SUPPLEMENT 1

HONG KONG MEDICAL JOURNAL 香港醫學雜誌

Volume 28 Number 1 **February 2022**

The official publication of the
Hong Kong Academy of Medicine and
the Hong Kong Medical Association

Health and Medical Research Fund

Research Dissemination Reports

醫療衞生研究基金

研究成果報告

Genetics
遺傳學

Cancer
癌症

Mental health
精神健康

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生殖健康

Renal system
腎臟系統

Respiratory system
呼吸系統

Eye
眼睛

28(S1)

HONG KONG MEDICAL JOURNAL 香港醫學雜誌

Volume 28 Number 1 **February 2022**

Supplement 1

ISSN 1024-2708

香港醫學專科學院出版社
HONG KONG ACADEMY OF MEDICINE PRESS

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Volume 28 Number 1 **February 2022**
SUPPLEMENT 1

Health and Medical Research Fund

Research Dissemination Reports

RENAL SYSTEM

RESPIRATORY SYSTEM

EYE

Editorial

Dissemination reports are concise informative reports of health-related research supported by the Health and Medical Research Fund administered by the Food and Health Bureau. In this edition, we present 13 dissemination reports of projects related to genetics, cancer, mental health, reproductive health, renal system, respiratory system, and the eye. 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.

Balanced chromosomal abnormalities are common genetic variations that are major contributors to human genetic variation. They occur in both healthy and diseased individuals in about one in every 500 (0.2%) newborns. Most cases are not associated with a pathological phenotype, but they have an increased risk of developing multiple congenital abnormalities, autism spectrum disorders or intellectual disability. Chung et al[1] used whole-genome sequencing with paired end sequencing to localise breakpoints of balanced chromosomal abnormalities at the nucleotide level to substantially improve diagnostic resolution, compared with karyotyping and other cytogenetic methods. Understanding gene disruption in this way can guide genetic counselling and allow accurate and personalised disease risk prediction.

Fibrosis and cirrhosis are risk factors for development of hepatitis B virus–related hepatocellular carcinoma (HCC). Liver biopsy is often used to assess liver fibrosis but concerns on its invasiveness and complications limit its use. Wong et al[2] evaluated the combined use of liver stiffness measurement based on transient elastography with enhanced liver fibrosis – a non-invasive assessment based on an algorithm of three serum biomarkers – to predict HCC in patients with chronic hepatitis B receiving antiviral treatment. The two-step algorithm could improve accuracy of predicting HCC, which could allow clinicians to better differentiate patients with hepatitis B virus for more specific surveillance, to improve survival and enhance use of resources.

Antenatal depression and postnatal depression affect maternal and infant health and effective interventions to reduce the risk of depression are required. Chan et al[3] developed and tested the effectiveness of a mobile application (iParent) to disseminate essential knowledge on pregnancy and infant care to pregnant women and to reduce maternal postnatal depression among 660 pregnant women in Hong Kong. They found that pregnant women who used the app reported significantly lower level of postnatal depression than those who received treatment as usual. The iParent app is a low-cost user-friendly alternative to the traditional resource-consuming antenatal classes.

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 (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.

Supplement editor

Dr Richard A Collins
Chief Scientific Reviewer
(Research Office)
Food and Health Bureau

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1. Chung BHY, Kan ASY, Chan KYK, et al. Analytical validity and clinical utility of whole-genome sequencing for cytogenetically balanced chromosomal abnormalities in prenatal diagnosis: abridged secondary publication. Hong Kong Med J 2022;28(Suppl 1):S4-7.
2. Wong GLH, Wong VWS. Dynamic change of LSM-HCC score and enhanced liver fibrosis score to predict hepatocellular carcinoma in chronic hepatitis B patients receiving antiviral treatment: abridged secondary publication. Hong Kong Med J 2022;28(Suppl 1):S11-3.
3. Chan KL, Leung WC, Tiwari A, Or KL, Ip P. Effectiveness of the iParent app for postnatal depression: a randomised controlled trial (abridged secondary publication). Hong Kong Med J 2022;28(Suppl 1):S17-8.

Analytical validity and clinical utility of whole-genome sequencing for cytogenetically balanced chromosomal abnormalities in prenatal diagnosis: abridged secondary publication

BHY Chung *, ASY Kan, KYK Chan, W Yang, MHY Tang, CCY Mak, GKC Leung

KEY MESSAGES

1. Whole-genome sequencing was superior to conventional karyotyping in detection of banding in nine of 10 cases. Improvement in detection could be confirmed by orthogonal methods.
2. Gene disruption was identified in two cases and led to definitive diagnoses for two families with proband affected by X-linked epilepsy (disruption of PCDH19) or microcephalic osteodysplastic primordial dwarfism type II (MOPDII). In the third case, gene disruption was identified, but variant of uncertain significance was concluded.
3. Using both short read (Illumina) and long read (Nanopore & PacBio) sequencing data together with bioinformatics tool (WhatsHap) can detect additionally the phasing of mutations.
4. Understanding the genomic mechanism of gene disruption secondary to balanced chromosomal abnormalities can guide the genetic counselling. It allows accurate and personalised disease risk prediction.

Hong Kong Med J 2022;28(Suppl 1):S4-7
HMRF project number: 05162986

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Introduction

Balanced chromosomal abnormalities (BCAs) are common genomic variations. Structural variants refer to a class of genomic variation at the chromosomal level, including copy number variations and balanced chromosomal rearrangements (eg, translocations and inversions), which involve changes in either localisation or orientation of a chromosomal segment without visible gain or loss of chromosomal material. BCA is a major contributor to human genetic variation. It occurs in both healthy and diseased individuals, affecting about one (0.2%) in every 500 newborns.[1] Although most cases are not associated with a pathological phenotype, these cases have an increased risk of developing multiple congenital anomalies, autism spectrum disorders, or intellectual disability.[1] In 370 000 women who underwent prenatal diagnosis by conventional cytogenetics, the risk for serious congenital anomalies was 6.1% for *de novo* reciprocal translocation, 3.7% for Robertsonian translocations, and 9.4% for inversions.[2] However, interpretation of BCAs by conventional cytogenetics is restricted by the resolution limit.

Conventional karyotyping is considered the standard for prenatal diagnosis of chromosomal disorders. It can detect numerical abnormalities as well as structural rearrangements (balanced or unbalanced) but is limited to a microscopic resolution of 3 to 10 Mb.[3] Whole-genome array comparative hybridisation can detect chromosomal imbalances at a resolution in the tens to hundreds of kilobases and can sometimes detect cryptic deletions and duplications associated with BCAs that are not detectable by karyotyping.[4]

Whole-genome sequencing (WGS) has emerged as a comprehensive diagnostic tool for the detection of a wide range of genomic changes. Paired-end sequencing refers to the massively parallel sequencing of both ends of the same DNA fragment by use of adaptors to generate short sequence reads. WGS performed using this method can localise BCA breakpoints at the nucleotide level. This can substantially improve the diagnostic resolution, compared with karyotyping and other cytogenetic methods.

Methods

Genomic DNA was extracted from the thawed cultured cells of the stored chorionic villi or amniotic fluid samples according to standard protocols. We followed the recommendations of the Laboratory Quality Assurance Committee of the American College of Medical Genetics and Genomics (ACMG) on the use of WGS for diagnostic purposes. We sequenced the 150-bp-paired ends of contiguous genomic DNA fragments of approximately 1 to 2 Kb. Sequencing using the Illumina HiSeq 2500

platform was performed by Macrogen or the Centre for Genomic Sciences, the University of Hong Kong.

We then interrogated the BCA breakpoints and determined whether they resulted in: (1) gene disruption (ie, whether the translocation breakpoint intercepts the open-reading frame of a gene that may result in termination of a gene transcript or translation of a fusion protein), (2) genomic imbalances (ie, cryptic deletion or duplication identified adjacent to the chromosome breakage), and (3) alteration of topologically associating domains (ie, long-range effects of cis-acting regulatory elements on protein-coding genes). Topologically associating domains are key elements of mammalian regulatory organisation and are conserved genomic conformations that partition the genome into megabase-sized compartments with frequent intra-domain regulation.

We retrospectively identified those with apparent *de novo* BCAs through the internal database of the Prenatal Diagnostic Laboratory, Tsan Yuk Hospital. Patients with multiple pregnancies, molar pregnancy, ectopic pregnancy, or complications associated with known environmental or teratogenic causes were excluded, as were those with isolated/ multiple major fetal structural abnormalities detected by ultrasonography. The presence of major structural abnormalities already affects the decision of termination of pregnancy. Ethics approval was obtained from the Institutional Review Board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster (UW 18-045).

Results

Ten patients with apparent *de novo* BCAs were identified: eight with reciprocal translocation and two with inversions (Table) Pair-ended short-read WGS identified the BCA and their breakpoints at single-nucleotide resolution. In five cases, extra DNA was available for validation using orthogonal methods (to confirm the WGS findings) including Sanger sequencing (n=4) and Nanopore sequencing and PacBio sequencing (n=1). The higher resolution of detecting breakpoints at the single-nucleotide level by WGS had improved detection of banding over karyotype in nine of ten cases and had the same banding detection as karyotype in one case.

WGS provided additional genomic information over conventional karyotype in genetic counselling in the following three cases.

Case 1 (subject 11F0523) was a product of intracytoplasmic sperm injection and showed a karyotype of 46,XX,t(18;19)(q12.2;q13.1)dn. Using WGS, we identified the breakpoints at chr18:29,652,147 and chr19:36,930,887, which were validated by Sanger sequencing (Fig 1). The translocation disrupted the gene *RNF125* at its last

TABLE. Comparison of the detection by Karyotype and WGS on 10 BCA cases

Case	Subject	Karyotype	Clinical indication for prenatal diagnosis	Banding (conventional vs whole-genome sequencing)	Breakpoints (GRCh37)	OMIM morbid gene	Orthogonal validation
1	11F0523	46,XX,t(18;19)(q12.2;q13.1)dn	Baby of intracytoplasmic sperm injection	18q12.2 vs 18.q12.1 19q13.1 vs 19q13.12	chr18:29,652,147 chr19:36,930,887	*RNF125*	Sanger
2	11F0621	46,XX,t(4;12)(q35;p13.1)dn	Maternal age	4q35 vs 4q35.2 12p13.1 vs 12p12.3	chr4:186,776,072 chr12:15,513,829	-	-
3	11F0959	46,X,inv(X)(p21q22.1)dn	Increased cardio-thoracic ratio	Xp21 vs Xp21.1 Xq22.1 vs Xq22.1	chrX:34,271,812 chrX:99,594,533	*PCDH19*	-
4	12F0839	46,XY,t(5;9)(q13;q32)dn	Bilateral prominent renal pelvis, borderline ventriculomegaly	5q13 vs 5q14.1 9q32 vs 9q32	chr5:78,899,784 chr9:117,899,085	-	-
5	12F1057	46,XY,t(1;4)(p21q21.1)dn	Maternal age	1p21 vs 1p21.2 4q21.1 vs 4q21.1	chr1:100,004,147 chr4:88,309,179	-	Sanger
6	13C0062	46,XX,t(8;11)(q22;q13)dn	Increased nuchal traslucency	8q22 vs 8q22.1 11q13 vs 11q13.1	chr8:97,086,794 chr11:65,540,889	-	Sanger
7	13C0266	46,XY,t(7;18;12)(q31;p11.3;q15)dn	Head shape roundish	7q31 vs 7q31.1 18p11.3 vs 18p11.31 12q15 vs 12q15	chr7:110493772 chr18:3766591 chr12:69794115	-	-
8	12F0050	46,XY,inv(21)q11.2q22.3)dn	Early-onset intrauterine growth retardation, mildly echogenic bowel	21q11.2 vs 21q11.2 21q22.3 vs 21q22.3	chr21:14,953,345 chr21:47,839,992	*PCNT*	PacBio, Nanopore
9	08F1641	46,XY,t(6;8)(p21.1;q24.1)dn	Echogenic focus in left ventricle, short femur, short humerus	6p21.1 vs 6p21.1 8q24.1 vs 8q24.21	chr6:45,841,060 chr8:126,545,471	-	Sanger
10	18T1192	46,XX,t(3;7,6)(q25;q36;q21)dn	Cleft lip	3q25 vs 3q25.32 6q21 vs 6q21 7q36 vs 7q36.3	chr3:157254970 chr6:106600067 chr7:158852085	-	-

exon. *RNF125* is associated with Tenorio syndrome (OMIM: 616260), which is an autosomal dominant disease characterised by overgrowth, macrocephaly, and intellectual disability syndrome. *RNF125* is a gene that is predicted to be tolerant to loss-of-function mutations (indicated by a low pLI score). In addition, all reported pathogenic variants in *RNF125* are missense mutations. According to the ACMG/AMP guideline, the variant was classified as a variant of uncertain significance. From the medical record, this pregnancy resulted in a healthy live-born with normal growth parameters.

Case 3 (subject 11F0959) was a female fetus with increased cardio-thoracic ratio who had a karyotype of 46,X,inv(X)(p21q22.1)dn. Using WGS, we identified the breakpoints at chrX:34,271,812 and chrX:99,594,533, which resulted in the disruption of exon 6 of *PCDH19*, a gene in which loss of function mutation was associated with X-linked early infantile epileptic encephalopathy (OMIM: 300088), which is known to affect mainly female. The carrier males are largely unaffected, except for minor psychiatric/behavioural abnormalities. According to the ACMG/AMP guideline for variant interpretation, the variant was classified as pathogenic. This pregnancy was terminated and therefore postnatal outcome was not assessed to compare with the molecular diagnosis. Nonetheless, we predicted that the baby would have a high chance of developing early-onset severe epilepsy causing encephalopathy, given the full penetrance of the condition.

Case 8 (subject 12F0050) was affected with severe intrauterine growth retardation (IUGR) of unknown aetiology during pregnancy. From prenatal diagnosis, a karyotype of 46,XY,inv(21)(q11.21q22.3)dn was identified, but this finding was not considered to be explaining the finding of IUGR. The couples decided to carry on the pregnancy, and the baby was delivered at 35 weeks. The baby showed features of IUGR, microcephalic, and mesomelic limb shortening. Microcephalic osteodysplastic primordial dwarfism type II (MOPDII) was suspected clinically. Initial NGS panel showed that there was one heterozygous frameshift mutation in *PCNT*:c.4633_4678delAGACAAGTGTTAAT p.(R1555Afs*6). Although the clinical presentations were compatible with MOPDII, the genetic diagnosis was incomplete because only one pathogenic variant was identified in an autosomal recessive condition. As *PCNT* was located on chromosome 22q11.21, we hypothesised that the *de novo* inversion might have disrupted the other *PCNT* allele not carrying the frameshift mutation. We identified one of the breakpoints of the inversion at chr21:47,839,992, which caused disruption of *PCNT*. However, the distance between the frameshift mutation and the inversion breakpoint is 20 kb apart, and thus we were unable to assess the phasing information of the two variants. Therefore, we decided to proceed to long-read sequencing using Nanopore and PacBio platforms to confirm the breakpoint of the inversion. Using a bioinformatic tool called WhatsHap, we combined the short- and long-read data to construct haplotypes that span across the frameshift mutation and the inversion breakpoint. The two variants were located in different haplotypes, indicating that they were located on different alleles (Fig 2). The molecular and clinical diagnosis of MOPDII was therefore substantiated. As the inversion was *de novo*, we informed the family of the low chance of recurrence in subsequent pregnancies.

Discussion

In contrast to karyotyping that detects BCA breakpoints at sub-band level, WGS can precisely detect breakpoints at single-nucleotide level and identify the disrupted genes. Combined with the ACMG/AMP 2015 guideline for variant interpretation in evaluating the pathogenicity of the genetic variants, we were able to provide a more

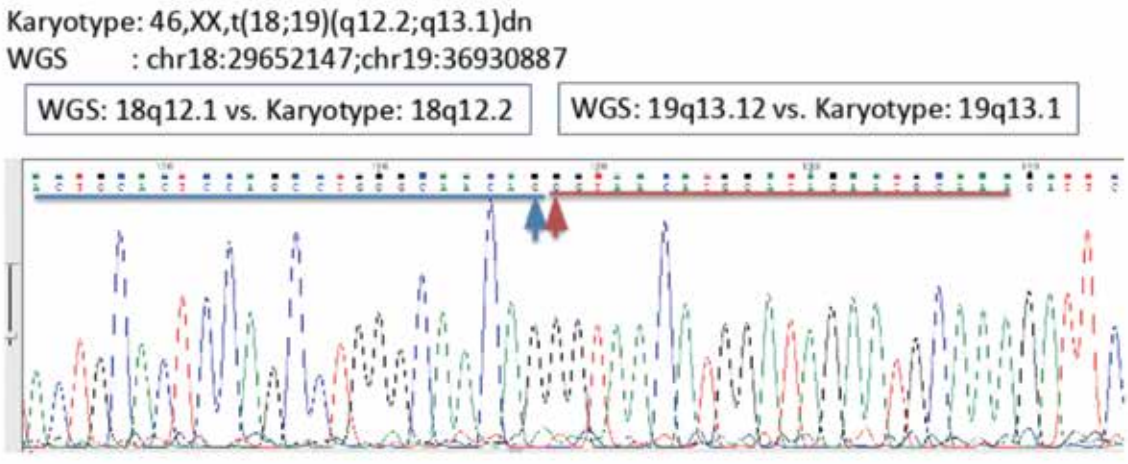
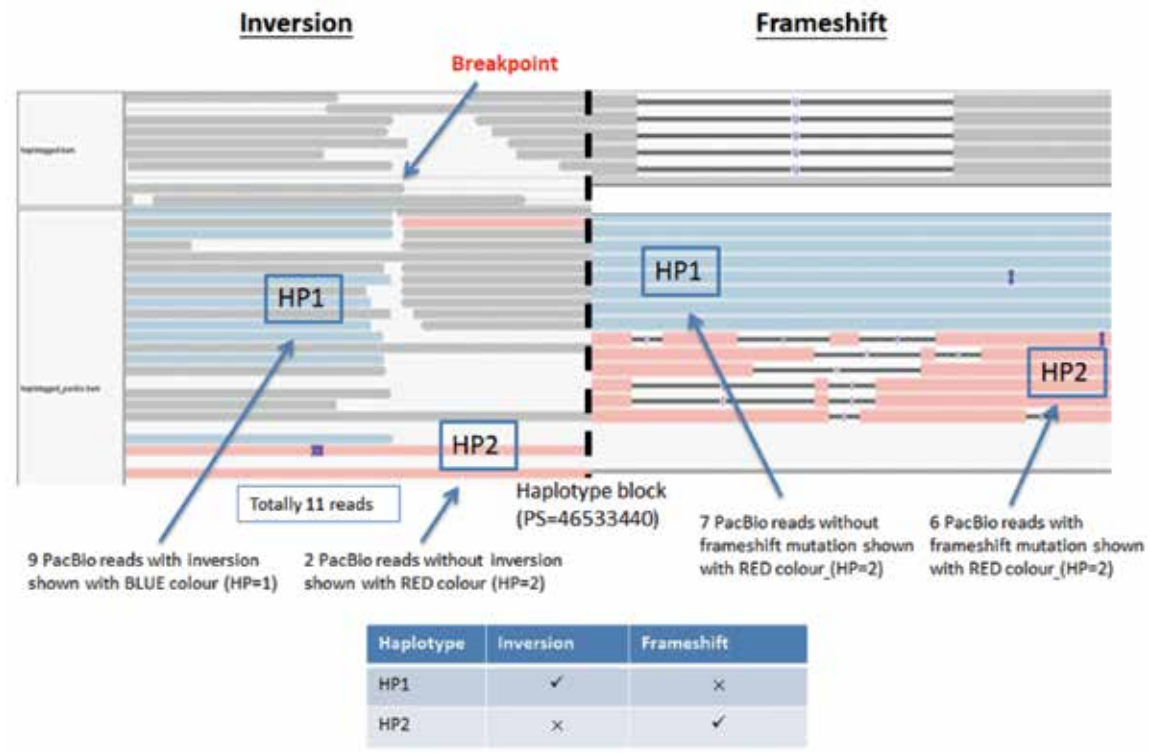


FIG 1. Sanger validation of case 1 (11F0523)



Haplotype	Inversion	Frameshift
HP1	✓	×
HP2	×	✓

FIG 2. Short read and long read of inversion on PCNT gene

precise and personalised genetic counselling on all ten cases, compared with the empiric risk of 6% to 9% recurrence risk. WGS showed improvement in detection of banding over karyotyping in nine of ten cases. This is consistent with a study stating "Revised the breakpoint localisation by at least one sub-band in 93% of subjects when compared to the karyotype interpretation".

We successfully set up the pipeline to analyse WGS data of both short-read (Illumina) and long-read sequencing data (Nanopore and PacBio) in the diagnosis of the case with MOPDII. With the limitation of comparatively large raw error rate (around 15%) of long-read sequencing (Nanopore and PacBio sequencing), the hybrid approach of using both short read and long-read sequencing is the most cost-effective and accurate method. The Illumina short-read platform provides high throughput and accurate sequencing of the single-nucleotide level, whereas the Nanopore and PacBio long-read platform provide easier structural variation and phasing detection. Illumina short-read WGS platform is good at detecting frameshift and structural variations but not good at detecting phasing, whereas Nanopore and PacBio long read is good at detecting structural variations but have limitations on detecting frameshift mutation and phasing owing to large raw error rate and not long enough reads at some cases. Combining both together with bioinformatics tools can effectively detect structural variations, frameshift mutation, and phasing.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#05162986). 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. Yu MHC, Chau JFT, Au SLK, et al. Evaluating the clinical utility of genome sequencing for cytogenetically balanced chromosomal abnormalities in prenatal diagnosis. Front Genet 2021;11:620162.

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Whole-genome sequencing of genetically undiagnosed euploid fetuses with increased nuchal translucency: abridged secondary publication

RKW Choy *, YE Cao, FM Lo, SW Cheung, Y Yang, TY Leung

KEY MESSAGES

1. We studied the genome-wide spectrum and frequency of genetic variants and spatial genomic organisation during early fetal development. We also investigated the variability of genomic variants associated with the increased nuchal translucency–related birth defects.
2. We implemented whole-genome sequencing to screen the candidate pathogenic variants that helped explain the congenital structural abnormalities for 15 trios in Hong Kong.
3. Compared with chromosomal microarray analysis and karyotyping, whole-genome sequencing provided an additional 20% (3/15) diagnostic yield.
4. Applying whole-genome sequencing in fetuses with increased nuchal translucency can comprehensively detect and delineate the various genomic variants that are causative to the diseases.

