SUPPLEMENT 6

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SUPPLEMENT 6

MEDICAL JOURNAL 香港醫學雜誌

Health and Medical Research Fund

Research Dissemination Reports

ORGAN DONATION AND TRANSPLANTATION

INJURIES AND ACCIDENTS

TRADITIONAL CHINESE MEDICINE

Editorial

Dissemination reports are concise informative reports of health-related research supported by the Health and Medical Research Fund administered by the Health Bureau. In this edition, we present 12 dissemination reports of projects related to cancer, public health, renal and urogenital system, infectious diseases, organ donation, injuries and accidents, and traditional Chinese medicine. In particular, research findings of three projects may provide insights to enhance clinical practices and help inform health policy formulation in Hong Kong.

Gynaecological problems are often investigated using pelvic ultrasound. Accurate prediction of a malignant mass enables appropriate referral to specialised care for follow-up and treatment. There are several different assessment tools available for interpreting ultrasound features including the International Ovarian Tumor Analysis (IOTA), Risk of Malignancy Index (RMI), and Risk of Malignancy Algorithm (ROMA). Chan et al[1] investigated whether ROMA/RMI could replace ultrasound assessment by an expert when IOTA outcome was inconclusive. They found that in Hong Kong women, the IOTA simple rules were 94% accurate in diagnosing pelvic mass malignancy by ultrasound when the IOTA rules were conclusive. The IOTA simple rules were more accurate than ROMA/RMI. In the 25% of cases that IOTA was inconclusive, addition of ultrasound assessment by an expert resulted in higher sensitivity, compared with addition of ROMA/RMI. The results suggest that pelvic masses detected by ultrasound should be assessed by IOTA rules first. Inconclusive results should be assessed by an expert first or by ROMA/RMI if expertise is not available.

Alcohol misuse is a major risk factor for mortality, morbidity, and disability. Kim et al[2] aimed to understand more about the prevalence of various first- and second-hand alcohol-related harms in Hong Kong adults and their associated factors. They found that 21.2% of drinkers experienced first-hand harms in the past year, whereas 18.2% of adults experienced second hand harms in the same period. Severe harms such as assault were rare, but public disturbance and lowered work productivity were common. The density of neighbourhood alcohol outlets was associated with being inconvenienced by inebriated drinkers. There was very little support for regulating alcohol outlets. The authors suggest targeting high-risk drinkers through happy-hour restrictions and minimum pricing regulations.

The first-line treatment for urinary incontinence in women is pelvic floor muscle training (PFMT). Lack of motivation and inability to contract the correct muscles may result in poor adherence to PFMT. A biofeedback device inserted vaginally can detect signals from voluntary muscle contraction, but it causes pain, discomfort, and hence refusal to continue treatment. In a pilot study, Kannan et al[3] compared a new non-invasive biofeedback device with the conventional device and no device on adherence to PFMT, pelvic floor muscle strength, safety, stress urinary incontinence symptoms, and other outcomes in women aged 35 to 60 years with mild to moderate stress urinary incontinence. They found that the new biofeedback device was well accepted and safe for PFMT and helped in strengthening pelvic floor muscles and reducing severity of urine loss after training.

Supplement editor

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Use of ultrasonographic rules and tumour marker HE4 level to predict malignancy of a pelvic mass: abridged secondary publication

KKL Chan *, VYK Chai, VYT Cheung, CKM Choi, MMY Chu, MKY Siu, KY Tse, HYS Ngan

KEY MESSAGES

1. In Hong Kong women, the International Ovarian Tumor Analysis (IOTA) simple rules are 94% accurate in diagnosing malignancy of a pelvic mass detected on ultrasound when the IOTA results are conclusive.
2. IOTA simple rules are more accurate than other assessment methods such as the Risk of Malignancy Index (RMI) and the Risk of Malignancy Algorithm (ROMA).
3. When results of IOTA simple rules are inconclusive in 25% of patients, addition of ultrasound assessment by an expert results in higher sensitivity than addition of ROMA or RMI, despite similar specificity and accuracy.
4. Our findings suggest that pelvic masses detected on ultrasound should be assessed by the IOTA simple rules first. If results are inconclusive, ultrasound assessment by an expert should be added. If expertise is not available, RMI or ROMA should be added to improve accuracy.

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Introduction

Pelvic ultrasound is commonly used to investigate gynaecological symptoms. Accurate prediction of a malignant mass enables appropriate referral to specialised care. The International Ovarian Tumor Analysis (IOTA) simple rules are based on assessment of various pre-defined benign and malignant ultrasound features.[1] The IOTA simple rules can be inconclusive in 25% of cases. In inconclusive cases, addition of ultrasound assessment by an expert is recommended. This study compared various combinations of methods in predicting malignancy of a pelvic mass: the IOTA simple rules with or without ultrasound assessment by an expert, the Risk of Malignancy Index (RMI),[2] or the Risk of Malignancy Algorithm (ROMA).[3] IOTA predicts malignancy by assessing the presence of five benign and five malignant ultrasound features. RMI predicts the risk of malignancy by assessing the menopausal status, ultrasound features, and the tumour marker CA-125 levels. ROMA uses levels of two tumour markers (HE4 and CA-125) to calculate the risk of malignancy. This study aims to determine whether ROMA/RMI can replace ultrasound assessment by an expert when IOTA outcome is inconclusive.

Methods

Consecutive women from one cancer centre and three general units who were scheduled for operation for a pelvic mass were recruited. Before surgery, the women underwent a pelvic ultrasound by a gynaecologist, and the pelvic mass was assessed according to the RMI and IOTA criteria. The risk of ovarian cancer was calculated using each all three methods: IOTA, RMI, and ROMA. For those with inconclusive IOTA results, ultrasound assessment by an expert was performed (Table 1). The final histology of the mass was obtained from the operative sample. The sensitivity, specificity, and accuracy for each combination of methods were

TABLE 1. Various methods to predict malignancy of a pelvic mass detected on ultrasound

Method	Detail
Ultrasound assessment by an expert	Most accurate method. Depends heavily on the level of expertise
International Ovarian Tumor Analysis simple rules	Ultrasound assessment using five benign and five malignant features. Simple to use by operators with basic training
Risk of Malignancy Index	Calculation of risk by five ultrasound features, menopausal status, and biomarker CA-125 level
Risk of Malignancy Algorithm	Calculation of risk by an algorithm that includes menopausal status, biomarkers CA-125 and HE4 levels. No ultrasound features involved.

compared with the McNemar test. The primary outcome was to determine the best method for predicting malignancy in women with inconclusive IOTA results. We assumed that the accuracy for ROMA was around 85%, and that the actual difference between the two methods was 5%, and that the range of non-inferiority was at 5%. A sample size of 160 subjects was expected to have 90% power to show non-inferiority between the two correlated accuracy rates. The one-sided non-inferiority test of two correlated proportions was used. A minimum of 640 women undergoing operation was needed.

Results

A total of 690 women with a histological/cytological diagnosis were included in the analysis (Table 2). Of them 519 (75.2%) had a conclusive IOTA result and 171 (24.8%) had an inconclusive IOTA result. Ultrasound assessment by an expert was more sensitive than the ROMA in diagnosing a malignant mass (81% vs 63%, P=0.009), with no significant difference in specificity (72% vs 73%) or accuracy (76% vs 68%). Among those with conclusive IOTA results, IOTA was more accurate than ROMA in diagnosing a malignant mass (94% vs 84%, P<0.001).

In 640 women with ovarian pathology, IOTA with ultrasound assessment by an expert was more sensitive than IOTA with ROMA (79.9% vs 73.2%, P=0.015, Table 3). Both IOTA with ROMA and ROMA alone were similarly sensitive (73.2% vs 74.3%) and were more sensitive than RMI alone (66.5%, P=0.030). Both IOTA with ROMA and IOTA with RMI as well as IOTA with ultrasound assessment by an expert were similarly accurate. Various combinations of IOTA with ultrasound assessment by an expert or ROMA or RMI were all more accurate than ROMA alone or RMI alone (89.2% vs 88% vs 88% vs 81.6% vs 84.2%, P=0.004 to P<0.001).

Both IOTA with ultrasound assessment by an expert and IOTA with ROMA were similarly sensitive in pre- and post-menopausal women (81% vs 79%) but both were more accurate in pre-menopausal women (92% vs 84%, P=0.009 and 90% vs 83%, P=0.017, respectively). Both ROMA alone and RMI alone were similarly sensitive and specific in pre- and post-menopausal women, but RMI alone was more accurate in pre-menopausal than post-menopausal women (87% vs 77%, P=0.003).

In 315 women from the cancer centre, IOTA with ultrasound assessment by an expert was more sensitive (83% vs 76%, P=0.033) but less specific (87% vs 91%, P=0.040) than IOTA with ROMA, with similar accuracy (85% vs 85%). Both ROMA alone and RMI alone were similarly sensitive, but RMI alone was more specific (87% vs 80%). In 325 women from the three general units, both IOTA with ultrasound assessment by an expert and IOTA with ROMA were similar in sensitivity, specificity, and accuracy. IOTA with ultrasound assessment by an expert or ROMA, or ROMA alone were more sensitive than RMI alone. IOTA with RMI was more sensitive than RMI alone (60% vs 42%, P=0.008).

TABLE 2. Characteristics of patients

Characteristic	One cancer centre*	Three general units*	Total*
No. of patient	341	349	690
Age, y	47 (18-85)	45 (19-89)	46 (18-89)
Menopausal status			
Post-menopausal	113	99	212
Pre-menopausal	228	250	478
Ovarian malignancy	112 (32.8)	30 (8.6)	142 (20.6)
Ovarian			
Benign	184	275	459
Endometriotic cyst	84	97	181
Dermoid	32	71	103
Serous/mucinous cystadenoma	34	57	91
Fibroma	5	7	12
Functional cyst	6	8	14
Hydrosalpinx	1	2	3
Mixed	3	1	4
Others/unspecified	19	32	51
Malignant	112	30	142
Serous	28	9	37
Mucinous	5	2	7
Clear cell	25	5	30
Endometrioid	18	7	25
Mixed	13	0	13
Sex cord stromal/germ cell	4	2	6
Metastatic	10	4	14
Others	9	1	10
Borderline	16	20	36
Malignant/borderline	1	0	1
Non ovarian			
Benign	10	23	33
Malignant	18	1	19
International Federation of Gynecology and Obstetrics staging			
I	36	15	51
II	11	4	15
III	23	2	25
IV	10	2	12
Unstaged	11	0	11
Inconclusive results of International Ovarian Tumor Analysis simple rules	105 (30.8)	66 (18.9)	171 (24.8)

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

TABLE 3. Diagnostic accuracy of five different methods in women with an ovarian pathology (n=640)

Method	Sensitivity*	Specificity*	Accuracy*	Histology malignant (misdiagnosed as low risk)*	Histology benign (misdiagnosed as high risk)*
International Ovarian Tumor Analysis simple rules (IOTA) + ultrasound assessment by an expert	79.9 (73.1-85.3)	92.8 (90.0-94.9)	89.2 (86.5-91.5)	20.1 (14.7-26.9)	7.2 (5.1-10)
IOTA + Risk of Malignancy Algorithm	73.2 (66.0-79.4)	93.7 (91.0-95.7)	88.0 (85.1-90.3)	26.8 (20.6-34)	6.3 (4.3-9)
IOTA + Risk of Malignancy Index	72.1 (64.8- 78.4)	94.1 (91.5- 96.0)	88.0 (85.1-90.3)	27.9 (21.6-35.2)	5.9 (4.0-8.5)
Risk of Malignancy Algorithm alone	74.3 (67.1-80.4)	84.4 (80.7-87.5)	81.6 (78.3-84.4)	25.7 (19.6-32.9)	15.6 (12.5-19.3)
Risk of Malignancy Index alone	66.5 (59.0-73.2)	91.1 (88.0-93.5)	84.2 (81.1-86.9)	33.5 (26.8-41)	8.9 (6.5-12)

* Data are presented as % (95% confidence interval)

Addition of IOTA improved the accuracy of ROMA alone or RMI alone.

Of 142 ovarian cancers, 51 were at stage 1. IOTA with ultrasound assessment by an expert was similar to IOTA with ROMA/RMI or ROMA alone in terms of sensitivity. All combinations of IOTA (with ultrasound assessment by an expert or ROMA or RMI) were more sensitive than RMI alone (81% vs 72% vs 70 % vs 58%, P=0.035 to P=0.003). ROMA alone was more sensitive than RMI alone (70% vs 58%), but the difference did not reach significance (P=0.061).

For 36 borderline tumours, all methods had poor sensitivity in diagnosing borderline tumours (36% to 57%). There was no significant difference between various methods.

Discussion

IOTA simple rules were more accurate than ROMA and RMI in diagnosing a malignant mass when the IOTA results were conclusive. When IOTA result is inconclusive in 25% of women, addition of ultrasound assessment by an expert enhances the accuracy the most.[1] In the present study, addition of ultrasound assessment by an expert increased sensitivity more than addition of ROMA, although differences in specificity and accuracy were not significant. We suggest that women with a pelvic mass detected on ultrasound should be first assessed using the IOTA simple rules by gynaecologists or radiologists. If the mass is at high risk of malignancy, woman should be referred to gynaecological oncologists for further assessment and management. Women with inconclusive results should be referred for an ultrasound assessment by an expert. If such an expert is not available, ROMA or RMI should be added to determine if the mass is malignant. Both ROMA and RMI require a blood test for tumour markers (HE4 and CA-125 for ROMA and CA-125 for RMI); it may be more cost-effective to add RMI than ROMA.

Various combinations of IOTA methods appeared to perform better in pre-menopausal women, consistent with a meta-analysis that reported a higher accuracy of IOTA simple rules in pre-menopausal women, likely owing to better diagnosis of endometriotic or dermoid cysts.[4] All methods were not sensitive in diagnosing borderline malignancies. This may reduce the overall performance of the tests. Borderline cases were excluded in other studies in the literature.

Other methods for predicting malignancy in pelvic masses include logistic regressions models, the IOTA simple rules risk model, and the ADNEX model. Ultrasound assessment by an expert has the best performance, but if such expertise is not available, the IOTA ADNEX model and the IOTA simple rule risk model are recommended.[5] The IOTA simple rules and RMI are most widely used in clinical practice in Hong Kong, mainly owing to the ease of use and recommendations by the Royal College of Obstetricians and Gynaecologists guidelines. We suggest using IOTA simple rules as the first step in assessing a pelvic mass, particularly in the general population setting. For those with inconclusive results, addition of ultrasound assessment by an expert is preferred, owing to a higher sensitivity. Nonetheless, addition of ROMA or RMI has similar accuracy.

Funding

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Disclosure

The results of this research have been previously published in:

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Long noncoding RNA profiling for prognostication in adult acute myeloid leukaemia: abridged secondary publication

HW Ip *, WF Tang, AYH Leung, H Sun, JCC So, JWH Wong

KEY MESSAGES

1. Whole transcriptome sequencing identified a 10-gene long noncoding RNA (lncRNA) prognostic score that has prognostic effects independent of standard European LeukemiaNet risk stratification in acute myeloid leukaemia (AML).
2. The lncRNA prognostic score facilitated more accurate risk stratification in favourable-risk and intermediate-risk AML patients.
3. External validity of the lncRNA prognostic score was demonstrated using large external datasets from Beat-AML and TCGA-LAML.
4. A clinical-grade capture-seq assay was devised and validated to facilitate cost-effective implementation of the lncRNA prognostic score in clinical laboratories.

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Acute myeloid leukaemia (AML) is a neoplastic disorder of stem cells of the myeloid lineage in the haemopoietic system, with diverse pathogenic mechanisms and highly variable clinical outcomes. Prognostication and guidance on clinical management are suboptimal in a large number of AML patients. We established an independent way to stratify risk among patients with AML by using long noncoding RNAs (lncRNA) expression profile measured by massively parallel RNA sequencing (RNA-seq). lncRNA is defined as a group of non-protein-coding RNAs, with transcripts longer than 200 nucleotides. Although lncRNA has been reported to have significant prognostic roles in AML, the implementation of gene expression profiles for AML prognostication has been hampered by the suboptimal external validity of the clinical trials, as studies tend to yield non-overlapping sets of prognostic lncRNA that may not be applicable outside the setting of the original trials. To circumvent this, this study included local discovery and validation datasets, while leveraging large external datasets for validation, to develop a reliable set of lncRNA biomarkers for patient prognostication applicable to patients with AML in Hong Kong.

The discovery cohort comprised 185 patients with newly diagnosed adult AML presented between 2007 and 2018. Ribosomal RNA-depleted and globin mRNA-depleted deep whole transcriptome sequencing was performed to delineate the lncRNA expression profiles in these patients. A 10-gene lncRNA signature was shortlisted by penalised regression from the discovery cohort and was shown to be prognostically significant on top of standard prognostic indicators such as patient age, white cell count at diagnosis, and European LeukemiaNet (ELN) risk stratification by genetic status.[1] This observation was confirmed by independent external data of large cohorts from The Cancer Genome Atlas (TCGA)[2] and Beat-AML.[3]

In clinical practice, one difficulty in managing patients with AML lies in the inaccuracy of predicting those patients with less favourable outcome in the ELN favourable-risk and intermediate-risk groups using current risk stratification tools for AML. For example, when our study cohort and the cohorts of Beat-AML and TCGA-LAML were risk-stratified by ELN 2017, non-significant difference in overall survival was shown between ELN intermediate-risk and adverse-risk groups. Less aggressive treatment strategies were often selected for these patients with underestimated disease risks, resulting in disease relapse and subsequent non-salvageable disease. Because the lncRNA prognostic score has independent prognostic significance on top of ELN risk stratification, a new classification system was devised based on a combination of ELN and the lncRNA score. Patients with AML were first classified by ELN 2017. If the lncRNA score was above a given threshold, patients would be categorised to a less

favourable prognostic category than the original ELN 2017 risk. Using this new classification, we successfully obtained a favourable-risk group that has clinical outcome consistently better than the ELN favourable-risk group across all three cohorts. Similarly, a better segregation was observed between the new intermediate-risk and adverse-risk groups, compared with the ELN intermediate-risk and adverse-risk groups.

In view of the significant clinical utility of our findings in the risk stratification of patients with AML, a capture-seq panel for massively parallel sequencing was designed to study the 10 lncRNA with prognostic relevance, along with reported fusion genes in leukaemias, genes with clinical significant expression profiles in haematological malignancies, and housekeeping genes. This panel provides a cost-effective one-stop testing platform for the diagnosis and prognostication of AML. In a correlation cohort (using 21 samples from the initial discovery cohort), the capture-seq assay had excellent correlations with the expression levels measured by whole transcriptome sequencing (r=0.63-0.93). To independently demonstrate the clinical utility of the capture-seq assay to measure the 10 lncRNA for prognostication in AML and calculate the lncRNA prognostic score, samples from 71 consecutive patients with AML who presented between 2019 and 2021 after the initial discovery cohort were retrospectively retrieved for testing. To use the original lncRNA prognostic score calculation, the lncRNA expression profile was converted from capture-seq scale to transcriptome scale by means of the regression coefficients obtained from the correlation cohort. The prognostic significance of the lncRNA class measured by capture-seq in AML was successfully validated. This provided evidence to substantiate the use of capture-seq for cost-effective measurement of the 10 lncRNA and calculation of the lncRNA score in Hong Kong for better risk stratification of patients with AML.

