of the intact hemisphere that may be inhibited normally is increased during paretic upper limb movement, resulting in suppression of the output from the damaged hemisphere.[4] However, bilateral movement training can facilitate the generation of identical motor commands in each cerebral hemisphere. This facilitates output from the damaged cerebral hemisphere and from normally inhibited ipsilateral pathways of the undamaged hemisphere in order to augment movement of the paretic upper limb.[4]

The BMCT involved practising repetitive movements in a computer-generated, non-immersive simulated environment. The training tasks were interactive, enriched, and task-orientated, and all movements were shown in real time at real speed as feedback. The virtual reality environment provides the opportunity for active learning, in which immediate feedback and task grading can optimise the motor learning process.[2] Repetitive and intensive task-specific training promotes cortical reorganisation and possibly contributes to functional recovery.[2] The subjects receiving BMCT thus demonstrated significant improvement in their movements, strength, and coordination, and in the functional use of their paretic upper limb.

In this study, there were no significant differences in the mean changes of SF-36 score between the two groups, likely because SF-36 is a generic outcome measure for assessing quality of life in stroke patients and the improvement may not be truly reflected in the changes of SF-36 score.

The findings of this study can only be generalised to stroke survivors fulfilling the study's inclusion criteria. Multicentre randomised trials are needed to confirm our findings. Moreover, patients' feedback, concentration, and motivation are all important aspects that require further investigation. The treatment effectiveness was assessed only up to 1 month after the treatment ended; the long-term effects and benefits remain unknown.

Conclusions

Sixteen sessions of 30-minute BMCT is superior to VDCT in improving motor control and functional use of the paretic upper limb after stroke. BMCT is a useful complement to conventional upper limb rehabilitation programmes for patients with subacute stroke.

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References

1. Cauraugh JH, Summers JJ. Neural plasticity and bilateral movements: a rehabilitation approach for chronic stroke. Prog Neurobiol 2005;75:309-20.
2. Saposnik G, Levin M; Outcome Research Canada (SORCan) Working Group. Virtual reality in stroke rehabilitation: a meta-analysis and implications for clinicians. Stroke 2011;42:1380-6.
3. Hijmans JM, Hale LA, Satherley JA, McMillan NJ, King MJ. Bilateral upper-limb rehabilitation after stroke using a movement-based game controller. J Rehabil Res Dev 2011;48:1005-13.
4. Morris JH, van Wijck F, Joice S, Ogston SA, Cole I, MacWalter RS. A comparison of bilateral and unilateral upper-limb task training in early poststroke rehabilitation: a randomized controlled trial. Arch Phys Med Rehabil 2008; 89:1237-45.

Aerobic dance for cognitive and physical functions and mood in older adults with cerebral small vessel disease: abridged secondary publication

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KEY MESSAGES

Aerobic dance is safe and effective for improving cognitive functions in older adults with cerebral small vessel disease.

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Introduction

Cerebral small vessel disease manifests as age-related white matter lesions and lacunar infarcts. It is associated with a plethora of age-related disabilities such as dementia, falls, depression, and impaired daily functioning.[1] Primary prevention by lifestyle intervention is therefore of utmost importance. Aerobic and strength training improves executive functions, memory, and general cognitive functions in community-dwelling adults with mild cognitive impairment. Dancing can be performed in older persons with physical and cognitive impairments. It is aerobic training and involves memory, attention, sensory stimulation, and social interaction. It is a good exercise for enhancing cognitive, mood, physical, and daily functions in individuals with cerebral small vessel disease.

In the present study, the primary objective is to investigate the effects of a 24-week aerobic dance training on cognition, mood, physical, and daily functions in stroke- and dementia-free community-dwelling persons with neuroimaging evidence of cerebral small vessel disease. The secondary objective is to examine whether the effects of aerobic dance training are mediated by changes in cerebral vasomotor reactivity (CVR).

Methods

This was a rater-blind randomised controlled study comparing the effects of aerobic dance with simple stretching and health education on cognition, mood, physical, and daily functioning for 36 weeks. A total of 110 community older adults were randomised in a close to 1:1 ratio into either the aerobic dance group or the control group.

Inclusion criteria were age ≥65 years, community dwelling, presence of significant cerebral small vessel disease (defined by the presence of ≥2 lacunar infarcts and/or a rating of ≥2 of white matter lesions using the age-related white matter changes scale on magnetic resonance imaging), presence of good temporal window on at least one side for transcranial Doppler ultrasonography, and written informed consent given.

Exclusion criteria were a history of stroke, dementia (determined by a score of lower than the education-adjusted cutoff on Mini-Mental State Examination for dementia or a history of dementia diagnosis), comorbidity with medical conditions affecting the central nervous system or cerebral white matter (such as multiple sclerosis, brain tumour, uncontrolled epilepsy, history of severe head injury, substance abuse), inadequately controlled psychiatric disorders affecting cognition and mood, and physical or sensory impediments hindering participation in cognitive assessment or exercise training.