Hong Kong Med J 2022;28(Suppl 1):S8-10
HMRF project number: 04152666

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Introduction

Detection of fetuses with increased nuchal translucency in routine first-trimester ultrasound screening has been widely used as a sensitive indicator for fetal chromosomal abnormalities and/or fetal structural anomalies such as congenital heart disorders or neurodevelopmental anomalies detected in later gestations. Fetuses with increased nuchal translucency and structural malformations are frequently contributed by genetic abnormalities and have poor prognoses. However, >80% of such cases do not obtain a causative result based on the routine prenatal diagnostic tests and hence genetic counselling and clinical management is challenging. In prenatal diagnosis, since 2010, chromosomal microarray analysis (CMA) has been the first-tier test for high-risk pregnancies to identify microscopic or submicroscopic copy number variations. However, this approach is limited by its resolution and inability to detect single-nucleotide variants and small insertions/deletions. Whole exome sequencing can provide genetic diagnoses for 9.1% to 32% of fetuses with a structural anomaly. Of these fetuses, whole-exome sequencing yielded diagnoses in 3.2% to 21% of the fetuses with increased nuchal translucency with/without structural malformations. Nonetheless, both whole-exome sequencing and CMA are unable to detect apparently balanced structural rearrangements (or structural variants); some of these rearrangements are disease-causing. The wide spectrum of genetic aetiologies in fetuses with increased nuchal translucency (ranging from single-base mutations to those affecting millions of base pairs and numerical disorders) warrants a holistic approach for comprehensive detection of the disease-causing genetic variants. Our previous studies have demonstrated the feasibility and potential diagnostic utility of low-pass whole-genome sequencing for detection of copy number variations and chromosomal structural rearrangements including balanced translocations and inversions in both clinical cohorts and presumably normal populations in the 1000 Genomes Project. By increasing the read depth to a minimum of 30-fold to detect single-nucleotide variants / small insertions/deletions, whole-genome sequencing enables comprehensive detection of various genomic variants, providing a platform for gene discovery and potential clinical application. We aimed to apply whole-genome sequencing to investigate genetic contributions to fetuses with increased nuchal translucency and structural malformations and to evaluate its potential clinical application.

Methods

First, 100 ng of genomic DNA from each sample was sheared to fragment sizes ranging from 300 to 500 bp by the Covaris S2 Focused Ultrasonicator.

Library construction including end repairing, A-tailing, adapter ligation, and PCR amplification was conducted subsequently. The PCR products were then heat-denatured to form single-strand DNAs, followed by circularisation with DNA ligase. After construction of the DNA nanoballs, paired-end sequencing with 100 bp at each end was carried out for each sample with a minimum read depth of 100-fold on the MGISEQ-2000 sequencing platform.

Interpretation of candidate pathogenic variants was based on the classification of potential Mendelian disease-causing variants described previously. According to the American College of Medical Genetics and Genomics recommendations, each variant was classified as benign, likely benign, uncertain significance, likely pathogenic, and pathogenic. Uncertain significance variants are further subclassified into 'favour benign' or 'favour pathogenic'. Variants with allele frequency of <1% or unknown in the database are rare and more likely to be disease causing. Candidate pathogenic variants are supposed to locate on the coding sequence, or splice site. Demonstration of the same mutation in phenotypically normal parents strongly suggests that the observed change in the index patient's genome is without clinical significance.

Results

In 15 trios with increased nuchal translucency (>3.5 mm) and negative results from karyotype and CMA, whole-genome sequencing provided an additional 20% (3/15) diagnostic yield, with two cases with pathogenic point mutations (Fig 1) and one case with cryptic insertions (Fig 2). Follow-up study further demonstrated the potential pathogenicity of an apparently balanced insertion that disrupted an OMIM autosomal dominant disease-causing gene at the insertion site.

Discussion

Applying whole-genome sequencing in fetuses with increased nuchal translucency can comprehensively detect and delineate the various genomic variants that are causative to the diseases. Importantly, prenatal diagnosis by whole-genome sequencing provided additional diagnostic yield, compared with routine protocols. Given a comparable turnaround time and less DNA required, whole-genome sequencing is useful in prenatal diagnosis, particularly in fetuses with increased nuchal translucency.

CMA can serve as first-tier genetic testing for prenatal diagnosis, whereas whole-genome sequencing can serve as a second-tier test in CMA negative patients. For fetuses with high nuchal translucency and structural malformations and at high gestational weeks (>20 weeks), whole-genome sequencing is suggested for a more plausible explanation.

Conclusion

Whole-genome sequencing is powerful to discover the candidate pathogenic variants for fetuses with high nuchal translucency and structural malformations. It has clinical application in Hong Kong.

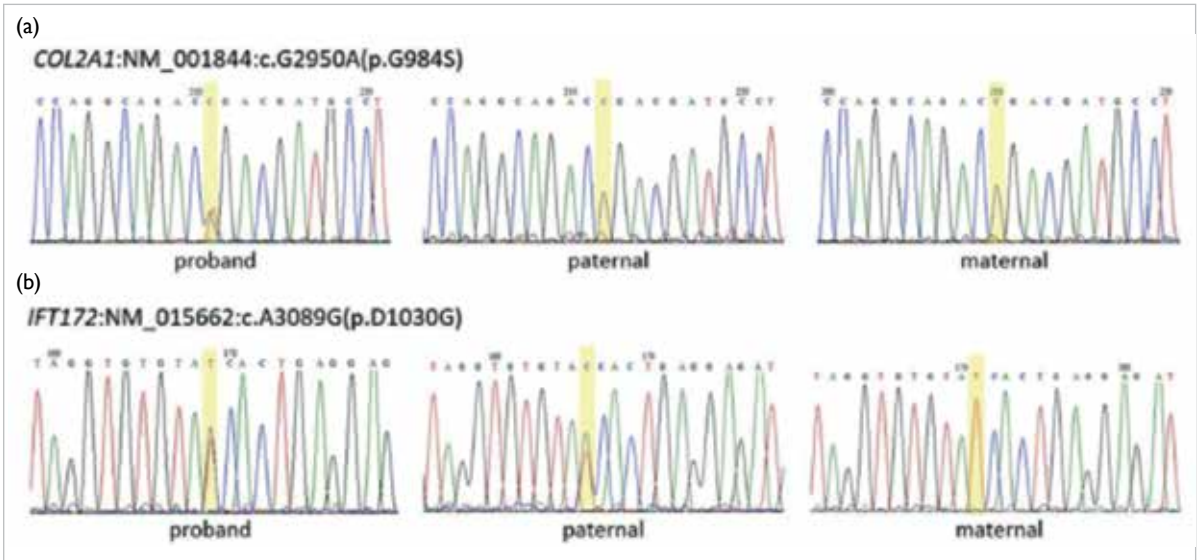

FIG 1. Comprehensive definition of the genetic aetiologies in 18NT0003. Whole-genome sequencing with parental confirmation reported: (a) a de novo heterozygous mutation NM_001844:c.G2950A(p.G984S) in COL2A1, (b) a paternal heterozygous mutation NM_015662:c.A3089G(p.D1030G) in IFT172.

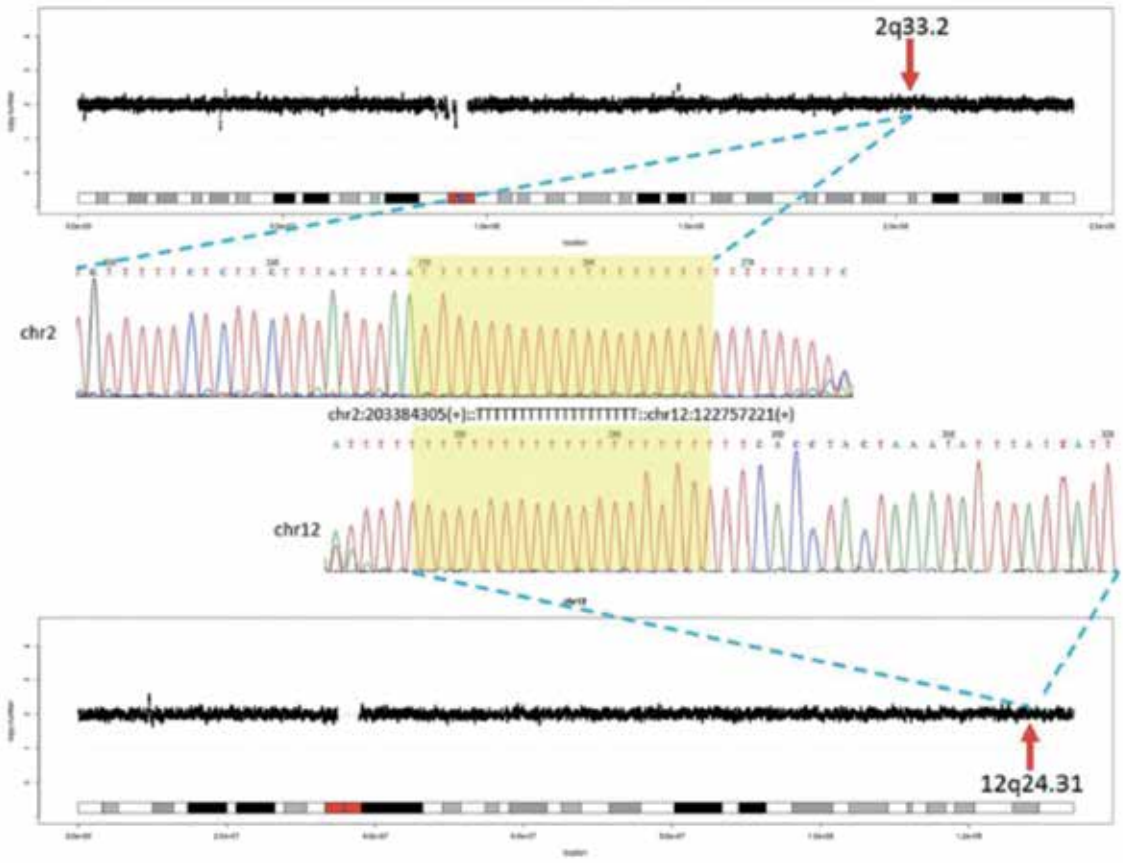

FIG 2. Distributions of copy ratios in chromosome 2 and chromosome 2 (top) and chromosome 12 (bottom). The inserted site in chromosome 2 and the rearranged segments in chromosome 12 are indicated by red arrows with the band numbers; both regions are copy number neutral. Black dots indicate the copy ratio in each window across different chromosomes. The X- and Y-axes represent the genomic coordinates and the copy ratios, respectively. Dotted grey lines indicate the copy numbers 0, 1, 3, and 4. Sanger sequencing results of the breakpoints are shown in the middle. Forward sequencing is shown on the top, whereas the reverse complementary sequencing result of the reverse sequencing is shown at the bottom. The breakpoint coordinates in chromosome 2 and chromosome 12 are shown with the aligned orientation, whereas the inserted sequence is remarked in between and highlighted in yellow in both Sanger sequencing results.

Acknowledgements

We thank the clinical colleagues for their support in recruiting cases. We also thank the patients for their supports and contributions and all other colleagues for their generous help and hard work.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#04152666). 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. Choy KW, Wang H, Shi M, et al. Prenatal diagnosis of fetuses with increased nuchal translucency by genome sequencing analysis. Front Genet 2019;10:761.

Dynamic change of LSM-HCC score and enhanced liver fibrosis score to predict hepatocellular carcinoma in chronic hepatitis B patients receiving antiviral treatment: abridged secondary publication

GLH Wong *, VWS Wong

KEY MESSAGES

1. A two-step algorithm combining LSM-HCC score and ELF score could improve the accuracy of predicting HCC in CHB patients after antiviral treatment.
2. The accuracy of predicting the risk of HCC after antiviral therapy may be further improved.
3. Clinicians could use this two-steps algorithm to better differentiate HBV patients and give a more specific surveillance method, which may save medical resources and help to decrease excessive medical care.
4. Better monitoring for CHB patients with the strategies of three-level prevention would improve the survival of patients and get economic benefits.
5. Further studies are needed to define the role of this algorithm to guide the need of intensity of HCC surveillance.
6. A prospective cohort study or a randomised controlled trial comparing this algorithm with existing recommendations would be warranted.

Hong Kong Med J 2022;28(Suppl 1):S11-3
HMRF project number: 13140651

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Introduction

Advanced fibrosis and cirrhosis are major risk factors of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC).[1] Liver biopsy is often used to assess liver fibrosis in clinical trials,[2] but its invasiveness and potential complications limit its use. Transient elastography is widely used to assess liver fibrosis because of its non-invasive nature and reproducibility.[3] Liver stiffness measurement (LSM) based on transient elastography has an accuracy of >90% in diagnosing cirrhosis; LSM is also an important prognostic tool to predict HCC.[4] Enhanced liver fibrosis (ELF) is another non-invasive assessment for liver fibrosis, based on an algorithm that comprises three serum biomarkers. Combining LSM and ELF improves diagnostic accuracy for liver fibrosis by reducing the number of patients falling into the grey zone of LSM.[5] We aimed to evaluate the accuracy of a two-step algorithm that combined the LSM-HCC score and the ELF score to predict HCC in patients with chronic hepatitis B (CHB) receiving antiviral treatment.

Methods

Since 2006, we have performed transient elastography for patients with CHB. We classified the patients according to the LSM-HCC score, which comprises four parameters (age, serum albumin and HBV DNA levels, and LSM) and is very sensitive to predict HCC risk in 3 years.[6] Patients who were classified as at intermediate or high risk of HCC (LSM-HCC score of 11-20 and 21-30, respectively) at the first examination were invited to undergo repeated transient elastography at least 3 years later to assess the dynamic change of the LSM-HCC score.

On the day of examination, fasting blood sample was collected and serum was stored at -80°C. Serum aminoterminal propeptide of type III procollagen, hyaluronic acid, and tissue inhibitor of matrix metalloproteinase type-1 were measured using a CE-marked random-access automated clinical immunochemistry analyser that performs magnetic separation enzyme immunoassay tests (ADVIA CentaurTM, Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

The ELF score was calculated using the algorithm recommended in the CE-marked assay (ELF=2.278+0.851 ln(hyaluronic acid)+0.751 ln(propeptide of type III procollagen)+0.394 ln(tissue inhibitor of matrix metalloproteinase type-1)].[7] The optimal cutoff values proposed by the manufacturer are 7.7 and 9.8 to stratify patients into none-to-mild fibrosis, moderate fibrosis, and severe

TABLE 1. Clinical characteristics of patients

	Baseline (n=453)*	Year 3 (n=453)*	P values
Male sex	337 (74.4)		
Age, y	51.7±10.3	54.7±10.3	
Body mass index, kg/m^2	24.8±3.7	25.2±4.0	0.761
Chronic alcohol use	18 (4.0)	21 (4.6)	0.743
Chronic smoking	39 (4.0)	33 (6.0)	0.539
Haemoglobin, g/dL	14.3±1.5	14.3±1.5	0.061
White cell count, ×10^9/L	6.1±1.9	6.0±2.2	0.591
Platelet count, ×10^9/L	180.6±66.1	170.8±55.7	<0.001
Albumin, g/L	42.6±3.6	43.4±3.7	<0.001
Total bilirubin, μmol/L	17.0±19.4	14.7±13.8	0.028
Alanine aminotransferase, IU/L	120.8 (41-115)	35.9 (22-42)	<0.001
American Association for the Study of Liver Diseases			
≤upper limit of laboratory normal	63 (13.9)	257 (57.1)	<0.001
>upper limit of laboratory normal	389 (86.1)	193 (42.9)	
Asian Pacific Association for the Study of the Liver			
≤upper limit of laboratory normal	112 (24.8)	330 (73.3)	<0.001
>upper limit of laboratory normal	340 (75.2)	120 (26.7)	
Creatinine, μmol/L	90.6±84.0	90.8±85.0	0.960
International normalised ratio	1.1±0.1	1.0±0.1	<0.001
Positive hepatitis B e antigen	155 (36.1)	97 (26.7)	<0.001
Hepatitis B virus DNA, log10 IU/mL	5.6±1.6	3.8±1.8	<0.001
Enhanced liver fibrosis panel			
Hyaluronic acid, ng/mL	163.9±201.4	102.9±159.9	<0.001
Propeptide of type III procollagen, ng/mL	14.8±14.6	9.6±4.3	<0.001
Tissue inhibitor of matrix metalloproteinase type-1, ng/mL	256.1±100.0	212.8±59.1	<0.001
Enhanced liver fibrosis score	10.24±1.16	9.47±1.06	<0.001
Transient elastography			
Liver stiffness measurement, kPa	14.0 (9.0-16.1)	8.7 (5.1-10.3)	<0.001
Interquartile range / liver stiffness measurement ratio	0.16±0.08	0.16±0.07	0.765
Success rate of acquisition, %	90.88±13.35	92.46±11.87	0.048
Aminotransferase platelet ratio index	3.33±20.65	0.62±0.49	0.024
Fibrosis-4 index	4.05±15.36	2.09±1.32	0.151
Forns index	8.53±1.95	9.21±1.45	0.235
Maintained virologic response	388 (85.65)		
Antiviral treatment			
Entecavir	414 (91.4)		
Tenofovir disoproxil fumarate	39 (8.6)		
Previous treatment exposure	53 (11.7)		
Antiviral treatment duration, months	47.2±13.3		

* Data are presented as mean ± standard deviation, median (range), or No. (%) of patients

fibrosis. In addition, LSM was performed using transient elastography (Fibroscan, Echosens, Paris, France). The LSM was considered reliable only if 10 successful acquisitions were obtained, and the ratio of interquartile range over LSM (IQR/LSM) was ≤0.3. The liver stiffness was expressed in kiloPascal (kPa).

Results

Of 1555 patients with CHB who underwent transient elastography between 2006 and 2008, 470 with intermediate and high risk of HCC (defined by LSM-HCC score) were invited to undergo repeated transient elastography at least 3 years later. Of them, 55 with HCC, 68 without antiviral treatment, 37 refusal to participate, and 11 with missing data were excluded. In addition, of 1072 patients with CHB who underwent transient elastography from 2009 onwards, 265 with intermediate and high risk of HCC were invited. Of them, 31 with HCC, 44 without antiviral treatment, 33 refusal to participate, and 3 with missing ELF data were excluded. The final number of patients recruited for repeated transient elastography and ELF test were 453.

Compared with the baseline results, the follow-up results had a lower platelet count (180.6±66.1 vs 170.8±55.7 ×10^9/L, P<0.001), higher serum albumin (42.6±3.6 vs 43.4±3.7 g/L, P<0.001), lower alanine aminotransferase (120.8 [IQR, 41.0-115.0] vs 35.9 [IQR, 22.0-42.0] IU/L, P<0.001), and lower HBV DNA (5.6±1.6 vs 3.8±1.8 log10 IU/mL, P<0.001) [Table 1].

Based on the LSM-HCC score, at baseline, 56.3% and 43.7% of patients were at intermediate- and high-risk of HCC, respectively. At the follow-up assessment, 56.3% of patients were at low risk, whereas 28.8% and 14.9% of patients were at intermediate- and high-risk of HCC, respectively. The difference in proportions of risk categories was significance (P<0.001). 71.4% of patients had improved LSM-HCC score, whereas 24.3% and 4.3% of patients had static and deteriorated LSM-HCC score, respectively.

Based on the ELF score, at baseline, 61.6%, 38.0%, and 0.4% of patients had severe, moderate, and mild liver fibrosis, respectively. At the follow-up assessment, the proportions were 31.3%, 66.2%, and 2.4%, respectively (P<0.001). ELF score improved, remained static, and deteriorated in 36.9%, 57.8%, and 5.3% of patients, respectively.

Data of other non-invasive markers, namely APRI, FIB-4, and Forns index were analysed in a subgroup of patients with complete parameters of the serum formulae. The changes in APRI, FIB-4, and Forns index results were of similar trend of ELF.

The cutoff of 20 in LSM-HCC score at baseline had 53.3% sensitivity and 57.4% specificity to predict HCC. The cutoff of 9.8 in ELF score at baseline had

75.6% sensitivity and 40.0% specificity. A combined LSM-HCC and ELF score had an improved sensitivity of 86.7% yet lower specificity of 29.7%, with the highest negative predictive value (95.3%), compared with that of ELF score (93.7%) and that of LSM-HCC score (91.8%). Using the combined LSM-HCC-ELF score, the number of patients with HCC missed was reduced (13%, 6 of 45 HCC patients), compared with the LSM-HCC score alone (47%, 21 of 45 HCC patients) and ELF score alone (24%, 11 of 45 HCC patients) [Table 2].

We therefore propose a stepwise algorithm combining LSM-HCC and ELF scores to predict HCC. Patients with intermediate risk, based on the LSM-HCC score, should be assessed using the ELF score. Of 255 patients with intermediate risk at baseline, according to the ELF score, 127 were classified as low or intermediate risk and 128 were classified as high risk at follow up. HCC occurred in 6 (4.7%) of 127 patients and 15 (11.7%) of 128 patients, respectively. Of 198 patients at high risk of HCC defined by LSM-HCC score, 24 (12.1%) had HCC.

TABLE 2. Liver stiffness measurement - hepatocellular carcinoma (LSM-HCC) score and enhanced liver fibrosis (ELF) score of patients

	Baseline*	Year 3*	P values
LSM-HCC score			<0.001
Low risk (0-10)	-	238 (56.3)	
Intermediate risk (11-20)	255 (56.3)	122 (28.8)	
High risk (21-30)	198 (43.7)	63 (14.9)	
Change in LSM-HCC score			
Improved	302 (71.4)		
Static	103 (24.3)		
Deteriorated	18 (4.3)		
ELF score			<0.001
None to mild fibrosis (<7.7)	2 (0.4)	11 (2.4)	
Moderate fibrosis (7.7-<9.8)	172 (38.0)	300 (66.2)	
Severe fibrosis (≥9.8)	279 (61.6)	142 (31.3)	
Change in ELF score			
Improved	167 (36.9)		
Static	262 (57.8)		
Deteriorated	24 (5.3)		

* Data are presented as No. (%) of patients

Discussion

The dynamic changes of risk of HCC in patients with antiviral treatment are mostly related to liver fibrosis regression and viral suppression, both of which result in reduced HCC risk.[5] The accuracy of predicting the risk of HCC after antiviral therapy may be further improved by this two-step algorithm, which can better differentiate patients with HBV and give more specific surveillance to save medical resources and decrease excessive medical care. Better monitoring of patients with CHB using the strategies of three-level prevention would improve patient survival and economic benefits.