This study supports the clinical usefulness of the lncRNA prognostic score, which paves ways for the introduction of expression profiling of lncRNA in clinical laboratories.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#05160046). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

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The targeted DNA-sequencing panel that provided baseline genomic data in 2016 to 2019 was supported by Hong Kong Blood Cancer Foundation. We thank clinical haematologists of Queen Mary Hospital, Pamela Youde Nethersole Eastern Hospital, Princess Margaret Hospital, and Tuen Mun Hospital for their support in biobanking for patients with AML.

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Berberine suppresses metastasis and recurrence of hepatocellular carcinoma by targeting circulating tumour cells: abridged secondary publication

CS Cheng, HY Tan, C Zhang, YT Chan, ZJ Zhang, K Man, MF Yuen, N Wang, Y Feng *

KEY MESSAGES

1. In mice with hepatocellular carcinoma with and without surgical resection, a combination of berberine and sorafenib potently improved the therapeutic efficacy and outcome without major adverse effects.
2. In our mouse model, berberine suppressed the in vitro viability and presentation of CD44+EpCAM+ circulating tumour cells (CTCs), as berberine is associated with inhibition of the invasiveness and re-attachment of CTCs and induction of cell apoptosis.
3. The inhibitory effect of berberine on CTCs was due to suppressed expression of CD44 and epithelial-mesenchymal transition pathways in CTCs.

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Introduction

The mechanism of haematogenous invasion and metastasis of hepatocellular carcinoma (HCC) is multi-factorial. Primary cancer–derived circulating tumour cells (CTCs) are the active source of HCC metastasis and recurrence.[1] CTCs disseminating from the primary lesion to the blood circulation is the intermediate stage of tumour metastasis.[2] In a meta-analysis of 23 papers, the presence of CTCs in HCC patients were strongly correlated with the relapse-free survival (relative risk=3.03, P<0.00001), overall survival (relative risk=2.45, P<0.00001), tumour, node, and metastasis stage (relative risk=1.30, P=0.03), tumour size (relative risk=1.36, P=0.006), vascular invasion (relative risk=1.99, P<0.0001), portal vein tumour thrombus (relative risk=1.73, P=0.0001), and serum alpha-fetoprotein level (relative risk=2.05, P=0.01).[3] CTCs are of clinical significance in HCC progression, especially metastasis and recurrence.

Berberine is a natural isoquinoline alkaloid from various medicinal plants such as *Coptis chinensis*. Berberine has anti-tumour effects on HCC.[4] Berberine is a potent blocker of intrahepatic and distant metastasis of HCC.[5] We postulate that berberine may inhibit metastasis and recurrence of HCC by suppressing CTCs.

Methods

In a mouse HCC model, we studied the efficacy and safety of a combination of berberine and sorafenib in improving treatment outcomes and preventing recurrence after surgical resection. In vitro inhibition of berberine on the cell viability, invasion, and re-attachment of CTCs were measured by relevant assays. Pathways related to the inhibitory effect of berberine were studied by a gain-of-function assay using CRISPR activation plasmid.

Results

In mice with sorafenib treatment alone, a marginal response was observed. However, co-treatment with berberine significantly improved the efficacy of sorafenib in a dose-dependent manner. Berberine treatment with or without sorafenib had minimal effect on the body weight of mice. The inhibitory effect on HCC progression was supported by the reduced plasma alpha-fetoprotein level and end-point tumour size. To determine whether sorafenib or berberine could suppress the CD44+EpCAM+ CTCs, CTC population was measured by flow cytometry. Sorafenib treatment alone did not affect the presentation of CTCs, but berberine treatment with or without sorafenib potently reduced CTCs levels in circulation. This suggested that co-treatment with berberine improved the sorafenib efficacy in reducing CTCs level. For safety, histological analysis of the liver, lung, and kidney showed no major tissue damage after berberine treatment with or without sorafenib. These observations indicated that berberine was effective and safe to improve sorafenib efficacy in HCC.

In postsurgical HCC mice with berberine treatment, plasma alpha-fetoprotein level and hepatic tumour burden were potently reduced, without body weight gain or loss. This suggested an inhibitory effect on tumour recurrence. Although a higher abundance of CTCs was observed in post-surgical mice, berberine treatment decreased the CTCs in the circulating system. For safety, histological analysis of the liver, lung, and kidney showed no major tissue damage after berberine treatment. This suggested that berberine was effective and safe to be an adjuvant therapy for HCC after surgical resection.

Berberine significantly reduced CD44 expression, which is the marker on the cell surface of CTCs. Expression of epithelial-mesenchymal transition, including Snail and Vimentin, was potently suppressed by berberine treatment. To determine the mechanism of action, we overexpressed CD44 in the parent HCC cells using CRISPR activation plasmid, which significantly recovered the expression of CD44 in the isolated CTCs treated with berberine. Recovery of CD44 expression potently nullified the inhibitory effect of berberine on Snail and Vimentin expression. This suggested that CD44 was the critical mediator involved in the action of berberine. Recovery of CD44 expression increased viability of CTCs upon berberine exposure, restored the invasion and re-attachment of CTCs, and inhibited cell apoptosis. This suggested that berberine regulated CTCs through CD44-mediated epithelial-mesenchymal transition pathways.

Discussion

In our mouse model, berberine suppressed the in vitro viability and presentation of CD44+EpCAM+ CTCs, as berberine inhibited the invasiveness and re-attachment of CTCs and induced cell apoptosis. Recovery of CD44 expression nullified the inhibitory effect of berberine on epithelial-mesenchymal transition expression in CTCs, restored invasion and re-attachment of CTCs, and inhibited cell viability. In HCC mice with and without surgical revision, combined treatment with berberine and sorafenib potently improved the therapeutic efficacy and outcomes without major adverse effects.

In patients with HCC not suitable for surgery, recurrence of HCC is common. Treatment option for advanced and/or recurrent HCC is limited, and distant metastasis is a risk factor for poor survival. Berberine is an affordable and safe natural compound; it provides an alternative treatment for HCC metastasis and recurrence by targeting CTCs.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#16172751). 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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2. Chen F, Zhong Z, Tan HY, Wang N, Feng Y. The significance of circulating tumor cells in patients with hepatocellular carcinoma: real-time monitoring and moving targets for cancer therapy. Cancers (Basel) 2020;12:1734.
3. Chen F, Zhong Z, Tan HY, et al. Uncovering the anticancer mechanisms of Chinese herbal medicine formulas: therapeutic alternatives for liver cancer. Front Pharmacol 2020;11:293.

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First- and second-hand harms of alcohol consumption in Hong Kong: a population-based study (abridged secondary publication)

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KEY MESSAGES

1. In Hong Kong, despite low per capita alcohol consumption levels, 18.2% of adults reported to have experienced second-hand harms in past year, whereas 21.2% of past-year drinkers reported to have experienced first-hand harms in past year.
2. Severe harms such as assault were rare, but public disturbance and lowered work productivity were common.
3. Neighbourhood alcohol outlet density was associated with being inconvenienced by inebriated drinkers.
4. There was extremely low support for regulating alcohol outlets. Hence, regular monitoring of these harms is suggested; future policy actions may target high-risk drinkers (younger drinkers and binge drinkers) through happy-hour restrictions and minimum pricing regulations.

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Introduction

Alcohol misuse is one of the top risk factors worldwide for mortality, morbidity, and disability. Alcohol misuse incurs heavy social and economic burden through costs of treatment, prevention, law enforcement, and lost productivity.[1] In addition to acute and chronic health conditions, alcohol misuse can cause impaired work performance, domestic violence, and public vandalism.[1] The prevalence of second-hand alcohol harms was high among western populations, but such data are limited in Asia.

In 2008, Hong Kong eliminated 30% tax on beer and 40% tax on wine as a means to boost the economy. This resulted in an increase in per capita alcohol consumption[2] and in the proportion of ever-drinkers in the population.[3] The Hong Kong government identifies reduction of alcohol-related harms as a priority,[4] but few alcohol regulations have been enacted to curb the increasing trend of alcohol use. There is a lack of understanding of alcohol-related harms in the public. This study aims to describe the prevalence of various first-hand and second-hand alcohol-related harms in Hong Kong adults and to identify factors associated with different harms.

Methods

Chinese Hong Kong permanent residents aged 18 to 74 years were the target population. Between August and December 2019, 3200 respondents were interviewed through telephone by trained interviewers using a pilot-tested structured questionnaire. The overall response rate among eligible households was 57.3%.

Data collected included respondent's sex, age, marital status, education attainment, employment status, housing type, district of residence, and monthly household income. Respondents were classified as past-year drinkers and ever drinkers when reported drinking in the past year and ever drinking, respectively, a full serving of alcohol. Past-year drinkers were asked about their past-year drinking frequency, location, and reason. Consistent with the World Health Organization's definition of heavy episodic drinking, binge drinking was defined as consuming ≥6 drinks on one occasion in the preceding 30 days. The four-item CAGE questionnaire was used to assess alcohol use disorder. Respondents were asked whether they had experienced second-hand harms in the past year in 24 items under four categories: friendship and social network, family, public, and work or school-related. Past-year drinkers were asked whether they had experienced first-hand harms in the past year in 32 items under the same four categories plus physical/mental and financial categories.

In addition, respondents were asked about their perception on the number of alcohol outlets and alcohol serving hours near their neighbourhood (should reduce/no change needed/should increase). Information on all on-premise alcohol outlet licences active on 7 November 2018 was obtained, as was information on 52 secondary planning units and boundary maps and demographic statistics of each unit.

TABLE 1. Prevalence of first-hand harms among past-year drinkers and second-hand harms among all respondents in past year by sex

Alcohol-related harm	Overall (n=949)*	Male (n=608)*	Female (n=341)*	P value
First-hand harm				
Physical and mental health harm				
Had short-term health problems like nausea or hangovers or sleep problems after drinking	12.1 (10.2-14.4)	12.0 (9.6-14.8)	12.3 (9.2-16.3)	0.89
Blacked-out or lost consciousness after drinking	4.7 (3.6-6.3)	5.3 (3.7-7.4)	3.8 (2.2-6.5)	0.32
Drinking contributed to smoking more than usual	1.9 (1.2-3.0)	2.3 (1.4-3.9)	1.2 (0.4-3.1)	0.32
Been told by a doctor that your drinking was worsening any chronic physical health problems like gastritis	1.0 (0.5-1.8)	0.8 (0.3-2.0)	1.2 (0.4-3.1)	0.73
Drinking contributed to long-lasting mood problems (eg, depression)	0.9 (0.5-1.8)	1.2 (0.5-2.4)	0.6 (0.1-2.3)	0.50
Had an adverse reaction with medications you were taking	0.8 (0.4-1.7)	1.0 (0.4-2.2)	0.6 (0.1-2.3)	0.72
Gambled more than usual after drinking	0.5 (0.2-1.3)	0.8 (0.3-2.0)	0.0	0.17
Had reproductive/sexual or pregnancy-related problems after drinking	0.2 (0.1-0.8)	0.3 (0.1-1.3)	0.0	0.54
Had to go to the hospital or emergency room after drinking	0.1 (0.01-0.7)	0.0	0.3 (0.04-0.2)	0.36
Used drugs (such as ecstasy) after drinking	0.1 (0.01-0.7)	0.2 (0.02-1.2)	0.0	1.00
Any health harms	15.7 (13.5-18.2)	16.1 (13.4-19.3)	15.0 (11.5-19.2)	0.64
Work-related harm				
Missed work/school or came in late the day after drinking	6.4 (5.0-8.2)	7.1 (5.3-9.4)	5.3 (3.3-8.2)	0.28
Had poorer performance/productivity, or got yelled at work or school after drinking	1.8 (1.1-2.9)	2.1 (1.2-3.7)	1.2 (0.4-0.3)	0.29
Felt drinking harmed career/studies	1.7 (1.0-2.7)	2.0 (1.1-3.4)	1.2 (0.4-0.3)	0.44
Had an injury at work/school after drinking	0.3 (0.1-1.0)	0.5 (0.2-1.5)	0.0	0.56
Drinking contributed to personal conflicts at work/school	0.1 (0.01-0.7)	0.2 (0.02-1.2)	0.0	1.00
Any work-related harms	7.5 (6.0-9.3)	8.4 (6.4-10.9)	5.9 (3.8-8.9)	0.16
Friendship and social network harm				
Did something very embarrassing after drinking that caused regret	4.3 (3.2-5.8)	4.3 (2.9-6.2)	4.4 (2.7-7.2)	0.93
Felt drinking harmed any of close friendships	1.7 (1.0-2.7)	2.0 (1.1-3.4)	1.2 (0.4-3.1)	0.44
Felt drinking harmed social life	1.5 (0.9-2.5)	2.1 (1.2-3.7)	0.3 (0.04-2.1)	0.02
Had a romantic/sexual encounter after drinking that caused regret later	1.2 (0.6-2.1)	1.3 (0.7-0.3)	0.9 (0.3-2.7)	0.76
Any social network harms	6.6 (5.2-8.4)	7.4 (5.6-9.8)	5.3 (3.3-8.2)	0.21
Public harm				
Got verbally harassed or yelled at in public after drinking	2.8 (2.0-4.1)	3.6 (2.4-5.4)	1.5 (0.6-3.5)	0.06
Felt unsafe in public places (eg, street or public transportation)	1.8 (1.1-2.9)	1.3 (0.7-2.6)	2.6 (1.4-5.0)	0.15
Had your property/personal belongings damaged/stolen while drinking	1.1 (0.6-1.9)	1.3 (0.7-2.6)	0.6 (0.1-2.3)	0.51
Been physically harassed, hit/pushed or injured after drinking	0.3 (0.1-1.0)	0.3 (0.1-1.3)	0.3 (0.04-0.2)	1.00
Been sexually harassed or inappropriately touched after you drank	0.2 (0.1-0.8)	0.2 (0.02-1.2)	0.3 (0.04-2.1)	1.00
Paid fines or got arrested after drinking	0.1 (0.01-0.7)	0.2 (0.02-1.2)	0.0	1.00
Been involved in a traffic accident after drinking	0.1 (0.01-0.7)	0.2 (0.02-1.2)	0.0	1.00
Any public harms	4.1 (3.0-5.6)	4.4 (3.1-6.4)	3.5 (2.0-6.1)	0.49
Family harms				
Had verbal argument with family/got yelled at home after drinking	2.1 (1.4-3.2)	2.6 (1.6-4.3)	1.2 (0.4-3.1)	0.16
Felt that it contributed to long-term bad feelings with family or caused disharmony in home-life	1.1 (0.6-1.9)	1.3 (0.7-2.6)	0.6 (0.1-2.3)	0.51
Been involved in physical argument at home after drinking	0.8 (0.4-1.7)	1.2 (0.5-2.4)	0.3 (0.04-2.1)	0.27
Felt that drinking interfered with family roles (eg, being a parent or sibling)	0.5 (0.2-1.3)	0.5 (0.2-1.5)	0.6 (0.1-2.3)	1.00
Any family harms	3.2 (2.2-4.5)	4.1 (2.8-6.0)	1.5 (0.6-3.5)	0.03

* Data are presented as % (95% confidence interval)

TABLE 1. (cont'd)

Alcohol-related harm	Overall (n=949)*	Male (n=608)*	Female (n=341)*	P value
Financial harms				
Drinking contributed to any financial harms	0.1 (0.01-0.7)	0.0	0.3 (0.04-0.2)	0.36
Any first-hand harm	21.2 (18.7-23.9)	21.7 (18.6-25.2)	20.2 (16.3-24.9)	0.59
Second-hand harm	n=3200	n=1418	n=1720	
Public harm				
Been inconvenienced by drunks vomiting, urinating or littering	9.2 (8.2-10.2)	10.3 (8.8-11.9)	8.2 (7.0-9.6)	0.04
Felt unsafe in public places (eg street or MTR)	5.6 (4.8-6.4)	5.9 (4.8-7.2)	5.3 (4.4-6.5)	0.52
Been kept awake at night or disturbed by drinkers	5.2 (4.4-6.0)	5.4 (4.4-6.7)	4.9 (4.0-6.1)	0.56
Been verbally harassed by drinkers in public	2.7 (2.2-3.3)	3.7 (2.9-4.8)	1.7 (1.2-2.5)	0.001
Had property/personal belongings damaged/stolen by drinker	0.5 (0.3-0.9)	1.0 (0.6-1.7)	0.1 (0.03-0.5)	<0.001
Been physically harassed, hit/pushed, or injured	0.5 (0.3-0.9)	0.9 (0.5-1.5)	0.2 (0.1-0.6)	0.01
Been involved in a traffic accident when someone else had been drinking	0.3 (0.1-0.5)	0.6 (0.3-1.2)	0.0	0.001
Been sexually harassed or inappropriately touched	0.3 (0.1-0.5)	0.3 (0.1-0.7)	0.2 (0.1-0.6)	0.83
Had to call the police or go to court because of some stranger's drinking	0.2 (0.1-0.4)	0.4 (0.2-0.9)	0.0	0.001
Any public harms	12.9 (11.8-14.1)	14.1 (12.4-15.9)	11.9 (10.5-13.5)	<0.001
Friendship and social network harm				
Had to go out of the way to help drunk friends	5.7 (4.9-6.5)	7.6 (6.3-9.0)	4.0 (3.2-5.1)	<0.001
Others' drinking harmed close friendships	2.1 (1.7-2.7)	3.1 (2.3-4.1)	1.2 (0.8-1.9)	<0.001
Others' drinking harmed the social life	1.8 (1.3-2.3)	2.6 (1.9-3.6)	1.0 (0.6-1.2)	0.001
Any social network harms	6.7 (5.8-7.6)	9.1 (7.8-10.7)	4.5 (3.6-5.6)	<0.001
Family harm				
Had worries or stress about the drinking behaviours of family members/relatives	3.8 (3.2-4.5)	4.3 (3.3-5.4)	3.4 (2.6-4.3)	0.19
Had to take care of any family/relatives who drank too much	2.6 (2.1-3.2)	3.2 (2.4-4.3)	2.0 (1.5-2.8)	0.03
Been involved in an argument, been yelled at or had to intervene in an argument at home after a family/relative was drinking	1.4 (1.1-1.9)	1.8 (1.2-2.6)	1.2 (0.8-1.8)	0.16
Family member's drinking caused major embarrassment/loss of face	1.4 (1.0-1.8)	2.0 (1.4-2.8)	0.9 (0.5-1.4)	0.01
Other people's drinking ever caused problems with own family members	1.2 (0.9-1.6)	1.5 (1.0-2.2)	0.9 (0.6-1.5)	0.15
Drinking of family member contributed to financial or legal problems	0.4 (0.2-0.7)	0.5 (0.3-1.1)	0.2 (0.1-0.6)	0.25
Been physically harassed, hit/pushed or suffered injury after a family member had been drinking (including drink-driving)	0.4 (0.2-0.7)	0.7 (0.4-1.3)	0.1 (0.02-0.5)	0.02
Any family harm	5.2 (4.5-6.0)	6.0 (4.9-7.4)	4.5 (3.6-5.6)	<0.001
Work-related harm				
Lost productivity due to drinkers at work/school	1.1 (0.8-1.6)	1.6 (1.1-2.4)	0.7 (0.4-1.2)	0.02
Experienced any type of mental stress from drinkers at work/school	0.6 (0.4-1.0)	1.1 (0.7-1.8)	0.2 (0.1-0.6)	0.003
Incurred job/school-related problems from drinking of others	0.6 (0.4-1.0)	1.1 (0.7-1.8)	0.2 (0.1-0.5)	0.001
Had financial losses due to other people's drinking at work/school	0.4 (0.3-0.7)	0.7 (0.4-1.3)	0.2 (0.1-0.5)	0.03
Got into personal conflicts at work/school from other's drinking	0.3 (0.2-0.6)	0.5 (0.3-1.1)	0.1 (0.03-0.5)	0.05
Had physical injury from drinkers at work/school	0.3 (0.2-0.6)	0.7 (0.4-1.3)	0.1 (0.01-0.4)	0.004
Any work-related harm	1.9 (1.6-2.4)	2.8 (2.1-3.8)	1.0 (0.7-1.7)	<0.001
Any second-hand harm	18.2 (16.9-19.5)	20.4 (18.4-22.5)	16.3 (14.6-18.1)	<0.001

The lifetime and past-year prevalence of alcohol-related harms was reported as percentages with 95% confidence interval (CI) by sex. Univariable logistic regression was conducted to identify factors associated with past-year experience of any first-hand and second-hand harms. Variables with a P value of <0.20 were included in the stepwise multivariable logistic regression. Neighbourhood alcohol outlet density was calculated by dividing the number of alcohol outlet licences within the respondent's secondary planning unit by the total population in the unit. Statistical significance was set at P<0.5.