Participants were assessed at baseline and weeks 12, 24, and 36 by a trained research assistant blinded to treatment allocation. Cognitive functions were assessed using the Montreal Cognitive Assessment, Colour Trails Test, Symbol Digit Modalities Test, Category Fluency Test, Modified Boston Naming Test, Rey Complex Figure Test, Digit Span Test, Hong Kong List Learning Test for global cognition, executive functions, psychomotor speed, and memory. Physical functions were assessed using the Mini-Balance Evaluation Systems Test (for dynamic balance), timed-up-and-go test (for functional mobility), and 6-minute walk test

(for walking capacity). Depressive symptoms were assessed using the 15-item Geriatric Depression Scale (GDS). Daily functions were assessed using the Lawton instrumental activities of daily living scale. Physical activities performed outside of training session were recorded using the International Physical Activity Questionnaire (IPAQ) to account for the potential confounding effects.

Transcranial Doppler ultrasonography was performed using a 2-MHz pulsed Doppler hand-held probe to insonate the middle cerebral artery through the temporal window above the zygomatic arch at a depth of 52-56 mm, and the vertebral artery through the occipital window at a depth of 64 mm. The pulsatility index (for vascular resistance of cerebral vessels) of the middle cerebral artery and vertebral artery and the breath-holding index (as a marker of CVR) were calculated.

Aerobic dance training was conducted in groups of five by a certified physiotherapist experienced in exercise training for older persons. The intervention lasted for 24 weeks, with 60 minutes of exercise per session, which included 10 minutes of warm-up, up to 40 minutes of dancing, and 10 minutes of cool-down. Participants were instructed to achieve 40% of their age-specific target heart rate (ie, heart rate reserve). Participants started doing the dance exercises for 30 minutes in month 1 and gradually progressed to 40 minutes to reach the target of 70% of heart rate reserve. Participants practised once per week in months 1 and 2, and twice per week in months 3 to 6. Participants were also asked to practise at home. In months 1 and 2, the dance consisted of rhythmical whole-body movement of weight shifting and repeated stepping in different directions (forward, backward, and sideways) and arm movements of multi-directional reaching and stepping. Progression was made to increase the speed and amplitude and complexity of the movements. Participants were asked to practise at home twice per week for 20 to 30 minutes. In months 3 to 6, the dance steps became more complex by adding lunge steps, tandem walking, and turning. Progression was made to increase the speed, amplitude, and complexity of the movement. Participants learned a new dance every 2 weeks. They were asked to practise at home for 30 to 40 minutes twice per week in weeks 1 and 3 each month, and 3 times per week in weeks 2 and 4 each month.

Participants in the control group (5 participants per group) received a weekly 3-hour programme containing stretching exercise, stress reduction, and health education on dementia and stroke prevention for 6 months. The total number of hours with staff contact in the control group was comparable to that in the intervention group (72 vs. 80 hours).

Between weeks 24 and 36, participants in both groups were not asked to comply with any exercise regimen, but they were free to exercise at their own will. This 12-week observation period was for assessing the sustainability effects of training.

Adverse events were recorded. Participants were asked about the occurrence of any adverse events at each clinical visit and were provided with a contact number to notify the study team about any occurrence of adverse events.

Group comparisons of baseline variables were conducted using independent sample *t* test, Chi-squared test, or Fisher's exact test where appropriate. An intention-to-treat analysis was used. Treatment effect was analysed using mixed effect model using analysis of covariance, with age, sex, years of education, and IPAQ score as covariates at baseline and at weeks 12, 24, and 36. Group effect size on outcomes was measured by the Cohen's *d* or Cohen's f^2 statistic, with 0.2, 0.5, and ≥0.8; 0.02, 0.25, and ≥0.4 indicating small, medium, and large effects, respectively. Performance on each cognitive test was analysed without being combined into a single composite score to determine the effects of intervention on specific cognitive domains. To examine whether the effects of treatment are mediated through changes in CVR, a standard mediation model was performed, with age, sex, years of education, change in IPAQ as covariates. Unstandardised indirect effects were computed for each of 5000 bootstrapped samples, and the 95% confidence interval was computed by determining the indirect effects at the 2nd and 97th percentiles. The mean pulsatility index and breath-holding index were calculated separately on the right and left hand for measuring CVR. Post-treatment effect between months 24 and 36 was examined using repeated measure analysis of covariance with treatment condition and time (outcome measures at months 24 and 36) being factors of interest. Pairwise deletion was used to handle missing data. Only the particular cases needed to test a particular assumption were eliminated in order to preserve more information. Alpha was set at 0.05 for all analyses. Statistical analyses were performed using SPSS (Windows version 21; IBM Corp, Armonk [NY], US).

Results

A total of 110 participants were randomised to either the aerobic dance group (n=54) or the control (stretching + education) group (n=56). 53 participants in the treatment group and 54 participants in the control group had at least one follow-up assessment. Fourteen (12.7%) participants withdrew from the study. There was no significant group difference at baseline in terms of demographics, vascular risk factors, cognitive functions, mood, physical functions, daily functions, physical activity, or CVR.