Conclusion

A two-step algorithm combining LSM-HCC score and ELF score could improve the accuracy of predicting HCC in patients with CHB after antiviral treatment.

Acknowledgements

We thank Prof Henry Lik-Yuen Chan for sharing his idea on the conception and design of the study and the development of methodology. We also thank Dr Lilian Y Liang for clinical and laboratory data collection and analysis, Mr Yee-Kit Tse for his support on statistical analysis.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#13140651). 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. Liang LY, Wong VW, Tse YK, et al. Improvement in enhanced liver fibrosis score and liver stiffness measurement reflects lower risk of hepatocellular carcinoma. Aliment Pharmacol Ther 2019;49:1509-17.
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Functional tumour volume and peritoneal carcinomatosis to identify suitable candidates for cytoreductive surgery in ovarian carcinoma: abridged secondary publication

EYP Lee *, ESK Hui, HYS Ngan

KEY MESSAGES

1. Peritoneal carcinomatosis (PC) is a negative predictor of achieving complete cytoreduction in ovarian carcinoma.
2. Functional peritoneal cancer index (fPCI) is a novel non-invasive score based on the functional tumour volume derived from diffusion-weighted imaging (DWI) and the number of critical sites affected by PC.
3. fPCI is highly correlated with surgical PCI derived from laparotomy.
4. fPCI could predict the likelihood of complete cytoreduction with high accuracy in ovarian carcinoma.

Hong Kong Med J 2022;28(Suppl 1):S14-6
HMRF project number: 03143616

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Introduction

The primary treatment for ovarian carcinoma is complete cytoreductive surgery (CRS). However, an upfront CRS is not always achievable due to the burden of peritoneal carcinomatosis (PC) and the disease extent. Neo-adjuvant chemotherapy followed by interval debulking surgery could be an alternative. PC is a negative predictor of achieving complete CRS. The best method to assess surgical resectability is by evaluating PC in the intraperitoneal cavity through laparotomy or laparoscopy. Peritoneal cancer index derived at surgery (sPCI) is a validated score, which measures the extent of PC. A high sPCI and involvement of critical sites by PC in and around the mesenteric vessels, bowel serosa, and porta hepatis reduce the likelihood of successful CRS.

Computed tomography is the most utilised non-invasive imaging modality in pre-operative evaluation of ovarian carcinoma and PC. A predictive model that incorporated information of PC size and distribution has been proposed to determine surgical resectability and predict the likelihood of achieving complete CRS. However, this predictive model is dependent on the radiologist's experience and a degree of subjectivity in the assessment, and hence it has not been generalisable to other cohorts.[1]

Diffusion weighted imaging (DWI) is a functional sequence on magnetic resonance imaging (MRI). It is superior to computed tomography in tumour characterisation, staging and depiction of PC. The derived apparent diffusion coefficient (ADC) reflects tumour cellularity and disease aggressiveness. Therefore, DWI has the potential in quantifying PC burden and in predicting the likelihood of surgical success.

The aims of our study were (1) to construct a new method in assessing PC tumour burden by incorporating functional tumour volume (FTV) derived from DWI and the PC spread pattern to form the functional peritoneal cancer index (fPCI); (2) to design a predictive model consisting of fPCI and clinical factors for predicting the likelihood of achieving complete CRS / interval debulking surgery; and (3) to investigate the relationship between tumour biology of PC and surgical resectability.

Methods

Patients with advanced (FIGO III/IV) or recurrent ovarian carcinoma were assessed with DWI before CRS / interval debulking surgery. Clinicopathological information including age, FIGO staging, and pre-surgical (time interval, <14 days) serum cancer antigen 125 (CA-125) level were documented.

Standard T2-weighted imaging was performed using a 3.0 T MRI platform for the abdominopelvic area in two planes with additional sagittal plane covering the pelvis. DWI was acquired with 3 *b* values (0, 400, 800 s/mm^2) in free breathing in the axial plane, with the same anatomical coverage as the conventional sequences. The ADC maps were constructed using Matlab R2019a (The MathWorks,

Natick, Massachusetts, USA).

During surgery, patients were assessed for PC burden using sPCI. The abdominopelvic cavity was arbitrarily divided into 13 regions (right upper quadrant, epigastrium, left upper quadrant, right flank, umbilical region, left flank, right lower quadrant, pelvis, left lower quadrant, mesentery and serosa in upper jejunum, lower jejunum, and upper ileum and lower ileum). A sPCI score of 0 was given when there was no PC. When PC was present, the largest one lesion was selected as the target lesion and was scored as 1 when <0.5 cm, 2 when 0.5-5.0 cm, and 3 when >5.0 cm. The immediate surgical outcome was dichotomised into complete and incomplete cytoreduction. The surgical duration and the number of surgical subspecialties involved were documented.

Volumes of interest for the calculation of FTV (VOIs-FTV) was drawn on all slices that the tumour existed in for all PC lesions on b=800 s/mm^2 and transferred to ADC maps. Using K-means clustering, the intermediate ADC cluster of each patient was regarded as the solid tumour component with high cellularity and was used for FTV calculation. The high ADC cluster was considered as normal or cystic tissues and low ADC cluster was regarded as fibrous or fat tissues, which were subsequently discarded.[2] A score of 0 to 3 was assigned to each region as in sPCI and additional points were given to each critical site involved by PC at the mesentery, serosa or porta hepatis to form the fPCI.

The largest PC lesion of each patient was chosen as the target lesion for the measurement of ADC. Volumes of interest for the measurement of ADC (VOIs-ADC) were drawn very strictly along the margin on all slices that the tumour existed on b = 800 s/mm^2 images and transferred to ADC maps. The average of ADC within VOIs-ADC was calculated.

The correlations were assessed by Pearson coefficient correlation (r). Univariate and multivariate logistic regressions were used to generate prediction model that included fPCI, sPCI or ADC as predictors combined with age, FIGO staging and pre-surgical CA125 level. The receiver operating characteristic (ROC) curves were generated. A P value of <0.05 was considered statistically significant.

Results

A total of 51 patients aged 20 to 82 (mean, 56) years with advanced or recurrent ovarian carcinoma were included in the analysis. Complete cytoreduction was achieved in 33 (64.7%) patients, and the remaining 18 (35.3%) patients had incomplete cytoreduction.

Significant correlations were found between fPCI and sPCI on patient-based analysis (r=0.774, P<0.001) and region-based analysis (r=0.777, P<0.001). The mean fPCI and sPCI were significantly higher in the patients with incomplete cytoreduction than patients with complete cytoreduction (10.00 vs 4.36 and 12.06 vs 5.70, respectively, both P<0.05); whereas the opposite was found in ADC (1.30 vs 1.52, P=0.040, Fig 1). Both fPCI and sPCI were moderately correlated with surgical duration (r=0.524, P<0.001 and r=0.632, P<0.001, respectively), but only modestly with the number of surgical subspecialties involved (r=0. 402, P=0.003 and r=0.396, P=0.004, respectively).

fPCI, sPCI and ADC were significant in univariate analysis (P<0.05). Age, staging, and pre-surgical CA125 levels were identified as independent prognostic variables. Multivariable models of fPCI, sPCI, and ADC, each including the previously identified 3 clinicopathological factors, could achieve accuracy of 92.2%, 80.4%, and 74.5%, respectively (area under ROC curves were 0.956, 0.909, and 0.788, respectively). There was no significant difference between fPCI and sPCI in predicting surgical outcome (P=0.202), but both outperformed ADC (fPCI vs ADC, P=0.007; sPCI vs ADC, P=0.034, Fig 2).

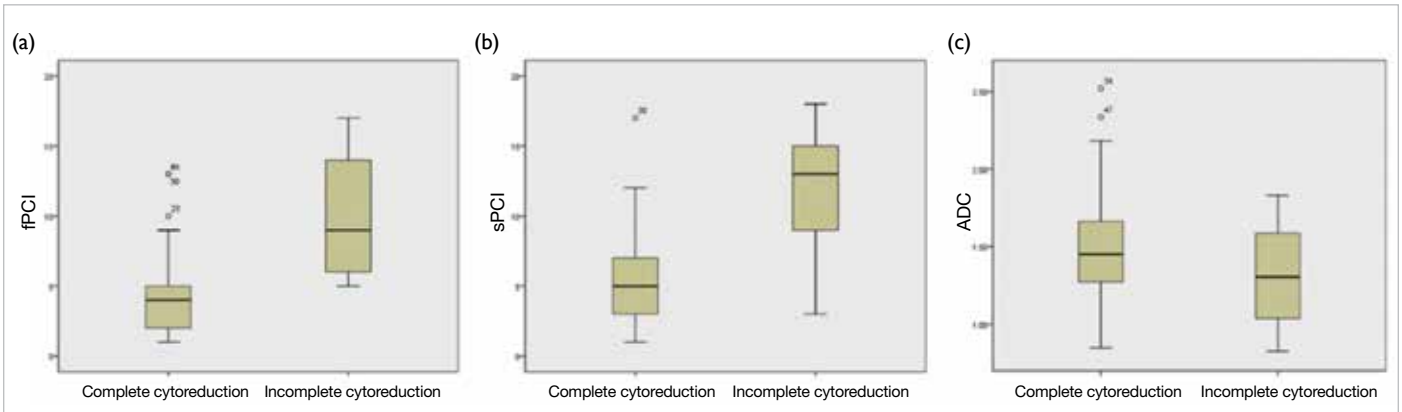

FIG 1. Comparison of (a) functional peritoneal cancer index (fPCI), (b) surgical peritoneal cancer index (sPCI), and (c) apparent diffusion coefficient (ADC) in patients with complete and incomplete cytoreduction.

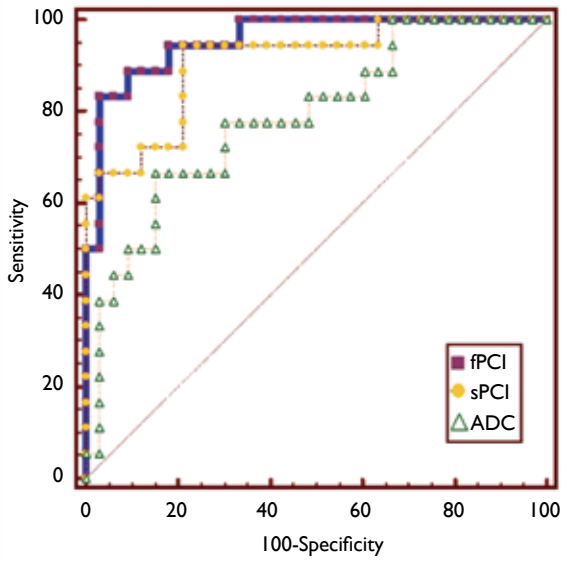

FIG 2. Receiver operating characteristic curves for functional peritoneal cancer index (fPCI), surgical peritoneal cancer index (sPCI), and apparent diffusion coefficient (ADC) in predicting tumour resectability.

Discussion

Our novel method of delineating PC using DWI is done by assessing PC tumour burden and disease spread and quantifying this information into an fPCI. A scoring system based on fPCI can predict the likelihood of complete cytoreduction in advanced and recurrent ovarian carcinoma.

K-means clustering has been shown to be able to differentiate malignant and benign tissues: the presence of benign fibrous tissues containing dense networks of collagen fibres and abundant collagen-producing fibroblastic cells would decrease the ADC value, whilst oedema, haemorrhage and cysts with hypocellular areas of loose oedematous connective tissue would result in a higher ADC value.[2] Hence, the intermediate ADC cluster that represented tumour with high cellularity was subsequently used to calculate the FTV.

fPCI has similar predictive value to sPCI in determining the likelihood of achieving complete cytoreduction.[3] fPCI has high agreement with sPCI.[4] In addition, we evaluated a semi-automated method in depicting tumour volume based on tumour cellularity. Considerations were given to critical sites and other clinicopathological factors that could impact surgical success.

Conclusion

fPCI, a non-invasive scoring system, can provide a quantitative means of evaluating tumour burden and disease spread. It has high correlation with sPCI in advanced or recurrent ovarian cancer. fPCI and ADC derived from DWI achieve a moderate-to-high accuracy in predicting success of cytoreduction, potentially aiding in patient's selection for cytoreduction and treatment stratification.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#03143616). 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:

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Effectiveness of the iParent app for postnatal depression: a randomised controlled trial (abridged secondary publication)

KL Chan *, WC Leung, A Tiwari, KL Or, P Ip

KEY MESSAGES

1. Pregnant women who used the iParent app reported a significantly lower level of postnatal depression than those who received treatment as usual after adjusting for demographic characteristics and baseline depression levels.
2. The iParent app is a low-cost, user-friendly alternative to the traditional, resource-consuming antenatal classes.

Hong Kong Med J 2022;28(Suppl 1):S17-8
HMRF project number: 15161101

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Introduction

Antenatal depression and postnatal depression (PND) affect maternal and infant health. Effective interventions to reduce the risk of depression related to pregnancy are necessary. Traditional interventions (regular antenatal check-ups and classes and other forms of pregnancy care provided by medical, nursing, or social work professionals) are useful to improve maternal and infant well-being.[1] Yet, these often involve a face-to-face meeting and hence are resource consuming, with limited numbers of recipients at a time.

Mobile health (mHealth) technologies have been widely applied in developed countries to reach out to families with pregnant women. These technologies include regular text messages, emails, platforms or forums for networking and knowledge exchange, and interactive smartphone apps, which may promote a better preparation for parenthood and a greater sense of social support.[2,3] A meta-analysis demonstrated moderate to large effect sizes of mHealth antenatal interventions to improve maternal mental health and physical health.[4] We developed the iParent app to disseminate essential knowledge for pregnancy and infant care to pregnant women. This study aimed to evaluate the effectiveness of the iParent app in reducing maternal PND in pregnant women in Hong Kong.

Methods

This study was a single-blind, prospective, randomised, two-armed (1:1) controlled trial conducted at Kwong Wah Hospital in late 2017 and 2018. First-time pregnant women with <24 weeks' gestation were recruited. Those who were younger than 18 years, unable to understand written Chinese or English, or unwilling to provide informed consent were excluded.

The iParent app comprised two main components: (1) essential materials for pregnancy and infant care in the form of short video clips and brief written passages with pictures, and (2) an interactive platform for pregnant women to seek private advice from obstetricians without time and space constraints. The materials were the same as those distributed in print form during traditional antenatal classes.

A total of 660 pregnant women were assigned at random to the intervention or control group using lots in a ratio of 1:1. Those in the intervention group were given access to the iParent app (with a specific login name and password) in addition to the treatment as usual (antenatal check-ups and the optional face-to-face classes held by midwives). The intervention lasted for about 20 weeks. Prompts were made via emails if the participants did not log in to the app. The control group received treatment as usual.

Participants were assessed at baseline (T1) for antenatal depression and demographic characteristics. Participants were contacted again approximately 4 weeks after childbirth (T2) for assessment of PND.

The primary outcome was the difference between the levels of antenatal depression at T1 and PND at T2. Antenatal and postnatal depressive symptoms were measured using the Chinese version

of the 10-item Edinburgh Postnatal Depression Scale (EPDS).[5] Total scores range from 0 to 30; higher scores indicate more severe depression. The Chinese EPDS has good reliability (Cronbach's alpha=0.84-0.85), Participants' age, education attainment, employment status, marital status, household income, and presence of diagnosed chronic physical and mental health conditions were also recorded.

Intention-to-treat principle was used. Missing data were treated by the last observation carried forward imputation method. To test the effectiveness of the iParent app in reducing depression, analysis of covariance was conducted to compare the differences in EPDS scores between the intervention and the control groups with adjustment of demographic characteristics. All analyses were performed using SPSS (Windows version 24; IBM Corp, Armonk [NY], US), with a significance level of 0.05.

Results

A total of 660 pregnant women (response rate, 82%) completed the T1 baseline survey and were evenly randomised to either the intervention group or the control group. At T2, 218 women in the intervention group and 225 women in the control group completed the follow-up survey (retention rate, 66% and 68%, respectively). No significant differences in demographic characteristics were found between those who completed both T1 and T2 surveys and those who withdrew.

The intervention and control groups were comparable in terms of maternal age (31.3±4.6 vs 31.2±4.5 years), being married or cohabiting with a partner (88% vs 92%), recipient of social security assistance (5% vs 3%), chronic physical health condition (5% vs 7%), and mental illness (1% vs 0%). The intervention group had a significantly greater reduction of EPDS scores (7.3±4.6 vs 7.2±4.6 at T1 and 5.3±4.4 vs 5.9±4.7 at T2, adjusted mean differences= -0.65 [95% confidence interval= -1.29-0.00, P<0.05).

Discussion

After approximately 20 weeks of using the iParent app in addition to the treatment at usual antenatal care services, pregnant women reported a significantly greater reduction in the severity of PND, compared with those with treatment as usual alone. Our findings support the usefulness of the iParent app as a low-cost alternative to traditional face-to-face educational or pregnancy care services. Through the iParent app, pregnant women and their partners could receive essential information about pregnancy and infant care without limitations on time and location. The mobility and portability of the app, together with the enhanced sense of connection with health professionals, might then promote better preparation for parenthood by providing a user-friendly platform to engage pregnant women and their partners.

Conclusion

The iParent app is useful as a low-cost, user-friendly means of antenatal care service.

Acknowledgements

The authors appreciate the contribution of the team of midwives at Kwong Wah Hospital for assisting with data collection.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#15161101). 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. Chan KL, Leung WC, Tiwari A, Or KL, Ip P. Using smartphone-based psychoeducation to reduce postnatal depression among first-time mothers: randomised controlled trial. JMIR Mhealth Uhealth 2019;7:e12794.

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Adjunctive light treatment in major depressive disorder among evening chronotype: a randomised controlled trial (abridged secondary publication)

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KEY MESSAGES

1. Home-based bright light therapy is a feasible adjunctive treatment with a highly tolerable adverse effect profile.
2. Home-based bright light therapy with gradual timing advance improves clinical outcome, with a quicker treatment effect, which is evident from a higher remission rate at week 2 of treatment and a higher cumulative remission rate for 5 months after treatment.
3. Adjunctive bright light therapy for patients with unipolar non-seasonal depression and evening-chronotype may be used in clinical practice to improve clinical outcomes.

Hong Kong Med J 2022;28(Suppl 1):S19-22
HMRF project number: 12131131

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Introduction

Major depressive disorder (MDD) is a common mental illness with significant morbidities and mortalities. In patients with MDD, the non-remission rate (as defined by a Hamilton Depression Rating Scale-17 [HRSD 17] score of ≥8 and the presence of a major depressive episode by the Mini International Neuropsychiatric Interview) was 42.3%, despite active treatment.[1] About 20% of subjects were evening chronotype as classified by the Morningness and Eveningness Questionnaire (MEQ), and they had more severe insomnia, worse depressive symptoms, and higher suicidality.[1] The evening-type patients also had higher non-remission rate (65.3%) than the morning-type (38.7%) or intermediate-type (35.9%). Non-remission of depression is associated with a range of adverse outcomes. A more intensive alternative or adjunctive treatment to improve the outcomes in this subgroup of patients is needed.

Light therapy has been demonstrated to be effective in two randomised controlled trials on non-seasonal depression.[2,3] Both studies showed that light therapy was associated with lower depressive symptoms score and a higher remission rate. Apart from improving mood symptoms, light is effective in phase-shifting the human circadian rhythm. Evening chronotype patients may be additionally benefited from the phase advancing effect of the light therapy. This study aims to examine the effectiveness of adjunctive light therapy with gradual advanced timing in evening-type patients with non-seasonal depression. We hypothesised that the bright light treatment (BLT) group would have a higher remission rate and a lower depression score than the dim red light (DRL) group.

Methods

Patients were recruited from the psychiatric outpatient clinic of the university-affiliated hospital. Ethical approval was obtained from the Joint CUHK-NTEC Clinical Research Ethics Committee (reference no: 2014.505-T) and the trial was registered with the Chinese clinical trial registry (ChiCTR-IOR-15006937).

This study was a home-based, randomised, assessor- and prescriber-blind trial. Usual psychotropic medications prescribed from the outpatient clinic, including antidepressants and hypnotics, and the prescriptions for the general medical conditions were allowed. Eligible subjects were randomly assigned to either the bright light therapy (BLT) group or the dim red light (DRL) group. Light therapy was prescribed for 30 minutes daily at their habitual wake time, which was based on the 1-week sleep diary before the start of treatment. Subjects were required to record the timing of light therapy daily. Compliance was defined on two levels: (1) day-compliance: the percentage of the number of days with light therapy over the past week, irrespective of the timing and (2) appropriately timed light therapy defined as >50% of the total weekly duration of light therapy received over the past treatment week with timing overlapped with or earlier than the prescribed time of light therapy.