Results

A total of 1480 men and 1720 women were included. The sample is comparable to Hong Kong general population in terms of sex, age, and district of residence.[5] The prevalence of ever and past-year drinkers was 61.6% and 41.1% among men and 31.6% and 19.8% among women, respectively (Table 1). The prevalence of past-year binge drinkers was 9.0% among men and 2.4% among women, whereas 16.5% of men and 4.7% of women drank at least weekly.

First-hand harms

Of past-year drinkers, 54.3% and 21.2% reported having experienced first-hand harms in their lifetime and in past year, respectively. The most common ones were health harms such as hangover (12.1%) and blackouts (4.7%), work-related harms such as absenteeism (6.4%), social network harms such as doing something embarrassing (4.3%). The least common harms were public harms and family-related harms.

Binge drinking, weekly drinking, and alcohol use disorder were associated with significantly higher risk of experiencing first-hand harms in past year (Table 2). In multivariable analysis, younger drinkers reported to have experienced significantly more first-hand harms (adjusted odds ratio [aOR]=2.64, 95% CI=1.63-4.28), particularly in health, work, and social network harms (aOR=1.92-6.80, P<0.05). Compared with married drinkers, single drinkers reported to have experienced significantly more public harms (aOR=2.25, 95% CI=1.12-4.52). Higher education attainment was independently associated with family harms (aOR=8.35, 95% CI=2.78-25.14) and social network harms (aOR=3.59, 95% CI=1.57-8.20). Sex, communal living, and work-related drinking were not associated with first-hand harms after adjusting for socio-demographic and drinking-related factors. Neighbourhood alcohol outlet density was not associated with any first-hand harms.

Second-hand harms

Of respondents, 45.3% and 18.2% reported to have had lifetime and past-year experience of second-hand harms, respectively. The most common ones were public harms such as being inconvenienced by drinkers (9.2%), feeling unsafe in public (5.6%), and sleeping disturbance (5.2%), followed by having to help drunk friends (5.7%) and stress about family member's drinking (3.8%) [Table 1]. Work-related harms were the least common (2.3%).

In multivariable analysis, experience of any second-hand harm in the past year was independently associated with younger age, higher education attainment, past-year drinking, and communal living (aOR=1.35-4.30, P<0.05, Table 3). Any past-year drinking, especially binge drinking, increased the risk for all categories of second-hand harms (aOR=1.63-14.28, P<0.05), whereas having higher education and communal living arrangements were independently associated with family and social network harms only. Neighbourhood alcohol outlet density was not associated with any second-hand harm, except being inconvenienced by inebriated bar patrons.

Perception on neighbourhood alcohol outlet regulation

38.4% of respondents supported reducing alcohol-serving hours, whereas only 4.2% of respondents supported reducing the number of neighbourhood alcohol outlets. However, 93.3% considered that there was no need to change. Those with past-year experience of any second-hand harms were significantly more supportive for reducing outlet density (OR=3.36, 95% CI=2.35-4.79).

Discussion

In Hong Kong, despite the low alcohol consumption culture, 18.2% of adults reported to have experienced second-hand harms in past year, whereas 21.2% of past-year drinkers reported to have experienced first-hand harms in past year. Although severe alcohol-related harms such as assault are uncommon, moderate levels of harms such as public inconveniences and lowered work productivity suggest a need for regular monitoring of these harms. Given the extremely low public support for regulating serving hours and density of alcohol outlets, future policy actions may target high-risk drinkers such as younger drinkers and binge drinkers by considering happy-hour restrictions and minimum pricing regulations. As public disturbances are the most common harm, further regulation on on- and off-premises license

TABLE 2. Factors associated with experiencing at least one first-hand harm in the past year (n=949)

	Any health harm		Any work-related harm	
	Univariable analysis†	Multivariable analysis‡	Univariable analysis†	Multivariable analysis‡
Sex				
Male	1.00	-	1.00	-
Female	0.92 (0.63-1.32)	-	0.68 (0.40-1.16)	-
Age, y				
55-74	1.00	1.00	1.00	1.00
35-54	0.85 (0.48-1.48)	0.82 (0.46-1.47)	1.03 (0.41-2.59)	0.94 (0.36-2.46)
18-34	1.94 (1.17-3.21)*	1.92 (1.15-3.22)*	3.31 (1.47-7.45)*	3.77 (1.63-8.75)*
Marital status				
Currently married	1.00	-	1.00	-
Single, never married	2.01 (1.39-2.89)*	-	3.18 (1.84-5.47)*	-
Divorced/widowed/separated	1.42 (0.47-4.29)	-	-	-
Education				
Secondary or less	1.00	-	1.00	-
Upper secondary non-degree	0.99 (0.54-1.84)	-	2.22 (1.06-4.69)*	-
University or above	1.21 (0.93-1.76)	-	1.56 (0.90-2.71)	-
Employment				
Unemployed	1.00	-	1.00	-
Employed	0.79 (0.55-1.15)	-	0.92 (0.55-1.54)	-
Monthly household income, HK$				
<25 000	1.00	-	1.00	-
25 000-49 999	0.95 (0.62-1.45)	-	1.52 (0.86-2.69)	-
≥50 000	0.74 (0.47-1.16)	-	0.59 (0.29-1.19)	-
Binge drinking	3.58 (2.44-5.26)*	3.23 (2.17-4.80)*	4.50 (2.73-7.42)*	2.36 (1.30-4.28)*
Weekly drinking	1.96 (1.37-2.79)*	-	3.25 (1.98-5.33)*	2.46 (1.35-4.46)*
Alcohol use disorder	2.93 (1.70-5.05)*	2.34 (1.31-4.16)*	5.74 (3.13-10.53)*	4.61 (2.38-8.94)*
Communal living	1.00 (0.38-2.64)	-	2.44 (0.91-6.56)	-
Work-related drinking	1.34 (0.75-2.38)	-	1.60 (0.64-3.35)	-
Alcohol outlet density in area of residence (by population)	0.97 (0.92-1.02)	-	0.88 (0.69-1.11)	-

* P<0.05

† Data are presented as odds ratio (95% confidence interval)

‡ Data are presented as adjusted odds ratio (95% confidence interval)

condition should be considered. The general public and policy makers should give more consideration to the extensive external effects of alcohol use and should facilitate social welfare to provide services to victims of first-hand and second-hand harms of alcohol consumption, as family harms (emotional stress from the drinking of family members) was most common.

There are limitations to this study. Our findings cannot directly compare with those of previous studies, as there is no international consensus on instruments to measure alcohol-related harms. Our questionnaire was developed for exploratory purpose and requires validation. The perception of harms is subjective. Our findings are prone to information bias from self-reported data. The slight over-representation of the non-working population in our sample may result in an underestimation of workplace harms prevalence. We did not ask about the perpetrator or context of second-hand harms, which warrants further research. Although respondents' support for regulating alcohol outlets was evaluated, more evidence on public opinion is needed to inform future alcohol policy.

Any public harm		Any family harm		Any social network harm		Any first-hand harm	
Univariable analysis†	Multivariable analysis‡	Univariable analysis†	Multivariable analysis‡	Univariable analysis†	Multivariable analysis‡	Univariable analysis†	Multivariable analysis‡
1.00	-	1.00	-	1.00	-	1.00	-
0.78 (0.39-1.57)	-	0.35 (0.14-0.91)*	-	0.70 (0.40-1.22)	-	0.91 (0.66-1.27)	-
1.00	-	1.00	-	1.00	1.00	1.00	1.00
0.68 (0.23-1.98)	-	0.92 (0.30-2.79)	-	2.09 (0.58-7.50)	1.67 (0.45-6.24)	0.98 (0.59-1.64)	0.97 (0.57-1.64)
1.82 (0.73-4.52)	-	1.37 (0.50-3.81)	-	7.55 (2.32-24.57)*	6.80 (1.99-23.28)*	2.49 (1.56-3.96)*	2.64 (1.63-4.28)*
1.00	1.00	1.00	-	1.00	-	1.00	-
2.50 (1.25-4.99)*	2.25 (1.12-4.52)*	2.31 (1.04-5.17)*	-	3.90 (2.11-7.18)*	-	2.44 (1.75-3.39)*	-
-	-	4.33 (0.89-21.17)	-	2.76 (0.59-12.8)	-	2.25 (0.91-5.57)	-
1.00	-	1.00	1.00	1.00	1.00	1.00	-
2.67 (1.11-6.43)*	-	7.59 (2.95-19.56)*	8.35 (2.78-25.14)*	5.12 (2.37-11.02)*	3.59 (1.57-8.20)*	1.63 (0.98-2.69)	-
1.10 (0.53-2.29)	-	1.20 (0.45-3.19)	1.50 (0.49-4.63)	2.29 (1.19-4.40)*	1.49 (0.73-3.03)	1.27 (0.90-1.78)	-
1.00	-	1.00	-	1.00	-	1.00	-
0.78 (0.40-1.53)	-	0.65 (0.31-1.38)	-	0.70 (0.41-1.19)	-	0.83 (0.60-1.16)	-
1.00	-	1.00	1.00	1.00	-	1.00	-
1.71 (0.79-3.69)	-	0.55 (0.24-1.24)	0.28 (0.10-0.75)*	0.97 (0.51-1.84)	-	1.14 (0.78-1.66)	-
0.68 (0.27-1.76)	-	0.36 (0.14-0.95)*	0.19 (0.06-0.59)*	1.11 (0.58-2.11)	-	0.73 (0.49-1.10)	-
3.29 (1.70-6.36)*	3.03 (1.55-5.90)*	3.57 (1.70-7.50)*	-	3.00 (1.75-5.13)*	2.43 (1.34-4.40)*	3.17 (2.22-4.52)*	2.34 (1.55-3.55)*
2.09 (1.10-3.97)*	-	3.46 (1.63-7.36)*	3.38 (1.45-7.88)*	2.09 (1.25-3.49)*	-	1.87 (1.36-2.56)*	1.47 (1.00-2.15)*
2.53 (1.02-6.27)*	-	14.28 (6.62-30.81)*	12.95 (5.33-31.33)*	7.53 (4.08-13.88)*	6.13 (3.09-12.14)*	3.17 (1.90-5.28)*	2.57 (1.49-4.43)*
-	-	2.10 (0.48-9.20)	-	4.26 (1.77-10.28)*	-	2.01 (0.95-4.23)	-
1.22 (0.42-3.52)	-	2.77 (1.10-6.99)*	-	1.86 (0.88-3.91)	-	1.41 (0.84-2.36)	-
0.48 (0.21-1.11)	-	0.99 (0.93-1.06)	-	0.98 (0.93-1.04)	-	0.96 (0.90-1.02)	-

Funding

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Disclosure

The results of this research have been previously published in:

1. Yu J, Sumerlin TS, Goggins WB, Dong D, Chung RY, Kim JH. First- and second-hand alcohol-related harms among urban Chinese: a population-based study from Hong Kong. Drug Alcohol Rev 2022;41:208-20.

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TABLE 3. Factors associated with experiencing at least one second-hand harm in the past year (n=3200)

	Any work-related harm		Any public harm		Any family harm		Any social network harm		Any second-hand harm	
	Univariable analysis†	Multivariable analysis‡	Univariable analysis†	Multivariable analysis‡	Univariable analysis†	Multivariable analysis‡	Univariable analysis†	Multivariable analysis‡	Univariable analysis†	Multivariable analysis‡
Sex										
Male	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
Female	0.36 (0.21-0.63)*	-	0.83 (0.67-1.02)	-	0.73 (0.54-1.00)	-	0.47 (0.35-0.63)*	-	0.76 (0.64-0.91)*	-
Age, y										
55-74	1.00	-	1.00	1.00	1.00	-	1.00	-	1.00	1.00
35-54	1.49 (0.73-3.02)	-	1.58 (1.19-2.08)*	1.43 (1.07-1.92)*	1.01 (0.67-1.53)	-	1.44 (0.95-2.18)	-	1.59 (1.24-2.03)*	1.35 (1.03-1.77)*
18-34	2.22 (1.12-4.43)*	-	2.16 (1.64-2.85)*	1.73 (1.28-2.34)*	1.72 (1.17-2.54)*	-	3.29 (2.24-4.83)*	-	2.74 (2.15-3.49)*	1.85 (1.40-2.44)*
Marital status										
Currently married	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	-
Single, never married	2.39 (1.40-4.09)*	1.25 (0.70-2.24)	1.71 (1.38-2.11)*	-	1.93 (1.40-2.66)*	1.17 (0.82-1.68)	3.12 (2.33-4.18)*	1.54 (1.12-2.13)*	2.02 (1.68-2.43)*	-
Divorced/widowed/ separated	4.13 (1.55-11.02)*	4.41 (1.58-12.29)*	0.98 (0.52-1.87)	-	2.61 (1.31-5.20)*	2.75 (1.35-5.62)*	3.51 (1.88-6.54)*	4.06 (2.07-7.97)*	1.08 (0.63-1.87)	-
Education										
Secondary or less	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00
Upper secondary non-degree	5.20 (2.66-10.15)*	4.22 (2.06-8.67)*	1.50 (1.04-2.17)*	-	4.57 (2.99-6.99)*	3.59 (2.28-5.66)*	3.52 (2.23-5.56)*	2.53 (1.54-4.14)*	2.17 (1.60-2.94)*	1.58 (1.13-2.20)*
University or above	2.27 (1.26-4.10)*	1.64 (0.86-3.16)	1.87 (1.50-2.34)*	-	2.20 (1.54-3.12)*	1.46 (0.98-2.17)	4.36 (3.19-5.96)*	2.58 (1.80-3.70)*	2.43 (2.00-2.96)*	1.41 (1.11-1.80)*
Employment										
Unemployed	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
Employed	1.62 (0.95-2.75)	-	1456 (1.18-1.79)*	-	1.63 (1.18-2.25)*	-	1.78 (1.33-2.38)*	-	1.62 (1.35-1.94)*	-
Monthly household income, HK$										
<25 000	1.00	-	1.00	1.00	1.00	-	1.00	-	1.00	1.00
25 000-49 999	1.42 (0.79-2.56)	-	1.34 (1.05-1.72)*	1.06 (0.82-1.37)	1.22 (0.84-1.77)	-	1.35 (0.97-1.89)	-	1.31 (1.07-1.62)*	0.87 (0.69-1.10)
≥50 000	1.96 (1.01-3.80)*	-	2.51 (1.92-3.29)*	1.72 (1.28-2.30)*	2.78 (1.90-4.08)*	-	3.14 (2.22-4.43)*	-	2.83 (2.24-3.59)*	1.35 (1.02-1.79)*
Past-year drinking status										
Abstainer	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Non-binge drinker	3.46 (1.83-6.53)*	2.80 (1.44-5.43)*	2.08 (1.66-2.61)*	1.63 (1.28-2.09)*	3.56 (2.52-5.01)*	2.85 (1.97-4.11)*	6.11 (4.38-8.52)*	4.23 (2.97-6.01)*	3.07 (2.52-3.75)*	2.32 (1.86-2.89)*
Binge drinker	16.92 (8.83-32.42)*	14.28 (7.27-28.07)*	3.27 (2.27-4.70)*	2.63 (1.81-3.83)*	6.68 (4.18-10.67)*	5.29 (3.23-8.67)*	15.40 (10.11-34.44)*	12.04 (7.76-18.69)*	5.60 (4.04-7.75)*	4.30 (3.05-6.06)*
Communal living	4.86 (1.69-14.00)*	-	1.97 (1.00-3.88)	-	4.14 (1.97-8.68)*	2.43 (1.11-5.30)*	5.20 (2.71-9.96)*	2.22 (1.10-4.52)*	3.72 (2.11-6.59)*	2.03 (1.11-3.72)*
Work-related drinking	6.37 (2.92-13.89)*	-	1.66 (0.95-2.90)	-	4.34 (2.42-7.77)*	-	4.55 (2.67-7.48)*	-	2.40 (1.51-3.83)*	-
Alcohol outlet density in area of residence (by population)	1.00 (0.96-1.05)	-	1.01 (1.00-1.03)	-	1.01 (0.99-1.03)	-	1.00 (0.98-1.03)	-	1.01 (1.00-1.02)	-

* P<0.05

† Data are presented as odds ratio (95% confidence interval)

‡ Data are presented as adjusted odds ratio (95% confidence interval)

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Projecting future temperature-related mortality in Hong Kong under climate change scenarios: abridged secondary publication

P Wang, EYY Chan, TC Lee, HW Tong, WB Goggins *

KEY MESSAGES

1. Age-standardised mortality rate (ASMR) was estimated to increase generally secondary to hot weather and decrease generally secondary to cold weather. The net change was estimated to be positive and increase during the entire century.
2. The increase in ASMR secondary to increasing heat and the decrease in ASMR secondary to decreasing cold among the population aged ≥75 years were both estimated to be higher than those among the younger population.

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Introduction

Climate change has brought about increases in mean annual temperatures, increases in the frequencies of hot days, and decreases in the frequencies of cold days worldwide. Mortality, particularly cardiovascular and respiratory mortality, is higher during periods of hot and cold weather,[1] but the nature of associations varies considerably among locales, owing to differences in climate and socioeconomic levels.

As increases in mean temperatures are expected to continue, these increases might affect population health outcomes including mortality. The increasing number of hot days is expected to increase heat-related mortality, whereas the decreasing number of cold days is expected to decrease cold-related mortality.[2] Therefore, if increases in heat-related mortality exceed decreases in cold-related mortality, the net impact of a long-term rise in mean temperatures could be increased mortality. If both the mean and variance of temperature increase, then both hot- and cold-related mortality could increase.[2]

Most studies use short-term exposure (temperature) – response (mortality) curves estimated from daily time-series studies. Discrepant results have been reported in various locations with different climatic, demographic, and socioeconomic conditions.[3] We aim to project future changes in temperature-related mortality secondary to climate change in Hong Kong.

Methods

Mortality data in Hong Kong from 1976 to 2018 were obtained from the Hong Kong Census and Statistics Department. Data on mean daily temperature, relative humidity, wind speed, and solar radiation were obtained from the Hong Kong Observatory, as were projections of daily temperature distributions for the years 2030-2039, 2050-2059, and 2090-2099. Daily pollutant levels (particulate matters <2.5 microns and <10 microns in diameter, ozone, and nitrogen dioxide) were obtained from the Hong Kong Environmental Protection Department. Deaths from external causes and cancer were excluded from analyses. The general circulation models with the minimum, 25th, 50th, and 75th percentiles, and maximum of the average annual mean temperatures during 2080 to 2099 across multiple general circulation models were chosen for the projection of future mortality. The final results were reported using the average of the five chosen general circulation models.