At week 12, no significant treatment effect

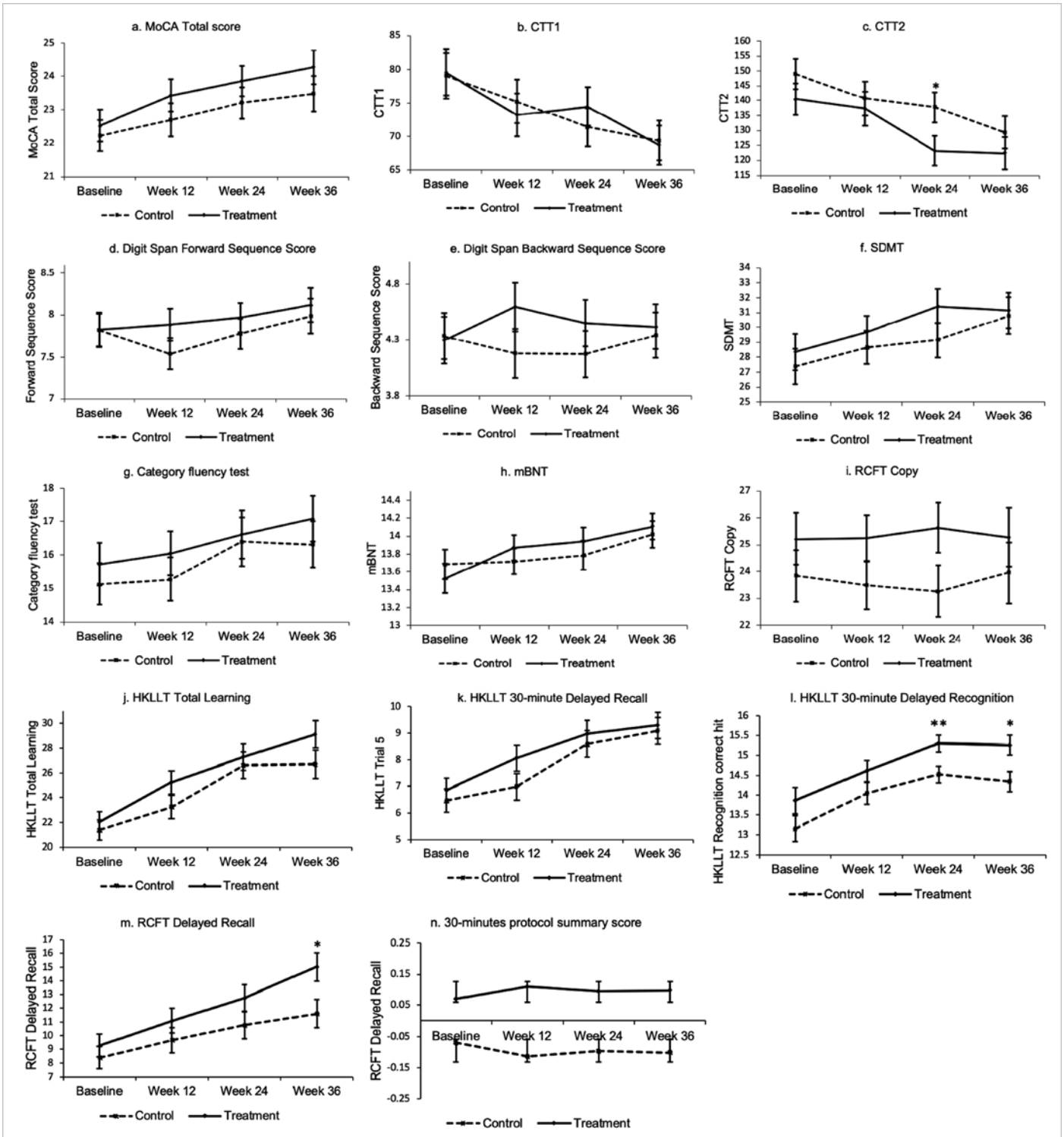

FIG 1. Group difference on cognitive functions at different time points. At week 24, the treatment group has better performance in executive functions measured by Colour Trails Test (CTT) and delayed recognition measured by Hong Kong List Learning Test (HKLLT). There is no group difference in other cognitive functions such as global cognition measured by Montreal Cognitive Assessment (MoCA), memory measured by Rey Complex Figure Test (RCFT), and processing speed measured by Symbol Digit Modalities Test (SDMT).

on cognitive functions was found. At week 24, the treatment group had better performance in executive functions and delayed recognition (Fig. 1). Effect size (Cohen's f^2) was 0.18 for delayed recognition and 0.39 for executive functions. There was no group difference in other cognitive functions.

At week 12, the treatment group had better performance in timed-up-and-go test. At weeks 24 and 36, there was no significant treatment effect on physical functions (Fig. 2).

