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研究成果報告

Diagnosis and Detection
診斷與檢測

Environment and Infection
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Evaluation of Interventions
介入性研究的評估

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EDITORIAL

We are delighted to bring you another series of dissemination reports of research projects supported by the *Health Services Research Fund* (HSRF)*, the *Health and Health Services Research Fund* (HHSRF) and the *Research Fund for the Control of Infectious Diseases* (RFCID). This edition features projects related to: diagnosis and detection; environment and infection; and the evaluation of interventions. Several projects are highlighted due to their significant findings, impact on health care delivery and practice, and/or contributions to health policy formulation in Hong Kong.

The key to effective communicable disease control and management is early identification. This is especially true for acute respiratory tract infections, which may be caused by many distinct viral and bacterial pathogens. Chan et al[1] developed polymerase chain reaction primer groups that could be used in a multiplex reverse-transcriptase polymerase chain reaction to detect 17 different respiratory pathogens. Using 50 nasopharyngeal aspirates positive for various respiratory viruses by conventional testing, the primer sets were optimised in cell culture preparations and then validated. The multiplex assays were 100- to 1000-fold more sensitive than the conventional tube culture. An additional advantage that may make the assay more applicable in clinical settings was the shortened turnaround time, which is often critical in the investigation and control of urgent outbreaks.

The 2003 severe acute respiratory syndrome (SARS) outbreak introduced a dilemma for clinicians with respect to the use of oxygen delivery devices (ODD). Improving patient oxygenation is an important factor when treating respiratory infections. However, ODD were thought to facilitate the spread of infectious organisms via air contaminated with respiratory secretions. Khaw et al[2] assessed the different practices adopted for oxygen therapy with respect to modified ODD used in Hong Kong public hospitals during the SARS epidemic and evaluated their performance using a human patient simulator. The most common modifications were the use of nasal cannulae covered with a surgical or N95 facemask. While there was lack of research data regarding the performance of the modified ODD, most health care workers interviewed considered that these modifications (made during the SARS epidemic) were effective in preventing disease transmission to themselves or their patients, without causing harm. This study also found that the modified ODD did not significantly increase airway resistance. These findings will provide a foundation for further assessment and development of modified ODD.

The UK Department of Health recommends that adults perform 30 minutes of moderate physical activity on 5 or more days per week. This minimum level can be accumulated throughout the day and does not need to be achieved in a single session. One simple means of achieving this recommendation cumulatively, is by walking throughout the day. Stair climbing is another way of extending this recommendation. Eves et al[3] evaluated the effects of different interventions designed to encourage stair climbing. Posters and banners were located at appropriate points between stairs and escalators in an outdoor setting (the Mid-Levels escalator system) and in an indoor air-conditioned shopping mall. Similar interventions have been shown to be useful in the US and UK. However, only one of the three stair climbing interventions tested in this study was found to have a significant effect on the proportion of pedestrians climbing stairs in Hong Kong. Further analyses indicated that an ingrained attitude to escalator use in the hot, humid and hilly environment of Hong Kong rather than negative perceptions about climbing stairs might be important factors determining physical activity. This study demonstrates the importance of the local environmental and cultural factors in affecting the outcome of lifestyle-related interventions, and should not be taken lightly.

We hope you find this selection of dissemination reports informative and enjoyable. These dissemination reports and the corresponding full project reports may be downloaded individually from the Research Fund Secretariat website (**http://www.fhb.gov.hk/grants**), where more information about the funds, including application procedures, can also be found.

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* The HSRF was succeeded by the *Health and Health Services Research Fund* (HHSRF) in 2002.

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A high-throughput detection method for hepatitis B virus mutations in the Chinese population

Key Messages

1. Arrayed primer extension (APEX) has been successfully developed as a high-throughput method for single nucleotide polymorphisms (SNPs) present in the hepatitis B virus (HBV) genome.
2. Co-existence of wild-type and mutant genotypes in one SNP was successfully identified by APEX, which was not identified by conventional DNA sequencing.
3. Thirty-three patients were involved in this study; APEX results showed A1762T and G1764A double mutations and G1896A mutation are the most prevalent SNPs present in HBV genomes.
4. APEX can be developed as a SNP screening tool to monitor other infectious agents.

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Introduction

Hepatitis B virus (HBV) is the infective agent responsible for the widespread liver disease in humans. According to the World Health Organization, about 2 billion persons worldwide have been infected with HBV; more than 350 million have been chronically infected, of whom three-quarters are Asian.[1] Chronic infection has been associated with a high risk of developing liver cirrhosis and hepatocellular carcinoma (HCC),[2] and kills one million persons each year.[1]

Evidence suggests that chronic HBV infection is associated with the development of point mutations or single nucleotide polymorphisms (SNPs) in the four open reading frames (ORFs) of the HBV genome, namely the C, S, P and X genes (Fig 1). Studies show that the development of SNPs in these ORFs is correlated with the occurrence of HCC. Therefore, different methods have evolved for the detection of HBV SNPs to monitor disease development. Conventional methods are generally not practical for detecting multiple SNPs. Also, for clinical and diagnostic analysis, simple techniques with high-throughput potential, high reproducibility, sensitivity, and specificity are required.