The mortality, meteorological, and pollutant data were used to update our previous work on long-term (annual) associations between hot and cold 'degree-days' and mortality.[4] Analyses were stratified by age groups (>75 vs ≤75 years). Annual frequency and severity of hot and cold weather were summarised using a degree-days approach, with annual hot degree-days (DDHOT) being defined as the sum of degree-days above the hot threshold, and annual cold degree-days (DDCOLD) as the sum of degree days below the cold threshold. For example, given a hot threshold of 28°C and a cold threshold of 25°C, a day with temperature of 29.8°C contributes 1.8°C to the annual DDHOT, whereas a day with temperature of 21.8°C contributes 3.2°C to the annual DDCOLD.

The projected distributions of daily temperatures were used to estimate the mean

annual DDHOT and DDCOLD for each decade under consideration. Together with the association estimated by the previous models, these projected temperatures were plugged into the estimated annual model to obtain projected future age-standardised mortality rates (ASMR). The baseline period was chosen as 2014 to 2018. Our annual generalised additive model is presented as: LN (ASMR) = β0 + βH × DDHOT + βC × DDCOLD + s(year of study, maximum degrees of freedom for the smooth term for trend = 8) + ε, where βH and βC are parameters describing the association between DDHOT/DDCOLD and ASMR. Estimated percentage changes in ASMR associated with particular changes in the distribution of hot (cold) degree days—Δ DDHOT(COLD)—were calculated from the relative risks from the generalised additive model as percentage change = (relative risk – 1) × 100, where relative risk = exp(βH × Δ DDHOT(COLD). Projections were expressed in terms of changes in ASMR rather than number of deaths. All analyses were conducted using the R statistical software 4.0.

Results

Between 1976 and 2018 in Hong Kong, 918 586 non-accidental and non-cancer deaths were recorded. Among which, 550 298 (59.9%) were deaths among those aged ≥75 years. The hot and cold threshold was 29.6°C and 26.4°C, chosen from May to April and November to October models that minimised the generalised cross-validation score, respectively.

ASMR was estimated to increase (attributable to excess hot days) and decrease (attributable to excess cold days), and the net change was estimated to be positive and increase, particularly after 2050 (Fig 1 and Table). There was no significant difference between representative concentration pathway (RCP) 4.5 and RCP6.0 projections, although net changes under RCP4.5 were estimated to be higher than those under RCP6.0, except for 2090 to 2099. The increase in ASMR in both hot and net effect under RCP8.5 was remarkably steep, with the net change from 0.12% in 2030s to 89.25% in 2090s.

Age was estimated to be a significant effect modifier (Fig 2). Under RCP2.6, 4.5, and 6.0, the increases in ASMR secondary to the increase of hot weather among those aged ≥75 years were estimated to be over two-fold relative to those aged ≤74 years (eg, 12.48% and 25.66% increase under RCP4.5, 15.24% and 31.35% increase under RCP6.0, and 67.42% and 174.64% increase under RCP8.5 in 2090s for those aged ≤74 and ≥75 years, respectively). In addition, larger decreases in ASMR secondary to the decrease of cold weather were estimated among those aged ≥75 years.

Discussion

Under the RCP4.5, 6.0, and 8.5 scenarios, ASMR was estimated to increase generally secondary to hot weather and decrease generally secondary to cold weather, and the net change was estimated to be positive and increase during the entire century. In addition, the increase in ASMR secondary to increasing heat and the decrease in ASMR secondary to decreasing cold among the population aged ≥75 years were both estimated to be higher than those among the younger population.

Projections on future deaths based on temperatures from climate models vary considerably

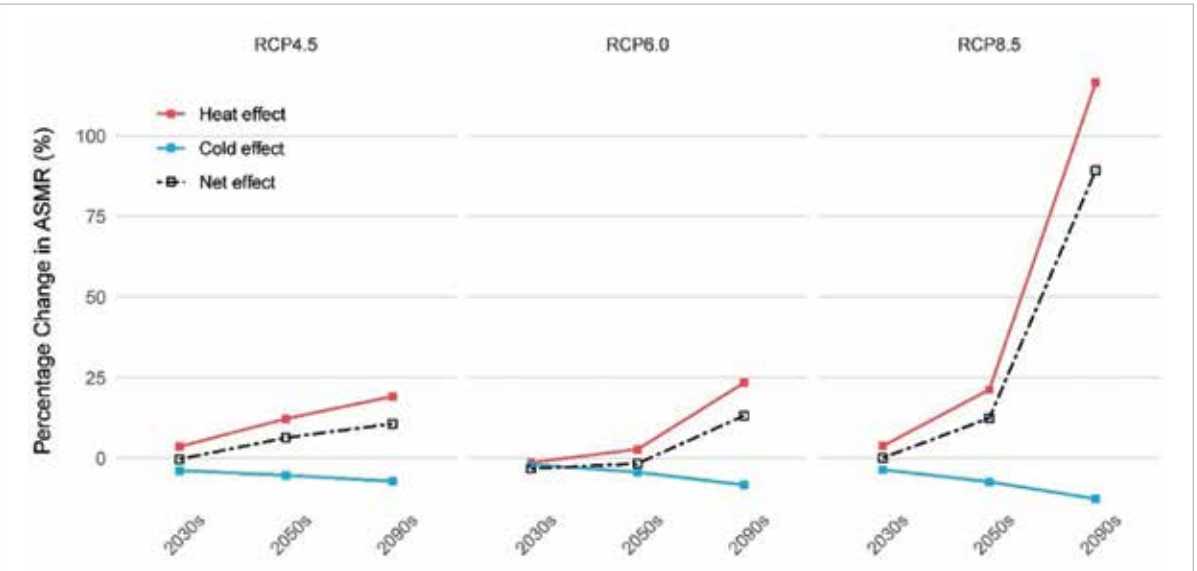

FIG 1. Average percentage changes in age-standardised mortality rate (ASMR) in Hong Kong during 2030s, 2050s, and 2090s under three climate change scenarios: representative concentration pathway (RCP) of 4.5, 6.0, and 8.5

TABLE. Projected percentage changes in age-standardised mortality rates in 2030s, 2050s, and 2090s in Hong Kong

Representative concentration pathway	% changes in age-standardised mortality rate								
	Lower bound of 95% CI			Mean			Upper bound of 95% CI		
	Hot degree-days	Cold degree-days	Net	Hot degree-days	Cold degree-days	Net	Hot degree-days	Cold degree-days	Net
4.5									
2030s	0.21	-2.10	-1.89	3.62	-3.78	-0.29	7.70	-5.41	1.87
2050s	0.72	-2.94	-2.24	12.28	-5.27	6.36	26.27	-7.52	16.78
2090s	1.08	-3.98	-2.95	19.18	-7.12	10.69	43.29	-10.14	28.77
6.0									
2030s	-0.09	-1.05	-1.15	-1.33	-1.91	-3.21	-2.36	-2.75	-5.04
2050s	0.16	-2.39	-2.23	2.78	-4.30	-1.64	5.98	-6.18	-0.57
2090s	1.32	-4.61	-3.36	23.42	-8.25	13.24	52.91	-11.73	34.96
8.5									
2030s	0.23	-1.97	-1.75	3.81	-3.55	0.12	8.08	-5.09	2.57
2050s	1.20	-4.08	-2.94	21.30	-7.30	12.44	48.13	-10.38	32.75
2090s	4.77	-7.12	-2.69	116.49	-12.58	89.25	399.28	-17.70	310.89

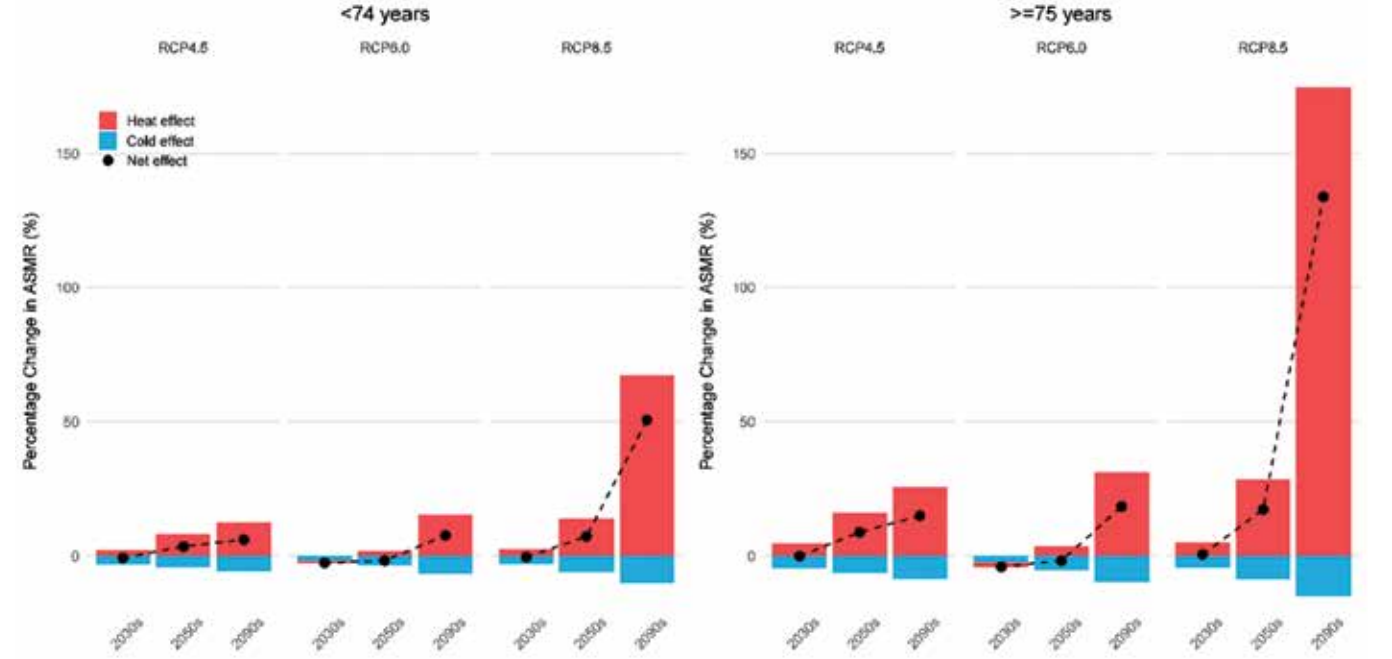

Fig 2. Average percentage changes in age-standardised mortality rate (ASMR) by age group in Hong Kong during 2030s, 2050s, and 2090s under three climate change scenarios: representative concentration pathway (RCP) of 4.5, 6.0, and 8.5

among locations.[3] Our results were in line with previous studies from other locations,[5] showing that the decrease in mortality secondary to decrease in cold temperatures in the future might not offset the increase in mortality secondary to increase in hot temperatures. We also found a negative net effect for all scenarios, but it was only reported for the lower bound of 95% confidence intervals of the estimated effects of the main model. In addition, the increasing trend by year and by climate change scenario was also partially supported by a global study on mortality projection, particularly in Southeast Asia,[3] where the climate is more similar to Hong Kong than to East Asia.

In a prediction study in the UK, older people aged ≥75 years are more sensitive to the future climate change.[5] However, even with the elevating temperatures in the coming decades in the 21st century, the impact of cold temperatures is estimated to outweigh the impact of hot temperatures.[5] Similarly, our study found that the increase in heat-attributed mortality rate secondary to increase in high temperatures could not be offset by the decrease in cold-attributed mortality rate secondary to decrease in low temperatures.

The current study has limitations. We used the annual data to project the future mortality secondary to climate change. Therefore, shift of seasonal pattern secondary to short-term climatological change could not be captured by our study. In addition, only 42 years were used for historical analysis and thus the sample size might not be sufficient for non-biased effect estimation. Furthermore, two important sources of uncertainty in estimates of future mortality were not accounted: adaptation assessment and potential demographic change. The integration of potential adaptation evaluation with long-term exposure-response association is required in future studies. Population change was not taken into consideration; future age structure was an essential factor influencing ASMR.

Conclusion

In the coming decades in Hong Kong, the ASMR was estimated to increase under medium and high emission scenarios secondary to climate change. Better urban planning strategy and public awareness should be promoted for the effective mitigation of future climate change.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#16170511). 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. Wang P, Tong HW, Lee TC, Goggins WB. Projecting future temperature-related mortality using annual time series data: an example from Hong Kong. Environ Res 2022;212:113351.

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A new biofeedback device to improve adherence to pelvic floor muscle training in women with urinary incontinence: a randomised controlled pilot trial (abridged secondary publication)

P Kannan *, G Cheing, B Fung, WC Leung, G Tang, R Chung, P Chan

KEY MESSAGES

1. The new biofeedback device is well accepted and safe for pelvic floor muscle training in women with stress urinary incontinence.
2. There are positive changes in the severity of urine loss and pelvic floor muscles strength after training with the new biofeedback device.

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Introduction

Pelvic floor muscle training (PFMT) is a first-line treatment for urinary incontinence (UI) in women. A lack of adherence to PFMT may result from an inability to contract the correct muscles and a lack of motivation. Therefore, a biofeedback device is commonly used with PFMT. The conventional biofeedback device involves insertion of a vaginal probe to pick up signals during voluntary contraction, but this causes discomfort or pain and refusal to treatment. A non-invasive biofeedback device with wearable electromyographic sensor was therefore developed. This study aims to compare the new biofeedback device with the conventional biofeedback device and no biofeedback device in terms of adherence to PFMT, retention rate, safety, stress UI symptoms, severity of urine loss, and pelvic floor muscles strength.

Methods

Non-pregnant women aged 35 to 60 years who were having mild to moderate stress UI (a score of ≤12 in the International Consultation on Incontinence Questionnaire–Urinary Incontinence Short Form [ICIQ-UI SF]) and had a mini-mental state examination score of ≥24 were recruited from Kwong Wah Hospital and two community centres in Hong Kong. Women were excluded if they were obese (body mass index of ≥30) or in the post-partum stage of <6 months or had severe pelvic organ prolapse, urine retention as an adverse effect of medications, incontinence secondary to other medical conditions or previous surgeries, complicated UI secondary to radiation to the pelvic region, mixed or urge UI, or severe psychological problems.

Eligible women were randomised by permuted blocks of three stratified by age and assigned to the new biofeedback device group (with the wearable electromyographic sensors attached to the perineal region), conventional biofeedback device group (with the conventional biofeedback probe inserted in the vagina), or control group (without any biofeedback device).

All participants underwent supervised PFMT once a week for 4 weeks, followed by unsupervised home exercises for 24 weeks. PFMT was performed in a lying position and progressed to sitting and then standing positions when participants were able to hold a contraction for 10 seconds in the previous position.

Assessors were blinded to the study. Outcomes were assessed at baseline and 4, 12, and 24 weeks. Primary outcome measures included adherence to exercise, retention rate, and safety. Exercise adherence was measured using a scale ranging from 0 (low adherence) to 10 (high adherence). Secondary outcome measures included the ICIQ-UI SF (for stress UI symptoms), the 1-hour pad test (for severity of urine loss), and the modified Oxford scale (for pelvic floor muscles strength).

Statistical analysis was performed on an intention-to-treat basis. Missing data were replaced with the last observation carried forward approach. Adherence to PFMT between groups was compared. Two-way repeated-measures analysis of variance was used to compare the three groups to determine the time × group interaction. Statistical significance was defined as a P value of ≤0.05.

Results

Of 60 women recruited, nine were excluded. 17 participants were assigned to each of the three groups. There was no significant difference in baseline characteristics between groups. After 24 weeks, in the new biofeedback device group, three participants reported adherence as high and 12 reported as moderate, whereas in the conventional biofeedback device and control groups, no participant reported adherence as high and 10 participants in the control group and four participants in the conventional biofeedback device group reported adherence as moderate. The retention rate was 100% in the new biofeedback device and control groups and 71% in the conventional biofeedback device group. No adverse events were reported in the new biofeedback device and control groups, but participants in the conventional biofeedback device group reported itching and blisters in and around the vaginal region (n=2), burning and painful urination (n=2), skin lacerations (n=2), and discomfort with vaginal probe insertion (n=1). Participants in the new biofeedback device group reported good device acceptance.

There was a significant effect in PFMT with the new biofeedback device than PFMT with the conventional biofeedback device or no biofeedback device on 1-hour pad test (both $P<0.01$), whereas PFMT with no biofeedback device was significantly more effective than PFMT with the conventional biofeedback device on severity of urine loss ($P<0.05$). There was a significant effect in PFMT with the new biofeedback device than PFMT with the conventional biofeedback device or no biofeedback device on ICIQ-UI SF score (both $P<0.01$), whereas there was no significant effect between PFMT with no biofeedback device and PFMT with the conventional biofeedback device on stress UI symptoms. The new biofeedback device was superior to the conventional biofeedback device and no biofeedback device in improving the modified Oxford scale score for pelvic floor muscles strength ($P<0.05$), whereas there was no significant difference between PFMT with no biofeedback device and PFMT with the conventional biofeedback device in improving pelvic floor muscles strength.

Discussion

Women with mixed stress UI were excluded, but they were unhappy being excluded and expressed that their urinary incontinence was severe (especially during coughing and sneezing). Therefore, we plan to recruit women with stress UI or stress-predominant mixed UI for future trials. The new biofeedback device is well accepted and safe for PFMT in women with stress UI. There were positive changes in the severity of urine loss and pelvic floor muscles strength after PFMT with the new biofeedback device.

Funding

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Carriage prevalence of antimicrobial resistance in Hong Kong: a longitudinal study (abridged secondary publication)

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KEY MESSAGES

1. The decreasing point prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E) in the general population from 54.8% (July 2018 to April 2019) to 43.4% (July 2019 to January 2020) suggests that the government's population-wide effort to combat antimicrobial resistance has been effective.
2. The high proportion (29.6%) of individuals who were colonised persistently (for a mean of 42.0 weeks) with ESBL-E is worrying. This suggests potential outbreaks of infections caused by ESBL-E. More understanding on the transient nature of ESBL-E colonisation enables better implementation of prevention strategies.
3. As an additional household member increased the odds of one carrying ESBL-E persistently by 16%; future study of household transmission of ESBL-E is warranted.
4. Owing to the continual presence of methicillin-resistant *Staphylococcus aureus* and carbapenem-producing Enterobacteriaceae in the community, an alert system should be in place to identify carriers discharged from healthcare settings, especially when the prevalence in the community remains low.

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Introduction

Antimicrobial resistance (AMR) is a major threat to public health worldwide. The United States Centers for Disease Control and Prevention identifies extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E), carbapenem-producing Enterobacteriaceae (CPE), and methicillin-resistant *Staphylococcus aureus* (MRSA) as the top drug-resistant threats.[1]

The epidemiology of ESBL-E carriage in Hong Kong community has not been studied. Hong Kong is a densely populated city and hence a potential reservoir for MRSA transmission. This study aims to estimate the colonisation rate of different types of antimicrobial-resistant bacterial strains and their temporal change across time in the community and to identify demographic characteristics and age-structured social mixing behaviours of individuals associated with antibiotic-resistant colonisation of ESBL-E.