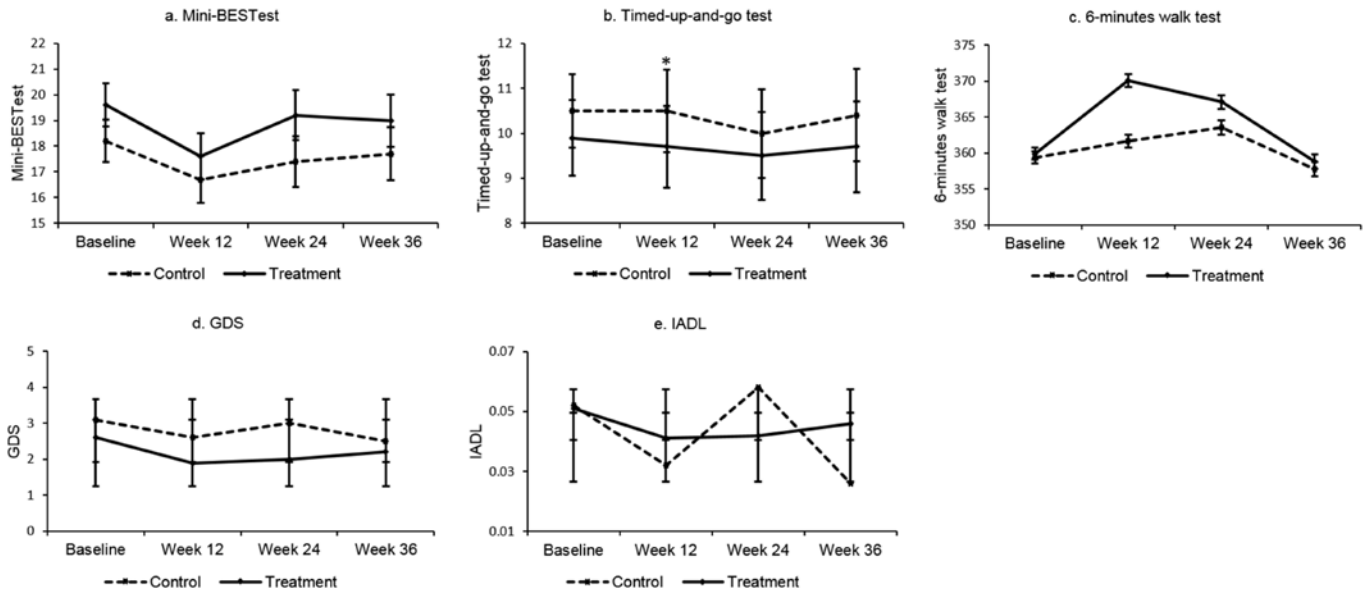

FIG 2. Group difference on physical functions, Geriatric Depression Scale (GDS), and Lawton instrumental activities of daily living scale (IADL) at different time points. At week 12, the treatment group has better performance in timed-up-and-go test. There is no significant group difference on GDS or Lawton instrumental activities of daily living scale at any time point.

There was also no significant group difference on GDS or Lawton instrumental activities of daily living scale at any time point.

There was no significant group difference on the pulsatility index or breath-holding index or IPAQ score at any time point. No mediation was found between aerobic dance training and cognitive functions, mood, or physical functions, after adjusting for age, sex, years of education, and change in IPAQ.

Post-treatment effects were found in memory (total learning and delayed recall) and GDS score.

A total of 26 adverse events were reported; 15 of them were serious adverse events. Only one event (plantar fasciitis) occurred in the treatment group was considered to be related to the intervention.

Discussion

The 24-week aerobic dance training improved performance across multiple cognitive domains. Moreover, the memory benefits lasted for at least 3 months after active treatment. Nonetheless, we did not find any clinically significant effects of treatment on physical functions, mood, or daily functions, nor a significant mediating role of CVR on the treatment effects on cognition.

In community older persons with cerebral small vessel disease, the 24-week aerobic dance training significantly improved memory and executive functions, with enduring memory benefits lasting at least 3 months. No benefits were found for physical functions, mood, or daily functions. Cognitive benefits of aerobic dance did not appear to be mediated by improvement in CVR. The aerobic dance is safe for older persons with cerebral small vessel disease.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#03140936). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

Reference

1. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol 2010;9:689-701.

Rapid movement therapy to improve balance recovery in stroke survivors: a randomised controlled trial: abridged secondary publication

RKY Tong *, KCC Cheng, M Junata, HS Man

KEY MESSAGES

1. Both rapid movement therapy and conventional balance training improved overall balance performance and lower limb motor function among stroke survivors.
2. Rapid movement training provided better balance recovery reaction in terms of stepping displacement.
3. However, the effect was not sustained at the 3-month follow-up. Therefore, regular training may be needed to retain the effects for a better balance recovery reaction.

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Introduction

To prevent falling, it is important to train the ability to maintain or recover the balance.[1] The balance recovery reactions can be achieved by controlling the position and the motion of the centre of mass of the body over the base of the support such as stepping and/or reaching-to-grasp a nearby object to rapidly increase the size of the base of the support.[2] These reactions are found to be impaired in stroke patients.[3] The aim of this study is to train stroke survivors to improve their balance and balance recovery reaction using the Kinect-based rapid movement therapy. The platform provides real-time feedback to the patient, generates a report for healthcare professionals to monitor patient progress, and can be utilised in patient homes or community centres.

Methods

This was a double-blinded randomised controlled trial with 3-month follow-up. A total of 32 patients with chronic stroke were randomly assigned to either rapid movement therapy (RMT) [n=16] or conventional balance training (CBT) [n=16]. Both interventions comprised 20 sessions of 60 minutes of training per day twice a week for around 7 weeks. RMT involved training of the legs in seven directions and of the hands in four directions (Fig. 1).

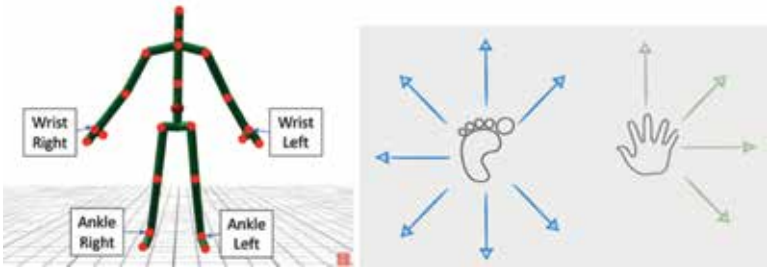

FIG 1. Microsoft Kinect skeleton tracking and the seven training directions for legs and four training directions for hands.