DNA microarray technology has been applied not only to study gene expression, but also for large-scale sequence analysis and mutation detection. Here we describe a DNA-based method for rapid detection of multiple mutations in a microarray format, known as arrayed primer extension (APEX). This method is based on the hybridisation of HBV DNA to an array of complementary DNA spotted on a glass surface, followed by the incorporation of fluorescent-labelled dideoxynucleotides mediated by an enzyme (Fig 2). Thus, APEX can be regarded as the miniature form of DNA sequencing. With prior optimisation, detection of known SNPs is possible in a single reaction by designing hundreds to thousands of short specific sequence of DNAs on the slide. One advantage of using APEX for detecting SNPs is the high signal-to-noise ratio, which allows rapid and accurate determination; APEX is a promising technique for large-scale analysis under optimal conditions. It may also be possible to apply this technique to detect SNPs in other bacterial or viral genomes.

Methods

This study was conducted from September 2004 to May 2006.

Selection of patients in the Chinese population for hepatitis B virus DNA extraction

The blood samples from HBV-infected patients with HBV DNA levels equal to or higher than 10^4 copies/mL were selected and the viral DNA were extracted. The DNA amount and quality was quantified and checked, and finally samples from 33 patients were selected.

Polymerase chain reaction for hepatitis B virus DNA amplification

The four ORFs of HBV were separately amplified from serum HBV DNA using polymerase chain reaction (PCR).

Preparation of short specific sequences of DNA and array printing

Short specific sequences of DNA for detecting the 30 SNPs in the four OFRs

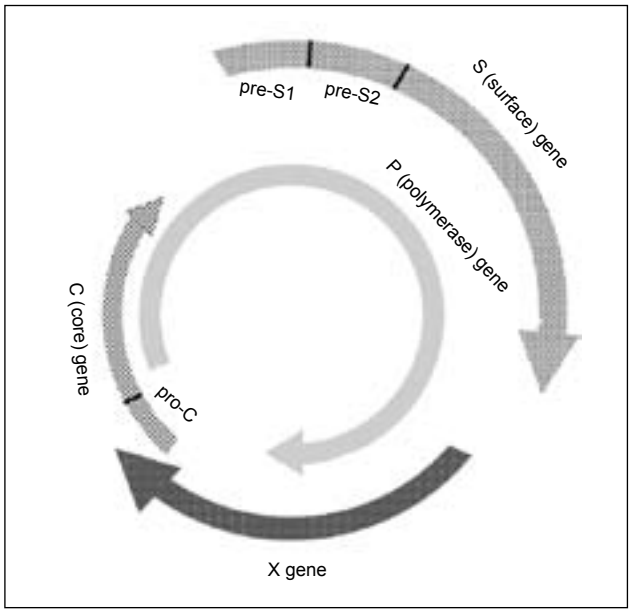

Fig 1. Schematic representation of the HBV genome[3]

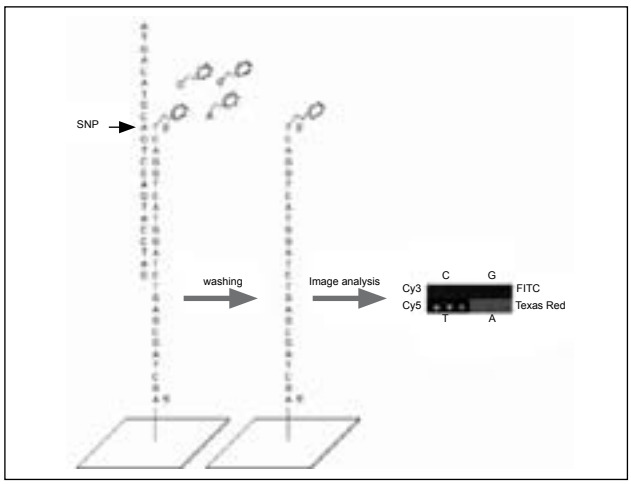

Fig 2. Principle of arrayed primer extension

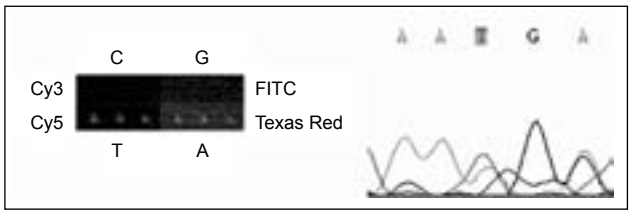

Fig 3. Typical comparisons of arrayed primer extension (APEX) single nucleotide polymorphism (SNP) genotyping and DNA sequencing

The coexistences of wild-type and mutant genotypes in one SNP of a single individual were successfully genotyped by APEX, but the same cases were not detected by DNA sequencing

were designed according to the genomic sequence of the HBV (GenBank accession no. NC_003977). Each short specific sequence was spotted in triplicate onto special glass slides.