Methods

Population-based household cohorts were invited consecutively through telephone until the sample size was reached. Random telephone dialling was used to top up the sample size if necessary. In round 1 conducted from 3 July 2018 to 27 April 2019, 1000 household members were expected to recruit. In round 2 conducted from 3 July 2019 to 3 January 2020, 750 household members were expected to recruit. Interested household members who were Hong Kong residents and resided in the selected households for at least 5 days a week were invited to the Prince of Wales Hospital or a site in Central for an interview about demographics, personal hygiene, and antibiotic use as well as for collection of nasal and handprint samples (by staff) and stool samples (previously collected by participants). Participants were compensated with HK$80 cash.

Nasal swabs were kept into nutrient broth with 7% salt for overnight incubation before subculture onto selective agar (ChromID MRSA, BioMérieux, France). Handprint samples of the non-dominant hand were collected onto the surface of selective agar (ChromID MRSA, BioMérieux, France), which were incubated at 37°C aerobically for 24 to 48 hours. Stool samples were collected within 24 hours of production and plated directly onto selective agar (ChromID ESBL and ChromID Carba SMART, BioMerieux, France) by cotton swab, streaking using 1 µl loop. After overnight culture at 37°C (ambient

air), one colony for each morphotype was picked and bacterial identification was performed using MALDI-TOF.

The prevalence of MRSA, CPE, and ESBL-E in round 1 and round 2 was determined. Colonisation status was defined as persistent carriage if a participant tested positive for bacteria in both rounds and as intermittent if tested positive in either round. For MRSA and CPE, the characteristics of their carriers and the corresponding household members were summarised. For ESBL-E, factors associated with their carriage were investigated. To account for within-household correlations, logistic regression analysis in the generalised estimating equation framework with an exchangeable correlation structure was applied to explore the risk factors for ESBL carriage. Explanatory factors included were demographics and personal characteristics (such as age, sex, household income, health conditions, antibiotic use in the past six months, contacts), household characteristics (such as size), and hospitalisations and social activities in the past 6 months. Variables included in the univariate analysis were based on a literature review. Chi-squared test, Fisher's exact test (for small counts), and Student's t-test were used as appropriate to test for associations with the outcome. The initial multivariate model included variables with a P value of <0.1 from the univariate analysis. A stepwise backward elimination technique was used to remove variables based on their P value from the likelihood ratio test. The final multivariate model was determined based on the one with the lowest Akaike information criterion. Adjusted odds ratios with 95% confidence intervals were presented. A statistical significance was set at P<0.05. Missing data were imputed using a multiple imputation technique.

TABLE 1. Characteristics and medical history of participants (n=1005)

Characteristic	Value*
Sex	
Male	416 (41.39)
Female	589 (58.61)
Age group, y	
3-49	234 (23.28)
50-59	237 (23.58)
60-90	534 (53.13)
Economic activity status	
Employee	389 (38.71)
Retired	386 (38.41)
Home-maker	120 (11.94)
Student, unemployed	50 (5.00)
Self-employed	26 (2.59)
Unknown	28 (2.79)
Educational attainment	
Primary or less	135 (13.43)
Secondary	502 (50.00)
Post-secondary	357 (35.52)
Unknown	11 (1.09)
Monthly household income, HK$	27500 (12500-45000)
Owns pets (cats, dogs, rabbits, hamsters, hedgehogs, fish, shrimp, turtles, parrots, bees, spiders)	187 (18.61)
Medical history	
History of chronic diseases	588 (58.5)
History of antimicrobial-resistant bacteria infection	12 (1.2)
Smoker (current/former)	121 (12.0)
Received antibiotic prescription in the past 6 months	472 (47.0)

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

Results

Of 2094 households invited, 814 agreed to be interviewed; the response rate was 38.9%. A total of 589 female and 416 male participants aged 3 to 90 (mean, 57.4) years were recruited (Table 1). 53.1% of participants were aged 60 to 90 years. 58.5% of participants had chronic diseases, with hypertension being the most common (23.7%), followed by hypercholesterolaemia (17.0%), diabetes mellitus (9.2%), and musculoskeletal diseases (9.2%). Twelve participants self-reported a history of an antimicrobial-resistant bacterial infection. 12% of participants were smokers, and 47% of participants had received antibiotics in the previous 6 months.

In round 1, 894 of 1005 participants provided stool samples. In round 2, 634 of 701 participants provided stool samples. The mean time elapsed between two rounds was 42 (range, 14.7-77.0) weeks. For MRSA, the prevalence was 1.69% in round 1 and 1.71% in round 2 and that of persistent carriage was 0.71% (95% confidence interval [CI]=0.23-1.66). For CPE, the prevalence was 2.13% in round 1 and 0.63% in round 2 and that of persistent carriage was 0%. However, for ESBL-E, among 607 participants who provided stool samples in both rounds, the prevalence of carriage was 54.8% (95% CI=51.5%-58.1%) in round 1 and 43.4% (95% CI=39.5%-47.3%) in round 2 and that of persistent carriage was 29.6% (95% CI=26.0%-33.4%) and that of intermittent carriage was 37.7% (95% CI=33.9%-41.8%); 199 subjects had no carriage in either round.

Factors associated with ESBL-E carriage in round 1 included sex (adjusted odds ratio [aOR]=1.34, 95% CI=1.03-1.72), economically active (aOR=1.44, 95% CI=1.09-1.91), receiving at least one course of antibiotics in the previous 6 months (aOR=1.59, 95% CI=1.16-2.18), visiting a clinic in the past 6 months (aOR=1.52, 95% CI=1.08-2.13), and having any close contacts on the day before the interview (aOR=1.48, 95% CI=1.09-2.02) [Table 2].

TABLE 2. Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E), carbapenem-producing Enterobacteriaceae (CPE), and methicillin-resistant *Staphylococcus aureus* (MRSA) in each round*

Bacteria	Round 1 (n=1005)	Round 2 (n=701)	Colonisation transition				
			Persistent carriage	Intermittent carriage			Neither
				Total	-ve to +ve	+ve to -ve	
ESBL-E	54.8 (51.5-58.1)	43.4 (39.5-47.3)	29.6 (26.0-33.4)	37.7 (33.9-41.8)	14.1 (11.5-17.1)	23.6 (20.3-27.2)	32.7 (29.0-36.6)
CPE	2.13 (1.28-3.30)	0.63 (0.17-1.61)	0 (0-0.60)	2.96 (1.76-4.63)	0.66 (0.18-1.67)	2.30 (1.26-3.83)	97.0 (95.4-98.2)
Staphylococcus aureus	23.4 (20.8-26.1)	21.3 (18.3-24.5)	12.7 (10.3-15.4)	19.5 (16.7-22.7)	8.56 (6.59-10.9)	11.0 (8.77-13.5)	67.8 (64.2-71.2)
MRSA	1.69 (0.99-2.69)	1.71 (0.89-2.97)	0.71 (0.23-1.66)	1.85 (0.99-3.15)	1.00 (0.40-2.05)	0.86 (0.31-1.85)	97.4 (96.0-98.5)
(%MRSA among *Staphylococcus aureus* +MRSA)	6.7	7.5	-	-	-	-	-

* Data are presented as % (95% confidence interval)

The odds of ESBL carriage was higher among male (aOR=1.80, 95% CI=1.23-2.64), those who visited a clinic or hospital in the past 6 months (aOR=1.21, 95% CI=1.03-1.43), and those who had a household member hospitalised in the past 6 months (aOR=1.13, 95% CI=1.01-1.28) [Table 3]. Household size was a risk factor for ESBL-E carriage, with the odds increasing by 16% for each additional household member.

TABLE 3. Logistic generalised estimating equation analysis of persistent carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae (n=607)

Variable	Univariate analysis, OR (95% CI)	Multivariate analysis, adjusted OR (95% CI)
Sex		
Female (reference)	-	-
Male	1.81 (1.23-2.66)	1.80 (1.23-2.64)
Visited a clinic/hospital in the past 6 months		
No (reference)	-	-
Yes	1.17 (0.99-1.38)	1.21 (1.03-1.43)
No. of household members	1.21 (1.05-1.39)	1.16 (1.01-1.34)
Any household member hospitalised		
No (reference)	-	-
Yes	1.14 (1.01-1.28)	1.13 (1.01-1.28)

Discussion

This population-based study assessed the background asymptomatic carriage of MRSA, CPE, and ESBL-E in the community. The prevalence of ESBL-E in the community was high, with *Escherichia coli* being the most common organism, and the prevalence of persistent carriage of ESBL-E was 29.6%. Colonisation of ESBL-E was a dynamic process from round 1 to round 2, with 14.1% (95% CI=11.5-17.1) acquired colonisation, compared with 23.6% (95% CI=20.3-27.2) lost colonisation. In contrast, the prevalence of CPE and MRSA in the community were low.

The significant decrease in the prevalence of ESBL-E carriage from 54.8% to 43.4% in the community suggests that the government effort in reducing AMR was effective. In 2017, Hong Kong Strategy and Action Plan on Antimicrobial Resistance was launched to improve awareness and understanding of antimicrobial resistance through effective communication, education, and training.

Future study of household transmission of ESBL-E is warranted, because an additional household member increased the odds of one carrying ESBL-E persistently by 16% (even after adjusting for having household members hospitalised), which is lower than the 23% to 25% reported in a study.[2] This may be due to the abundance of ESBL-E in the community.

The prevalence of persistent carriage of ESBL-E of 29.6% is worrying, which is lower than the 40% reported in a study.[3] With the average time elapsed between two rounds being 42.0 weeks, the rate of acquiring colonisation was 14.1% and that of clearance was 23.6%. The median time to clear ESBL-E colonisation was reported to be 6.6 months,[3] and the period to gradual clearance of ESBL-E was reported to be 12 to 54 weeks.[4] The different colonisation rate in our study may be masked by several cycles of colonisation and clearance. Further study on the transient nature of colonisation with more frequent rounds of recruitment is warranted.

Owing to the persistent presence of MRSA and CPE in the community, an alert system should be in place to identify carriers after discharged from healthcare settings, especially when the prevalence in the community remains low. MRSA and CPE used to be found only in hospitals. For CPE,

plasmid-mediated genes in hospitals are effective in spreading in the community. Such spread was exacerbated by patients' persistent carriage of CPE after discharge (mean time to clearance is 387 days). With an increasing rate of hospital-acquired CPE in Hong Kong,[5] strategic control measures are needed. Similar situation applies to MRSA.

There are limitations to the present study. Only two rounds of recruitment across a mean of 42 weeks were conducted. The observed transient rate of colonisation or clearance may be masked by multiple cycles. More rounds of recruitment should have been performed. Not all members of a household were enrolled and thus the effect of household transmission may not have been assessed. Not all sources of AMR transmission such as dietary intake (the prevalence of ESBL-E and CPE in pig and fish samples from wet markets was high[5]) were studied given limited resources. Caution should be exercised when extrapolating the findings from this study to the current situation of AMR because of the knock-on effect of COVID-19 pandemic on AMR.

Funding

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Disclosure

The results of this research have been previously published in:

1. Kwok KO, Chan E, Chung PH, et al. Prevalence and associated factors for carriage of Enterobacteriaceae producing ESBLs or carbapenemase and methicillin-resistant Staphylococcus aureus in Hong Kong community. J Infect 2020;81:242-7.

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Proteomics-based approach for target discovery in Zika virus infection: abridged secondary publication

HM Tun *, S Sanyal

KEY MESSAGES

1. The proteomics-based approach is effective in rapid identification of functionally relevant targets in virus infections.
2. Deubiquitylases that are specifically activated upon Zika/dengue virus infection are key regulators of immune activation and virus secretion via Src-family kinases (SFK) function.
3. Genetic and pharmacological inhibition of deubiquitylases and SFKs can effectively attenuate production of viral progenies.
4. A biochemical screening strategy combined with in vivo models can provide powerful means of developing drug screening platforms.

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Introduction

Ubiquitylation can dramatically change protein expression and activity.[1,2] Alteration in cellular ubiquitylation is recurrent in most virus infections caused by reorganisation of host intracellular networks that need to be rapidly installed. Among the enzymes of the ubiquitylation machinery, deubiquitylases (DUBs) and E3-ligases regulate critical signalling pathways at the intersection of host immunity and viral pathogenesis. We aim to identify and characterise druggable targets of the ubiquitylation pathway in Zika infection.

Methods

A combination of mass spectrometry, biochemistry, and virology was applied to address how enzymes of the ubiquitylation pathway affect Zika virus infection. A reporter for ubiquitin—HA-Ubvme and E2 charged with HA-Ubvme—was used to isolate deubiquitylases and E3 ligases from mock and infected samples for identification using mass spectrometry. Hepatocytes were treated with perfringolysin O, a pore-forming toxin, to permeabilise the plasma membrane and facilitate delivery.[3,4] Purified reporters were added to permeabilised cells, which were Zika or mock-infected. The function of the highest scoring candidates in Zika infection was validated and characterised. Otub1 and Ataxin-3 were identified to be associated with the RIG-I signalling pathway and the Src-family kinases (SFK), respectively. The SFKs in turn regulated secretion of viral progenies via an unconventional mechanism derived from autophagosomes triggered specifically upon infection. Genetic deletions were generated using the CRISPR/Cas9 strategy and their function characterised in Zika infection. The efficacy of a set of inhibitors as possible therapeutic candidates for targeting the SFKs and the deubiquitylases was evaluated in in vitro and in vivo models.

Results

To identify enzymes of the ubiquitylation machinery that are activated upon Zika virus infection, we applied a ubiquitin charged E2 probe to isolate all activated ubiquitylation enzymes from infected cells (Fig 1). We used a quantitative proteomics screen combined with activity-based E3 ligase profiling to identify E3-ligases that were activated upon infection. The screen revealed that while antigen presentation was suppressed, membrane associated RING-CH (MARCH) family E3-ligases were enriched in Zika and dengue-infected primary human monocytes. Several MARCH E3 ligases emerged as significant hits from the proteomics screen, particularly MARCH9 and 2, which also co-purified with MHC-I and II from dengue-infected cells. The MARCH E3 ligases share sequence homology and domain organisation with the herpesvirus K3 family of E3 ligases that are known to downregulate MHC. We therefore hypothesise that Zika co-opts MARCH E3 ligases to suppress MHC-I and II through similar strategies as the K3 proteins to escape T-cell surveillance.

To isolate DUBs from Zika and IAV-infected cells, mock and virus infected cells were permeabilised using perfringolysin O, and activated

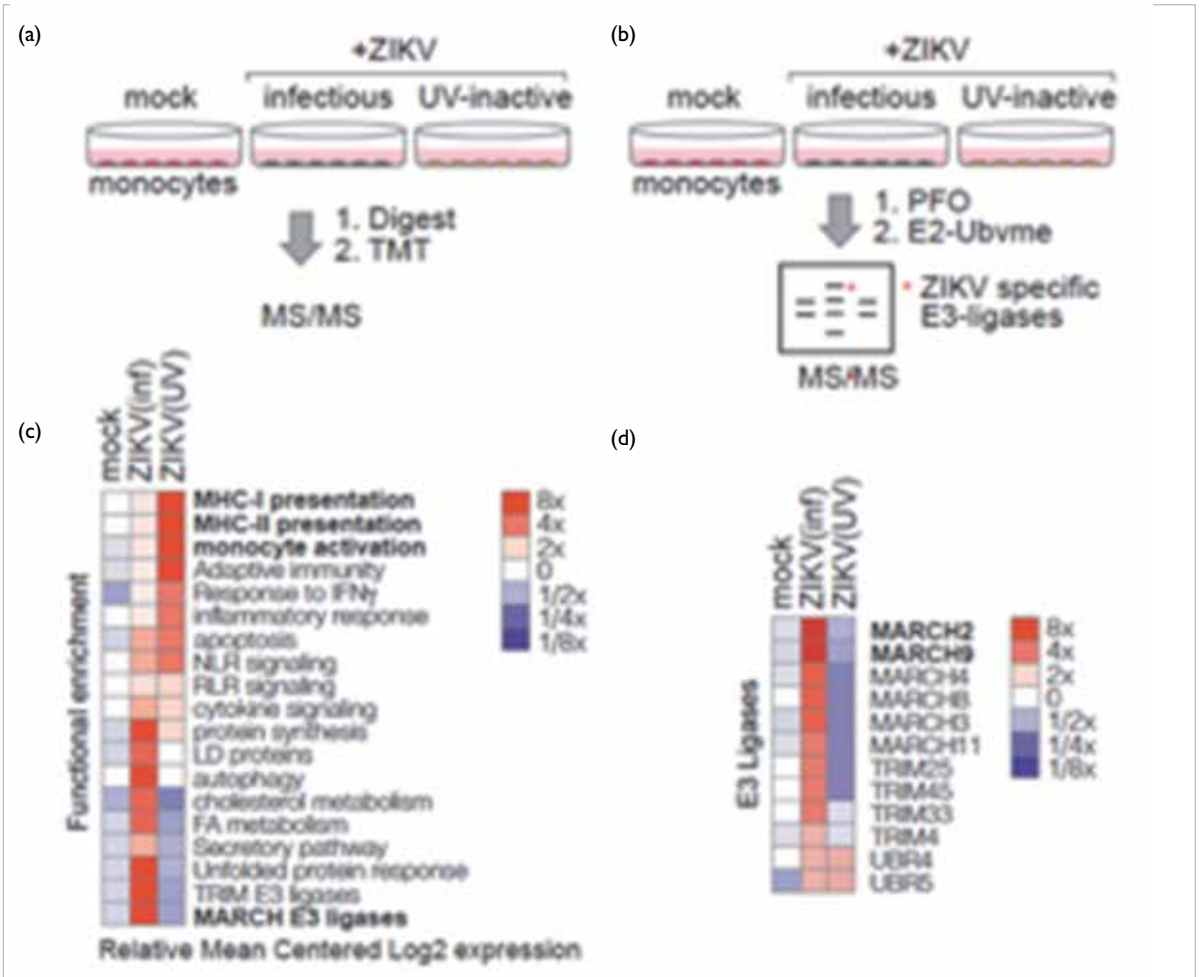

FIG 1. Quantitative proteomics screens showing decreased antigen presentation and increased MARCH E3-ligases in Zika-infected monocytes: (a) mock and virus-infected (infectious/UV-inactivated) monocytes are either digested and TMT-labelled or (b) exposed to permeabilisation with a pore forming toxin, perfringolysin O, and treated with an E2 probe (HA-E2-Ubvme) to isolate E3-ligases. Differentially activated E3-ligases are isolated on anti-HA beads from cells exposed to no virus (mock), infectious Zika (inf) or UV-inactivated Zika (UV). Protein bands resolved by SDS-PAGE and Coomassie staining are digested and subjected to mass spectrometry. (c) Quantitative differences in peptide abundance are analysed between mock, infectious Zika, and UV-inactivated Zika. Pathway enrichment analyses reveal significantly lower antigen presentation and monocyte activation (in bold) correlated with upregulation of E3 ligases in cells exposed to infectious Zika, compared with inactivated controls. (d) The set of E3 ligases are isolated, with highest scoring candidates being the MARCH family of E3 ligases.