Outcome measures were Activities Balance Confidence Scale, Activities of Daily Living Scale, Berg Balance Scale, Fugl-Meyer Assessment, and Timed Up and Go Test. Fall assessment involved 'lean-and-release' and included kinematic measures of movement onset time, movement completion time, mediolateral step displacement, anteroposterior step displacement, XY stepping displacement, and number of steps. Assessments were performed at baseline, end of the intervention, and 3 months after the intervention ended.

Results

Using intention-to-treat analysis and two-way repeated-measures ANOVA, both RMT and CBT improved overall balance performance (Berg Balance Scale) and lower limb motor function (Fugl-Meyer Assessment) among stroke survivors. Post-hoc analysis (univariate 2-way ANOVA) showed that RMT resulted in significantly better improvement in anteroposterior stepping displacement ($F(1.840, 44.172)=3.551$, $P=0.041$) and XY stepping displacements ($F(1.812, 43.485)=3.586$, $P=0.040$), compared with CBT. In addition, RMT resulted in significant improvement in both the range of motion and movement completion time (Fig. 2). Larger range of motion means that stroke survivors can reach and step further to recover their balance. Faster movement time means that stroke survivors

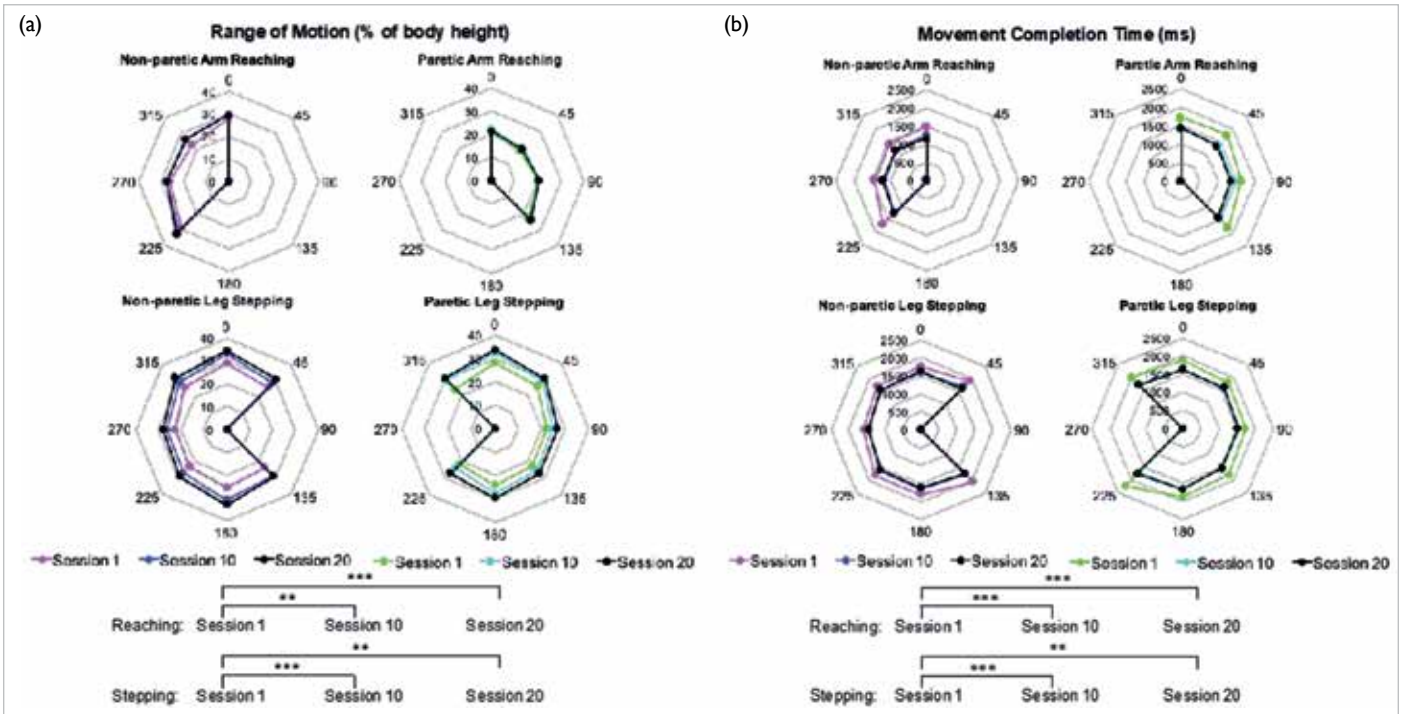

FIG 2. Improvement of (a) range of motion and (b) movement completion time after stepping and reaching training.

can complete the grasping and stepping balance reaction in a shorter time. However, the effect was not sustained at the 3-month follow-up. Therefore, regular RMT is needed to retain the effects for a better balance recovery reaction.

Conclusions

Both RMT and CBT improved overall balance performance and lower limb motor function among stroke survivors. RMT requires less manpower than CBT. RMT resulted in better balance recovery reaction in terms of anteroposterior and XY stepping displacement. However, the effect was not sustained at the 3-month follow-up. Therefore, regular RMT is needed to retain the effects for a better balance recovery reaction.