Template preparation for APEX

The purified PCR products were enzymatically fragmented.

Arrayed primer extension

Under optimal conditions, the fragmented PCR products were allowed to bind to the short complementary sequence of DNA in the presence of four different fluorescent-labelled dideoxynucleotides and DNA polymerases. A standard APEX reaction (but without an HBV PCR product) was used to check if there were self-extending oligonucleotides or non-specific background signals.

Image capture and analysis

The slides were scanned using a confocal laser scanner. The intensity of each spot on the microarray was quantified and subsequently analysed. The specificities and sensitivities were calculated according to the following equations:

Specificity = number of true negatives / (number of true negatives + number of false positives)

Sensitivity = number of true positives / (number of true positives + number of false negatives)

DNA sequencing

The sequences of the PCR products were determined by DNA sequencing, which acted as the 'gold standard'.

Results

Single nucleotide polymorphisms genotyping

This study involved 33 patients infected with HBV. The results were compared with the 'gold standard' DNA sequencing data for method validation. Typical comparisons of APEX SNP genotyping and DNA sequencing on different nucleotides are shown in Fig 3. Among the 30 SNPs present on the whole HBV genome, specificities ranged from 100 to 66% and sensitivities from 100 to 59%.

Coexistence of wild-type and mutant genotypes in a single nucleotide polymorphism

The coexistences of wild-type and mutant genotype in one SNP of a single individual were successfully genotyped by APEX. However, the same cases were not discernable by DNA sequencing (Fig 3). These cases happened most frequently on SNP G1896A in the C gene (9 of 33 patients), while there was no significant co-existence for other SNPs.

Prevalence study by arrayed primer extension

According to the APEX results, the most frequent mutation (23 of 33) was G1896A in the C gene. All patients carried the G1896A mutant SNP, which creates a stop codon TAG in codon 28 of the precore region. The other 'hotspots' were A1762T and G1764A double mutations present in the precore region, in 22 and 20 patients respectively. In addition, some frequent mutations are also present in the C

gene. These included A1979G (codon 27), A2159G (codon 87), A2189C (codon 97) and C2288A (codon 130), which comprised 18%, 27%, 27%, and 30% of the respective mutations. For the P gene, the SNPs of A739G and G741T which lead to lamivudine resistance in the YMDD motif were only found in three and two patients respectively. For the S gene, one patient expressed A530G and another carried a G546A mutation, showing that these were minor mutations. For the X gene, the eight nucleotide deletions (nt 1768 -1775) commonly present in the COOH terminal were not found in our pool of samples. However, 33% (11/33) of our patients had T1464C/G mutations.

Discussion

Liver cancer is the third commonest cause of cancer mortality in Hong Kong, of which HBV infection is one of the major causes. Many studies show that SNPs present on the HBV genome correlate with liver cancer development. Therefore, large-scale population studies to determine the prevalence of SNPs, its natural course, and response to treatment are urgently needed. Using the APEX strategy to detect mutations in the HBV genome will be applicable in this regard, as it allows multiplex screening of different SNPs in a single experiment. By designing short specific sequences of DNA, different SNPs in the HBV genome could be genotyped accordingly. One feature of APEX is the design of a redundant set of short specific sequences of DNA against the same SNPs, to ensure the highest statistical significance and the correct interpretation of signals. Binding efficiency of HBV DNA to short complementary sequences of DNA is increased by fragmentation. In the presence of DNA polymerases, fluorescent-labelled ddNTPs corresponding to the SNPs are extended accordingly. The labelled short specific sequence of DNA is subsequently visualised by a laser confocal scanner. The advantage of APEX is that all the SNPs present in an ORF can be detected in a single-step reaction.

According to our results, the most common co-existence of wild-type and mutant SNPs within individual patients are A1762T, G1764A and G1896A present in the C gene. However, these coexistences are undetectable by DNA sequencing. This is due to the ambiguity in base-calling by the instrument, during the presence of double peaks. This limitation affects the reliability of direct sequencing for the identification of nucleotides in these SNPs, at least under automated conditions. The coexistence of wild-type and mutant genotypes may imply the transition stage of chronic infection or that one viral species is dominant over the others.[3] Thus, the detection of both dominant and non-dominant viral species by APEX, which can be missed by DNA sequencing, might be significant for monitoring disease development.

The APEX results also showed that A1762T, G1764A and G1896C are the most frequent SNPs present in the precore/basal core promoter region as well as the whole HBV genome. The presence of G1896A mutation causes suppressed external core antigen (HBeAg) secretion. As HBeAg is a major humoral and cellular target, HBeAg negativity is regarded as an indicator of immunological clearance of HBV and rapid viral replication. The A1762T and G1764A double mutations suppress the transcription of precore mRNA and arrest HBeAg production. Therefore, the detection of this double mutation is useful for monitoring disease development.