DUBs were isolated with Ub-vme carrying a TAMRA or HA-tag (Fig 2). Ub-vme reactive material from control and infected cells were resolved by SDS-PAGE and visualised by fluorescent scanning or enriched on anti-HA beads first and detected by silver staining. Potential candidates were identified by trypsin digestion, mass spectrometry, and spectral counting of peptides on immunoprecipitated material and validated in lysates from infected cells. Candidates identified included Usp25,[3] Usp15,[4] and DUBA, which displayed reduced expression in virus-infected cells and high Ubvme reactivity in IFN-I treated samples, in agreement with a previous study.[5]

Among the DUBs identified in the Ubvme screen, we identified Ataxin-3, which regulates the expression of SFKs in flavivirus infection. We therefore screened for activated SFKs in Zika and dengue-infected cells. There are at least nine members of SFKs expressed in different combinations in all mammalian cells.[6] To identify those that are activated upon Zika infection, we immunoprecipitated phosphorylated SFKs using anti-pSFK antibodies from cell lysates prepared from mock or Zika-infected cells. Phosphorylated SFKs and associated cellular factors were isolated, resolved by SDS-PAGE, and detected by silver staining (Fig 3). The lanes were sliced into 2-mm sections and subjected to trypsin digest for identification by mass spectrometry. We identified three members: Src, Fyn, and Lyn, and several SFK-regulated substrates in Zika-infected samples. Several co-

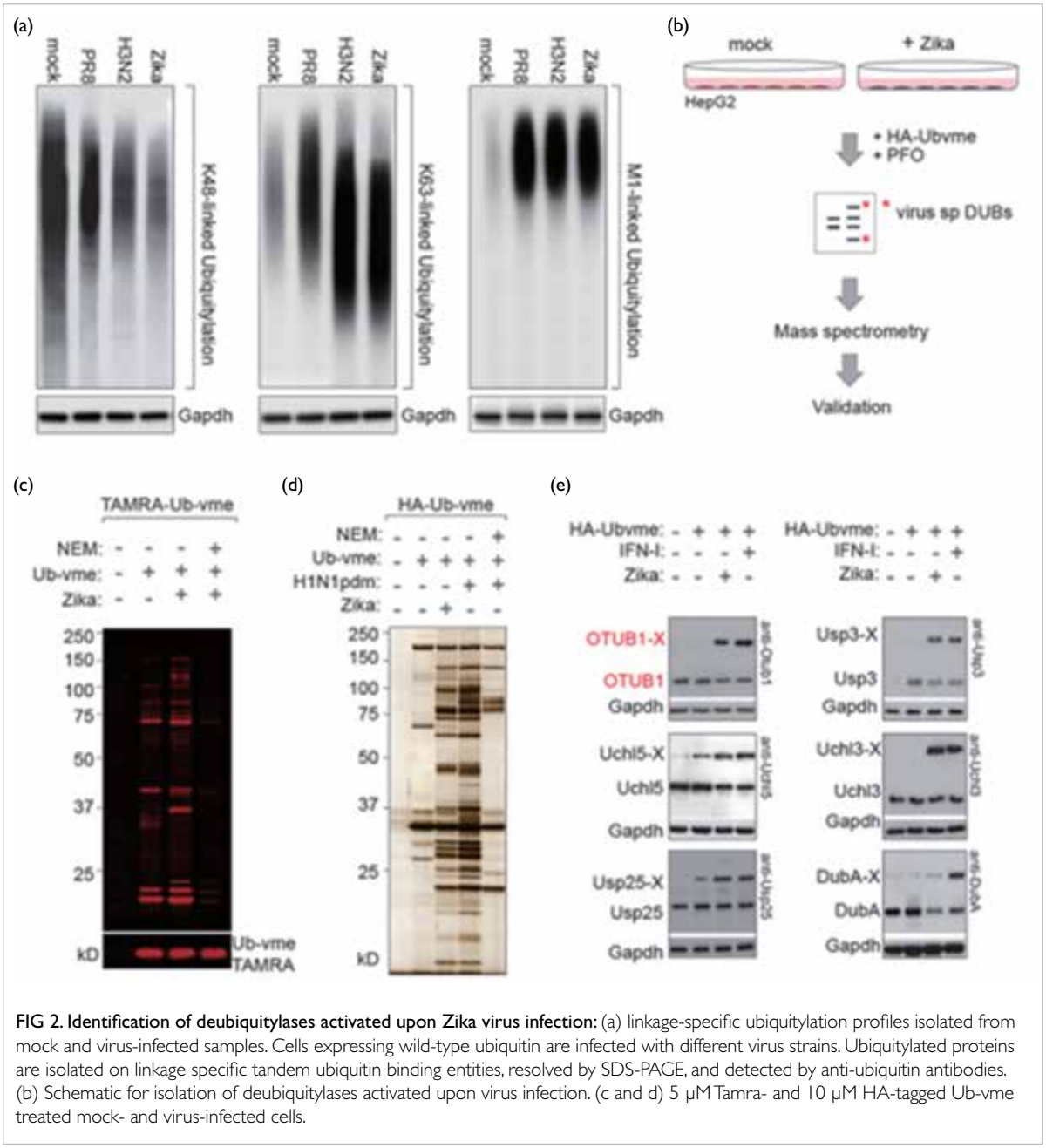

FIG 2. Identification of deubiquitylases activated upon Zika virus infection: (a) linkage-specific ubiquitylation profiles isolated from mock and virus-infected samples. Cells expressing wild-type ubiquitin are infected with different virus strains. Ubiquitylated proteins are isolated on linkage specific tandem ubiquitin binding entities, resolved by SDS-PAGE, and detected by anti-ubiquitin antibodies. (b) Schematic for isolation of deubiquitylases activated upon virus infection. (c and d) 5 μM Tamra- and 10 μM HA-tagged Ub-vme treated mock- and virus-infected cells.

immunoprecipitating proteins were components of the secretory pathway, ER/Golgi resident proteins, and vesicular transport machinery including the KDELRs, which have been characterised as important for dengue secretion.[7]

The three SFKs displayed high levels of expression in susceptible cell lines as confirmed by immunoblotting. To confirm their activation, we immunoprecipitated phosphorylated proteins from mock and Zika virus-infected Vero cells using anti-p-tyrosine-antibodies. Eluates from immunoprecipitated material were analysed by Western blotting with specific antibodies against the selected kinases: Src, Fyn, and Lyn. Although expression levels of total SFKs were comparable in mock and infected samples, a significant increase was noted in their phosphorylated form in eluates from Zika virus-infected samples, compared with mock. We used a reciprocal strategy where kinases from mock and Zika-infected samples were first immunoprecipitated on specific antibodies followed by immunoblotting with anti-phospho-tyrosine antibodies to confirm these results. To further quantitate increases in specific SFK activation, we used the Milliplex Map 8-plex SFK activation kit using the Luminex technology to confirm specific activation of Lyn, Src, and Fyn in lysates prepared from dengue and Zika-infected cells.

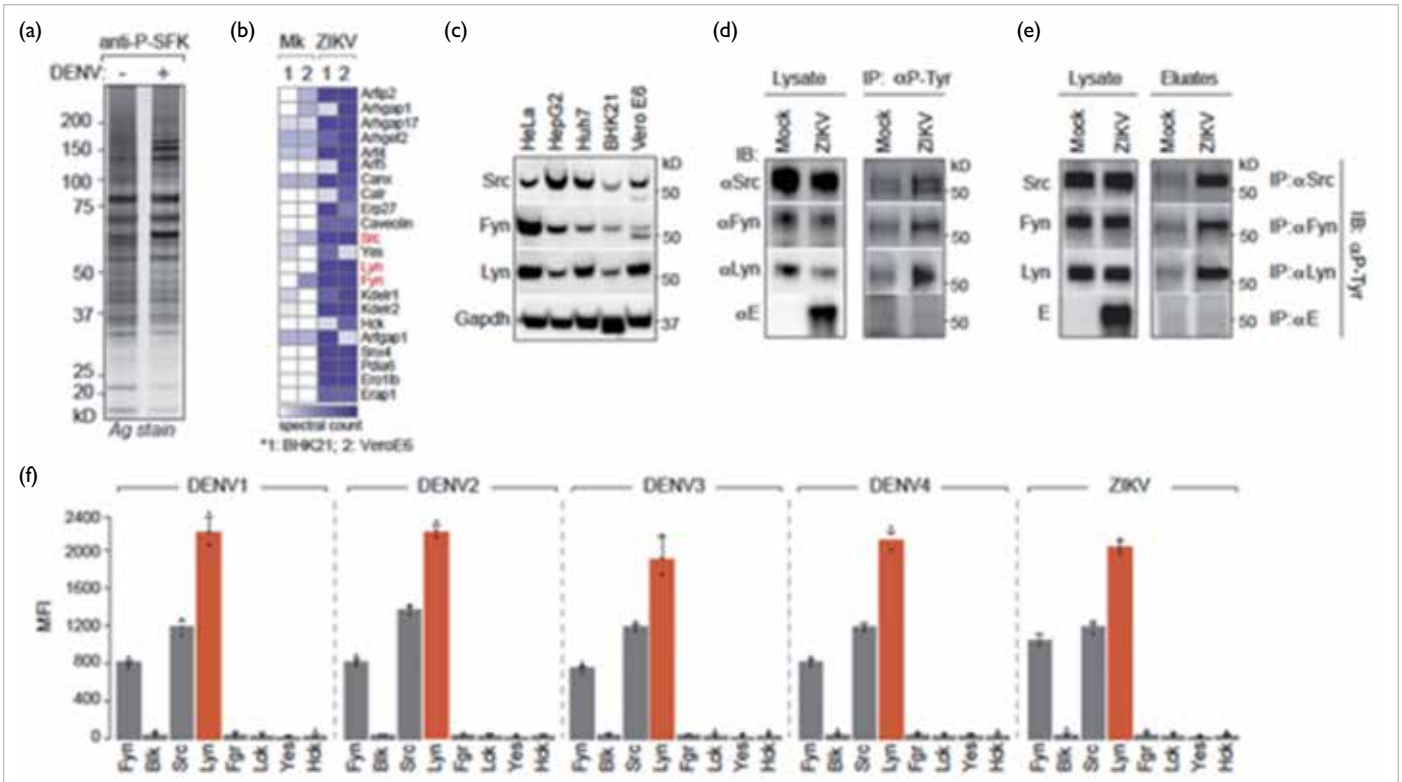

FIG 3. Flavivirus infection triggers activation of three specific SFKs: (a) large-scale immunoprecipitation of activated SFKs is performed on anti-phospho-SFK antibodies from mock and Zika-infected BHK21 and Vero E6 cells (MOI 2, 24 h). Isolated proteins are resolved by SDS-PAGE and detected by silver staining. (b) Entire lanes on gels are sliced into 2-mm sections and subjected to trypsin digest. The peptide mix is processed and analysed by an LTQ Orbitrap mass spectrometer (c) Protein expressions of Lyn, Fyn, and Src kinases are validated in lysates prepared from indicated susceptible cell types. (d) Activation of Lyn, Fyn, and Src upon Zika infection is measured by immunoprecipitating on anti-phospho-tyrosine antibodies and then by immunoblotting with specific SFK antibodies. (e) A reciprocal immunoprecipitation on specific anti-SFK antibodies followed by immunoblotting with anti-phospho-tyrosine antibodies. (f) Activation of SFKs is measured in lysates prepared from Zika-infected cells with the Milliplex MAP 8-plex assay kit. Red bars indicate Lyn activation, and error bars indicate mean±standard deviation from three biological replicates.

Discussion

The ubiquitylation machinery is extensively used during virus infections. Zika infected samples provide proof-of-concept results in underscoring the importance of targeting host factors for development of antivirals that can be administered, especially in virus infections that do not have effective vaccines. ZIKV infection activates different classes of enzymes, particularly DUBs, E3-ligases, and SFKs. Targeting autophagy-dependent secretion specifically triggered during virus infection may prevent host toxicity while selectively blocking secretion of virus progenies. Inhibitors to selective autophagy may provide broad-spectrum protection against infections by positive stranded RNA viruses.

Funding

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Next-generation sequencing for deducing donor mismatched human leukocyte antigen typing from urine of kidney transplant recipients: abridged secondary publication

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KEY MESSAGES

1. Donor human leukocyte antigen (HLA) typing is crucial for the diagnosis of antibody-mediated rejection. It facilitates prompt medical intervention to salvage the graft from failure. Recipients' urine sample is proven to be a valuable non-invasive source for the deduction of donor HLA typing.
2. Next-generation sequencing (NGS) can help resolve the donor mismatched HLA typing at high resolution and reveal additional HLA loci that were not determined during transplant workup.
3. The NGS protocol can complement the polymerase chain reaction–single specific primer method to determine the presence of donor-specific antibody and define the specificity of donor typing at allelic level.
4. Loss of donor chimerism in recipient urine sample was associated with failure in detecting donor HLA allele by NGS.
5. Further optimisation of donor DNA quantity, amplification, and NGS data analysis is warranted to enhance the detection of donor DNA material in recipient urine sample.

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Introduction

Human leukocyte antigen (HLA) plays an integral part in immune surveillance and recognition of self and non-self antigens. HLA mismatches can affect the outcome of allogeneic transplantation. Owing to complexity and diversity of HLA, determination of antigenic specificity of HLA has always been challenging. Owing to the limited availability of specific antibody sera, HLA typing by serological method has been replaced by DNA-based typing. Polymerase chain reaction–based methods have limitations of tedious workflow, low throughput, un-phased data, and ambiguity. Whereas next-generation sequencing (NGS) provides a high throughput and comprehensive HLA typing without uncertainty. High-resolution typing information and expansion of additional HLA loci enable clinicians to fine-tune immunosuppressive treatment regimen, facilitate better monitoring of graft survival, and minimise complications related to the immune system.

Kidney transplantation is the most cost-effective modality for end-stage kidney diseases. It enables better quality of life and has superior survival outcomes. However, there is a severe shortage of kidney donors in Hong Kong. Many patients opt to undergo transplantation outside of Hong Kong and then return to Hong Kong for subsequent immunosuppressive therapy and follow-up care. In the event of graft failure, renal replacement therapy and retransplant pose a heavy burden on the healthcare system.

With the use of new immunosuppressive regimen to control T-cell alloimmunity, the episode of T-cell-mediated acute rejection has dramatically reduced. Nonetheless, antibody-mediated rejection still plays a major role in acute and chronic allograft rejection. Development of de novo donor-specific antibody (DSA) also leads to acute or chronic allograft rejection. With the improvement in sensitivity and specificity of antibody identification, DSA can be detected in early emerging stage. For patients who underwent kidney transplantations in Hong Kong, DSA can be promptly identified with known donor information to facilitate early clinical intervention by adjusting the immunosuppressant dosage and minimising expensive treatments.

To determine the donor HLA typing and enable the detection of DSA to facilitate clinical management, we developed a protocol using conventional HLA

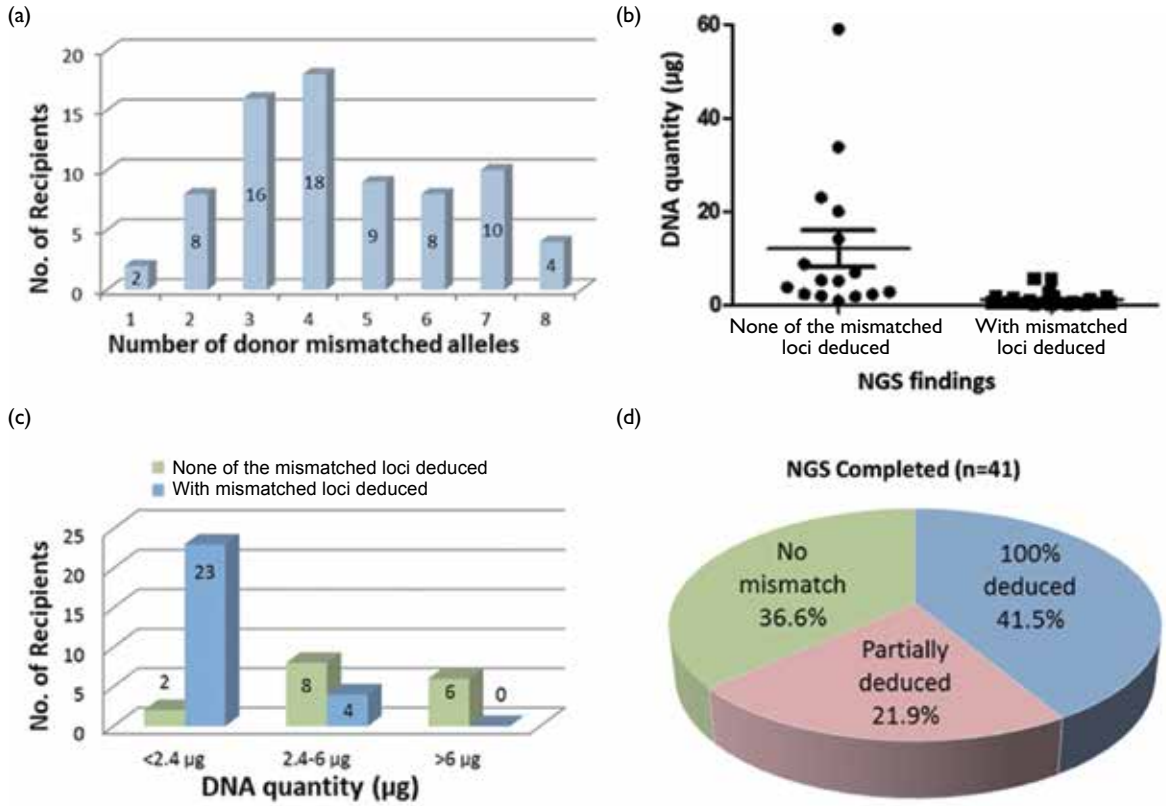

FIG. (a) Distribution of the number of mismatched human leukocyte antigen (HLA) between the recipients and the donors, (b) the association between DNA quantity and next-generation sequencing (NGS) findings of all samples, (c) distribution of the number of recipients with different amount of urine DNA quantity and the NGS results, and (d) results of deduction of donor HLA typing from recipient urine samples

typing to deduce donor mismatched HLA typing from recipient urine sample.[1] In our previous project, we have demonstrated that donor mismatched HLA typing can be successfully deduced from recipient urine samples and achieves a DSA diagnostic rate comparable with that in patients with known donor typing. However, ambiguities were found owing to the limitation of the polymerase chain reaction–single specific primer (PCR-SSP) method; some of the antigens were masked. In addition, poor DNA quality and/or inadequate amount of DNA render deduction of donor mismatched antigen unfeasible. Therefore, this study aimed to evaluate NGS for deduction of mismatched donor HLA information from recipient urine samples.

Materials and methods

A total of 75 patients who underwent kidney transplantations between 2016 and 2021 in Hong Kong with known donor HLA information and with more than one HLA mismatch at HLA-A, -B, -DRB1 or -DBQ1 loci were included. Patients with zero mismatch donor were excluded. The mean number of mismatched HLA was 4.4 alleles (Fig a).

Non-invasive urine HLA typing was deduced from the DNA material extracted from fresh early morning urine of recipients. HLA typing was performed by a NGSgo HLA typing kit (GenDx, The Netherlands) according to manufacturer instructions. The deduced mismatched donor HLA typing from the urine sample was compared with the patient's own HLA typing and known donor typing transplantation in Hong Kong. Data were analysed with the NGSMultiAnalysis tool.

Results

A total of 41 urine DNA samples with optimum DNA yield and quality were subjected to NGS analysis. The DNA quantities of the urine samples were significantly higher in those who failed to retrieve donor typing than those who could deduce donor HLA typing (P=0.0142, Fig b). The overall success rate for deducing donor mismatched HLA typing from urine samples was 63.4%. Low DNA quantity of the urine sample was associated with better result of deduction of the donor mismatched typing (Fig c). One plausible explanation could be the domination of recipient DNA in the chimeric urine

sample. Most of the amplicon and sequencing reads were dominated by the recipient DNA and hence the donor DNA failed to be detected.