Acknowledgements

We thank the participants, research assistants, and student helpers for their contribution.

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This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#12131911). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

Disclosure

The results of this research have been previously published in:

1. Junata M, Cheng KCC, Man HS, Wang X, Tong RKY. Development and evaluation of a Kinect-based rapid movement therapy training platform for balance rehabilitation. Annu Int Conf IEEE Eng Med Biol Soc 2018;2018:2345-8. doi: 10.1109/EMBC.2018.8512822

References

1. Stewart Williams J, Kowal P, Hestekin H, et al. Prevalence, risk factors and disability associated with fall-related injury in older adults in low- and middle-income countries: results from the WHO Study on global AGEing and adult health (SAGE). BMC Med 2015;13:147.
2. Marigold DS, Eng JJ, Dawson AS, Inglis JT, Harris JE, Gylfadóttir S. Exercise leads to faster postural reflexes, improved balance and mobility, and fewer falls in older persons with chronic stroke. J Am Geriatr Soc 2005;53:416-23.
3. Geurts AC, de Haart M, van Nes IJ, Duysens J. A review of standing balance recovery from stroke. Gait Posture 2005;22:267-81.

Antioxidative effect of Gastrodiae Rhizoma–containing herbal formula in PC12 cell model: abridged secondary publication

Z Liu, CH Ko, CF Ng, HL Wong, JF Zhang, PK Lam, WS Poon, PC Leung *

KEY MESSAGES

1. The IC_{50} of hydrogen peroxide (H_2O_2) was 61.9 μM on PC12 cells.
2. Cell viability after co-treatment with Gastrodiae Rhizoma–containing herbal formula (DCXF) and H_2O_2 at all tested concentrations was similar to that with H_2O_2 treatment alone, suggesting that the antioxidative effect of DCXF is weak.
3. The antioxidant potential of DCXF was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. DCXF dose-dependently inhibited the DPPH radical at concentrations of 0.3125 to 20 mg/mL, compared with the control (0 mg/mL DCXF). However, the DPPH radical scavenging activity of DCXF was three orders of magnitude weaker than vitamin C.

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Introduction

Free radical formation and oxidative damage are main contributors to the pathophysiology of traumatic brain injury. Following the primary mechanical injury, the multiple interrelated secondary injuries cascade (including the loss of ionic homeostasis, glutamate excitotoxicity, mitochondrial dysfunction, and microvascular disruption) and result in the free radical formation such as hydrogen peroxide (H_2O_2), nitric oxide, and peroxynitrite.[1,2] The oxidative stress induced by uncontrolled free radicals may in varying degrees results in peroxidation of cellular and vascular structures, protein oxidation, cleavage of DNA, inflammatory, blood-brain barrier dysfunction, oedema formation, impairment of cerebral vascular function, apoptotic and necrotic neuronal cell death, and eventually neurologic disorders.[3,4]

The neuroprotective effects of Gastrodiae Rhizoma–containing herbal formula (DCXF) and the anti-oxidative activities of the individual herbs of DCXF and its main pharmacological active compounds (TMP, *E*-ferulic acid, *Z*-ligustilide, and gastrodin) have been extensively studied. However, the anti-oxidative effect of DCXF has not been reported. We used the H_2O_2-induced rat pheochromocytoma PC12 cell model and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay to evaluate the anti-oxidative effects of DCXF. The findings may be used to predict the potential therapeutic effect of DCXF on traumatic brain injury and establish groundwork for in vivo studies.

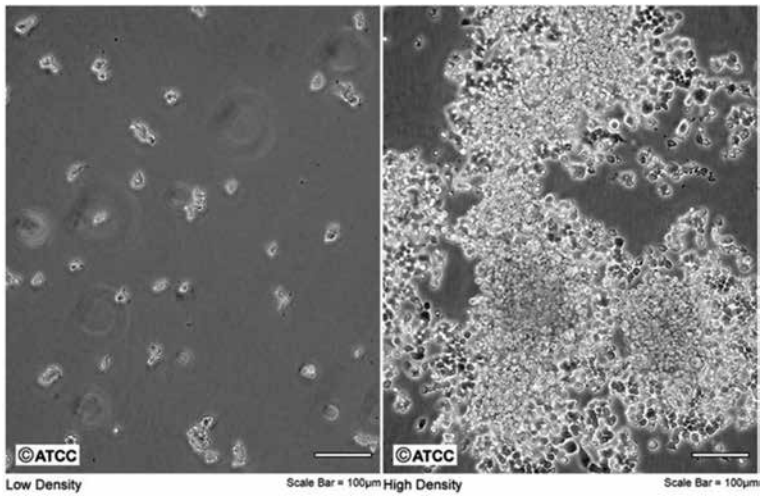

FIG 1. The morphology of PC12 cells in low and high density (adopted from American Type Culture Collection).