In addition, some frequently encountered SNPs present in the C gene may disturb the level of HBcAg expression, causing evasion of the host's immune clearance and thus directly affect the activity of HBV-induced liver disease.

For the S gene, no significant SNPs were detected in our samples. However, since the SNPs present in this region lead to HBV replication in vaccinated patients, the detection of SNPs present on the S gene will be beneficial for vaccine development.

For the P gene, although no significant SNPs were detected in our samples, the detection of SNPs present in it was crucial for patients who receive lamivudine therapy during the course of HBV infection. Lamivudine is a reverse-transcriptase inhibitor and has antiviral activity against HBV. However, recent studies reported the emergence of lamivudine-resistant HBV during treatment. Therefore the detection of lamivudine-resistance–related SNPs in this region can be an alternative means of monitoring the development of lamivudine-resistant strains of HBV.

For the X gene, re-sequencing by APEX was used to detect the presence of HBx COOH-terminal in our study. The design of an APEX array for comparison sequencing is straightforward and complementary to the gene of interest.[4] According to our results, none of the patients had HBV X gene COOH-terminal deletions. However, studies demonstrated that the HBx COOH-terminal is responsible for controlling host cell viability and proliferation. In addition, a prevalent HBV X gene mutant T1464C/G present in Taiwanese patients with liver cirrhosis and HCC was also included in our system. Approximately 33% (11/33) of patients had the T1464C/G mutation. Development of this mutant might represent a strategy of the virus to escape immune surveillance, so a reliable genotyping method will be beneficial for studying the process of multiple-step hepatocarcinogenesis.

In conclusion, the simultaneous and high-throughput screening of SNPs in the HBV genome by APEX enables large-scale diagnostic analysis, which is an alternative to genotyping by DNA sequencing.

Acknowledgements

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A clinical prediction rule for diagnosing severe acute respiratory syndrome in the emergency department

Key Messages

1. Several features increased the likelihood of severe acute respiratory syndrome (SARS): previous contact with a patient with SARS, fever, myalgia (muscle aches), malaise (feeling unwell), abnormal chest radiograph, and abnormal lymphocyte and low platelet counts. Age older than 65 years or younger than 18 years, sputum production, abdominal pain, sore throat, runny nose, and high neutrophil count decreased the likelihood of SARS.
2. We derived a risk index that used data easily obtained in emergency departments, and identified patients with low and high likelihood of SARS during an outbreak.
3. Study data were obtained by reviewing medical records. Some patients may have had symptoms and findings that were not recorded in the records. Characteristics that identify patients with a high likelihood of SARS may differ in settings that are not large outbreaks.

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Introduction

Severe acute respiratory syndrome (SARS) was a disease that affected many countries, with more than 8000 cases reported globally, and carried with it a substantial morbidity and high case mortality.[1] It appears unlikely that SARS can be effectively eradicated after the 2003 global epidemic and conceivably it has achieved endemicity in various regions in southern China and elsewhere.

We sought to develop a clinical prediction rule for diagnosis that would accurately identify patients with SARS in the emergency department setting during an outbreak, and to validate the predictive accuracy of this rule.

Methods

This study was conducted from June 2004 to February 2005.

Study design

Retrospective analysis of patient records from two hospitals in Hong Kong using a two-step coefficient-based multivariable logistic regression scoring method, with internal validation by bootstrapping.

Sample size

A total of 2649 patients seen at two Hong Kong triage clinics during the 2003 SARS epidemic were studied. There were 1274 ($n_{non\text{-}SARS}$=897, n_{SARS}=377) consecutive patients from the United Christian Hospital, and 1375 ($n_{non\text{-}SARS}$=1191, n_{SARS}=184) consecutive patients from the Prince of Wales Hospital.

Study instruments

We reviewed medical records of these patients who visited SARS triage clinics in the emergency departments of these two Hong Kong hospitals in 2003. Specifically, we reviewed standard forms that had been used to record patient symptoms at the presentation, physical findings, chest radiography, and blood laboratory results. We also reviewed records to see which patients had blood test results that confirmed SARS. We analysed these data to determine which presenting features were associated with increased and decreased probabilities of diagnosing SARS. Then, we used a risk index to score characteristics that helped identify or rule out SARS, and tested the ability of the risk index to correctly identify SARS patients.

Main outcome measures

Points were assigned on the basis of history, physical examination, and simple investigations obtained at presentation. The outcome measure was a final diagnosis of SARS, as confirmed by World Health Organization laboratory criteria.