TABLE 1. Baseline characteristics, medication use, and sleep parameters of the dim red light group and bright light treatment group

	Dim red light group (n=45)*	Bright light treatment group (n=46)*	P value
Age, y	45.2±12.0	47.4±11.5	0.38
Female sex, %	88.9	69.6	0.02
Duration of depression, y	13.5±11.2	13.9±11.0	0.86
Hamilton Rating Scale for Depression	19.9±5.90	18.6±7.63	0.35
Insomnia Severity Index	16.8±5.56	17.7±6.43	0.49
Hamilton Anxiety Rating Scale	22.9±9.80	20.8±11.4	0.27
Morningness Eveningness Questionnaire	34.7±7.05	36.0±6.56	0.34
Beck Scale for Suicide Ideation	11.5±7.06	11.8±5.65	0.85
Chalder Fatigue Scale	20.3±6.34	20.2±7.51	0.81
Short-Form 36 Health Survey	266.2±99.2	306.2±106.5	0.07
Hospital Anxiety and Depression Scale	22.4±6.08	20.4±6.22	0.13
Young Mania Rating Scale	0.80±1.45	0.83±1.52	0.93
Expectation score	59.1±17.1	62.9±21.7	0.35
Medications, %			
Antidepressants	77.3	76.1	0.89
>1 type of antidepressant	31.8	23.9	0.40
Antipsychotics	22.7	26.1	0.71
Mood stabilisers	4.5	13.0	0.16
Benzodiazepines	40.9	45.7	0.65
Hypnotics	29.5	26.1	0.71
Sleep parameters			
Time to go to bed	01:24±02:00	01:18±01:39	0.80
Time to sleep	02:03±1:59	01:53±1:26	0.63
Wake up time	09:37±2:23	09:31±2:22	0.98
Time getting up from bed	10:29 ±02:00	10:21±02:02	0.77
Time in bed	8:59±1:25	9:07±1:43	0.72
Wake after sleep onset	0:37±0:40	0:35±0:45	0.98
Actual sleep time	7:38±1:30	7:40±1:39	0.92
Sleep efficiency	0.85±0.13	0.84±0.11	0.86
Sleep midpoint	5:50±2:02	5:41±1:45	0.72

* Data are presented as mean standard deviation or % of patients

The timing of light therapy was gradually advanced 30 minutes each week if the subject was able to reach 50% appropriately timed light therapy until a desirable wake time was achieved. If the subject was not able to adhere to the prescription, the timing would be kept the same and no advancement would be made. A prescriber reviewed the sleep diary and light therapy record weekly. An independent clinical assessor assessed the patients at baseline and at each follow-up (weekly during the 5-week treatment period, and at 1 week, 1 month, 2 months, and 5 months after treatment). Both the prescriber and assessor were blinded to the group allocation. The study subjects were instructed not to reveal their allocated treatment to both prescriber and assessor.

The primary outcomes measures were the rate of remission (as defined by HRSD 17 scoring ≤7) and the change of HRSD 17 score. Secondary outcomes included anxiety symptoms measured by Hamilton Anxiety Rating Scale, Hospital Anxiety and Depression Scale, insomnia symptoms by Insomnia Severity Index, suicidal ideation by Beck Scale for Suicide Ideation, fatigue by Chalder Fatigue Scale, and quality of life by Short Form-36 Health Survey. Adverse outcomes were monitored by a modified adverse event checklist. Young Mania Rating Scale was used to monitor for possible hypomanic/manic symptoms. At baseline, subjects were asked to give an expectation score towards treatment efficacy in reducing depressive symptoms on a Likert scale from 0 to 100; higher scores indicated higher positive expectations.

All analyses were based on a modified intent-to-treat model: subjects with at least one follow-up assessment were included. Chi squared analysis and *t* tests were used to compare baseline characteristics. Treatment effects on the outcome variables were analysed using repeated measure ANOVA. All tests were based on a 0.05 level of significance. The differences between the DRL group and BLT group in terms of rates of remission and response were tested by binary logistic regression. Statistical analyses were performed using SPSS (IBM Corp, Armonk [NY], US).

Results

The DRL and BLT groups were comparable in terms of baseline clinical characteristics, medication use, and sleep parameters, except that there was a female preponderance in the DRL group (88.9% vs 69.6%, P=0.023, Table 1).

Compared with the DRL group, the BLT group had higher (but not significantly) remission and response rates at all time points, except that at week 2 of treatment the remission rate was significantly higher (37.0% vs 13.3%, Table 2) based on both unadjusted regression model (P=0.012) and adjusted regression model (P=0.034) controlled for the baseline HRSD 17 score, age, sex, and season of enrolment. From baseline to 5 months after treatment, all clinical symptom measures had a reduction of score over time (P<0.001 for time), except for Young Mania Rating Scale and Short Form-36. However, the differences in the changes of these clinical measures between the two groups for 5 months were not significant (P>0.05 for time × intervention).

Using Kaplan-Meier curve analyses, cumulatively 31 (67.4%) of patients in the BLT group achieved remission of depression (HRSD17 score

TABLE 2. Change in clinical measures between bright light treatment (BLT) group and dim red light (DRL) group

	Intervention period						Follow-up period after treatment				P value for time	P value for time × intervention
	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5	1 week	1 month	2 month	5 month		
Remission, %												
DRL	0	13.3	13.3	20.0	24.4	20.0	28.9	22.2	20.0	26.7		
BLT	0	15.2	37.0	39.1	34.8	32.6	37.0	34.8	23.9	32.6		
Hamilton Depression Rating Scale											0.001	0.41
DRL	19.8±5.91	16.3±7.68	15.2±8.04	14.9±8.10	13.6±7.48	14.0±7.53	13.4±7.80	14.7±7.69	15.2±8.14	13.7±8.01		
BLT	18.6±7.63	16.2±7.30	12.1±7.91	11.5±7.72	12.0±8.67	11.8±7.66	10.7±7.41	11.0±7.28	12.6±7.12	12.2±8.26		
Hamilton Anxiety Rating Scale											0.001	0.51
DRL	22.9±9.80	19.0±10.4	17.8±10.2	19.4±10.8	17.6±9.94	17.6±9.23	17.4±10.7	18.8±10.7	18.0±11.0	17.4±10.7		
BLT	20.4±11.4	19.2±10.0	16.2±11.7	15.3±10.5	16.3±10.4	15.6±10.1	14.3±9.55	15.0±9.51	16.6±10.4	16.4±10.9		
Young Mania Rating Scale											0.66	0.86
DRL	0.80±1.45	0.75±1.35	0.64±1.17	0.91±1.33	0.73±1.19	0.80±1.48	0.80±1.86	0.64±1.31	1.04±2.12	0.93±1.66		
BLT	0.82±1.52	0.76±1.51	0.93±2.58	0.94±2.23	0.93±2.05	1.26±2.90	1.22±2.59	1.21±2.59	1.28±2.53	0.98±2.18		
Insomnia Severity Index											0.001	0.33
DRL	16.8±5.56	17.0±6.52	15.7±5.95	15.4±6.67	15.1±6.70	15.4±6.92	15.3±7.07	15.3±6.47	14.8±6.61	15.0±6.73		
BLT	17.7±6.43	16.7±6.40	15.6±6.62	15.1±6.08	15.4±5.98	14.5±7.08	14.1±6.60	14.1±7.04	14.8±7.09	14.3±6.28		
Hospital Anxiety and Depression Scale											0.001	0.22
DRL	22.4±6.08	22.1±6.28	21.7±6.60	21.6±6.26	20.6±6.79	20.2±6.47	21.2±6.83	21.4±7.30	21.4±6.38	21.6±6.45		
BLT	20.4±6.22	19.2±7.98	18.7±6.99	18.4±7.79	17.0±8.27	17.1±7.82	16.6±7.76	16.6±8.21	17.0±8.07	17.9±8.44		
Beck Scale for Suicide Ideation											0.001	0.38
DRL	11.5±7.06	10.7±6.89	9.98±6.98	10.3±7.15	9.43±6.59	9.24±6.91	9.76±6.98	9.48±6.97	9.81±7.12	9.43±6.80		
BLT	11.8±5.65	10.1±5.95	10.1±5.95	8.95±6.00	8.62±6.38	8.52±6.37	8.71±6.16	8.76±6.90	8.83±6.19	9.02±6.68		
Chalder Fatigue Scale											0.001	0.09
DRL	20.4±6.33	-	-	-	-	18.5±7.57	-	19.5±7.14	18.7±7.18	18.7±7.55		
BLT	20.0±7.50	-	-	-	-	16.8±7.81	-	15.5±7.52	17.3±8.10	16.1±6.83		
Short Form-36 Health Survey											0.06	0.58
DRL	244.1±92.9	-	-	-	-	266.7±114	-	272.8±156	267.7±127	272.6±145		
BLT	316.2±116	-	-	-	-	355.2±161	-	333.2±139	326.1±147	349.9±144		

≤7) at any follow-up, whereas 21 (46.7%) patients in the DRL group achieved remission (P=0.04, log rank test, Fig).

Discussion

In evening-chronotype patients, the overall remission rate was lower than that reported in other bright light trials,[2,4] consistent with our previous finding that evening-chronotype tended to be associated with a higher rate of non-remission of depression.[1] The rate of remission was significantly higher in the BLT group than in the DRL group (37.0% vs 13.3%) at week 2 in both unadjusted and adjusted models. This indicates that BLT has a quicker anti-depressant effect. Survival analysis also revealed significantly higher cumulative remission rate in the BLT group than in the DRL group (67.4% vs 46.7%).

The improvement in clinical symptom measures was greater (but not significantly) in the BLT group than in the DRL group. Several reasons may explain the lack of significant difference between groups. First, the relative short duration of light therapy may not be adequate to discern the

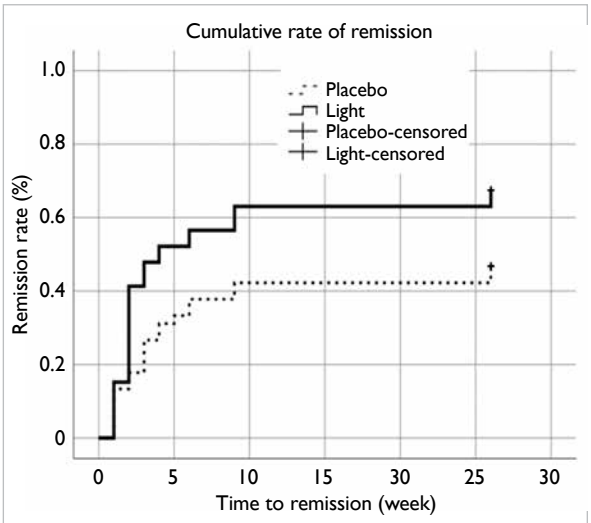

FIG. Kaplan-Meier curve analysis on depression remission between bright light treatment group and dim red light group

full effect of BLT. The duration of treatment that effectively achieved remission was 6 to 8 weeks.[2,3] In patients with bipolar depression, the remission rate greatly increased by 30% only after 4 weeks of treatment.[3] Likewise, a longer duration of each light therapy session may improve treatment outcomes. Second, the DRL group is not a pure placebo group. This group also underwent a gradual advance in light therapy and had a small advancement in their time to sleep, wake-up time, and sleep midpoint. The sleep advancement of the DRL group was comparable to that of the BLT group. This gradual advance may signify a stabilisation of sleep-wake rhythm; subjects were able to maintain a more regular and gradually advanced sleep-wake cycle. With an earlier wake time, subjects might be exposed to more environmental sunlight beneficial for the mood condition. Third, we used a more stringent definition of compliance, taking into account both the number of days with light therapy and adherence to the exact treatment timing. The overall compliance in terms of the number of days with light therapy was 66.3% and 73.5% in the DRL and BLT groups, respectively, whereas the compliance rate in other studies (albeit without precise definition) was 80% to 90%.[2-4] The inability to detect the outcome differences may be attributed to the lower compliance rate. The relatively lower compliance could be related to the recruitment of exclusive evening-type subjects who were known to be associated with poorer self-regulation.[5] Nevertheless, BLT was highly tolerable, with few adverse events. No subject had a hypomanic or manic swing. BLT is a viable adjunctive treatment, with no drug-drug interactions and with few contra-indications.

Conclusion

Adjunctive BLT with gradual timing advancement is useful in patients with non-seasonal depression and evening-chronotype, with a quicker and potentially augmented antidepressant effect.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#12131131). 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. Chan JW, Lam SP, Li SX, et al. Adjunctive bright light treatment with gradual advance in unipolar major depressive disorder with evening chronotype: a randomized controlled trial. Psychol Med 2020:1-10.
2. Chan JW, Chan NY, Li SX, et al. Change in circadian preference predicts sustained treatment outcomes in patients with unipolar depression and evening preference. J Clin Sleep Med 2021 Sep 21.

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Perioperative prophylactic internal iliac artery balloon occlusion for prevention of postpartum haemorrhage in placenta praevia: a randomised controlled trial (abridged secondary publication)

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KEY MESSAGE

Prophylactic internal iliac artery balloon occlusion during caesarean section did not reduce the postpartum haemorrhage and had no effect on the maternal and neonatal morbidity for patients with placenta praevia.

Hong Kong Med J 2022;28(Suppl 1):S23-6
HMRF project number: 03140236

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Introduction

Placenta praevia (PP) is a major cause of massive postpartum haemorrhage (PPH) and maternal mortality. 5.3% of patients with PP required hysterectomy, which is 30 times higher than those without PP.[1] The prevalence of PP and its related complications including placenta accreta spectrum have increased because of the increasing caesarean section (CS) rate, resulting in an increased cost of obstetric care.[2]

Preoperative placement of internal iliac artery (IIA) catheters followed by intraoperative arterial balloon occlusion is a popular prophylactic method to prevent PPH. After caesarean delivery of the baby and clamping of the umbilical cord, the intra-arterial balloons are inflated to occlude the arteries. They do not arrest blood flow to the uterus completely but decrease the pulse pressure distal to the occlusion site. By reducing the rate of blood loss, the haemostatic procedures could be easier and shorter. The intra-arterial catheters also allow therapeutic embolisation to be performed if bleeding persists. There are case series reporting the use of prophylactic balloon catheter insertion in PP and accreta spectrum.[3] In a randomised controlled trial on this approach in placenta accreta spectrum, the beneficial role of balloon occlusion was not supported, and procedure-related adverse effects were noted in 15.4% of cases.[4] Evidence of IIA on PP is not yet available. Hence the current study aims to determine whether prophylactic IIA balloon occlusion during CS for PP can reduce PPH and other maternal morbidity such as blood product transfusion, need of hysterectomy, and intensive care unit admission.

Methods

This was an open-label randomised controlled trial conducted at a university hospital in Hong Kong. This trial was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (CRE ref. no.: 2015.260-T). Pregnant women who were diagnosed to have a low-lying placenta in the mid-trimester morphology scan were scheduled for ultrasonography at 34 weeks of gestation to determine the placental location. Pregnant women who were diagnosed to have PP at 34 weeks and required CS were invited to participate. Exclusion criteria were (1) age of <18 years, (2) mentally handicapped or severely ill, (3) fetus diagnosed with in-utero death, (4) contraindication for IIA balloon occlusion, (5) unstable clinical condition in which patients could not be sent to interventional radiology suite for pre-operative internal IIA catheter placement, (6) unable to understand English or Chinese to give consent, and (7) ultrasonic features suggestive of placenta accreta spectrum. PP was defined as lower placenta edge within 2 cm from the internal os, and the grading of PP was defined according to the Royal College of Obstetricians and Gynaecologists guideline.[2] Participants were reassessed within 1 week before the operation, during which transvaginal ultrasonography was repeated to double-check the distance between the placental edge and the internal os. CS was arranged for women between 37 and 38 weeks of gestations if PP persisted at this gestation. Those with placental edge >2 cm away from the internal os were allowed to undergo vaginal delivery. Eligible participants were randomly assigned to either IIA balloon occlusion (occlusion group, n=20)

or standard management (control group, n=20) at 1:1 ratio.

Those randomised to the occlusion group underwent IIA balloon catheter placement in the interventional radiology suite by an interventional radiologist before CS, as described in the literature.[4] They were then sent to the operating theatre for CS. After the baby was delivered, the occlusion balloons were inflated immediately. The placenta was then delivered and CS was continued in the usual manner. After haemostasis was achieved, the balloon was deflated. If bleeding could not be well controlled and uterine artery embolisation was indicated, the balloons were left inflated until embolisation was performed by the interventional radiologist. Otherwise, the vascular sheaths were left in situ for 12-24 hours after surgery in case emergency embolisation was required. All CS of both groups were performed by a qualified obstetrician with experience and expertise in operating with PP.

The primary outcome was the reduction in intrapartum blood loss in the occlusion group. Secondary outcomes included any drop of haemoglobin level on day 1 and day 3 after the operation, the amount of blood products transfused, the incidence of hysterectomy, maternal complications (including renal failure, ischaemic liver, disseminated intravascular coagulation, and adult respiratory distress syndrome), days of stay in the hospital after the operation, admission to an intensive care unit, and maternal death.

The sample size calculation was based on a reduction of blood loss by half in the IIA balloon occlusion group as compared with the control group. Based on a previous report[3] and our observation, the average blood loss in patients undergoing caesarean sections for PP was 800-1000 mL (standard deviation, 300 mL). To detect a difference of 400 mL blood loss, with a power of 80% and a significance level of 5%, 10 subjects were required in each arm. 20% more subjects were added to the sample size because of the skewed distribution of blood loss. The sample size was increased to 16 in each arm to allow abandoning of the procedure because of unforeseen emergency. Statistical analysis was performed using the intention-to-treat (ITT) method and was repeated using the on-treatment approach.

Results

The two groups were comparable in terms of baseline characteristics, except that the occlusion group had more previous CS than did the control group (60.0% vs 10.0%, P=0.019, Table 1). Four women in the occlusion group did not have the planned IIA procedure. Three of them required emergency CS because of severe antepartum haemorrhage before the planned IIA balloon catheter insertion. They were included in the ITT analysis but were excluded from the analysis by the on-treatment method. The remaining one was reassessed at 36 weeks and showed that the placental edge was 2 cm from the internal os. She opted for a trial of vaginal delivery and thus was excluded.

In the ITT analysis, the two groups were comparable in terms of intraoperative blood loss and

TABLE 1. Baseline characteristics of the occlusion group and the control group.

Baseline characteristic	Occlusion group (n=20)*	Control group (n=20)*	P value
Maternal age, y	35.3 (31.5-37.6)	36.6 (32.7-39.1)	0.289
Gestational age, wk	36.6 (35.2-37.2)	36.1 (34.8-37.7)	0.968
Nulliparous	10 (50.0)	10 (50.0)	0.999
Previous caesarean section	6 (60.0)	1 (10.0)	0.019
Body mass index at caesarean section, kg/m^2	27.0 (24.9-29.4)	26.9 (24.5-29.7)	0.862
Assisted reproduction pregnancy	1 (5.0)	2 (10.0)	0.999
Active smoker	4 (20.0)	1 (5.0)	0.342
Gestational diabetes	3 (15.0)	4 (20.0)	0.999
Pregnancy-induced hypertension	2 (10.0)	0 (0)	0.487
Placenta praevia grade at recruitment			0.999
I & II	5 (25.0)	6 (30.0)	
III & IV	15 (75.0)	14 (70.0)	
Placental site			
Anterior	7 (35.0)	4 (20.0)	0.479
Non-anterior	13 (65.0)	16 (80.0)	
Antepartum haemorrhage before procedure	11 (55.0)	10 (50.0)	0.752

* Data are presented as median (interquartile range) or No. (%)

all other maternal and neonatal outcomes, except that the use of carboprost was significantly higher in the occlusion group than in the control group (36.8% vs 5.0%, P=0.020, Table 2). After excluding the three cases that required an emergency CS before the planned IIA procedure, the results remained the same, except that the occlusion group had a significantly lower base excess than the control group (-5.10 [-9.13 to -2.98] vs -2.90 [-5.00 to -1.00], P=0.017), but the difference was not clinically significant.

TABLE 2. Intra-operative, post-operative, neonatal, and maternal outcomes analysed using the intention-to-treat method.

Outcome	Occlusion group (n=19)*	Control group (n=20)*	P value
Intra- and post-operative outcome			
Intra-operation blood loss, mL	1451 (1024-2388)	1454 (888-2300)	0.945
Postpartum haemorrhage ≥1500 mL	9 (47.4)	10 (50.0)	0.869
Postpartum haemorrhage ≥1000 mL	15 (78.9)	15 (75.0)	0.999
Length of surgery, minutes	49 (30-62)	37 (30-51)	0.204
Blood transfusion at operation	11 (57.9)	10 (50.0)	0.621
Transfusion of packed red blood cells	11 (57.9)	10 (50.0)	0.621
Units of packed red blood cells transfused	2 (0-4)	0.5 (0-2.75)	0.550
Transfusion of platelets	4 (21.1)	5 (25.0)	0.999
Units of platelets transfused	0 (0-4)	0 (0-3)	0.945
Transfusion of fresh frozen plasma	2 (10.5)	4 (20.0)	0.661
Units of fresh frozen plasma transfused	0	0	0.411
Transfusion of cryoprecipitate	1 (5.3)	1 (5.0)	0.999
Units of cryoprecipitate transfused	0	0	0.999
Syntocinon infusion during operation	17 (89.5)	20 (100.0)	0.231
Misoprostol during operation	7 (36.8)	5 (25.0)	0.501
Carboprost during operation	7 (36.8)	1 (5.0)	0.020
Intrauterine balloon during operation	0	1 (5.0)	0.999
Compression suture during operation	6 (31.6)	4 (20.0)	0.480
Uterine artery or internal iliac artery ligation during operation	2 (10.5)	0	0.231
Uterine artery embolisation after operation	2 (10.5)	0	0.231
Hysterectomy	0	0	-
Maternal outcome			
Post-operative hospital stay, d	4.0 (3.0-6.0)	3.0 (3.0-4.0)	0.070
Intensive care unit admission	0	1 (5.0)	0.999
Rebleeding after operation	0	0	-
Relaparotomy	0	0	-
Maternal death	0	0	-
Occlusion-related complications	0	0	-
Other maternal complications (adult respiratory distress syndrome, renal failure, ischaemic liver and disseminated intravascular coagulation)	0	0	-
Neonatal outcomes			
Birthweight, g	2690 (2530-2980)	2895 (2673-3190)	0.149
Male	10 (52.6)	11 (55.0)	0.882
Apgar score <7 at 5 minutes	0	1 (5.0)	0.999
Umbilical cord arterial pH	7.29 (7.26-7.30)	7.29 (7.27-7.32)	0.459
Umbilical cord arterial base excess	-4.50 (-8.93 to -2.3)	-2.90 (-5.0 to -1.0)	0.109
Neonatal intensive care unit admission	1 (5.3)	1 (5.0)	0.999

* Data are presented as median (interquartile range) or No. (%)

Discussion

Our study is the first randomised controlled trial on the use of prophylactic IIA balloon occlusion in women with PP undergoing CS. It showed that IIA balloon occlusion did not reduce the obstetrics blood loss during CS or had any significant effect on the maternal or perinatal outcomes.