Of the 26 samples with deduced HLA mismatch typing, 17 (65.4%) could detect all the mismatched alleles and nine (34.6%) could detect partial donor mismatched alleles (Fig d). The deduced HLA typing was 99.9% specific and matched with the donor typing in record. 15 (36.6%) of 41 urine samples had no mismatched donor HLA typing deduced, despite the mean number of mismatched alleles of 4.4. The number of mismatched between the three groups was not significantly different (P>0.05). This suggests that the probability of detecting deduced mismatch donor HLA typing was independent from the number of mismatched HLA alleles between the recipients and the donors.

The possible cause of failure in the deduction could be loss of donor chimerism in the urine sample. Only 0% to 4% of donor chimerism was observed from samples that failed in the deduction of donor mismatched HLA typing. However, this was independent to the time lapse after transplantation. Percentage of donor chimerism in individual mismatched allele could be determined by counting the sequence reads of the alleles. The mean number of donor alleles detected was 7.6% (range, 1.9%-25.1%). Samples with higher percentage of donor chimerism were likely to have 100% deduction of mismatched HLA alleles.

To determine whether NGS could resolve the ambiguity and quantity insufficiency issue of the PCR-SSP method, samples of recipients with unknown donor typing were retrieved. Mismatched HLA typing could be detected with a mean level of donor DNA of 5.6% (range, 2.5%-12.1%). In two samples, donor mismatched A*02:03 allele could be discriminated from the recipient A*02:07/A*02:06 allele, which could not be done so in the PCR-SSP method. Mismatched of all the eight loci have also been revealed. These results suggested that NGS could resolve the ambiguity and quantity insufficient issue of the PCR-SSP method and that additional loci could be typed simultaneously with less amount of DNA in urine sample.

Discussion

This study showed that mismatched donor HLA typing could be deduced with 99.9% specificity. However, owing to loss of donor DNA chimerism in the recipient urine samples, some donor mismatched HLA alleles were unable to be detected, probably masked by the predominant recipient DNA material. The sensitivity of detecting the donor HLA from the urine samples was 63.4%.

The superb resolution of NGS over the PCR-SSP method in terms of ambiguity resolution and typing accuracy for antibody antigen assay enables interpretation of the presence of DSA. NGS can deduce donor typing of some new HLA alleles such as HLA-C and HLA-DPB1, which plays a role in antibody-mediated rejection.[2] The additional donor HLA typing information facilitates decision making in prompt medical intervention and hence prolong graft survival.

The current NGS workflow requires high quality of template DNA, but DNA from the urine sample is usually fragmented with small molecular size, which results in a lower detection rate, compared with the PCR-SSP method. Alternative amplification or enrichment strategy may enable detection of scarce donor material in the urine sample. Further optimisation of NGS workflow and data analysis is warranted to enhance the detection of scarce donor HLA in recipient urine sample.

NGS is a viable alternative to deduce donor mismatched HLA typing from the recipient urine. It can resolve the donor typing at high resolution level and reveal the typing of additional allele simultaneously. This information is crucial for DSA interpretation and facilitation of prompt medical intervention to avoid the cost of treating chronic rejection.

Funding

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Multicomponent intervention to promote expression of organ donation wish to family members: a randomised controlled trial (abridged secondary publication)

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KEY MESSAGES

1. An intervention integrating online videos and motivational interviewing through telephone significantly increased expression of organ donation wish to family members, compared with only receiving a text message related to organ donation.
2. However, the increase in expression of organ donation wish to family members did not increase the rate of new organ donation registration.
3. Participants were satisfied with the motivational interviewing session and considered the narrative stories and information in the videos the most useful.
4. Most participants discussed the organ donation issue with family members during family gatherings.

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Introduction

Hong Kong has one of the lowest rates of deceased organ donation (OD) in the world.[1] In a Hong Kong survey in 2019, only 31.7% of the general population were willing to donate organs.[2] In Hong Kong, consent of the next-of-kin (eg spouse/child/parent/sibling) is required for OD of deceased persons regardless of the deceased person's decision. However, 76.6% of the general population were unaware of their family members' wish on posthumous OD.[2] Therefore, communication with family members about OD is as important as registration. We developed a multicomponent intervention (by integrating e-health strategies and motivational interviewing[3]) and evaluated its efficacy in facilitating expression of OD wish to family members. In addition, we explored potential mediation effects of self-efficacy and perceived barriers/facilitators in expression of OD wish to family members.

Methods

Between December 2019 and January 2021, 500 Hong Kong Cantonese-speaking residents aged 18 to 70 years who had intentions to donate organs after death but did not express OD wish to family members were recruited using respondent-driven sampling via random telephone numbers (n=131) or online platforms (n=369).

Participants were randomly allocated to the intervention (n=254) or control (n=246) group by six block randomisations. Participants in the intervention group watched two short online videos (sent via social media) containing OD-related information, testimonials from an organ recipient and their family member, and narrative stories related to people's concerns about OD. Then, they underwent a 15-minute motivational interviewing session through telephone by a trained research staff to discuss the barriers/facilitators of expressing OD wish to family members and to resolve the ambivalence over expression of OD intent. The intervention materials were designed based on theoretical concepts in the Health Belief Model and the Social Cognitive Theory. Participants were provided with pictorial e-messages predesigned by the research team to initiate OD-related conversations through social media. Participants in the control group received a text/Whatsapp message highlighting the importance of OD and expression of OD wish to family members in Hong Kong.

The primary outcome was the participants' self-reported expression of OD wish to family members. Secondary outcome was the prevalence of new OD registration. Perceived usefulness and satisfaction of the intervention components were evaluated among participants in the intervention group. All participants were followed up at 2 months through

TABLE 1. Expression of organ donation wish to family members and new organ donation registration between the intervention and control groups

	Intervention group (n=254)	Control group (n=246)	Relative risk (95% CI)	Absolute risk reduction (95% CI)	No. needed to treat	Adjusted odds ratio (95% CI)	P value
Expression of organ donation wish to family member at 2-month follow-up	57.5%	41.5%	1.39 (1.56-1.67)	0.16 (0.07-0.24)	6.24	1.88 (1.31-2.69)	0.001
New organ donation registration at 2-month follow-up	10.2%	8.1%	1.15 (0.81-1.61)	0.021 (-0.03 to 0.07)	47.48	1.37 (0.74-2.54)	0.32

telephone surveys. Each participant who completed the survey was given a HK$50 supermarket coupon. Absolute risk reduction (ARR), relative risk (RR), and their 95% confidence interval (CI) at follow-up were derived. Two-sided tests were used, and P values of <0.05 were considered statistically significant.

Results

Among the participants, 62.6% were aged 18 to 35 years, 66.9% were women, 20.3% had attained a secondary education or below, and 33.8% were currently married/cohabitating with someone. The intervention and control groups were comparable in terms of sociodemographic and psychosocial variables, except that significantly more participants in the intervention group were female and had a religious affiliation. Thus, sex and religion were adjusted for in subsequent analyses.

At baseline, all participants did not express their OD intent. At the 2-month follow-up, more participants in the intervention than control group reported expression of OD wish to family members (57.5% vs 41.5%, RR=1.39, 95% CI=1.56-1.67, ARR=0.16, number needed to treat=6.24, Table 1). However, the two groups did not differ significantly in new OD registration (RR=1.15, 95% CI=0.81,1.61, ARR=0.02, Table 1). Respectively among 144 and 106 participants in the intervention and control group who expressed their OD intent, 60.2% and 59.8% did so in family gatherings, 25.3% and 31.3% in face-to-face private talks, and 5.5% and 1% through our pre-designed pictorial messages; parents were the most reported informed family members (44.5% and 44.1%) [Table 2].

TABLE 2. Expression of organ donation wish to family members by participants

Expression of organ donation wish	Intervention group (n=146)	Control group (n=102)
Family member		
Parent(s)	65 (44.5)	45 (44.1)
Sibling(s)	19 (13.0)	12 (11.8)
Spouse	27 (18.5)	32 (31.4)
Other (eg children)	35 (24.0)	13 (12.7)
Method		
Pre-designed pictorial messages	8 (5.5)	1 (1.0)
Face-to-face talk with family members	37 (25.3)	37 (31.3)
Bringing up the topic during family gathering	88 (60.2)	61 (59.8)
Watching organ donation information with family	8 (5.5)	3 (2.9)
Others (eg talking on the phone)	5 (3.4)	5 (4.9)

The mediation effects of intervention on expression of OD wish to family members were not significant in terms of perceived barriers (indirect effect= -0.01, standard error=0.03, 95% CI= -0.06-0.04) and self-efficacy (indirect effect= -0.02, standard error=0.30, 95% CI= -0.09-0.02). After adjusting for the mediators, the direct effect of intervention (versus controls) on expression of OD wish to family members remained significant (effect=0.92, standard error=0.23, 95% CI=0.47-1.37, P<0.001).

On a scale of 0 (lowest) to 10 (highest), participants in the intervention group were highly satisfied with the intervention components (range, 6.67-7.43) and considered the information delivered useful (range, 6.49-7.35) [Table 3]. Specifically, they were satisfied with the motivational interviewing session and the narrative stories in the videos and considered them useful for expression of OD wish to family members.

Discussion

The multicomponent intervention significantly increased expression of OD wish to family members, despite no significant increase in new OD registration. Compared with pamphlets and posters, videos provide supplementary technical information (eg perceived misconceptions) and enhance emotional connection through narratives and testimonials of other donors/recipients of OD.[4]

A short single session (15 minutes) of motivational interviewing, even delivered by telephone, can effectively change behaviours such as smoking and drinking.[3] Similarly, we applied motivational interviewing to identify and discuss culturally relevant barriers/facilitators with the participants. Motivational interviewing session

TABLE 3. Evaluation of intervention components in a scale of 0 (lowest) to 10 (highest) by participants in the intervention group (n=196)

	Video 1	Video 2	Motivation interviewing session	Predesigned photos/ e-messages	Overall
Usefulness of information	7.22±1.58	7.35±1.41	7.03±1.53	6.49±1.39	**7.32 (1.51)**
Usefulness for organ donation intent expression	6.80±1.74	6.64±1.94	6.91±1.63	6.27±1.56	**7.18 (1.53)**
Level of satisfaction	7.32±1.47	7.43±1.41	7.42±1.39	6.67±1.27	**7.47 (1.37)**

facilitated expression of OD wish to family members.

Although we provided pre-designed social media messages to participants to express their OD wish to family members, most participants preferred to discuss the posthumous OD issue through face-to-face family talk regardless of group allocation. This suggested that watching videos and motivational interviewing were sufficient to motivate participants to discuss OD issues with family members.

Despite an increase in expression of OD wish to family members, there was no significant change in perceived barriers and self-efficacy, which are determinants of behaviours according to the Health Belief Model. The model may not be the best fit for understanding expression of OD intent and OD registration in the Hong Kong population. Future studies are warranted to determine if other psychosocial factors are associated with the intervention effect on the expression of OD intent. To enhance OD registration, more efforts should be made to proactively provide immediate and complete opportunities to passive-positive donors (ie those who have the intent to donate organ but have not completed donor registration).[5] One strategy could be provision of a direct link to the OD registration website together with our intervention components.

This study has limitations. The sample consisted of higher proportions of highly educated and younger individuals than the general population and thus our findings may not be generalisable to the general population. It was novel to apply motivational interviewing to facilitate expression of OD intent in Hong Kong with opt-in legal contexts. Whether our findings are applicable to other cultural and opt-out contexts has yet to be confirmed.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#15160631). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

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A prediction model for return to work after injury in Hong Kong: abridged secondary publication

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KEY MESSAGES

1. In Hong Kong, 54% of injured patients were able to return to work within 12 months.
2. Factors independently associated with return to work within 12 months of injury were length of hospital stay of ≤8 days, discharge home directly, non-heavy physical work of job nature, higher educational level, and better 1-month health status.
3. Our prediction model for return to work within 12 months achieved an area under the receiver operating characteristic curve of 0.850.
4. Future studies should focus on the external validation of this prediction model and interventions that could potentially modify return-to-work outcomes.

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Introduction

Trauma is a leading cause of death and disability worldwide. In Hong Kong, trauma ranks sixth as a cause of death for all age groups. The aim of trauma care for the injured person is to restore patients to the best possible health status and, if applicable, to return to work (RTW). This study aims to evaluate the RTW status of Hong Kong adult patients 1 year after moderate and major trauma, and to derive a reliable prediction model for RTW.

Methods

Patients aged ≥18 years who were working or seeking employment before admitted with moderate and major trauma to the Prince of Wales Hospital, Queen Elizabeth Hospital, Tuen Mun Hospital, or Princess Margaret Hospital in Hong Kong between 2017 and 2019 were recruited. Patients with isolated hip or pathological fractures were excluded, as were those with an injury severity score of 1 (very minor injuries).

The primary outcome was the RTW status. Secondary outcomes were health-related quality of life assessed by the Short Form-12 and the EQ-5D-5L. Functional outcome was assessed using the extended Glasgow Outcome Scale (GOSE). Pain was assessed using a numeric rating scale ranging from 0 to 10. Patients were assessed in person or through telephone on admission, at discharge/30 days after injury, and at 3, 6, 9, and at 12 months after injury. The RTW status was censored, and the earliest RTW was used, no matter whether the patient was still at work at 12 months.

Chi-squared test or Fisher's exact tests was used for categorical variables, whereas the t-test was used for continuous variables. All tests were two tailed. A P value of <0.05 was considered statistically significant. Univariate logistic regression was performed, with variables including patient characteristics, injury-related parameters, hospital treatment received, and clinical outcomes at discharge and 1-month post injury. Variables with $P<0.25$ were included in the multivariable logistic regression analysis. The prediction model was then established, and the relative weighting calculated. The model was tested using the area under the receiver operating characteristic curve, which reflects the discriminative ability of the model.

Results

A total of 1115 trauma patients were recruited. Their median age was 47 years and 81% of them were men (Table 1). 3.4% of patients had a history of psychiatric illness, and 38% had a pre-existing comorbidity. The median working experience of patients was 8 years; 67% of patients had physically demanding jobs before injury. 68% of injuries were work related. Blunt trauma was most common (89%). The two commonest causes of injury were traffic crashes (43%) and falls (30%). The median

TABLE 1. Characteristics of patients

Variable	Prince of Wales Hospital (n=401)*	Queen Elizabeth Hospital (n=359)*	Tuen Mun Hospital (n=319)*	Princess Margaret Hospital (n=36)*
Age, y	48 (34-58)	46 (33-58)	49 (37-58)	44 (30.8-54.3)
Sex				
Male	319 (79.6)	280 (78)	272 (85.3)	28 (77.8)
Female	82 (20.4)	79 (22)	47 (14.7)	8 (22.2)
Ethnicity				
Asian	398 (99.3)	356 (99.2)	316 (99.1)	36 (100)
Non-Asian	3 (0.7)	3 (0.8)	3 (0.9)	0 (0)
Trauma type				
Non-blunt	54 (13.5)	37 (10.3)	26 (8.2)	2 (5.6)
Blunt	347 (86.5)	322 (89.7)	293 (91.8)	34 (94.4)
Mechanism of injury				
Traffic	170 (42.4)	159 (44.3)	127 (39.8)	21 (58.3)
Fall	129 (32.2)	103 (28.7)	88 (27.6)	11 (30.6)
Penetrating	36 (9)	30 (8.4)	22 (6.9)	2 (5.6)
Burn	17 (4.2)	7 (1.9)	4 (1.3)	0 (0)
Others	49 (12.2)	60 (16.7)	78 (24.5)	2 (5.6)
Work-related injury				
No	259 (64.6)	261 (72.7)	209 (65.5)	34 (94.4)
Yes	142 (35.4)	98 (27.3)	110 (34.5)	2 (5.6)
Psychiatric disease				
No	391 (97.5)	341 (95)	311 (97.5)	34 (94.4)
Yes	10 (2.5)	18 (5)	8 (2.5)	2 (5.6)
Pre-existing comorbidity				
No	287 (71.6)	126 (35.1)	265 (83.1)	18 (50)
Yes	114 (28.4)	233 (64.9)	54 (16.9)	18 (50)
Injury severity score	10 (5.00-20.50)	10 (6.0-21.0)	9 (4.0-17.0)	12 (4.3-18.8)
Abbreviated Injury Scale for head				
<3	264 (65.8)	240 (66.9)	239 (74.9)	26 (72.2)
≥3	137 (34.2)	119 (33.1)	80 (25.1)	10 (27.8)
Operation performed				
No	204 (50.9)	138 (38.4)	151 (47.3)	17 (47.2)
Yes	197 (49.1)	221 (61.6)	168 (52.7)	19 (52.8)
Intensive care unit admission				
No	302 (75.3)	318 (88.6)	245 (76.8)	15 (41.7)
Yes	99 (24.7)	41 (11.4)	74 (23.2)	21 (58.3)
Intensive care unit length of stay	0 (0.00-0.00)	0 (0.0-0.0)	0 (0.0-0.0)	1 (0.0-2.0)
Length of hospital stay	6.4 (3.20-12.10)	10 (5.0-17.0)	9 (5.0-18.0)	8.5 (5.0-19.0)
Extended Glasgow Outcome Scale on discharge				
Good recovery	319 (79.8)	330 (91.9)	273 (85.8)	33 (91.7)
Moderate/severe disability	81 (20.3)	29 (8.1)	45 (14.2)	3 (8.3)
Discharge destination				
Home	316 (78.8)	230 (64.1)	270 (84.6)	29 (80.6)
Non-home	85 (21.2)	129 (35.9)	49 (15.4)	7 (19.4)
30-day mortality				
No	401 (100)	359 (100)	318 (99.7)	36 (100)
Yes	0 (0)	0 (0)	1 (0.3)	0 (0)
No. of years worked	9 (2.0-23.0)	7.5 (2.5-20.0)	8 (2.0-20.0)	6.5 (2.5-20.0)

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

TABLE 1. (cont'd)

Variable	Prince of Wales Hospital (n=401)*	Queen Elizabeth Hospital (n=359)*	Tuen Mun Hospital (n=319)*	Princess Margaret Hospital (n=36)*
Job nature				
Heavy physical work	242 (63)	250 (70.8)	207 (66.6)	25 (69.4)
Medium physical work	80 (20.8)	57 (16.1)	51 (16.4)	4 (11.1)
Low physical work	62 (16.1)	46 (13)	53 (17)	7 (19.4)
Monthly individual income, HK$				
<20 000	184 (56.4)	177 (51.9)	133 (50.4)	15 (48.4)
≥20 000	142 (43.6)	164 (48.1)	131 (49.6)	16 (51.6)
Education level				
Primary	82 (20.4)	64 (17.9)	59 (18.5)	5 (13.9)
Secondary	241 (60.1)	219 (61.2)	202 (63.3)	20 (55.6)
Post-secondary	78 (19.5)	75 (20.9)	58 (18.2)	11 (30.6)
Living status				
Alone	60 (15)	46 (12.8)	46 (14.4)	7 (19.4)
With family	340 (85)	313 (87.2)	273 (85.6)	29 (80.6)
Compensation				
No	118 (36.8)	88 (26.2)	64 (25.5)	6 (31.6)
Yes	203 (63.2)	248 (73.8)	187 (74.5)	13 (68.4)
Pre-injury physical component summary	55.1±4.7	56.4±4.6	55.7±4	56±3.4
Pre-injury mental component summary	54.6±6.5	54.5±5.2	54.6±6.4	53.6±6
Pre-injury EQ-5D-5L	1±0.1	1±0.1	1±0.1	1±0
1-month extended Glasgow Outcome Scale				
<6	240 (72.9)	286 (85.4)	231 (86.2)	14 (87.5)
≥6	89 (27.1)	49 (14.6)	37 (13.8)	2 (12.5)
1-month physical component summary	38.8±10.9	33±10.4	35.4±10	34.6±11.3
1-month mental component summary	50±10.4	52.6±8.4	48.8±12.3	49.4±14.1
1-month numeric rating scale for pain				
<1	66 (20.9)	50 (16.1)	27 (10.4)	0 (0)
≥1	250 (79.1)	260 (83.9)	233 (89.6)	15 (100)
1-month EQ-5D-5L	0.6±0.3	0.6±0.3	0.5±0.3	0.5±0.3

injury severity score was 10. The Abbreviated Injury Scale score for head was ≥3 in 31% of patients. 54% of patients underwent surgery, and the median length of hospital stay was 8 days. 21% of patients were admitted to intensive care unit. On discharge, 86% of patients reported good recovery and 76% were discharged directly home. 70% patients had applied for compensation after injury.