Results

Several features increased the likelihood of SARS—previous contact with a patient with SARS, fever, myalgia (muscle aches), malaise (feeling weak), abnormal chest radiograph, and abnormal lymphocyte and low platelet counts. Age older than 65 years or younger than 18 years, sputum production, abdominal

Table 1. Multivariable predictors of SARS diagnosis and associated risk scoring system for step 1

Characteristic	Points assigned*	Beta regression coefficient (95% confidence interval) $n_{estimation,\ step1}$=2039
Age-group (years)		
<18	-1	-0.15 (-0.55 to 0.25)
18-64	0	Reference
≥65	-5	-0.81 (-1.21 to -0.41)
Contact history		
Yes	8	1.14 (0.86 to 1.42)
No	0	Reference
Presence of symptoms on presentation		
Fever	15	2.18 (1.83 to 2.53)
Myalgia	3	0.40 (0.13 to 0.67)
Malaise	3	0.47 (0.21 to 0.72)
Sputum production	-4	-0.63 (-0.94 to -0.32)
Abdominal pain	-8	-1.24 (-1.82 to -0.66)
Sore throat	-5	-0.67 (-0.98 to -0.37)
Rhinorrhoea	-4	-0.55 (-0.87 to -0.23)

* Cutoff threshold for total point score (with a pre-specified sensitivity of 0.99: ≥ –3 indicates high-risk group whereas < –3 indicates low-risk group

Table 2. Multivariable predictors of SARS diagnosis and associated risk scoring system for step 2

Characteristic	Score assigned*	Beta regression coefficient (95% confidence interval) $n_{estimation,\ step2}$=1053
Age-group (years)		
<18	-1	-0.25 (-0.86 to 0.36)
18-64	0	Reference
≥65	-6	-1.54 (-2.13 to -0.94)
Contact history		
Yes	7	1.66 (1.20 to 2.13)
No	0	Reference
Fever	5	1.32 (0.82 to 1.83)
Sputum production	-4	-0.91 (-1.34 to -0.48)
Chest X-ray		
Normal	0	Reference
Haziness	8	1.91 (1.45 to 2.36)
Pneumonia (unilateral lesion, bilateral lesion)	8	1.98 (1.39 to 2.56)
Lymphocyte count ($x10^9$/L)		
Low (<1.5)	5	1.29 (0.89 to 1.70)
Normal (1.5-4)	0	Reference
High (>4)	5	1.24 (-0.81, 3.29)
Neutrophil ($x10^9$/L)		
Low (<2.0)	4	0.98 (0.073, 1.88)
Normal (2.0-7.5)	0	Reference
High (>7.5)	-5	-1.29 (-1.79, -0.79)
Platelets ($x10^9$/L)		
Low (<150)	5	1.16 (0.70, 1.61)
Normal (150-400)	0	Reference
High (>400)	-5	-1.12 (-2.87, 0.62)

* Cutoff threshold for total point score (with a pre-specified sensitivity of 0.95: ≥8 indicates high-risk group whereas <8 indicates low-risk group

pain, sore throat, runny nose, and high neutrophil count decreased the likelihood.

In step 1 of the clinical prediction rule, age in years (18-64 vs ≥65) and contact history were independently associated with a final diagnosis of SARS. In addition, the presence of three cardinal symptoms (fever, myalgia, and malaise) and the absence of sputum production, abdominal pain, sore throat, and rhinorrhoea were each independently associated with a final diagnosis of SARS (Table 1). None of the vital signs achieved statistical significance in the stepwise multivariable model and were therefore excluded. A total of 11% of the cohort with a total score of less than the threshold of -3 was assigned to the low-risk group, and did not proceed to step 2.

In step 2, in addition to four of the nine factors identified in step 1, four radiographic/laboratory findings (chest radiograph, lymphocyte count, neutrophil count, and platelet count) were each independently associated with a final diagnosis of SARS. Myalgia, malaise, abdominal pain, sore throat, and rhinorrhoea no longer achieved statistical significance in step 2 after inclusion of the investigations. The point scoring system shown in Table 2 was used to quantify the magnitude of association of each of these eight factors with SARS. A total score of 8 or greater would qualify the patient as being at high risk for SARS, with a pre-specified sensitivity of 95% overall. A total of 8% of

those considered in step 2 were further assigned to the low-risk category.

Using an internal validation procedure, application of the rule achieved an optimism-corrected sensitivity of 0.90, a specificity of 0.62, and an area under the receiver-operating characteristics (ROC) curve of 0.85.

Discussion

Our findings suggest that a simple model that uses clinical data at the time of presentation to an emergency department during an acute outbreak can predict the incidence of SARS and provide a practical diagnostic decision aid. The clinical prediction rule achieved high sensitivity and area under the ROC curve, which were maintained on internal validation by bootstrapping. This finding is important because of the high case-fatality ratio of SARS and potential public health hazards associated with its misdiagnosis. In addition, the rule could rule out SARS in a substantial proportion of persons presenting to an emergency department.

Ma et al[2] validated our rule on SARS data from Taiwan and showed that our rule performed very well, with a sensitivity of 98.8%, and a specificity of 52.0%. These results confirm the generalisability of the algorithm beyond Hong Kong to another urban population affected by SARS.

Before recommending the adoption of this clinical prediction rule by public health authorities in their SARS management plans, we must address several potential limitations. First, the analysis was based on data from retrospective chart review, and, therefore, the accuracy and completeness of information, as documented in the medical records, would influence the validity of the results.