The study design of randomised controlled trial avoided selection bias and minimised confounding factors. However, the nature of the treatment made double blinding unfeasible. Nonetheless, intra-operative blood loss was measured objectively. Thus, the median blood loss of 1400 to 1500 mL in our study was higher than that in another study.[3] The sample size of 20 per group was nearly double that of a RCT for placenta accrete (12 per group).[4] Our sample size was estimated to detect a difference of 400 mL blood loss, which is considered clinical significant. The median blood loss in both groups was within the range of 1400 to 1500 mL; it is not likely that further study with a larger sample size can demonstrate a clinically significant difference. Nonetheless, a study with a larger cohort may help to identify any predictive factor for the success or failure of IIA prophylaxis. Although there were more women with previous CS in the occlusion group, it is not likely to be a confounding factor in blood loss, as placenta accreta spectrum was excluded in our study. Although four cases in the occlusion group did not have the assigned treatment, the result was the same in terms of the intention-to-treat analysis or the on-treatment analysis.

In a retrospective study comparing 42 women with PP managed with IIA balloon occlusion and 26 women without PP, the incidence of heavy blood loss (≥1000 mL) during CS was significantly lower in the former group (38% vs 69%), although the median amount of blood loss was not significantly different (800 mL vs 1000 mL).[3] However, our results did not support their findings. IIA balloon occlusion did not add any beneficial effect because surgical haemostasis was adequate for most of the PP cases, and collateral circulation such as ovarian arterial supply could not be controlled by IIA occlusion.

Infra-renal intra-aortic balloon has been proposed for control of obstetric haemorrhage. A retrospective study comparing pre-CS aortic balloon occlusion and IIA balloon occlusion suggested that the former may be a better prophylactic choice for patients with PP; the median blood loss was reduced from 3750 mL with IIA occlusion to 1600 mL with aortic occlusion.[5] However, the blood loss was not significantly different in patients with accrete or increta. Yet RCT is required to investigate the value of aortic occlusion in accreta spectrum or PP.

Safety of IIA catheterisation-related complications is a major concern. In our study, no patient had catheterisation-related complication. IIA balloon occlusion appears to be a safe treatment for women with PP.

Conclusion

Prophylactic IIA balloon occlusion for patients with PP undergoing CS did not reduce PPH or had any effect on maternal or neonatal morbidity. Although perioperative placement of internal iliac artery occlusion balloon is a safe and minimally invasive procedure, unnecessary radiological intervention should be avoided.

Acknowledgements

We thank the medical staff and midwives from antenatal clinic and labour ward at the Prince of Wales Hospital for facilitating this study.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#03140236). 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. Yu SCH, Cheng YKY, Tse WT, et al. Perioperative prophylactic internal iliac artery balloon occlusion in the prevention of postpartum hemorrhage in placenta previa: a randomized controlled trial. Am J Obstet Gynecol 2020;223:117.e1-117.e13.

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Combined electroacupuncture and auricular acupuncture to alleviate pain after gynaecological abdominal surgery: a randomised sham-controlled trial (abridged secondary publication)

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KEY MESSAGE

Perioperative acupuncture is safe and effective in alleviating pain after gynaecological laparotomy. However, a large sample size trial is warranted to confirm the efficacy. Hong Kong public hospitals may consider providing inpatient perioperative acupuncture services to such patients.

Hong Kong Med J 2022;28(Suppl 1):S27-30
HMRF project number: 13142951

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Introduction

Postoperative pain affects recovery. Although analgesics such as opioids are effective in lowering postoperative pain, they have adverse effects such as dizziness, nausea, and vomiting. Acupuncture is effective in reducing pain after abdominal surgeries. However, perioperative inpatients are not able to opt for acupuncture service in Hong Kong. This study aims to assess the safety, feasibility, efficacy, and cost-effectiveness of adding acupuncture to the usual postoperative pain control plan for patients with gynaecological laparotomy.

Methods

A total of 72 adults who planned to undergo a laparotomy with a midline or horizontal incision for gynaecological diseases were recruited between 12 October 2016 and 6 November 2018 from Queen Mary Hospital or Kwong Wah Hospital (Fig). We excluded those who were very ill, taking analgesics for chronic pain, having recent acupuncture experience, or not suitable for receiving acupuncture (eg heart diseases, bleeding disorders or skin lesions).

Participants were randomised evenly into either the acupuncture group or the sham control group. Block randomisation using random block sizes (4-8) was conducted. Only the acupuncturist could read the allocation code just before the first treatment. Usual care was provided to all participants. Standard protocols for anaesthesia, surgery, and postoperative pain management were recommended. Acupuncture and sham acupuncture were administered within 2 hours before surgery, immediately upon arrival at the ward, and then once a day during hospitalisation up to 5 days after surgery.

The semi-standardised acupuncture treatment protocol included electroacupuncture and auricular acupuncture. Five fixed basic acupoints (bilateral LI4, ST36, SP8, SP10, and LR3) were used in all sessions, and four to five selective points were chosen by the acupuncturist in every session. An electric-stimulator (4 Hz, continuous wave) produced electrical current to stimulate four pairs of acupoints (preoperatively: bilateral LI4-ST36, bilateral ST25, CV4-CV12; postoperatively: acupoints across the wound, bilateral ST36) for 30 minutes. For auricular acupuncture, filiform needles (0.22×15 mm) were needled on the auricular points and retained for 30 minutes in the preoperative session. Adhesive tape was also applied to the body acupoints. In the acupuncture sessions after surgery, intradermal needles (0.20×1.5 mm) were inserted, rather than filiform needles. The intradermal needles were retained and replaced every 3 days.

The sham control group received sham acupuncture with the same procedures and schedule, except that sham acupoints were located at 1-2 cun beside the true acupoints and were stimulated by non-invasive sham acupuncture devices.

The primary outcome measure was pain at rest measured by a numerical rating scale (NRS) for 5 days

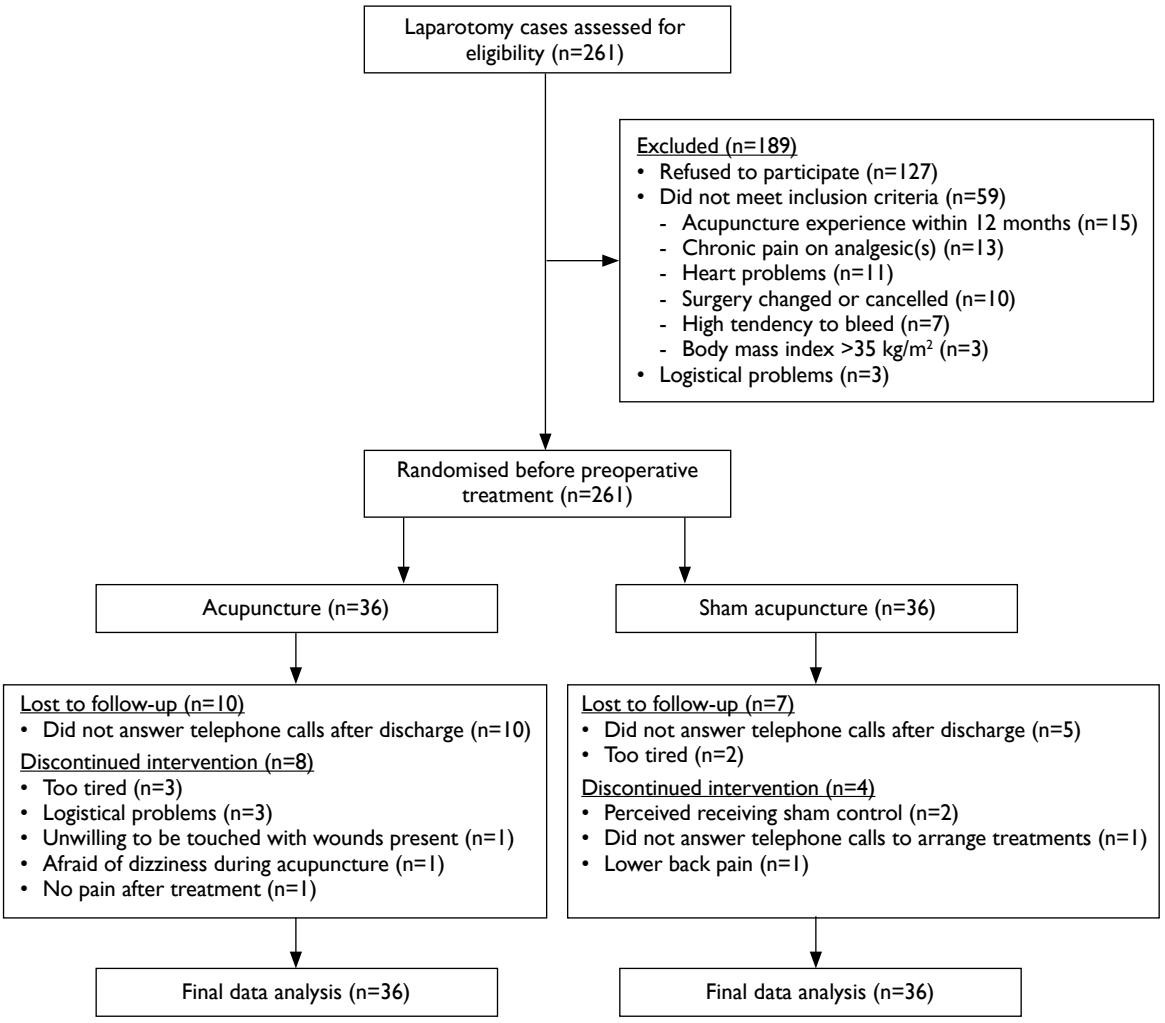

FIG. Patient recruitment flowchart

after surgery. Secondary outcomes included pain while coughing, analgesics consumption, quality of life, time to recovery, the incidence of opioid-related adverse effects, patients' satisfaction, the patients' perception of the credibility of the treatment, and the blinding success. The pain NRS scores and patient-controlled analgesia attempts (tries/ goods) were taken hourly for the first 6 hours after surgery, then every 4 hours up to day 2, every 6 hours up to day 3, and then once a day. All other outcomes were measured once a day. The last measurement was taken on day 5 after surgery. Patients who were discharged <5 days after surgery were followed up with telephone calls.

The sample size was calculated based on the effect size (pain at rest for 5 days after surgery) calculated from an electroacupuncture study for postoperative pain control.[1] R (version 3.6.0) was used for statistical analyses. We adopted the intention to treat approach and verified the results with a post hoc complete case analysis. Missing data were imputed through linear interpolation, whereas those time-independent, baseline, and last missing outcomes were imputed by multiple imputation 10 times. Independent two-sample t test or linear regression models adjusted for regressors such as type of incision were used to compare the continuous variables. Chi squared test or a multinomial logistic regression model adjusted for similar regressors were used to compare the categorical variables. Survival analysis was also conducted for the time to recovery variables. All tests were two-tailed, and the statistically significant level was set at 0.05.

Health economics analysis was based on the perspective of the health service providers on the direct medical costs. The healthcare costs were estimated by the number of hospital stay multiplied by the day cost taken from the Hong Kong government's Gazette, whereas the intervention costs were measured by the time of preparation and

TABLE 1. Pain scores across time

	Missing rate, %			Intention to treat analysis					Complete case analysis				
	Acupuncture group	Sham acupuncture group	Total	Acupuncture group, mean	Sham acupuncture group, mean	Mean difference	95% confidence interval	P value	Acupuncture group, mean	Sham acupuncture group, mean	Mean difference	95% confidence interval	P value
Baseline													
Rest	5.6	5.6	5.6	5.4	4.8	0.6	-1.4 to 2.3	0.604	5.5	4.7	0.7	-1.8 to 2.220	0.813
Cough	11.1	13.9	12.5	6.7	6.2	0.5	-2.2 to 1.8	0.826	6.7	6.3	0.4	-3.0 to 1.3	0.450
At 22 hours													
Rest	13.9	11.1	12.5	2.6	4.0	-1.4	-2.7 to -0.2	0.029	2.5	3.9	-1.4	-3.0 to -0.0	0.044
Cough	22.2	19.4	20.8	5.2	6.3	-1.1	-2.9 to 0.4	0.119	5.1	6.3	-1.2	-3.8 to 0.0	0.050
At 96 hours													
Rest	11.1	13.9	12.5	1.2	1.7	-0.5	-1.4 to 0.6	0.423	1.2	1.7	-0.5	-1.8 to 0.6	0.314
Cough	13.9	16.7	15.3	3.9	3.6	0.3	-1.1 to 1.8	0.608	4.0	3.6	0.4	-1.5 to 1.7	0.906

implementation of 'acupuncture' treatment and the number of consumables used. All the costs were adjusted to 2018 prices by referring to the Hong Kong Consumer Price Index. The health outcome for cost-effectiveness analysis was the quality-adjusted life years (QALY) measure for 5 days after surgery, using SF-6D and EQ-5D-5L (secondary). Cost-reduction analysis was performed, as the difference between the QALY of the 2 groups was close to 0. Sensitivity analyses were performed in four scenarios with variation in the cost and health outcomes and by bootstrapping (1000 times). The probability of the simulated ICER being cost-effective (threshold: HKD 149838) was estimated.

TABLE 2. Net savings of the acupuncture group in different scenarios of post-laparotomy patients

Scenarios	Mean ± standard deviation total cost, HKD		Net savings, HKD
	Acupuncture group	Sham acupuncture group	
Sham acupuncture	26 014.6±8695.0	30 419.7±9604.7	4405.1
No additional treatment	26 014.6±8695.0	26 491.7±9270.2	477.1

Results

The acupuncture group and the sham control group were similar in terms of characteristics and baseline data, except that the sham control group comprised more patients who received a vertical incision (P=0.029).

With reference to the suggested postoperative analgesic protocol, respectively in the acupuncture group and the sham control group, four and five patients received patient-controlled analgesia, oral paracetamol, and celecoxib, whereas 25 and 27 patients were given both the preoperative and the first postoperative treatments, with a mean of 4±2 and 5±1 in total.

Compared with the sham control group, the acupuncture group had smaller (but not significantly) area under the curve of pain at rest across the 5 days after surgery (285.1±182.6 vs 232.7±210.0, 95% confidence interval [CI]= -144.5 to 63.7, P=0.439, Table 1).

At 22 hours after surgery, the mean NRS pain score at rest was clinically significantly smaller in the acupuncture group than in the sham control group (2.6 vs 4.0, 95% CI=-0.2 to 2.7, P=0.029, Table 1). No significant difference was observed at the other two time points (baseline and 96 hours).

Patients in the two groups shared a similar perception of the credibility of acupuncture. As shown by the Bang Blinding Index, participants in the sham control group were blinded ($BI_{sham\ acup}$ = -0.0472, 95% CI=-0.3015 to 0.2071), but participants in the acupuncture group were not blinded (BI_{acup} = 0.4028, 95% CI=0.1972-0.6084).

There were two missing needle accidents but no serious adverse event. There were four and one events of numbness or pain at needle insertion site in the respective groups and one event of nausea during the preoperative treatment in the acupuncture group.

The QALYs of the two groups were similar. The total cost of each patient was lower in the acupuncture group than in the sham control group (HKD 26014.6±8695.0 vs HKD 30419.7±9604.7, P=0.042, Table 2). The acupuncture group saved HKD 477.1 when the cost of the sham treatment was assumed to be HKD 0 (Table 2). In the complete case analysis and intention to treat analysis, the differences in QALY between the two groups, measured using SF-6D or EQ-5D-5L, across 5 days or 4 days were close to zero. The probability of acupuncture to be more cost-effective than sham acupuncture was 94.9%.

Discussion

The acupuncture group demonstrated a clinically significant superiority in terms of pain at rest over 5 days (mean difference=1.4, 95% CI=-0.2 to -2.7, P= 0.029, post hoc analysis). In the health economic analysis, a saving of HKD 4405.1 per patient was observed in the acupuncture group, compared with the sham control group. No serious adverse events occurred.

The present study used a less frequent postoperative acupuncture protocol (once a day) than a post-hysterectomy pain study[2] (every 4 hours then tapered off) but still observed a significant reduction of the pain at rest. This may be due to the combined use of electroacupuncture and auricular acupuncture.

The present study reported a potential in saving of HKD 477.1 to 4405.1 per patient by adding the acupuncture treatment, compared with a study that reported a net saving of about HKD 42.6 (Euro 4.77) per patient by using an app to follow up patients after surgery.[3]

Conclusion

Perioperative acupuncture treatment was not dominantly superior to sham acupuncture in managing post-laparotomy pain, as evaluated by the area under the curve of pain at rest for 5 days after surgery. However, the acupuncture group showed a significant reduction in pain at rest at 22 hours after surgery.

Acknowledgements

We thank Dr WC Leung (Kwong Wah Hospital), Dr TC Liu (Kwong Wah Hospital), Dr TW Leung (Kwong Wah Hospital), and Dr PWS Ma (Queen Mary Hospital) for their help in patient recruitment and analgesic protocol execution. We thank Dr VCH Chung (The Chinese University of Hong Kong), Dr S Wong (Queen Elizabeth Hospital), and Dr S Zhang (Hong Kong Baptist University) for serving as the Data and Safety Monitoring Board members. We thank Dr H Zhi (The University of Hong Kong) for her advice on the statistical analysis. We thank Dr KC Lo (The University of Hong Kong) and Ms LL Yam (The University of Hong Kong) for performing the acupuncture treatments. We thank Ms PY Chan (The University of Hong Kong), Ms L Lau (Queen Mary Hospital), Ms WK Choi (Queen Mary Hospital), and Ms WL Leung (Kwong Wah Hospital) for their help in recruiting the patients and collecting the outcomes. We thank all participants and nurses at Queen Mary Hospital and Kwong Wah Hospital for the kind support.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#13142951). 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. Lam WL, Yeung WF, Wong MK, et al. Combined electroacupuncture and auricular acupuncture for postoperative pain after abdominal surgery for gynecological diseases: study protocol for a randomized controlled trial. Trials 2018;19:8.

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Modified urine typing to enhance clinical management in kidney transplant patients with unknown donor human leukocyte antigen typing: abridged secondary publication

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KEY MESSAGES

1. Availability of donor human leukocyte antigen (HLA) typing is crucial for early diagnosis of antibody-mediated rejection (AMR) and prompt medical intervention to salvage the graft from failure. Recipients' urine samples are valuable for deduction of donor HLA typing.
2. In 727 urine samples collected from recipients of kidney transplantations, donor mismatched HLA antigens were successfully deduced from 79.0% of the samples.
3. Anti-HLA IgG antibodies against HLA Class I and Class II antigens were detected in 27.9% of the patients. Presence of donor-specific antibody (DSA) was found in 11.1% of the patients. The DSA correlation rate was comparable to that in patients who received transplantations in Hong Kong with known donor typing.
4. With early detection of DSA, AMR were under control in 88.5% patients. Allograft failure with histologic AMR was found in 11.5% of patients before the commencement of this study.
5. This protocol can complement earlier transplant recipients with incomplete donor typing of the newly defined antigens such as HLA-C and -DQ.

Hong Kong Med J 2022;28(Suppl 1):S31-4
HMRF project number: 13142121

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Introduction

Kidney transplantation is the most cost-effective treatment for end-stage kidney diseases. Its survival outcomes is superior to other treatment modalities. However, there is a severe shortage of kidney donors in Hong Kong. Many patients have their transplantations performed outside Hong Kong because of the better accessibility to kidney transplantation. These patients eventually return to Hong Kong healthcare system for subsequent clinical management, including immunosuppressive therapy and follow-up care. In case of graft failure, these patients will get back on the waiting list for renal replacement and pose a burden on the healthcare system.

New immunosuppressive regimens and advancements in drug efficacy in controlling T-cell alloimmunity have reduced episodes of T-cell-mediated acute rejection considerably. Nonetheless, antibody-mediated rejection (AMR) still plays a major role in acute and chronic allograft rejection. Development of de novo donor-specific antibody (DSA) also leads to chronic allograft rejection. With the improvement in both sensitivity and specificity of antibody identification, DSA could be detected in its early stage in patients who received kidney transplantations locally. The test results facilitate early clinical intervention by adjusting the immunosuppressant dosage and minimising expensive treatments.

Currently, more than two-thirds of the transplant patients received their kidney transplantations outside Hong Kong, with unknown donor human leukocyte antigen (HLA) typing and specificities of the anti-HLA antibodies. There is an urgent need to determine the donor HLA typing and DSA for local clinical management. We developed a protocol using a convention HLA typing

method to deduce donor mismatched HLA typing from recipient allograft biopsy.[1] To circumvent the requirement of biopsy, we developed a novel protocol using urine sample to facilitate correlation of DSA (Fig 1).[2]

We evaluated the significance of using urine sample to determine donor HLA typing in DSA correlation. We aimed to achieve a comparable DSA correlation rate for patients with unknown donor typing as that of patients who received transplantations locally. Early medical intervention improves the graft survival rate and reduces the cost of treating acute and chronic humoral rejection.

Methods

A total of 727 patients who received kidney transplantations outside Hong Kong with unknown donor HLA information were recruited. Recipient HLA typing was determined using LIFECODES Luminex-PCR-SSO typing kit (Immucor, Stanford, CT, USA). Allograft donor HLA typing was deduced from DNA extracted from fresh kidney allograft biopsies using our previously established protocol[1] with CTS-PCR-SSP tray kit (University of Heidelberg, Germany). Urine HLA typing was deduced from DNA extracted from fresh early morning urine. HLA typing was performed according to our established protocol.[2]

Sera were collected from post-transplanted patients. For the control cohort of local transplant patients, prospective sera samples were collected according to the protocol of Guarding Recipient Against Failed Transplant programme of the Hospital Authority.

The Luminex bead-based solid-phase immunoassay was used to detect and characterise IgG antibodies against HLA class I and II antigens. LABScreen Mixed kits, Single Antigen kits (One Lambda, CA, USA) and LIFECODES LSA Kits (Immucor, CA, USA) were used where appropriate.