Overall, 607 (54%) patients had RTW within 12 months of injury. Compared with those who did not RTW, patients who RTW were younger (44 vs 51 years, P<0.001) and had a lower injury severity score (10 vs 11.5, P<0.001), a shorter length of hospital stay (6 vs 11 days, P<0.001), fewer surgery performed (46% vs 65%, P<0.001), and fewer intensive care unit admissions (15% vs 28%, P<0.001).

Patients who RTW had a lower proportion of pre-injury heavy physical work (57% vs 79%, P<0.001), a higher proportion of attaining post-secondary education (28% vs 11%, P<0.001), and a lower proportion of primary level education as highest educational attainment (12% vs 27%, P<0.001). They also had fewer work-related injuries (23% vs 42%, P<0.001) and fewer pre-existing comorbidities (35% vs 41%, P<0.034). With respect to GOSE, 92% of patients who RTW reported good recovery on discharge, whereas 78% of patients who did not RTW had good recovery. In addition, 84% of patients who RTW were discharged home directly, but this was the case in only 66% of patients who did not RTW.

Fewer patients had applied for compensation in the RTW group than in the non-RTW group (63% vs 81%, P<0.001). There were no differences in pre-

TABLE 2. Factors associated with return to work within 1 year of injury

Variable	Univariate analysis		Multivariable analysis			
	Odds ratio (95% CI)	P value	Before backward		After backward	
			Adjusted odds ratio (95% CI)	P value	Adjusted odds ratio (95% CI)	P value
Age, y						
18-34	1		1			
35-50	0.71 (0.51-0.98)	0.04	0.88 (0.52-1.5)	0.64		
>50	0.47 (0.35-0.64)	<0.001	0.75 (0.42-1.35)	0.34		
Sex						
Female	1					
Male	0.92 (0.69-1.25)	0.60				
Trauma type						
Non-blunt	1					
Blunt	0.99 (0.68-1.45)	0.97				
Mechanism of injury						
Traffic	1		1			
Fall	0.8 (0.61-1.06)	0.13	0.74 (0.43-1.29)	0.29		
Penetrating	0.78 (0.5-1.23)	0.29	0.84 (0.39-1.79)	0.64		
Burn	1.11 (0.51-2.41)	0.80	1.04 (0.28-3.89)	0.96		
Others	0.65 (0.46-0.91)	0.010	0.84 (0.45-1.59)	0.60		
Work-related injury						
No	1		1		1	
Yes	0.43 (0.33-0.55)	<0.001	0.47 (0.28-0.78)	0.003	0.41 (0.28-0.61)	<0.001
Psychiatric disease						
No	1		1			
Yes	0.53 (0.28-1.04)	0.06	1 (0.31-3.28)	1.00		
Pre-existing comorbidity						
No	1		1			
Yes	0.77 (0.6-0.98)	0.030	0.91 (0.61-1.36)	0.65		
Injury severity score	0.97 (0.96-0.98)	<0.001	1 (0.98-1.02)	0.90		
Abbreviated Injury Scale for head						
<3	1					
≥3	1.08 (0.84-1.4)	0.55				
Operation performed						
No	1		1			
Yes	0.45 (0.36-0.58)	<0.001	0.8 (0.51-1.24)	0.31		
Intensive care unit admission						
No	1		1			
Yes	0.46 (0.34-0.61)	<0.001	0.87 (0.51-1.47)	0.5		
Length of hospital stay						
≤8 days	1		1		1	
>8 days	0.35 (0.28-0.45)	<0.001	0.52 (0.34-0.81)	0.004	0.48 (0.33-0.72)	<0.001
Extended Glasgow Outcome Scale on discharge						
Good recovery	1		1			
Moderate/severe disability	0.3 (0.21-0.43)	<0.001	0.86 (0.45-1.64)	0.65		
Discharge destination						
Home	1		1		1	
Non-home	0.36 (0.27-0.48)	<0.001	0.63 (0.38-1.06)	0.08	0.55 (0.35-0.85)	0.007
No. of years worked	0.99 (0.98-1)	0.24	1.01 (0.99-1.03)	0.41		

TABLE 2. (cont'd)

Variable	Univariate analysis		Multivariable analysis			
	Odds ratio (95% CI)	P value	Before backward		After backward	
			Adjusted odds ratio (95% CI)	P value	Adjusted odds ratio (95% CI)	P value
Job nature						
Heavy physical work	1		1		1	
Medium physical work	2.6 (1.85-3.64)	<0.001	1.81 (1.02-3.21)	0.043	1.86 (1.07-3.24)	0.029
Low physical work	3.13 (2.16-4.53)	<0.001	1.54 (0.81-2.93)	0.19	1.62 (0.87-3.03)	0.13
Monthly individual income, HK$						
<20 000	1					
≥20 000	0.97 (0.75-1.26)	0.84				
Education level						
Primary	1		1		1	
Secondary	2.06 (1.5-2.84)	<0.001	1.57 (0.93-2.67)	0.09	1.61 (1-2.6)	0.05
Post-secondary	5.6 (3.69-8.5)	<0.001	3.63 (1.61-8.19)	0.002	3.91 (1.86-8.25)	<0.001
Living status						
Alone	1		1			
With family	1.49 (1.06-2.08)	0.021	1.32 (0.75-2.34)	0.34		
Compensation						
No	1		1			
Yes	0.42 (0.31-0.57)	<0.001	0.91 (0.58-1.44)	0.70		
Pre-injury physical component summary	1.01 (0.98-1.04)	0.54				
Pre-injury mental component summary	0.98 (0.96-1)	0.11	0.99 (0.96-1.03)	0.65		
Pre-injury EQ-5D-5L	0.44 (0.06-3.11)	0.41				
1-month extended Glasgow Outcome Scale						
<6	1		1		1	
≥6	46.03 (16.91-125.27)	<0.001	41.32 (5.51-309.81)	<0.001	40.22 (5.46-296.39)	<0.001
1-month numeric rating scale for pain						
<1	1		1			
≥1	0.42 (0.28-0.62)	<0.001	1.12 (0.6-2.11)	0.72		
1-month physical component summary						
≤34	1		1		1	
>34	4.01 (3-5.37)	<0.001	1.91 (1.22-3)	0.005	1.86 (1.21-2.88)	0.005
1-month mental component summary						
≤49	1		1		1	
>49	3.13 (2.35-4.17)	<0.001	2.86 (1.9-4.29)	<0.001	2.91 (1.96-4.33)	<0.001
1-month EQ-5D-5L						
≤0.49	1		1		1	
>0.49	5.65 (4.23-7.55)	<0.001	1.51 (0.97-2.37)	0.07	1.5 (0.97-2.31)	0.07

injury health status (physical component summary [PCS] and mental component summary [MCS] of Short Form-12 and the EQ-5D-5L) between the two groups, but the 1-month health status were better in patients who RTW than in those who did not RTW. A higher proportion of patients had GOSE of ≥6 (upper moderate disability) in the RTW group than in the non-RTW group at 12 months after injury (32% vs 1%, P<0.001).

In the multivariable logistic regression analysis, RTW within 12 months of injury were independently associated with non-work-related injury, length of hospital stay of ≤8 days, discharge home directly, non-heavy physical work of job nature, higher educational level, and better 1-month health status (Table 2). A prediction model was established using

TABLE 3. Proposed prediction model for return to work within 12 months of injury

Variables	Score
Non-work-related injury	2
Job nature	
Medium physical work	1.5
Light physical work	1
Education level	
Secondary	1
Post-secondary	3.5
Length of hospital stay of <9 days	2
Discharge directly home	1.5
1-month extended Glasgow Outcome Scale of >5	9
1-month physical component summary of >34	1.5
1-month mental component summary of >49	2.5
1-month EQ-5D-5L of >0.49	1
Score range	**0-24.5**
Area under the receiver operating characteristic curve (95% CI)	**0.850 (0.824-0.875)**

these factors; the area under the receiver operating characteristic curve was 0.850 (95% confidence interval=0.824-0.875) for discriminating RTW and not RTW (Table 3).

Discussion

Predictors for not RTW within 12 months of injury were primary education levels, heavy physical work, work-related injury, length of hospital stay of ≥9 days, not discharge directly home, poorer health-related quality of life measures, and poorer functional outcome at 1 month following injury.

For those who RTW at 12 months, 63% returned to original work at full capacity, 26% returned with reduced work capacity, and 12% changed job nature. In the Victorian State Trauma Registry cohort,[1] 51.6% of respondents had early and sustained RTW, 15.5% had delayed RTW, 13.3% failed RTW, and 19.7% did not RTW. Predictors of delayed and no RTW included having a manual occupation and injuries sustained in motor vehicle crashes. Older age and receiving compensation predicted both failed and no RTW patterns. Severity of injury and treatment factors were not significant predictors for RTW status.

In our prediction model, higher education level and non-manual labour occupations were predictors for RTW, as were length of hospital stay of <9 days, discharge home directly, and 1-month scores of PCS, MCS, EQ-5D-5L, and GOSE. Three-month pain and physical functioning scores have also been suggested to be important.[2]

Although receiving compensation was not a predictor, non-work-related injury was a predictor for RTW in our study, which may remove disincentive for recovery through indirectly receiving compensation and benefits.[3,4]

It is important to routinely collect data relating to longer term outcomes including RTW. Future studies should investigate the role of early dedicated rehabilitation interventions on 1-year RTW rate.

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Effects of electroacupuncture on postoperative cognitive dysfunction: a preclinical study (abridged secondary publication)

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KEY MESSAGES

1. Electroacupuncture reduces cognitive impairment and phosphorylation of tau in a mouse model of postoperative cognitive dysfunction.
2. The protective effects of electroacupuncture are comparable to that of ibuprofen.

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Introduction

Postoperative cognitive dysfunction (POCD) is a common complication following surgery and hospitalisation. After general anaesthesia and surgery, patients may experience impairment in attention, concentration, executive function, memory, visuospatial ability, and psychomotor speed over a period ranging from weeks to years. These changes in cognition can put patients at higher risks of postsurgical complications (eg, misunderstanding wound care and drug treatment instruction).

No specific pathophysiological mechanism can fully explain POCD, owing to its multifactorial nature. Possible explanations include inflammatory responses induced after surgical procedures, excessive neuronal apoptosis, increased oxidative stress, free radical injury, and synaptic changes. Among these, inflammation seems to have a major role in POCD. Both systemic- and neuroinflammation, particularly in the hippocampus, triggered by peripheral surgery trauma or anaesthesia have been proposed for the cognitive deficits. Elevated levels of pro-inflammatory cytokines (such as IL-6, IL-1β, and TNF-α) after surgery may be related to POCD. Morphological changes and activation of microglia after surgery have been reported in preclinical studies. It is proposed that microglia mediate multiple aspects of neuroinflammation and can trigger cognitive dysfunction through multiple signalling pathways.

In our previous study, ibuprofen (a non-steroidal anti-inflammatory drug) could attenuate systemic inflammation and cognitive impairment in mice that had undergone laparotomy. Ibuprofen is commonly used for postoperative pain management, but it can cause gastric bleeding and renal damage and hence not suitable for prolonged use. Non-pharmacological approach for POCD is preferred because of adverse effects of medications, especially in older patients with comorbidities.

Acupuncture and electroacupuncture (EA) have been used to treat neurological and mental disorders. Acupuncture before surgery has been reported to reduce the incidence of POCD and suppress systematic inflammation. This study aims to assess the effects of postoperative EA on cognition and related pathology of mice following laparotomy under general anaesthesia.

Methods

12-week-old male C57BL/6N mice (weighing 25±3 g) were randomly assigned to four groups: sevoflurane alone (control), laparotomy alone, laparotomy + EA, and laparotomy + ibuprofen (positive control). Under anaesthesia with sevoflurane for 20 minutes, a longitudinal midline incision was made in the abdomen. The intestine was exteriorised and generally rubbed for 1 minute and then put back into the abdominal cavity. The muscle layers and skin were then closed and sutured. Analgesic was given for 3 days after laparotomy.

EA treatment was started 2 days after laparotomy to allow sufficient rest. Mice were physically restricted using a custom-made nylon net. Acupuncture needles were inserted horizontally at the Baihui (DU20) and Zusanli (ST36). Electrical stimulation was generated using an EA apparatus set to disperse waves of 1 and 20 Hz. The 7 days and 14 days protocols were used, in which EA treatment lasts for 20 minutes daily for 5 or 12 days, respectively. These time points represent the early and middle postoperative periods. Mice in the

control, laparotomy alone, and ibuprofen groups were fixed in place for 20 minutes using similar apparatus but without EA. Mice in the ibuprofen group were fed with ibuprofen (60 mg/kg/day) for 5 or 12 days.

Behavioural changes were assessed using the (1) novel object recognition test for hippocampal-dependent memory, (2) open field test for general locomotor activity and anxiety levels, (3) Y-maze test for associated memory, and (4) puzzle box test for executive functions and cognitive ability.

Mice were euthanised after assessment. The plasma and brain were harvested; the brain was dissected into the frontal cortex and hippocampus. Real-time polymerase chain reaction, western blot analysis, immunohistochemical staining (according to the Accu-OPTIClear protocol), and Milliplex cytokine assays were performed to assess pathological changes in mice.

Results

Electroacupuncture attenuated cognitive impairment induced by laparotomy

To understand the short- and medium-term effects of EA, cognitive performances of mice were assessed at different time points in the 7-day and 14-day protocols. In the Y-maze test, the number of errors in early and medium time points significantly increased in mice in the laparotomy-alone group than in the control group, whereas those received EA made fewer errors than those in the laparotomy-alone group. This suggested an improvement in associated memory. In the novel object recognition test, mice in the laparotomy-alone group displayed reduced ability to differentiate the novel object from the old object, which suggested an impairment in recognition memory, whereas mice that had received EA for 12 days showed significant improvement. In the puzzle box test, mice in the laparotomy-alone group showed significantly impaired problem-solving ability, short-term memory, and long-term memory during the middle postoperative period, but these cognitive changes were attenuated by EA. In most behavioural tests, the effects of EA were similar or slightly worse than that of ibuprofen.

For the general health condition of mice after laparotomy, an immediate weight loss was observed, which persisted for 2 weeks and could not be reversed by EA. The locomotor activity was reflected by the total distance travelled in the arena in the open field test. The anxiety- or depression-like behaviour was reflected by the time spent in outer zone during the open field test. There was no significant difference in the anxiety- or depression-like behaviour between groups. These suggested that the cognitive changes were unlikely to be caused by diminished physical ability or anxiety or depression levels.

Electroacupuncture attenuated laparotomy-induced tau phosphorylation

Tau is an essential protein for maintaining the stability of microtubules. Post-translational modification of tau, such as increased phosphorylation, can occur in pathological situations including Alzheimer disease and results in tau aggregation and generates toxicity. To determine if EA can reduce such pathological changes, we used western blot analysis to assess changes of the tau phosphorylation. Laparotomy induced phosphorylation of tau (AT180, AT8, p-Tau 404, p-Tau 396) in the hippocampus and frontal cortex of mice 14 days after surgery. These changes were partly attenuated by EA. The effects of EA on attenuating tau phosphorylation slightly differed between the hippocampus and the frontal cortex.

Western blot analysis was used to examine any change of tau- and stressed related kinases in the early postoperative period (7 days post-surgery). We found that EA attenuated the activation of GSK3β (at tyrosine 216) and reduced the phosphorylation of JNK after laparotomy in both the hippocampus and the frontal cortex. However, there were no significant changes in the JAK/STAT signalling proteins.

We examined the effects of EA on synaptic proteins, which play key roles in neurotransmission. In the hippocampus, there was no significant change in the levels of synapsin-1, synaptophysin, or NMDAR2B receptor 14 days after laparotomy. EA also did not show any significant effect on the expression of these proteins. In the frontal cortex, a significant reduction of NMDAR2B was found 14 days after laparotomy. However, EA could not reverse this reduction. This suggested that cognitive impairment after laparotomy might not be related to the expression levels of the detected synaptic proteins.

Effects of electroacupuncture on attenuating neuroinflammation

To study the effects of EA on neuroinflammation, we assessed the morphological changes of microglia (detected by Iba-1) and astrocyte (detected by GFAP) in the hippocampus of the mice. There were increased levels of Iba-1 and GFAP in CA1 region of the hippocampus 14 days after laparotomy. Microglia changed their morphology to amoeboid-like shape, with retraction of the fine processes. In astrocytes, the processes showed more ramification (hypertrophy). All these were attenuated in mice that received EA treatment. To determine if cytokines were involved, we examined expression of mRNA for inflammatory cytokines in the hippocampus and frontal cortex 7 days and 14 days after laparotomy. There were no significant changes in the levels of IL-1β, TNF-α, MCP-1, IL-6, IL-10 and IL-8 in the brain in the two time points. However, there were

mild yet significant increased levels of TNF-α and IL-10 in the hippocampus 14 days after laparotomy. EA attenuated the increase of IL-10. In the peripheral circulation, there were increased levels of IL-6 and IL-10 in the plasma 7 days after laparotomy. These changes seemed to be attenuated by EA despite not significantly.

Discussion

Postoperative EA could attenuate the cognitive impairment after laparotomy. This effect was not prominent at the early postoperative phrase, in which EA improved the performance of mice in the Y-maze test but not the novel object recognition test. This may be related to the number of EA received before the tests were conducted. When the number of EA treatment was increased in the 14-day protocol, a positive treatment effect of EA was found in both tests. These suggests that a single/few EA treatment is unlikely to attenuate the cognitive impairment. This is not consistent with the finding in a study that a single session of EA during or after surgery is sufficient to reduce the incidence of POCD in patients.[1] It seems that EA before/during surgery provides more beneficial effects than EA after surgery. Our findings cannot be compared directly with those of other animal studies, as different POCD models such as partial hepatectomy or splenectomy are used.[2-4] We believe that laparotomy without removal of any parts of internal organs is a relatively milder surgery. Our data suggested that EA could suppress stress kinases and reduce tau-pathology, but its effect on attenuating neuroinflammation was less prominent.

Conclusion

EA can attenuate cognitive dysfunctions and some neuropathological changes in the brain of mice after laparotomy. The protective effect of EA is slightly lower than that of ibuprofen. EA can attenuate the activation of microglia and astrocyte in the brain. However, other mechanisms, both peripherally and in the brain, may also exist to explain the observed benefits. Postoperative EA can be a viable option for the management of POCD.

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