Second, this rule was derived by using data from an acute outbreak; in this situation, the prevalence of SARS at the time of presentation was very high. Therefore, the prediction rule may not apply to isolated cases during the interepidemic period.

Third, the rule was constructed from dichotomous or categorical variables to facilitate use in practice. This may oversimplify the way physicians interpret the predictor variables. Therefore, as with all clinical practice guidelines, our rule should not supersede the physician's judgement in equivocal or borderline cases.

On a more practical level, health care providers should remember the usual limitations associated with practice guidelines and must maintain a high level of clinical suspicion, especially in the case of SARS and when isolation wards can still cope with admitting more patients. This decision tool will be most useful in a large epidemic when the health system's surge capacity is being overwhelmed by the number of patients seeking care.

Ultimately, the generalisability of the findings should be validated further prospectively, if SARS returns. In the meantime, we believe our prediction rule will provide the best evidence-based guidelines for the triage and management of patients suspected to have SARS when presenting to emergency departments and primary care settings.

Acknowledgements

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Formulation of a multiplex-reverse-transcription-polymerase-chain-reaction–based screening protocol to facilitate rapid clinical diagnosis of respiratory tract infections

Key Messages

1. Use of multiplex polymerase chain reaction (PCR) assays can improve the diagnostic yield in terms of overall sensitivity and spectrum of detection for respiratory tract infections.
2. The broad coverage and rapid turn-around achieved by multiplex PCR allows prompt measures in response to serious respiratory tract infections.
3. Implementation of multiplex PCR testing to routine diagnostics is recommended.

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Introduction

Respiratory tract infection accounts for a major proportion of illnesses seen in hospital settings. The outbreak of severe acute respiratory syndrome (SARS) and experience with avian influenza underscore the importance of prompt diagnosis for prompt initiation of specific measures, including isolation, contact tracing, and other public health measures. A wide range of viruses and bacteria can cause respiratory infections with presentations almost indistinguishable from SARS and avian influenza, especially in the initial stage when the patient first presents to hospital. Confirming the diagnosis of 'less severe' infections is equally important from the perspective of excluding serious conditions. Given the albeit small chance of co-infection, an ideal diagnostic approach should have the capacity of identifying multiple pathogens at the same time.

The ultimate goal of this study was to improve the performance and efficiency of laboratory diagnosis for acute respiratory tract infections. We therefore set out to devise, optimise, and evaluate a rapid diagnostic scheme for the simultaneous detection of common causative agents responsible for human respiratory tract infections.

Methods

This study was conducted from February 2005 to April 2006. Multiplex polymerase chain reaction (PCR) assays were designed to detect influenza A H1N1, H3N2 and H5N1; influenza B; parainfluenza 1, 2, 3, and 4; respiratory syncytial virus A and B; rhinovirus, enteroviruses, human coronavirus OC43, 229E and SARS-CoV; human metapneumovirus; *Mycoplasma pneumoniae*; *Chlamydia pneumoniae; Legionella;* and adenovirus.

Primer design

Multiple consensus regions of each organism were determined. Sequences of 10-20 representative strains of each pathogen were downloaded and aligned using Clustal X (http://bips.u-strasbg.fr/en/Documentation/ClustalX/) to verify sequence variability, and to select potential regions for primer design. Primer sets producing amplicon sizes that could be differentiated by agarose gel electrophoresis were identified for further evaluation and optimisation.

Multiplex polymerase chain reaction assay design and optimisation

The optimisation was first conducted using cell culture grown preparations, or when not available, clinical specimens known to contain the target agents as templates. For enteroviruses, the commonly encountered serotypes including Coxsackie A9, B1, B2, B3 and B5, Echo 7, 11, 30, EV71, and Polio 1 were included in all the evaluation processes. Different combinations of primer concentrations (range, 0.01-1.0 μM) for each were evaluated. During the optimisation experiments, a cocktail of 4 to 5 pairs of primers were used throughout, so as to better assess primer-primer interactions, which are often

the most critical technical issues to resolve. Templates containing single as well as mixtures of target organisms were used to assess detection of single as well as multiple infections. The potential for cross-amplification on human DNA/RNA carried in clinical samples was assessed by testing the primer cocktails against 50 negative clinical samples. This approach was also used to identify primer pairs for monoplex confirmatory PCR assays.

Thermal cycler selection

To provide the shortest possible turn-around time, a 'fast' thermal cycler (Applied Biosystems Fast PCR machine, US) was used. When coupled with the DNA polymerase contained in the Fast PCR Master Mix (Applied Biosystems GeneAmp, US), a 35-cycle PCR assay can be completed within 35 minutes, compared to approximately 180 minutes for ordinary cyclers. All multiplex PCR assays were optimised to fulfil the manufacturer's recommendation that a two-step cycling with annealing at 64°C be used. As for the confirmatory PCR assays, a three-step cycling on an ordinary thermal cycler using the hot start T*aq* polymerase (HotStarT*aq*, Qiagen, Germany) was used; as these conditions allow a more flexible choice of primers.