Donor mismatched HLA antigens from urine or biopsy sample's DNA were analysed by subtracting the recipient's own HLA typing from the PCR-SSP banding pattern as described.[1,2]

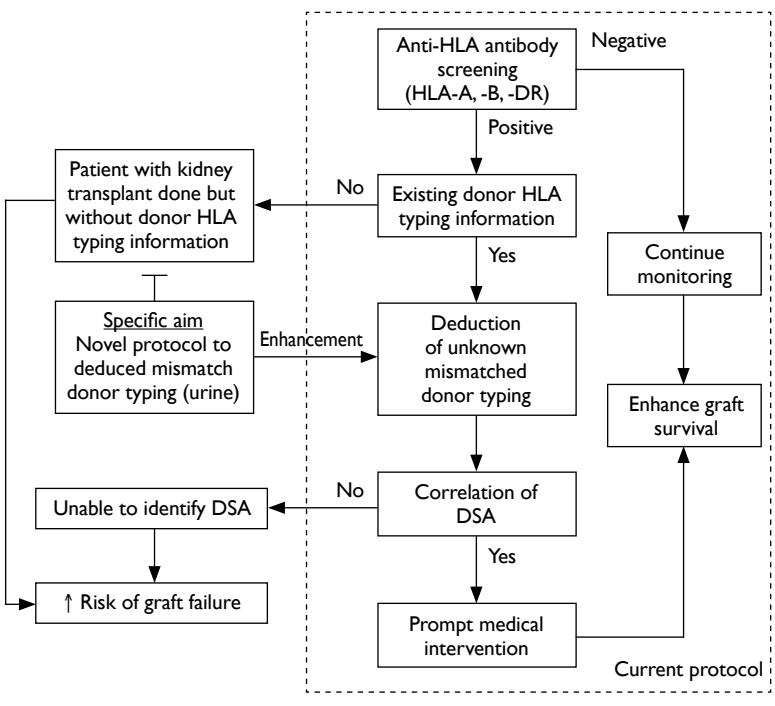

FIG 1. Clinical management protocol for kidney transplant recipients with unknown donor human leukocyte antigen (HLA) typing to facilitate the correlation of donor-specific antibodies (DSA), the diagnosis of antibody-mediated rejection, and early medical intervention.

Results

The 727 patients received kidney transplantations from China (98.6%) and other Asian countries (1.4%) including Taiwan, Cambodia, and Pakistan between 1 April 1989 and 31 March 2018. 80.5% of them received their transplantation <10 years. DNA extracted from the urine samples showed that most had acceptable purity. CTS-PCR-SSP typing was performed for all samples, except for 10.9% with low DNA yield or poor A260/280 ratio (DNA issue). The urine samples were typed for HLA-A, -B, -DR, and -DQ. Additional HLA-C or -DQA1was added if indicated.

Donor mismatched HLA typing was found in 79.0% of the urine samples, whereas no mismatched HLA typing was found in 7.3% of the urine samples, which could be due to absence of HLA mismatched between donors and recipients or total chimerism in the urine samples. 100% concordance rate in the deduced donor typing between both urine and allograft biopsy samples was observed in the same 20 recipients. This suggests that our method was accurate in deducing matched donor HLA antigens.

Anti-HLA IgG antibodies were detected in the serum samples of 203 (27.9%) of 727 recipients (Fig 2). With the availability of deduced donor mismatched HLA antigen information, the presence of DSA was detected in 81 (39.9%) of 203 antibody-positive recipients (11.1% of all recruited recipients). Of the 81 antibody-positive DSA recipients, 72 (88.9%) were against HLA Class II antigens, 16 (19.8%) were against HLA Class I antigens, and 7 (8.6%) were against both HLA Class I and II antigens. 66.7% (54/81) of the antibodies were against newly defined HLA-DQ antigens, consistent with a recent report.[3] Nonetheless, the presence of DSA was unable to confirm in 4.7% of the recipients, owing to poor quality of urine DNA or presence of ambiguities of PCR-SSP.

Recipients who received kidney transplantations in Hong Kong were prospectively monitored. They were compared with those who had transplantation outside Hong Kong from 2013

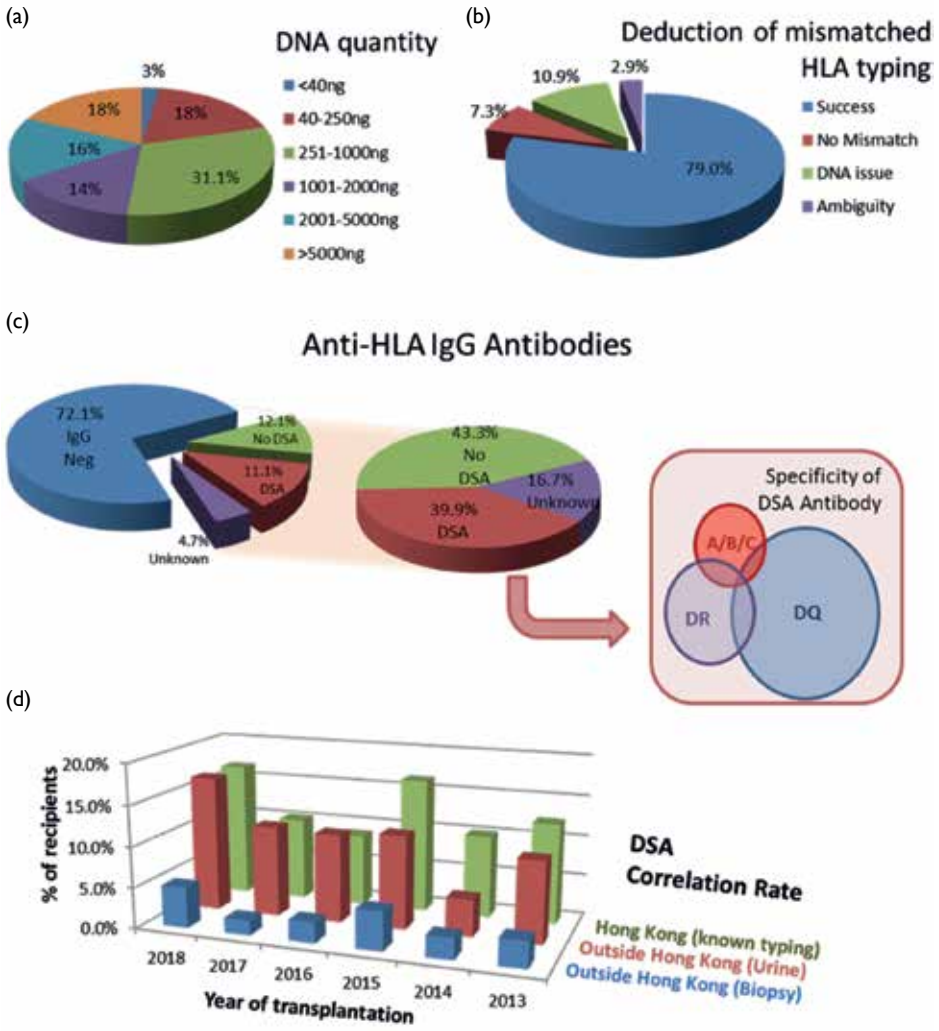

FIG 2. (a) DNA quantity of urine samples, (b) donor human leukocyte antigen (HLA) typing of urine samples, (c) anti-HLA IgG antibody of serum samples in 727 recipients. Of 203 recipients with positive anti-HLA IgG antibodies, 81 were detected to have donor-specific antibodies (DSA). (d) The correlation rate was comparable between the local cohort with known HLA typing and the outside Hong Kong cohort with unknown HLA typing who underwent urine HLA typing (P=0.852) but was significantly lower in those with unknown donor typing who relied on typing deduction from allograft biopsy (P<0.001).

to 31 March 2018. The two groups were comparable in terms of sex and follow-up period but differed significantly in terms of patient age and donor type (Table). The two groups were comparable in the detection rate of anti-HLA IgG antibodies (24.4% vs 25.6%) and average DSA correlation rate (9.6% vs 11.8%). However, for those with unknown donor typing relying only on typing deduction from allograft biopsy, the DSA correlation rate significantly dropped from 9.6% to 3.3% (P<0.001). This indicated that urine DNA could be a feasible alternative to facilitate the detection of DSA and aid clinical diagnosis.

Of 26 recipients with DSA detected, 23 (88.5%) had stable graft function with active clinical interventions such as close monitoring, adjustment of immunosuppressant regimen, biopsy diagnosis, and intravenous immunoglobulin treatment. In three (11.5%) of the 26 recipients with DSA detected, antibodies already existed before the commencement of this study while no HLA information was available when the antibody first appeared. Plasmapheresis and intravenous immunoglobulin treatment were given to the patients at a late stage; however, the damage could not be reverted and resulted in allograft lost with histologic AMR. Although DSA were found in 50 (11.8%) of 422 recipients in the local cohort, prompt clinical intervention was initiated and no AMR-related allograft failures were reported. These results suggested that prompt DSA correlation is important to guide clinical management in preventing AMR-related allograft failure.

Discussion

We used a non-invasive method to deduce

TABLE. Comparison of recipients who underwent transplantation between 2013 and 2018 outside Hong Kong or within Hong Kong

	Outside Hong Kong	Hong Kong	P value
Age, y			<0.0001
Range	23-80	6-77	
Mean	54.53	45.9	
Median	56	48	
No. (%) of patients			0.155
Male	168	237	
Female	103	183	
Donor type			<0.0001
Living	6 (2.2%)	83 (19.8%)	
Deceased	251 (92.6%)	337 (80.2%)	
Unknown	14 (5.2%)	0	
Transplantation year			0.068
2018 as of 31/3/2018	12	24	
2017	36	78	
2016	46	79	
2015	61	79	
2014	66	79	
2013	50	81	
Total	**271**	**420**	
Follow-up duration, months			
Range	10-73	10-73	
Mean	44.1	41.5	
Median	46	43	
Antibody status			0.788
IgG negative	204 (75.0%)	314 (74.4%)	
IgG positive	66 (24.4%)	108 (25.6%)	
Donor-specific antibody	26 (9.6%)	50 (11.8%)	0.748
No donor-specific antibody	34 (12.5%)	58 (13.7%)	
Unknown	7 (2.6%)	-	
Outcome			
Antibody-mediated rejection related graft failure	3	0	

mismatched donor HLA typing from the recipient urine sample using conventional PCR-SSP method. Donor mismatched HLA typing was successfully deduced in 79% of the recruited subjects. The accuracy of using urine to deduce donor mismatched HLA typing was confirmed by allograft biopsies. Around 10% DSA correlation rate was achieved and was comparable with that for the locally transplant recipients with known donor typing.

In the past, DSA correlation could only be made when impaired renal function was indicated. At the time of the diagnosis of presence of DSA or histologic AMR, the allograft damage cannot be reverted and may result in allograft loss. Therefore, availability of donor HLA information is important in clinical diagnosis and management.

In the present study, only recipients who received transplantations between 2013 and 2018 were included in the outcome analysis, because prospective anti-HLA antibody screening was introduced in 2013. Therefore, the sample size is limited, and the incidence of AMR within 5 years is relatively low. Long-term studies to review allograft survival are needed.

Deduction of donor mismatched HLA typing was hampered by technical limitations, including DNA quantity requirement and resolving power of the conventional PCR-SSP method for HLA typing. Newer techniques with higher resolution and sensitivity are warranted. Next-generation sequencing–based HLA typing has superior resolving power and higher sensitivity. It enables detection of additional loci such as HLA-C, -DQ, or even -DP, with increasing clinical significance in antibody-mediated rejection.[4]

Conclusion

Recipient's urine sample is accurate in deducing matched donor HLA antigens. It circumvents the need for renal biopsy and facilitates the detection of DSA and aid clinical management of kidney transplant patients. Our protocol has been implemented since October 2018.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#13142121). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html). This study was also supported by the Guarding Recipient Against Failed Transplant programme of the Hospital Authority Annual Plan.

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Lymphocyte subset profile and clinical phenotype in lupus nephritis: abridged secondary publication

DYH Yap *, TM Chan, S Yung, S Wong

KEY MESSAGES

1. Distinct B cell subsets and relevant cellular signatures are related to relapses in lupus nephritis.
2. Cyclophosphamide and mycophenolate induction confer different changes in lymphocyte subsets and cytokines in patients with active lupus nephritis.

Hong Kong Med J 2022;28(Suppl 1):S35-7
HMRF project number: 03143866

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Introduction

Lupus nephritis is characterised by repeated nephritic flares intercalated with periods of quiescence. Repeated nephritic flares lead to attrition of nephron mass and hence compromise long-term renal outcomes.

Perturbation in lymphocyte subsets and the relevant serum cytokine profiles is worth investigating. Patients with systemic lupus erythematosus exhibit an expanded memory B cells and plasma cells in the peripheral blood as well as shrinkage in the number of naïve B cells.[1] Disturbed T-cell homeostasis plays an essential role in the development of systemic lupus erythematosus; T-helper cells and regulatory T-cells (T_{Reg}) appear to be the more crucial subsets involved.[1] Cytokines such as IL-2, IL-4, IL-6, IL-10, IL-18, and IFN-γ are more involved in the Th1/Th2 and T_{Reg} balance, whereas IL-6, IL-17, IL-21, and IL-23 are more involved in Th17 subsets.[1] B cell subsets are significantly influenced by the B lymphocyte activation factor (BAFF), IL-6, and IL-21.[1] Both cyclophosphamide (CTX) and mycophenolate mofetil (MMF) are established initial treatment for severe lupus nephritis; CTX induction is associated with fewer relapses than MMF, but the underlying mechanism remains to be elucidated.[2]

Methods

We compared the lymphocyte subsets and serum cytokine profiles between patients with multiple relapses (MR) [ie, ≥3 within 36 months, unrelated to treatment non-compliance] and those with no relapse (NR). Clotted blood samples during quiescent disease were obtained for analysis of lymphocyte subsets and serum cytokine levels. Healthy subjects were recruited as controls. B cell signatures (miRNA148a, BACH1, BACH2, and PAX5) were measured in the serum and B cells.

Patients with biopsy-proven active class III/IV±V lupus nephritis (both treatment-naïve and relapsed patients were eligible) from two renal units (Queen Mary Hospital and United Christian Hospital) were randomised to receive CTX or MMF, together with corticosteroids, as induction treatment. The commencing dose of prednisolone was 0.8 mg/kg/day and tapered to reach 7.5 mg/day at approximately 6 months. MMF was initiated at 1 g twice a day for the first 6 months and then tapered according to clinical status. CTX was administered orally at 2 mg/kg/day for 6 months and then substituted with oral azathioprine at 1.5 mg/kg/day.

Exclusion criteria were having class II or pure class V lupus nephritis, having received calcineurin or proliferation signal inhibitors or biological therapies in the preceding 12 months, having received CTX or MMF at a daily dose >500 mg in the preceding 12 months, having other medical conditions with associated immunological abnormalities or requiring treatment with corticosteroids or immunosuppressive medications, having leucopoenia (white cell count $<3.5 \times 10^9$/L) secondary to lupus activity, and being pregnant or lactating. Serial blood and urine samples were assessed for lymphocytes subsets and serum cytokine levels. Patients were followed at 12-week intervals for 24 months to monitor for relapse.

Results

A total of 48 patients with stable lupus nephritis (24 in the MR group and 24 in the NR group) were included for analysis (Table 1). Circulating naïve B cells were significantly lower in MR patients than NR patients (0.7% [0.1%-14.1%] vs 4.0% [0.4%-24.6%],

TABLE 1. Clinical characteristics of those with multiple relapses or with no relapse in lupus nephritis

	Multiple relapses group (n=24)*	No relapse group (n=24)*	P value
No. of female:male	20:4	21:3	0.683
Age, y	48.7±6.6	51.4±11.3	0.314
Duration of follow-up from last nephritic episodes, months	118.9±79.9	133.2±81.9	0.594
Class of lupus nephritis			
Class III/IV	11 (45.8)	17 (70.8)	0.079
Class III/IV± V	13 (54.2)	7 (29.2)	0.089
Maintenance treatment:			
Prednisolone alone	6 (25.0)	12 (50.0)	0.140
Prednisolone + mycophenolate mofetil	11 (45.8)	6 (25.0)	0.230
Prednisolone + azathioprine	7 (29.2)	6 (25.0)	0.750
White cell count, $\times 10^9$/mL	5.2±1.8	5.8±2.3	0.383
Serum C3, mg/dL	80.0±29.5	83.0±20.9	0.699
Anti-dsDNA, IU/mL	62.7±89.8	42.2±67.0	0.865
Estimated glomerular filtration rate, mL/min/1.73 m^2	59.6±29.2	75.6±21.6	0.070
Serum albumin, g/L	39.3±5.1	42.5±2.2	0.017
Urine protein excretion, g/D	0.6±1.0	0.2±0.3	0.01

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

TABLE 2. Serum cytokine levels in patients with multiple relapses or with no relapse

Serum cytokine levels, pg/mL	Multiple relapses group*	No relapse group*	P value
B cell activating factor	1338.0 (1059.9-1627.1)	1252.7 (809.8-1664.9)	0.555
IL-6	203.7 (121.6-405.2)	195.0 (129.4-221.4)	0.598
IL-21	9.7 (6.4-19.4)	6.7 (3.1-8.8)	0.121
IFN-α	26.9 (22.9-30.1)	27.7 (25.8-40.5)	0.555
IFN-γ	33.5 (24.1-279.1	64.3 (23.1-216.7)	1.000
IL-2	2150.7 (1577.7-8053.5)	2304.3 (1709.7-7041.9)	0.877
IL-4	41.0 (34.2-43.8)	41.8 (34.9-52.8)	0.555
IL-10	6.7 (6.0-7.6)	6.9 (6.3-7.2)	0.926
IL-17	350.8 (49.1-502.2)	141.5 (23.1-506.5)	0.475
IL-18	212.7 (191.1-313.4)	211.1 (153.3-486.1)	1.000
IL-23	12.3 (11.1-14.4)	11.9 (10.9-16.2)	0.828

* Data are presented as median (range)

P=0.017). The two groups were comparable in terms of percentage of circulating memory B cells (0.6% vs 1.0%) and plasma cells (0.2% vs 0.3%). The memory-to-naïve B cell ratio was significantly higher in MR patients than NR patients (0.8 [0.1-9.0] vs 0.2 [0.1-2.0], P=0.024). The percentage of circulating T cell subsets (Th1, Th2, Th17, and Treg) did not differ between the two groups. Lymphocyte subsets showed no association with serum anti-dsDNA antibody or C3 levels in both groups. Serum levels of BAFF, IL-2, IL-4, IL-6, IL-10, IL-17, IL-18, IL-21, IL-23, IFN-α, and IFN-γ were similar in both groups (Table 2).

We investigated the relevant B cell signatures in serum and B cells to account for the changes in the circulating B cell subsets profile. MR patients showed significantly higher serum miRNA-148a expression than did NR patients or healthy controls (1.0±0.0 vs 0.7±0.2 vs 9.4 ± 6.9 fold difference, p<0.001). miRNA-148a expression was significantly higher in memory B cells in MR patients than NR patients or healthy controls (1.0±0.0 vs 0.8±0.3 vs 5.8±1.7 fold difference, p<0.001). BACH1, BACH2, and PAX5 expressions were also significantly lower in memory B cells from MR group.

18 patients with active class III/IV±V lupus nephritis were randomised to CTX group (n=8) or MMF group (n=10). The overall renal response was comparable at 6 months (62.5% vs 80.0%, P=0.41, Table 3). Only one patient in the CTX group had renal relapse. After 24 weeks, the CTX group showed numerically higher circulating cytotoxic T cells (51.2% [43.3%-53.7] vs 23.0% [22.5%-52.0%], P=0.700] and lower Th1 cells (1.0% [1.0%-2.0%] vs 6.4% [3.6%-8.7%], P=0.700). There was no significant difference in other B cell subsets and T-helper cells. After 24 weeks, in the CTX group, MMF induction was associated with significantly lower serum BAFF (855.0 [714.8-1094.7] pg/mL vs 2175.0 [1721.6-2786.2] pg/mL, P=0.016) but significantly higher IFN-γ (553.6 [353.2-756.6] pg/mL vs 26.5 [18.9-29.9] pg/mL, P=0.032). There was no significant difference in the serum levels of IL-2, IL-4, IL-6, IL-10, IL-17, IL-18, IL-21, IL-23, and IFN-α.

Discussion

We demonstrated that during disease quiescence, MR patients had a higher memory-to-naïve B cell ratio and reduced circulating naïve B cells than NR patients had. Changes in B cell subset profile in MR patients may be related to repeated exposure to autoantigens, which promotes differentiation of naïve B cells to more mature B cell subpopulations. Memory B cells from patients with systemic lupus erythematosus also show reduced FcγRIIb expression, which leads to increased Ca^{2+} influx and diminished inhibitory signals for memory B cell activation.[3,4] We did not observe any significant difference in the frequency of circulating plasma cells in MR and NR patients. This may be because plasma cells in the bone marrow and circulating plasma cells are present in very low quantities. In addition, we did not observe any significant change in T lymphocyte subsets profile between MR and NR patients. Pro-inflammatory and anti-inflammatory cytokines (such as BAFF, IL-2, IL-4, IL-6, IL-10, IL-17, IL-18, IL-21, IL-23, IFN-α, and IFN-γ) are secreted by B and T cells to promote B cell maturation

and survival, immunoglobulin switching, defective apoptotic cell clearance, and Th1 differentiation. This may be explained by the collection of samples during quiescent disease.

We demonstrated that serum miRNA-148a level was significantly higher in memory B cells in MR patients than in NR patients. This was accompanied by a decrease in BACH1, BACH2, and PAX5 expression in B cells. Increased miRNA-148a expression can inhibit *Gadd45a*, *Bim*, and *PTEN* and prevent apoptosis of immature B cells, resulting in enhanced B lymphocyte autoreactivity. A recent GWAS meta-analysis identified BACH2 as a novel susceptibility locus in Chinese lupus patients. Other studies reported that BACH2 expression was reduced in B cells isolated from patients with systemic lupus erythematosus, and that transfection of BACH2 into B cells from these patients suppressed their proliferation and promoted apoptosis.

Our results suggested that patients receiving CTX induction showed numerically lower percentage of circulating Th1 cells but higher percentage of cytotoxic T cells. Our findings of lower circulating Th1 and IFN-γ in the CTX group supported the finding of a lower risk of relapse in patients receiving CTX induction.[2] CTX induction in some high-risk ethnic groups was reported to be associated with increased renal fibrosis and considerable incidence of chronic kidney disease.[5] Our finding of higher circulating cytotoxic T cells might provide some explanation for the more renal parenchymal damage. MMF induction was reported to be associated with earlier reduction of circulating plasma cells, but CTX induction was reported to confer preferential depletion of naïve B cells and pre-switched memory B cells. We reported significantly lower BAFF levels in patients receiving MMF induction, which might translate into less efficient B cell repopulation and proliferation during disease quiescence.