Materials and methods

The rat pheochromocytoma PC12 cells (Fig. 1) were obtained from American Type Culture Collection. The cells were grown in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated horse serum, 5% (v/v) FBS, and 1% (v/v) penicillin/streptomycin solution. The cell culture was maintained in poly-L-lysine-coated tissue culture flasks at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

MTT assay was used to select a suitable H_2O_2 concentration to induce oxidative stress with about 50% cell death, and to assess the toxicity and protective effect of DCXF on cell viability in the H_2O_2-induced PC12 cell model. Briefly, PC12 cells were seeded in 96-well plates pre-coated with poly-L-lysine at a density of 2×10^4 cells per well. After incubated for 24 hours, the cells were treated with H_2O_2 at concentration ranging from 0 to 200 μM or with DCXF at concentration ranging from 0 to 2000 μg/mL for 24 hours. The culture medium was then removed and MTT solution was added to each well. After further incubation for 4 hours, the incubation solution was aspirated and the dark blue formazan crystals were solubilised in DMSO. Then, the absorbance was detected at 570 nm using a microplate reader.

To determine the cytoprotective activity of DCXF on H_2O_2-induced PC12 cells, 60 μM H_2O_2 was selected as the concentration of H_2O_2-induced oxidative stress on PC12 viability. PC12 cells were seeded in 96-well plates at a density of 2×10^4 cells per well and incubated for 24 hours. Then the cells were treated with indicated concentrations of DCXF with or without 60 μM H_2O_2. The control group cells were added with medium without H_2O_2. After further incubation for 24 hours, the cell viability was then measured by MTT method. All data were expressed as a percentage of untreated groups, which were expressed as 100%.

The radical scavenging activity of the samples was determined by DPPH assay as previously described, with slight modification.[5] The chemicals' antioxidative activity can be evaluated according to the reduction of a stable DPPH radical by solution of antioxidants. The colour of DPPH in solution can be altered from deep violet to colourless or pale yellow when neutralised, which causes a decrease in absorbance and can be measured spectrophotometrically. Briefly, 100 μL of different dilutions of DCXF were mixed with 1000 μL DPPH (0.075 mM methanolic solution), followed by incubation for 30 minutes in the dark at room temperature. The absorbance of each sample was read at 517 nm using a microplate reader. Vitamin C was used as standard antioxidant compound for the comparison of IC_{50} (inhibition of 50% DPPH radical) with DCXF. All determinations were performed in triplicates. Percentage of inhibition of the DPPH radical was calculated using the following equation: inhibition of DPPH (%) = (1 - absorbance of samples (or) standard / absorbance of control) × 100.

Multiple group comparisons were made using one-way ANOVA with Dunnett post-hoc test using GraphPad Prism 6. Each experiment was repeated at least three times in triplicate or as indicated. Differences were considered statistically significant when $P<0.05$.

Results

H_2O_2 at the concentration of 37.5 to 200 μM significantly decreased the cell viability in a dose-dependent manner, compared with the untreated control group (0 μM H_2O_2) [Fig. 2a]. IC_{50} of H_2O_2 was 61.9 μM, and hence 60 μM H_2O_2 was chosen for subsequent experiments. DCXF had no cytotoxic effect on PC12 cells at all tested concentrations of 0 to 2000 μg/mL, compared with the untreated control group (0 μg/mL DCXF) [Fig. 2b]. 60 μM H_2O_2 could cause about 50% cell death, compared with the untreated control group (0 μg/mL DCXF, 0 μM H_2O_2). However, there was no significant change in the cell viability after treatment with DCXF at all tested concentrations, compared with the H_2O_2-treated alone (Fig. 2c), suggesting that the anti-oxidative effect of DCXF is weak in the H_2O_2-induced PC12 cell model.

The antioxidant potential of DCXF was evaluated by the DPPH assay. DCXF dose-

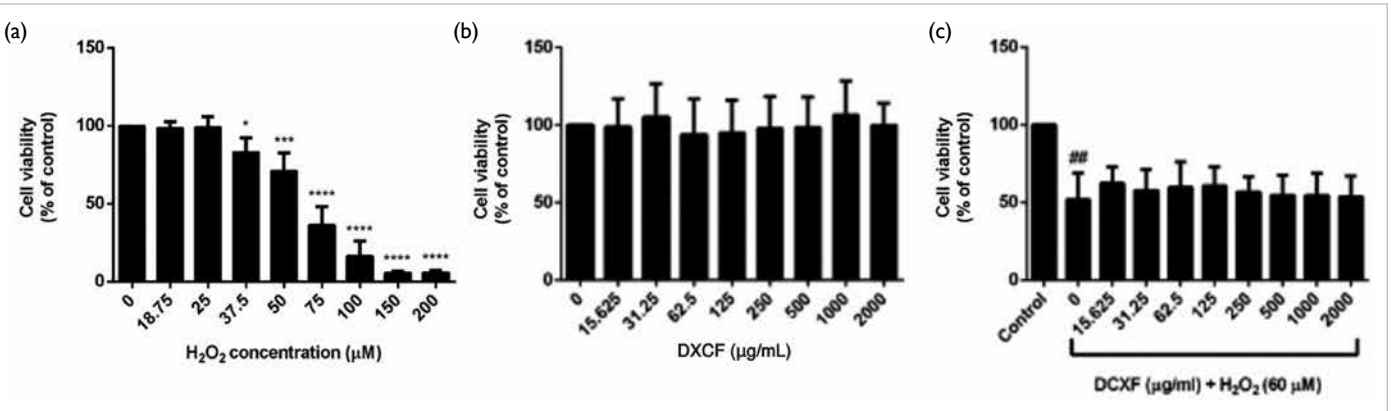

FIG 2. (a) Hydrogen peroxide (H_2O_2)–induced cytotoxicity on PC 12 cells. (b & c) Effect of Gastrodiae Rhizoma–containing herbal formula (DCXF) for 24 h with or without 60 μM H_2O_2 on viability of PC12 cells. Cell viability is determined by MTT assay.