Field evaluation with clinical specimens

To evaluate the performance of the multiplex PCR assays when applied to routinely collected clinical specimens, 303 nasopharyngeal aspirate samples collected from patients who were admitted to the Prince of Wales Hospital for suspected respiratory tract infections were subjected to the multiplex PCR and routine isolation.

Nucleic acid extraction

All 50 nasopharyngeal aspirates selected for extraction evaluation were confirmed positive for respiratory viruses by routine isolation. The results of end-point dilution testing indicated that the QIAamp MinElute Virus Spin Kit (Qiagen, Germany) that extracts both viral RNA and DNA in combination was most effective. This kit was used to extract RNA/DNA throughout the study.

Reverse transcription

The Superscript III Reverse Transcriptase (Invitrogen, US) was chosen based on previous experience, and further optimised for this study. Briefly, the reaction was carried out in a 20-μL reaction mix containing 10 units of reverse transcriptase, 4 units of RNase OUT, 0.5 mM dNTP, 0.5 mM DTT, 2.5 ng random primers, and 8 μL of the extracted preparation derived from the clinical specimen. The reaction mix was subjected to thermal conditions of 65°C for 5 minutes, 4°C for 1 minute, 25°C for 5 minutes, 50°C for 50 minutes, and finally 37°C for 20 minutes.

Multiplex polymerase chain reaction

The optimal primer combinations were as follows. Group 1 comprised influenza A and B group–specific and subtype H1N1, H3N2, H5N1-specific primers; group 2 comprised parainfluenza 1, 2, 3 and 4; group 3 comprised respiratory syncytial virus A and B, rhinovirus, and enterovirus; group 4 comprised human coronavirus OC43, 229E and SARS-CoV, and human metapneumovirus; and group 5 comprised *M pneumoniae*, *C pneumoniae, Legionella* and adenovirus.

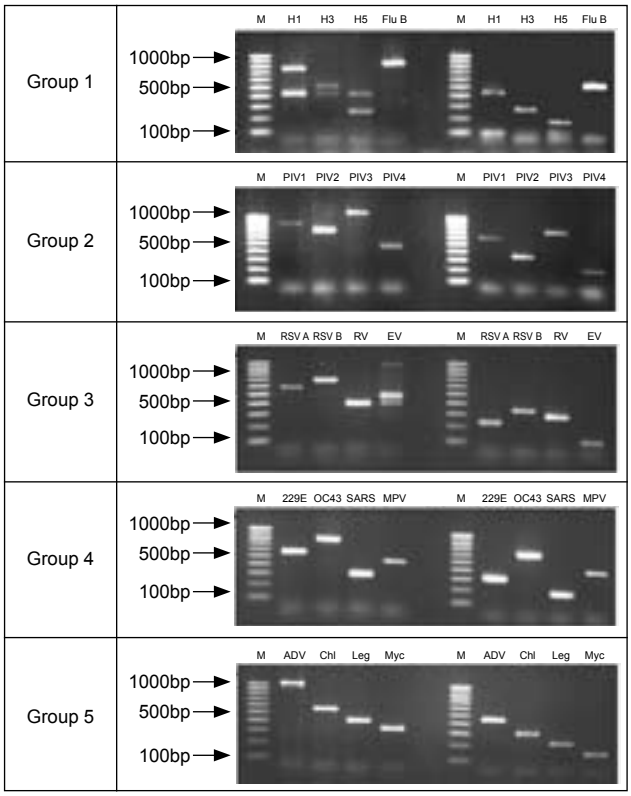

Fig. Agarose gel electrophoresis of multiplex polymerase chain reaction products

M denotes marker, Flu influenza, PIV parainfluenza, RSV respiratory syncytial virus, RV rhinovirus, EV Enterovirus, MPV metapneumovirus, ADV adenovirus, Chl *Chlamydia pneumoniae*, Leg *Legionella*, and Myc *Mycoplasma pneumoniae*

All multiplex PCR assays were conducted using the GeneAmp Fast PCR Master Mix (Applied Biosystems, US) in a 20-μL reaction. Two microlitres of the cDNA preparation were used as templates for the first round of PCR for groups 1 to 4, whereas 8 μL of the extracted preparation was used for group 5. An 0.2-μL aliquot of first-round PCR product was used as template for the second round.

The thermal cycling was carried with the Applied Biosystems Fast PCR machine (Applied Biosystems, US) with an initial denaturation at 95°C for 10 seconds, then 30 cycles of denaturation at 95°C for 1 second and annealing/extension at 64°C for 40 seconds, followed by a final extension at 72°C for 1 minute. The cycling conditions were the same for groups 1 to 4, whereas 35 cycles of denaturation at 95°C for 5 seconds and annealing/extension at 64°C for 40 seconds were used for group 5.