TABLE 3. Baseline clinical characteristics and circulating lymphocyte subsets profiles in patients with active lupus nephritis receiving cyclophosphamide or mycophenolate mofetil induction

	Cyclophosphamide group (n=8)*	Mycophenolate mofetil group (n=10)*	P value
No. of female:male	7:1	9:1	0.867
Age, y	41.3±12.7	44.6±16.2	0.639
Lupus nephritis class III/IV ± V	8	10	1.000
White cell count, $\times 10^9$/L	10.4±5.5	8.5±3.2	0.392
Serum creatinine, μmol/L	133.9±72.8	80.1±38.3	0.203
24-hr urine protein, g/D	2.2±0.8	2.7±1.4	0.414
Anti-dsDNA, IU/mL	144.8±132.4	221.8±118.7	0.277
Serum C3, mg/dL	67.1±20.5	49.8±13.0	0.093
Circulating lymphocyte subsets before induction treatment			
Naïve B cells, %	9.9 (2.1-23.1)	10.8 (1.0-24.4)	1.000
Memory B cells, %	5.3 (4.1-5.9)	8.8 (4.7-10.6)	0.343
Plasma cells, %	0.12 (0.05-1.58)	0.29 (0.04-0.52)	0.886
CD8+, %	47.2 (36.7-60.1)	25.0 (17.3-40.0)	0.629
Th1, %	3.3 (1.7-4.1)	8.9 (6.4-10.0)	0.229
Th2, %	1.1 (0.7-2.2)	0.3 (0.2-0.4)	0.057
Th17, %	0.9 (0.3-2.3)	0.8 (0.5-1.2)	1.000
Treg, %	1.0 (0.6-2.2)	3.5 (2.3-4.5)	0.114

* Data are presented as mean ± standard deviation or median (range)

Acknowledgements

We thank all members of the research team and the patients for participation.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#03143866). 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. Yap DY, Yung S, Lee P, Chan TM. Circulating lymphocyte subsets and disease relapse in lupus nephritis. J Am Soc Nephrol 2016;27(Suppl):15.
2. Yap DY, Tang CSO, Lee P, et al. Relationship between B cell signatures and disease flare in lupus nephritis patients. J Am Soc Nephrol 2017;28(Suppl):583.
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WhatsApp groups to promote childhood seasonal influenza vaccination: a randomised control trial (abridged secondary publication)

Q Liao *, R Fielding, DYT Cheung, J Lian, WWT Lam

KEY MESSAGES

1. Communication about seasonal influenza vaccination (SIV) in a WhatsApp group has no significant effect on promoting parents to take children for SIV but significantly promoted their perceived self-efficacy in taking children for SIV.
2. In the WhatsApp groups, participants mainly shared their negative experience or views about SIV including their concerns over vaccine safety or adverse effects, concerns over vaccine effectiveness, and negative opinions of vaccination.
3. The group moderator played a main role in sharing knowledge/information and in shifting the discussion about SIV from negative experience/views to positive experience/views.

Hong Kong Med J 2022;28(Suppl 1):S38-41
HMRF project number: 16150752

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Introduction

Seasonal influenza vaccination (SIV) in young children remains low in Hong Kong.[1] Sending vaccination reminders through mobile phone was considered effective in promoting vaccination uptake in children, but the effect size was small.[2] Current vaccination reminders mainly contain information on the risks of influenza infection, benefits of SIV, and doctors' recommendations for vaccination,[2] which may be insufficient to address concerns over risks of SIV, an important impediment to SIV.[1] Parental decision-making for children's vaccination can be greatly influenced by other parents' vaccination decisions. Others' behavioural choices provide important behavioural cues for social learning or imitation, indicating social approval and safety of a behavioural choice.[1,3] Therefore, encouraging positive experience and information sharing among parents in addition to providing vaccination reminders could promote parents to take their children for SIV. WhatsApp is an easily accessible platform for parents to share this kind of information.

Time constraints are likely to increase decision-maker reliance on heuristic cues for decision-making,[4] but the effect may depend on whether parents will use the positive or negative cues for facilitating their decision making for children's SIV. In Hong Kong, parents are recommended to take their children who are eligible to receive SIV in October each year. Vaccination uptake before the winter influenza season (from January to March) is strongly recommended because it takes around 2 weeks to produce immunity after vaccination. Therefore, decision making for vaccination is naturally time-constrained, and the optimal time for children vaccination is 2 weeks before the winter influenza season.

This preliminary study aims to test the effect of delivering vaccination reminders and communicating risks and benefits of SIV through WhatsApp groups. Specifically, it aims to test the effect of the WhatsApp groups on promoting childhood SIV uptake, the effect of including the time constraint components (the remaining time for optimal timing of SIV) into the weekly vaccination reminders on promoting childhood SIV uptake, and parental acceptability.

Methods

This study only targeted mothers because they were the main decision makers for their children's immunisation in most Hong Kong families. Inclusion criteria were mothers who had at least one child aged 6 to 72 months, was able to communicate in Chinese or Cantonese and read and type Chinese, and was able to access the internet via mobile phone and willing to use WhatsApp. Mothers were excluded if their eligible children had medical contraindications for immunisation. Mothers were pre-recruited from previous population-based telephone surveys and advertisements. They were invited by trained telephone interviewers to participate in the study. Using block randomisation with a ratio of 5:2:2, they were randomly allocated into the control group or one of the two social-networking intervention (SNI) groups: receiving weekly vaccination reminder with or without time constraint components (SNI+TC or SNI-TC). Participants were asked to complete a ~10-minute baseline assessment.

The vaccination reminders comprised three messages: message 1 introduced the childhood SIV subsidy scheme and doctors' recommendation for children's SIV; message 2 was about risks of seasonal influenza to children, benefits and safety of SIV; and message 3 was about the remaining days for optimal

timing of SIV for children (for SNI+TC group only). Between October and December 2017, vaccination reminders were sent to the intervention groups at afternoon times of different weekdays once per week. A total of eight vaccination reminders were sent over 8 weeks. A total of four WhatsApp groups were established including two SNI-TC groups and two SNI+TC groups, each comprised ~40 participants. The moderator also sent messages on a weekly basis to encourage positive experience and information exchange, vaccination planning, and information seeking. The moderator also addressed mothers' questions, concerns, or misunderstandings about influenza and influenza vaccination. The WhatsApp groups were closed by the moderator by the end of December 2017. All respondents were contacted again between April and May 2018 for a follow-up assessment on children's SIV uptake, perceptions related to SIV, and opinions about using social networking intervention to promote childhood SIV uptake.

Generalised estimating equations (GEE) logistic regression model was conducted to examine the effect of social-networking intervention and the effect of including time constraint into vaccination reminders on children's SIV uptake. GEE was also used to examine changes in perceptions regarding influenza and SIV by intervention arm. If participants had more than one child aged 6 to 72 months, the youngest one's SIV uptake was used as the main outcome in the GEE logistic regression model. Content analysis was conducted to analyse all posts in the WhatsApp group to understand how participants interacted with each other and the moderator during the communication process. Each post was coded according to the following categories: role (moderator or participant), format (text, picture, emoji, or hyperlink), cyber support (eg, sharing views, experiences, or emotions), and discussion topics (eg, vaccine effectiveness, vaccine safety, adverse effects). A coding scheme was entered into the QRS Nvivo 12.0 and independently coded by two researchers. Opinions about the benefits and barriers of the implemented interventions were summarised to determine the acceptability and usefulness of the interventions.

Results

A total of 205, 80, and 80 mothers were allocated to the control, SNI-TC, and SNI+TC groups, respectively. The three groups were comparable in terms of demographics, target child's characteristics, and the past-12-month SIV uptake. At the follow-up, 174, 60, and 57 participants completed the outcome assessment, with a dropout rate of 15.1%, 28.7%, and 25.0%, respectively.

Respectively in the three groups, 37.4%, 33.3%, and 38.3% of the participants reported that all their target child(ren) received SIV, whereas their youngest target child's SIV uptake was similar: 37.9%, 33.3%, and 38.3%, respectively. GEE logistic regression models revealed that the WhatsApp groups had no significant effect on children's SIV uptake but had significant effect on promoting parents' perceived self-efficacy in taking children for SIV in the SNI-TC group (OR=2.57, 95% CI=1.06-6.23) and the SNI+TC group (OR=2.39, 95% CI=1.13-5.06) [Fig 1]. In addition, including the time constraint component into the vaccination reminders did not have significant effect on children's SIV uptake.

Of 434 mothers' posts, 52.1% were coded as sharing experience/views, 27.4% as seeking information/opinions, 24.4% as sharing knowledge/information, and 15.2% as emotional exchange. Approximately 44.7% of the experience/views shared were coded as negative, including concerns over vaccine safety, adverse effects, and effectiveness, as well as negative opinions of vaccination. Participants mainly sought information/opinions about vaccine safety or adverse effects, medical eligibility of SIV, first SIV for children, vaccination clinic or cost, and vaccine effectiveness. Of the knowledge/information shared by participants, 45.3% was about vaccination clinics and cost and 17.9% was about the medical eligibility of SIV (Table).

Of 203 moderator's posts, 70.9% were coded as sharing knowledge/information, 20.7% as encouraging information and experience sharing, 10.3% as encouraging vaccination planning, 9.9% as encouraging information seeking, 6.9% as sharing experience/views, and 3.9% emotional exchange. Most knowledge or information shared by the moderator was on vaccine effectiveness (20.8%), vaccination clinic and cost (18.8%), and vaccine safety or adverse effects (17.4%). Although participants mainly shared about their negative experience/views/emotions regarding SIV at the beginning of the discussion, the moderator's involvement resulted in more frequent posts about sharing positive experience/views, sharing knowledge/information, and sharing positive emotional exchange (Fig 2).

Of participants in the SNI groups, 81.2% reported no concerns with the intervention. Among those who reported being a bit or somewhat/very concerned, the most common concerns were receiving misinformation, privacy concerns, and receiving irrelevant information. 79.4% of participants agreed or strongly agreed that the information could

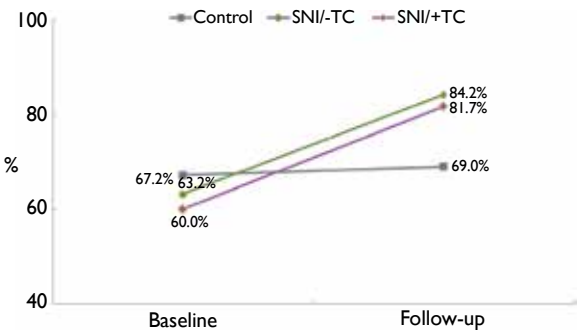

FIG 1. Change in perceived self-efficacy in taking children for seasonal influenza vaccination in the control group, the social-networking intervention (SNI) group with time constraint component (SNI+TC), and the SNI group without time constraint component (SNI-TC).

TABLE. Discussion topics and quotes of posts from participants

Discussion topics	No. (%) of posts	Quotes
Vaccination decision or plan	134 (30.9)	
Positive	69 (15.9)	I will take my child for flu vaccination.
Negative	40 (9.2)	I won't take my child for flu vaccination because there is still some negative news.
Uncertain	25 (5.8)	Then, should I take my child for flu vaccination? I am considering (whether to take my child for flu vaccination)
Vaccination clinics and costs	63 (14.5)	Dr Ng Chin Nang at Kwai Fong, trivalent vaccine is free and quardrivalent vaccine cost HK$60. My child just took the vaccination yesterday and they still have some available vaccines.
Vaccine safety and adverse effects	62 (14.3)	
Concerns over vaccine effectiveness	40 (9.2)	Is it true that all family members should receive influenza vaccination once one member of the family receives the flu vaccination (otherwise it can be worse)?
Clarify misperception or providing information for vaccine safety/ adverse effects	6 (1.4)	It is the misinformation that vaccination can cause autism. This rumour has been dismissed many years before.
Mixed/neutral	16 (3.7)	What can be the side effects of flu vaccination?
Vaccine effectiveness	51 (11.8)	
Concerns over vaccine effectiveness	26 (6.0)	Now there are too many viruses/bacteria and they change very quickly. This time, we take the flu vaccination for this virus but then there may be another new virus. How can we ensure that the vaccination is effective?
Clarify misperception or providing information for vaccine effectiveness	15 (3.5)	Although there is mismatch, the vaccine is still effective for preventing influenza H1N1 or influenza B viruses
Mixed/neutral	15 (3.5)	Can flu vaccination help to avoid severe complications? Different children may have different reactions to the flu vaccination.
Medical eligibility of vaccination	40 (9.2)	I thought to take my daughter for flu vaccination today but she has running nose and some cough. Is it OK for her to take vaccination?
Vaccination experience	33 (7.6)	
Positive	16 (3.7)	My child has taken the flu vaccination and he still feels very good now.
Negative	12 (2.8)	My elder daughter took the flu vaccination for once but got more and severe sicknesses that year. Since then, she has never taken flu vaccination.
Mixed or neutral	5 (1.2)	My two sons have taken the flu vaccination. One is three years old. He was given injection at the hip and he said no pain. Another is seven years old. He was given injection at arm. He said it was very painful and the pain lasted for two days.
Opinions or general attitudes relating to vaccination	26 (6.0)	
Negative	23 (5.3)	Vaccination=Injecting bacteria/viruses into our body
Positive	1 (0.2)	It is an additional protection for our children.
Mixed or uncertain	2 (0.5)	Is it necessary to take flu vaccination if my child is always healthy?
First-time flu vaccination	20 (4.6)	I would like to ask: it is my baby's first flu vaccination. The doctor said he needs to take two doses of vaccines. Then what's the maximum time interval between the two vaccinations?
Influenza risks and consequences	20 (4.6)	But my daughter got flu as soon as she entered pre-nursery school in early September. Then, she had been hospitalized for three days and had to be quarantined.
Eligibility of receiving vaccination subsidy and application procedure	12 (2.8)	In fact, is it only family with low income can receive the vaccination with subsidy?
Influenza vaccine (trivalent/quardrivalent etc.)	10 (2.3)	Which is better? The quadrivalent vaccine or the trivalent one? Which one do you choose? The quadrivalent vaccine or the trivalent one?
Timing of vaccination	10 (2.3)	Is it better to take my child for flu vaccination after he enters a school?
Other influenza preventive measures	6 (1.4)	Actually, I used natural methods, herb oil and food therapy to help my child prevent flu
Mechanism of flu vaccination	4 (0.9)	In fact, it is because that it is to inject the viruses into your body to help your body generate antibody. Therefore, some can get a fever after taking vaccination.
Treatment of influenza	3 (0.7)	I heard that many people got flu vaccination cannot recover without treatment and had to take Tamiflu but Tamiflu has a lot of side effects.

improve their understanding about the risks of influenza and benefits of influenza vaccination for children, but only 59.8% agreed that the information was useful to make children's vaccination decision, and 19.7% reported that information was not sufficient. 87.2% of participants were satisfied with the information provided by the moderator. 84.6% of participants were willing to recommend other mothers to participate in the discussion group.

Discussion

The WhatsApp group was not effective to promote children's SIV uptake, likely because the interventions

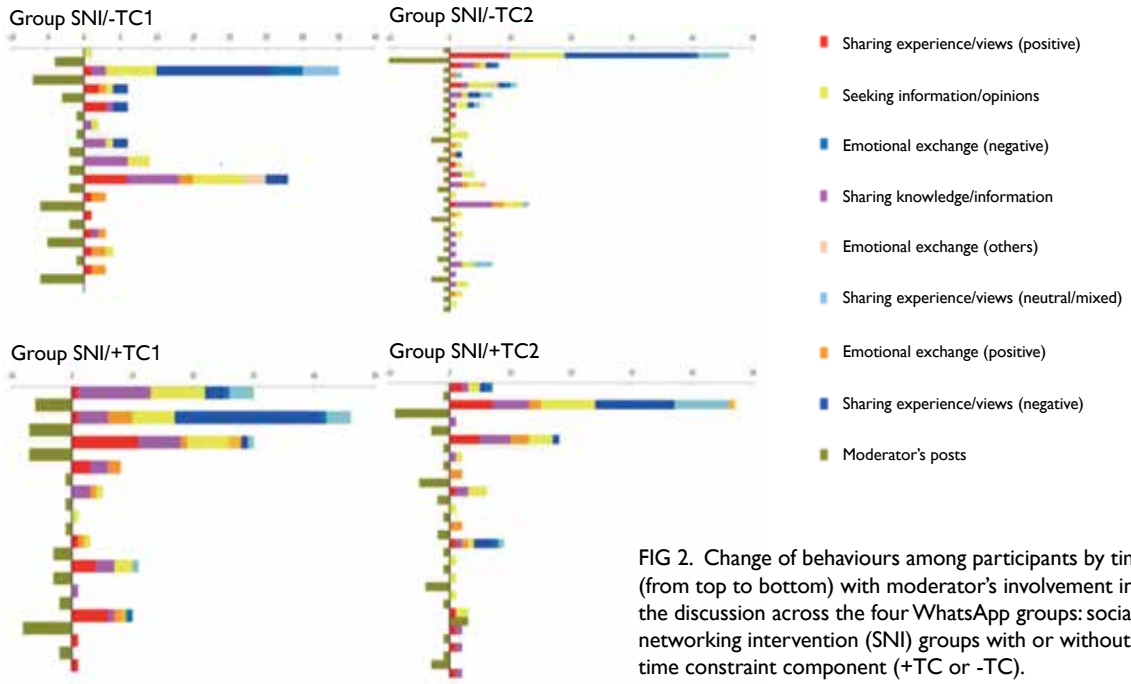

FIG 2. Change of behaviours among participants by time (from top to bottom) with moderator's involvement in the discussion across the four WhatsApp groups: social-networking intervention (SNI) groups with or without time constraint component (+TC or -TC).

did not adequately address participants' concerns over vaccine safety, adverse effects, and effectiveness, as well as negative opinions of vaccination. Incorporating a time constraint component into the vaccination reminder did not have a significant effect on changing children's SIV uptake. This is likely because the time constraint component was not highly valued and that the weekly change in the remaining time for optimal timing of vaccination was not easily noticed by participants. Participants shared various concerns over SIV which seemed to link to their belief that SIV could weaken human immunity, general distrust in how the vaccine strain was estimated and a perception that vaccination is not a natural preventive measure. The WhatsApp group significantly promoted parents' perceived self-efficacy in taking their children for SIV, which is likely to motivate future SIV uptake.[1] However, peer information support (eg information about vaccination clinic and cost and medical eligibility of SIV) may be mainly useful for mothers who had considered taking their child for SIV but should not be able to capture the attention of those who had strong negative views about SIV. The information shared in the WhatsApp groups were mainly useful for improving participants' understanding about SIV but not adequate for some to make vaccination decision. However, the interaction between moderator and participants indicates that health professional active participation and responses can create a more positive discussion about vaccination. This is an important direction for combating vaccine hesitancy in the future.

This study had several limitations. First, our study only recruited participants who used WhatsApp and thereby not be representative for the target population. Second, a WhatsApp group specified for discussing influenza vaccination may automatically exclude those who were not interested in the topic, causing in-group biases. Third, this is a preliminary study and thereby the sample size was not sufficient with a small effect size.

Acknowledgements

We thank Mr Koon Ken Fung for assisting in development of vaccination reminders, testing of the questionnaire, data collection, and data entry. We also thank Miss Ripple Yuan for her assistance in qualitative data analysis. We also thank MVA Hong Kong Ltd for recruiting subjects and conducting baseline telephone interviews.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#16150752). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

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Obstructive sleep apnoea and continuous positive airway pressure therapy for patients with non-alcoholic fatty liver disease: abridged secondary publication

DSC Hui *, SSS Ng, GLH Wong, WCW Chu, A Chan, VWS Wong

KEY MESSAGES

1. Of 226 patients with non-alcoholic fatty liver disease (NAFLD) who underwent home sleep test, 222 had evidence of obstructive sleep apnoea (OSA) with respiratory event index (REI) of ≥5/hour.
2. Both therapeutic and subtherapeutic continuous positive airway pressure therapy (CPAP) had similar effects on non-invasive markers of liver fat, steatosis, and fibrosis after 6 months of treatment. Percentage change in weight after 6 months correlated with the change in transient elastography controlled attenuation parameter, which is a marker of liver fat (B=3.249, SE=0.873, 95% Wald CI=1.538-4.960, P<0.001).
3. CPAP alone is unlikely to alter NAFLD activities in patients with concomitant OSA. The additional role of weight reduction through lifestyle modification deserves further investigation.

Hong Kong Med J 2022;28(Suppl 1):S42-5
HMRF project number: 13140801

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Introduction

Obstructive sleep apnoea (OSA) is associated with metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). OSA may play a role in the progression of hepatic steatosis and the development of steatohepatitis through chronic intermittent hypoxia leading to systemic inflammation, increased oxidative stress, insulin resistance, and dyslipidaemia.[1] This study aimed to screen for OSA in patients with biopsy-proven NAFLD who were followed up at the Hepatology Clinic and assess the effect of auto continuous positive airway pressure therapy (CPAP) versus a control group on subtherapeutic treatment over 6 months with reference to NAFLD activities. We hypothesised that OSA should be common among patients with NAFLD in HK and correction of hypoxemia with nasal CPAP treatment might improve the activities of NAFLD in those with concomitant OSA.

Methods

Patients with biopsy-proven NAFLD were screened for OSA using a home sleep study. The following conditions were excluded as the underlying cause of liver disease in this cohort: a history of excessive alcohol consumption (>30 g/day for men and >20 g/day for women), secondary causes of hepatic steatosis (such as chronic use of systemic corticosteroids), positive hepatitis B surface antigen, anti-hepatitis C virus antibody, or histological evidence of other concomitant chronic liver diseases.[2]

Patients were initially assessed at the respiratory clinic using the Epworth sleepiness score with symptoms evaluated before the home sleep study using the Embletta device.[3] OSA syndrome was defined as a respiratory event index (REI) of ≥5/hour during sleep plus daytime sleepiness or two of the following symptoms: choking or gasping during sleep, recurrent awakenings from sleep, unrefreshed sleep, daytime fatigue, and impaired concentration. REI was defined as the total number of respiratory events scored ×60 divided by monitoring time.

Those with biopsy-proven NAFLD who had REI of ≥5/hour on home sleep test were offered a 30-minute CPAP trial at 4cm$H_2$0 before randomisation into either the auto CPAP group (in auto-adjusting pressure mode ranging 4-20 cm$H_2$0) or subtherapeutic CPAP group (pressure fixed at 4cm$H_2$0).