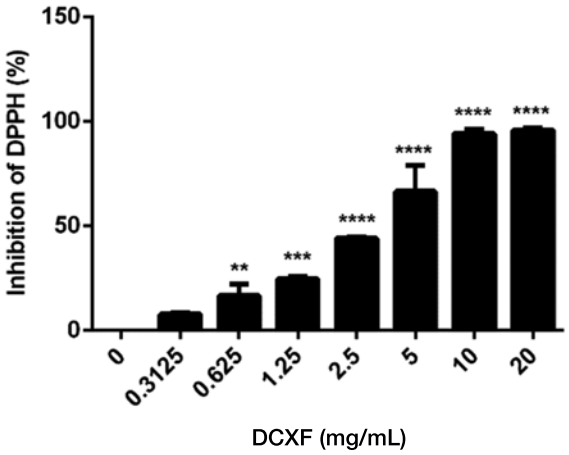

FIG 3. The antioxidant potential of Gastrodiae Rhizoma–containing herbal formula (DCXF) on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

dependently inhibited the DPPH radical at concentrations of 0.3125 to 20 mg/mL, compared with the control (0 mg/mL DCXF) [Fig. 3]. However, the DPPH radical scavenging activity of DCXF (IC_{50}=3.4 mg/mL) was three orders of magnitude weaker than vitamin C (IC_{50}=2.8 μg/mL) [data not shown], indicating that DCXF had a very weak antioxidant effect.

Discussion

PC12 cells have been extensively applied to study neurotoxicity (eg, cellular H_2O_2 toxicity), neurosecretion, neuronal differentiation, neuronal function, and neurodegeneration owing to their similarity with sympathetic neurons and their reversible differentiation response to nerve growth factor. Moreover, PC12 cells are widely used for neuroprotection studies of various neurological disorders such as traumatic brain injury, Parkinson disease, and Alzheimer disease. H_2O_2 is the major cause of oxidative stress and diffuses readily through cells and tissues, which can cause proteins oxidation, lipid peroxidation, DNA strand breakage, base modification, and eventually cell death via apoptosis or necrosis. H_2O_2 involves in the pathogenesis of various neurological disorders and has been used as an inducer of oxidative stress to elucidate the neuroprotective mechanisms of anti-oxidative therapeutics.[4] Moreover, the H_2O_2-induced PC12 cell model has been validated in analysing the neurological apoptosis and the therapeutic mechanisms of antioxidants.

The result of the DPPH chemical method demonstrated that DCXF had very weak antioxidant activity. In contrast to our previous studies, the aqueous extract of Gastrodiae Rhizoma provided neuroprotective effect on beta-amyloid-induced toxicity in PC12 cells and drosophila models, and demonstrated to attenuate locomotor deficit and reduce the inflammation on controlled cortical impact–induced traumatic brain injury rat model. The presence of Chuanxiong Rhizoma in DCXF may mitigate the antioxidative effect and reinforce the anti-inflammatory effects. Therefore, we will focus on the anti-inflammatory effects of DCXF.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#12134111). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

Disclosure

The results of this research have been previously published in:

1. Liu ZK, Ng CF, Shiu HT, et al. Neuroprotective effect of Da Chuanxiong Formula against cognitive and motor deficits in a rat controlled cortical impact model of traumatic brain injury. J Ethnopharmacol 2018;217:11-22.
2. Liu ZK, Ng CF, Shiu HT, et al. A traditional Chinese formula composed of Chuanxiong Rhizoma and Gastrodiae Rhizoma (Da Chuanxiong Formula) suppresses inflammatory response in LPS-induced RAW 264.7 cells through inhibition of NF-κB pathway. J Ethnopharmacol 2017;196:20-8.
3. Ng CF, Ko CH, Koon CM, et al. The aqueous extract of rhizome of Gastrodia elata Blume attenuates locomotor defect and inflammation after traumatic brain injury in rats. J Ethnopharmacol 2016;185:87-95.

References

1. Bains M, Hall ED. Antioxidant therapies in traumatic brain and spinal cord injury. Biochim Biophys Acta 2012;1822:675-84.
2. Cornelius C, Crupi R, Calabrese V, et al. Traumatic brain injury: oxidative stress and neuroprotection. Antioxid Redox Signal 2013;19:836-53.
3. O'Connell KM, Littleton-Kearney MT. The role of free radicals in traumatic brain injury. Biol Res Nurs 2013;15:253-63.
4. Abdul-Muneer PM, Chandra N, Haorah J. Interactions of oxidative stress and neurovascular inflammation in the pathogenesis of traumatic brain injury. Mol Neurobiol 2015;51:966-79.
5. Li KK, Shi XG, Yang XR, Wang YY, Ye CX, Yang ZY. Antioxidative activities and the chemical constituents of two Chinese teas, Camellia kucha and C. ptilophylla. Int J Food Sci Technol 2012;47:1063-71.

AUTHOR INDEX

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