Baseline measurements included subjective sleepiness based on the Epworth sleepiness score, intrahepatic triglyceride (IHTG) level measured by proton magnetic resonance spectroscopy, liver stiffness assessment measured by fibroscan, routine liver function, and serum cytokeratin-18 fragment. The primary outcome was the difference in changes in IHTG after 6 months of either treatment.

Secondary outcomes included changes in Epworth sleepiness score, transient elastography controlled attenuation parameter (a marker of liver fat), liver stiffness assessment, serum cytokeratin-18 fragment, and objective CPAP usage.[2]

Inclusion criteria were age 20 to 80 years, symptoms of OSA with home embletta REI of ≥5/hour, and NAFLD diagnosed by liver biopsy. Exclusion criteria were (1) unstable cardiovascular diseases (eg recent unstable angina, myocardial infarction, stroke, or transient ischaemic attack within the previous 6 months or severe left ventricular failure), (2) neuromuscular disease affecting or potentially affecting respiratory muscles, (3) moderate-to-severe respiratory disease (ie, breathlessness affecting activities of daily living) or documented hypoxemia or awake SaO_2 <92%, (4) psychiatric disease that limits the ability to give informed consent or complete the study, (5) professional drivers, and (6) gross structural abnormalities (large nasal polyps, gross nasal turbinate hypertrophy or septal deviation, and enlarged 'kissing' tonsils).

Sample size calculation was based on the use of IHTG as the primary endpoint and our previous studies. The mean IHTG in patients with NAFLD was reported to be 12%±6%.[2,4] Patients with NAFLD on usual care had IHTG decreased by 2% in 12 months.[4] An IHTG of 5% was the threshold to define NAFLD; a 7% decrease in IHTG was considered clinically meaningful. Assuming the change in IHTG was -7% in the auto CPAP group and -3% in the subtherapeutic CPAP group, a sample size of 48 patients per arm was expected to achieve 90% power in detecting the difference at a 5% significance level. To allow a dropout rate of 20%, a total of 120 patients were needed.

Data were analysed on an intention-to-treat basis followed by treatment per protocol. For comparisons between the 2 groups at each time point, unpaired t test was used for normally distributed variables and Mann-Whitney U test for non-normally distributed variables. To compare the measurements before and after CPAP treatment, paired t-test was used for normally distributed variables and Wilcoxon's signed rank test for non-normally distributed variables. Two-factor ANOVA (group vs time) with repeated measures on the factor time (baseline minus treatment) was used to test for the effect of CPAP vs conservative treatment. The effect of CPAP treatment and other metabolic parameters including BMI on IHTG was evaluated by multivariable analysis using the linear regression model.

TABLE 1. Baseline demographics of the auto continuous positive airway pressure therapy (CPAP) group and the subtherapeutic CPAP group

Baseline demographic	Auto CPAP group (n=60)*	Subtherapeutic CPAP group (n=60)*	P value
Age, y	54.62±10.37	54.75±9.71	0.943
Weight, kg	77.03±15.52	78.53±14.79	0.408
Body mass index, kg/m^2	28.62±4.47	28.72±4.22	0.783
Neck circumference, cm	39.84±10.64	38.98±3.58	0.288
Waist circumference, cm	98.13±10.97	98.19±11.02	0.815
Hip circumference, cm	103.49±8.79	103.90±8.39	0.631
Epworth sleepiness score	9.18±5.62	9.80±5.24	0.364
Respiratory Event Index	30.38±22.41	33.50±20.58	0.209
Minimum SaO_2 %	77.02±9.59	76.47±8.58	0.423
% of total sleep time with SaO_2 <90%	10.96±17.31	10.64±16.44	0.607
Duration of SaO_2 <90%, min	42.46±65.51	40.51±61.74	0.775
Oxygen desaturation index$_3$	31.48±22.36	33.84±19.88	0.268
Liver stiffness, kPa	6.47±2.46	6.91±3.59	0.789
Transient elastography controlled attenuation parameter, dB/m	319.63±45.53	316.82±41.67	0.717
Fat peak	0.17±0.13	0.16±0.10	0.951
Liver fat, %	13.54±8.99	13.20±7.53	0.951
Total protein, μmol/L	77.08±3.66	76.58±3.99	0.228
Albumin, g/L	39.25±3.00	38.78±3.17	0.419
Total bilirubin, μmol/L	10.63±3.94	11.82±5.96	0.571
Total alkaline phosphatase, IU/L	73.20±23.69	73.40±24.31	0.844
Alanine aminotransferase, IU/L	50.22±41.51	44.28±29.22	0.551
Serum cytokeratin-18 segment	243.58±240.94	229.63±205.32	0.846

* Data are presented as mean ± standard deviation

Results

Of 243 patients with NAFLD who underwent home sleep test, ten failed the test (poor signal [n=8], unable to sleep [n=2]) and seven met exclusion criteria and were withdrawn (hypoventilation [n=2], hepatitis B [n=2], lung cancer [n=1], drivers [n=2]). Of the remaining 226 patients, 222 had REI of ≥5/hour and 198 of them agreed to proceed to a 30-minute CPAP trial at 4 cmH_20. Of the 198 patients, 64 refused further CPAP use and 14 refused randomisation. Finally, 120 patients were equally randomised into either the auto CPAP group or the subtherapeutic CPAP group. The two groups were comparable in terms of baseline demographics, severity of OSA (REI, min SaO_2, % of total sleep time with SaO_2<90%, oxygen desaturation index$_3$ [ODI_3], which is the number of times per hour of sleep that the blood oxygen level dropped by at least 3% from baseline), and NAFLD indices (IHTG, liver stiffness, transient elastography controlled attenuation parameter, serum cytokeratin-18 segment, and liver function blood test parameters (Table 1).

Transient elastography controlled attenuation parameter (a marker of liver fat) correlated with markers of OSA severity including REI (r=0.203,

P=0.026), min SaO_2 (r= -0.167, P=0.068), % of total sleep time with SaO_2 <90% (r=0.265, P=0.003), and ODI_3 (r=0.214, P=0.019). Other fatty liver parameters had no significant correlation with markers of OSA severity (Table 2).

After 6 months of treatment, the objective CPAP usage of the auto CPAP and subtherapeutic CPAP groups was 4.37±2.14 and 3.84±2.29 hours, respectively (P=0.191). The 95th centile pressures were 11.09±1.59 and 4.00±0.00 cmH_20, respectively

TABLE 2. Correlations between obstructive sleep apnoea severity (OSA) markers and non-alcoholic fatty liver disease (NAFLD) markers

OSA marker	Liver stiffness, kPa	Transient elastography controlled attenuation parameter, dB/m	Fat peak	Liver fat, %	Total protein, µmol/L	Albumin, g/L	Total bilirubin, µmol/L	Alkaline phosphatase, IU/L	Alanine amino-transferase, IU/L	Serum cytokeratin-18 segment
Respiratory event index										
Pearson correlation, r	0.057	0.203	0.062	0.069	0.108	0.014	0.094	-0.160	0.027	0.114
P value (2-tailed)	0.538	0.026	0.503	0.453	0.240	0.876	0.309	0.082	0.767	0.214
Minimum SaO_2, %										
Pearson correlation, r	-0.141	-0.167	-0.033	-0.046	0.020	-0.024	-0.096	0.105	-0.034	-0.123
P value (2-tailed)	0.123	0.068	0.724	0.622	0.830	0.793	0.297	0.256	0.713	0.181
% of total sleep time with SaO_2 <90%										
Pearson correlation, r	0.069	0.265	0.176	0.185	0.026	0.117	0.130	-0.152	0.069	0.119
P value (2-tailed)	0.456	0.003	0.062	0.050	0.782	0.214	0.167	0.107	0.466	0.209
Oxygen desaturation $index_3$										
Pearson correlation, r	0.077	0.214	0.099	0.105	0.135	0.041	0.086	-0.136	0.049	0.114
P value (2-tailed)	0.404	0.019	0.298	0.266	0.153	0.664	0.365	0.148	0.608	0.227

TABLE 3. Intention-to-treat analysis of the auto continuous positive airway pressure therapy (CPAP) group and the subtherapeutic CPAP group

Variable	Baseline			Month 6			P value (month 6 vs baseline)		Within-group difference	Between-group difference (95% CI)	P value
	Auto CPAP group (n=60)*	Subtherapeutic CPAP group (n=60)*	P value	Auto CPAP group (n=60)*	Subtherapeutic CPAP group (n=60)*	P value	Auto CPAP group	Subtherapeutic CPAP group			
Epworth sleepiness score	9.18±5.62	9.80±5.24	0.364	6.33±4.66	7.42±4.40	0.087	<0.001	0.001	-2.85±4.88	-0.47 (-2.23 to 1.30)	0.653
Weight, kg	77.03±15.52	78.53±14.79	0.408	77.47±14.91	78.36±14.68	0.567	0.229	0.330	-2.38±4.89	0.99 (-0.43 to 2.41)	0.105
Liver stiffness, kPa	6.47±2.46	6.91±3.59	0.789	6.50±2.25	6.69±3.47	0.787	0.914	0.333	0.84±4.28	0.24 (-0.42 to 0.90)	0.250
Transient elastography controlled attenuation parameter, dB/m	319.64±45.53	316.82±41.67	0.717	316.07±43.15	306.45±43.84	0.283	0.490	0.031	-0.17±3.64	6.80 (-6.97 to 20.57)	0.170
Fat peak	0.17±0.13	0.16±0.10	0.935	0.18±0.12	0.18±0.15	0.585	0.139	0.588	0.03±1.92	-0.01 (-0.04 to 0.02)	0.375
Liver fat, %	13.54±8.99	13.20±7.47	0.944	14.21±8.32	13.83±9.23	0.582	0.149	0.675	-0.22±1.72	0.04 (-1.82 to 1.90)	0.296
Total protein, µmol/L	77.08±3.66	76.58±3.99	0.228	76.37±3.83	76.60±4.28	0.987	0.218	0.993	-3.57±39.84	-0.73 (-1.92 to 0.45)	0.390
Albumin, g/L	39.25±3.00	38.78±3.17	0.419	38.65±2.65	38.33±3.60	0.698	0.160	0.182	-10.37±36.24	-0.15 (-1.02 to 0.72)	0.956
Total bilirubin, µmol/L	10.63±3.94	11.82±5.96	0.571	9.95±3.59	11.47±5.29	0.184	0.074	0.310	0.01±0.08	-0.33 (-1.51 to 0.84)	0.757
Total alkaline phosphatase, IU/L	73.20±23.69	73.40±24.31	0.844	72.80±22.64	74.33±25.37	0.921	0.566	0.762	0.02±0.09	-1.33 (-6.77 to 4.10)	0.823
Alanine amino-transferase, IU/L	50.22±41.51	44.28±29.22	0.551	41.55±28.25	43.53±30.62	0.823	0.027	0.369	0.67±5.45	-7.92 (-17.14 to 1.31)	0.312
Serum cytokeratin-18 segment	243.58±240.94	229.63±205.32	0.846	206.22±193.77	237.50±224.46	0.505	0.208	0.659	0.63±4.79	-45.23 (-110.24 to 19.78)	0.466

(P<0.001) and the residual REI was 3.62±2.3 and 10.88±13.01 per hour, respectively (P<0.001). The two treatment groups were comparable in terms of primary and secondary endpoints based on the intention-to-treat approach (Table 3) and treatment per protocol.

Regression analysis showed that percentage change in weight after 6 months correlated with the change in transient elastography controlled attenuation parameter, which is a marker of liver fat (B=3.249, SE=0.873, 95% Wald CI=1.538-4.960, P<0.001).

Discussion

One study reported that Apnea-Hypopnea Index, ODI, min SaO_2, and % of sleep time with SaO_2 <90% were independent predictors of NAFLD, whereas the most correlated parameter for the severity of NAFLD was the duration of hypoxia during sleep.[6] Our study findings support these correlations of OSA severity with transient elastography controlled attenuation parameter (a marker of liver fat).

Several randomised controlled trials with different treatment duration have showed no significant impact of CPAP in patients with OSA and NAFLD. A meta-analysis concluded that CPAP did not significantly contribute to the improvement in liver histology, liver steatosis, liver fibrosis, and aminotransferase levels,[7] consistent with our findings.

In view of the high (27.3%) prevalence of NAFLD in Hong Kong population[2] and the high frequency of OSA in patients with NAFLD, it is of great interest to examine the role of lifestyle modification[4] on top of CPAP for those with OSA and/or NAFLD using non-invasive assessment of NAFLD activities.

Conclusion

Although transient elastography controlled attenuation parameter correlated with markers of OSA severity, therapeutic and subtherapeutic CPAP had similar effects on non-invasive markers of liver fat, steatosis, and fibrosis. CPAP alone is unlikely to alter NAFLD activities in patients with concomitant OSA. The additional role of weight reduction through lifestyle modification deserves further investigation.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#13140801). 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. Ng SSS, Wong VWS, Wong GLH, et al. Continuous positive airway pressure does not improve nonalcoholic fatty liver disease in patients with obstructive sleep apnea. A randomized clinical trial. Am J Respir Crit Care Med 2021;203:493-501.

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Neuroprotective effects of wolfberry in normal tension glaucoma: abridged secondary publication

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KEY MESSAGES

1. Normal tension glaucoma can progress in the absence of high intraocular pressure.
2. Neuroprotection supplements the therapeutic effect of anti-glaucoma medications.
3. Daily oral wolfberry food supplement for 2 years does not show additional beneficial effect in the treatment of normal tension glaucoma.

Hong Kong Med J 2022;28(Suppl 1):S46-7
HMRF project number: 01121206

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Introduction

We conducted a prospective randomised control study to investigate the neuroprotective effect of wolfberry supplement in normal tension glaucoma (NTG), which is a progressive optic neuropathy that occurs under normal (<21 mmHg) intraocular pressure (IOP). Contributing factors may include nocturnal hypotension, increased blood viscosity, and genetic factors. NTG is associated with migraine headaches, Raynaud phenomenon, and diffuse cerebral ischaemia. Similar to other types of glaucoma, NTG results in progressive and irreversible retinal ganglion cell death that manifests as visual field loss and thinning of the retinal nerve fibre layer (RNFL). The progressive damage of the optic nerve is not caused by high IOP. Despite this, reduction of the IOP slows down the progression of the disease, but in many cases the disease still progresses despite a low IOP.

Wolfberry (*Lycium barbarum*) has been proven in rats to promote retinal ganglion cell survival against elevated IOP.[1,2] The neuroprotective effects of wolfberry are partly due to modulating the activation of the retinal microglia.[3] In animal studies, wolfberry protects neurons from ischaemic and toxic stresses to the brain.[4,5] This study aims to investigate the neuroprotective effects of oral wolfberry supplement in NTG.

Methods

This study was conducted in the eye clinic of the Grantham Hospital. Ethics approval was obtained prior to the commencement of the study. Informed consent was obtained from each participant. Patients with NTG who required anti-glaucoma treatment were invited to participate. NTG was defined as glaucomatous cupping that resulted in general or focal neuroretinal rim thinning, disc haemorrhage, or intereye cup/disc ratio asymmetry of >0.2, and visual field defects, with an IOP consistently <21 mmHg. Diagnosis of NTG was made based on the Goldmann tonometer for IOP, gonioscopy examination of the drainage angle, cup-disc ratio assessment, visual field test using the Humphrey Visual Field Analyzer, and optical coherence tomography of the RNFL thickness using the Heidelberg Spectralis system. For the visual field test, the 24-2 Swedish Interactive Thresholding Algorithm full-threshold Humphrey visual field was used. Medical records and IOP levels of eligible subjects were reviewed before commencement of ocular-hypotensive treatment. Only those with two consecutive pre-treatment IOP of <21mmHg were invited to participate.

Participants were assigned randomly to the control group (topical anti-glaucoma medications) or the wolfberry group (taking 20 wolfberry dry seeds per day) in addition to topical anti-glaucoma medications, which include prostaglandin analogue, carbonic anhydrase inhibitor, beta-blocker, and alpha-agonist. These drugs were used in the above sequence as monotherapy or in combination.

All participants were followed up every 4 months for 2 years. In each visit, they underwent ophthalmic tests including visual acuity test, IOP measurement, gonioscopic examination of the drainage angle, and cup-disc ratio assessment. They attended the eye clinic every 6 months for visual field test and RNFL thickness measurement.

The primary outcome measures were change in the mean RNFL thickness and change in the mean deviation (MD) in the visual field 24-2 tests. The visual field test result with fixation loss, false positive and negative values <20% for the test was considered reliable. For the RNFL measurement, images with signal strength <8 were discarded. The mean RNFL thickness in each of the superior, inferior, nasal, and temporal quadrants was calculated. The secondary outcome measures were the IOP and the cup-disc ratio.

Results

Between December 2014 and December 2016, 113 patients were recruited. Of them, 30 withdrew from the study and 83 (44 in treatment group, 39 in control group) completed the 2-year follow-up. The two groups were comparable in terms of the demographics and baseline clinical parameters (Table 1).

The rates of visual field loss were evaluated by the parameter MD over time through linear mixed models. The rates of RNFL loss over time were evaluated by the mean thickness of RNFL of four quadrants by linear mixed models, in which the average evolution of the outcome variable (ie, MD and RNFL thickness) is described using a linear function of time. Random intercepts and random slopes introduce subject and eye-specific deviations from this average evolution. The model can account for the fact that different eyes may have different rates of visual field loss over time, while also accommodating correlations between both eyes of the same individual. Slopes for individual eyes were estimated by best linear unbiased predictions. Covariates of age, baseline IOP, and baseline average cup-to-disc ratio were adjusted in the mixed linear models. Changes of both the MD and the RNFL thickness were not significant after controlling for age, laterality, baseline MD, baseline IOP, and average cup-disc ratio (Table 2).

Discussion

NTG can develop and progress even in the absence of a persistently elevated IOP. Unstable blood perfusion to the optic nerve may play a role in the pathogenesis. Patients with systemic hypotension, migraine, or obstructive sleep apnoea are at higher risk of developing NTG. Although NTG is IOP-independent, reduction of IOP is effective in slowing down progression of NTG in most cases. Factors that cause unstable blood perfusion to the optic nerve can hardly be altered. Neurologists aim to develop pharmacological agents to protect the nerve and brain tissues. Wolfberry (*Lycium barbarum*) has been proven in a rat model to promote retinal ganglion cell survival against elevated IOP.[1,2] Wolfberry modulates the activation of the retinal microglia to exert its neuroprotective effects.[3] However, this study failed to demonstrate an additional benefit of daily consumption of wolfberry for 2 years in terms of visual field MD and RNFL thickness.

There were limitations to this study. Failure to show a significant difference may be due to the short follow-up period, as glaucoma is a slow progressive disease and may take 5 to 10 years to see the therapeutic effect of the wolfberry supplement. In addition, the sample size may be underpowered. In sample size calculation, it was assumed that 20% of cases would have fast progression with visual field index change >3 dB/year. A more accurate sample size calculation should have been adopted, taking into account the percentage of fast progression in practice. Subjects often decided not to join the study once they knew they were randomised into the control group. This resulted in a relatively long enrollment period. The drop-out rate was high (26.5%), owing to compliance to the treatment protocol. Subjects in the treatment group might not adhere to the dosage of the prescribed oral wolfberry, and subjects in the control group might have taken self-purchased wolfberry. The initial design was to supply wolfberry tablets and placebo tablets. However, owing to the strict regulations for clinical trials, no commercial drug firm agreed to manufacture wolfberry tablets. Therefore, wolfberry dry seeds were used. In addition, dry seeds contained numerous fibres and the dosage was difficult to quantify. The quality of the seeds varies at different times of the year; this may influence their effect. A larger-scale double-blind randomised controlled study using quantified tablets that contain consistent ingredient concentration can increase the recruitment rate and minimise the dropout rate. A longer follow-up for at least 5 years is needed to show any neuroprotective effect of wolfberry.

TABLE 1. Demographic and clinical characteristics of study subjects

Characteristic	Control group (n=39)	Treatment group (n=44)	P value
Age, y	69	74	0.069
Right eye	36	41	
Left eye	31	41	
Visual field baseline	-8.63±7.72	-8.78±6.46	0.896
Visual field mean deviation change	-1.34±3.51	-0.56±2.46	0.136
Retinal nerve fibre layer thickness			
At baseline			
Superior	84.98±20.30	82.65±16.77	0.449
Nasal	65.71±10.82	64.62±10.00	0.529
Temporal	58.20±13.92	59.78±13.03	0.482
Inferior	74.22±19.61	75.41±17.34	0.698
Overall	70.78±12.16	70.61±10.28	0.930
Change			
Superior	-0.15±4.96	-0.14±5.55	0.984
Nasal	-0.43±5.54	0.04±5.83	0.623
Temporal	0.34±3.99	0.52±5.19	0.818
Inferior	0.19±7.94	-0.36±5.22	0.608
Overall	-0.01±4.06	0.01±3.43	0.969
Intraocular pressure at baseline, mmHg	13.69±2.99	13.21±2.58	0.311
Average cup-disc ratio at baseline	0.79±0.76	0.77±0.10	0.285
Vertical cup-disc ratio at baseline	0.79±0.08	0.77±0.10	0.252

* Data are presented as mean or mean±standard deviation

TABLE 2. Multivariable models assessing the changes of visual field in mean deviation between groups over time adjusting for age, laterality, baseline intraocular pressure, and baseline cup-disc ratio

Parameter	Visual field mean deviation change		Retinal nerve fibre layer thickness change	
	Coefficient (confidence interval)	P value	Coefficient (confidence interval)	P value
Groups	-0.66 (-2.2 to 0.91)	0.315	0.34 (-0.11 to 0.78)	0.14
Baseline intraocular pressure	-0.018 (-0.30 to 0.26)	0.84	-0.11 (-0.18 to -0.046)	0.001
Average cup-disc ratio	-2.4 (-11 to 5.9)	0.51	-5.26 (-8.1 to -2.4)	<0.001
Age	-0.029 (-0.081 to 0.023)	0.27	-0.21 (-0.42 to -0.00023)	0.048

Acknowledgements

We thank Mr Keith Leung for his work throughout this study and Dr Li Qing for her statistical analysis of the data.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01121206). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

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