Organism identification

Results of PCR were analysed by electrophoresis using 1.5% agarose with ethidium bromide staining. The identity of amplification products was determined by their sizes (Fig).

Table. Multiplex polymerase chain reaction (PCR) assays compared with conventional isolation

Organism	No. (%) of positive specimen (n=303)		P value*
	Nested multiplex PCR	Conventional isolation	
Any infection	147 (48.5)	61 (20.1)	<0.001
Single infection	140 (46.2)	61 (20.1)	<0.001
Influenza A	19 (6.3)	15 (5.0)	0.480
Influenza A H1	17 (5.6)	NA†	
Influenza A H3	2 (0.7)	NA	
Influenza A H5	0	NA	
Influenza B	10 (3.3)	9 (3.0)	0.820
Parainfluenza virus type 1	19 (6.3)	14 (4.6)	0.371
Parainfluenza virus type 2	6 (2.0)	1 (0.3)	0.123
Parainfluenza virus type 3	3 (1.0)	2 (0.7)	1.0
Parainfluenza virus type 4	2 (0.7)	0	0.499
Respiratory syncytial virus	8 (2.7)	5 (1.7)	0.400
Respiratory syncytial virus group A	5 (1.7)	NA	
Respiratory syncytial virus group B	3 (1.0)	NA	
Rhinovirus	16 (5.3)	NA	
Enterovirus	3 (1.0)	0	0.249
Human coronavirus OC43	16 (5.3)	NA	
Human coronavirus 229E	3 (1.0)	NA	
SARS-CoV	0	0	1.0
Human metapneumovirus	15 (5.0)	NA	
Mycoplasma pneumoniae	5 (1.7)	NA	
Legionella	0	NA	
Chlamydia pneumoniae	0	NA	
Adenovirus	15 (5.0)	15 (5.0)	1.0
Coinfection	7 (2.3)	0	0.015
Influenza A and *Mycoplasma pneumoniae*	1 (0.3)	NA	
Influenza A H1 and *Chlamydia pneumoniae*	1 (0.3)	NA	
Influenza A H3 and human coronavirus 229E	1 (0.3)	NA	
Influenza A H3 and parainfluenza virus type 2	1 (0.3)	T2‡	
Human metapneumovirus and *Mycoplasma pneumoniae*	2 (0.7)	NA	
Human metapneumovirus and parainfluenza virus type 4	1 (0.3)	NA	

* By Chi squared test or Fisher's exact test as appropriate

† NA denotes organisms not isolated/differentiated by conventional isolation

‡ Parainfluenza virus type 2 isolated

Results

Sensitivity and specificity

The nested multiplex PCR assays were found to be 100- to 1000-fold more sensitive than conventional tube culture. The detection limit of group 5 multiplex PCR for *Legionella* and *M pneumoniae* were $2.3x10^3$ and $6.7x10^4$ colony forming units/mL, respectively. The evaluation using 50 clinical specimens containing known organisms did not reveal any cross-amplification.

Field specimen evaluation

Study subjects and specimens

A total of 303 nasopharyngeal aspirate specimens were collected, with 235 from paediatric patients aged 1 month to 5 years (mean, 2 years). The other 68 were from elderly patients aged from 65 to 107 years old (mean, 65 years).

Performance of multiplex polymerase chain reaction versus isolation

Of the 303 specimens, 61 (20%) were positive by conventional virus isolation with 15 influenza A, 15 adenovirus, 14 parainfluenza virus type 1, 9 influenza B, 5 respiratory syncytial virus, 2 parainfluenza virus type 3, and 1 parainfluenza virus type 2. No coinfection was found by conventional virus isolation. All these 61 isolation-positive specimens were also found to be positive by multiplex PCR, and with the corresponding detected viruses (Table). Altogether, 147 specimens were positive by the nested multiplex PCR. The positivity rate was significantly higher than that of conventional isolation (48.5% vs 20.1%, P<0.001; Table). Of the 140 single infections detected by multiplex PCR, 55 were not detected by conventional isolation. Of the seven (2.3%) coinfections revealed by multiplex PCR, six were negative by conventional virus isolation. As for the cultivable organisms, no statistically significant difference in positivity rates between multiplex PCR and conventional isolation was observed (Table). Although a clear agarose gel electrophoresis result was obtained for all positive specimens, for the purpose of this study, all multiplex PCR positive results were confirmed by separate PCR testing using alternative primer pairs.

Discussion

While molecular techniques provide superior analytical sensitivity to conventional isolation, this gain may not be reflected in clinical sensitivity. In settings where clinical specimens are collected and maintained in good quality, the amount of virus present may well be enough for detection by 'less sensitive' conventional methods. Our data are in line with this. Despite there being a higher sensitivity for multiplex PCR, the difference was not statistically significant when the cultivable viruses were compared.

Our finding that for multiplex PCR the overall positivity rate was double that of conventional isolation, was due to the broad spectrum of detection offered by the former. Previous studies targeting as many as nine different respiratory pathogens have been reported.[1] The current study included 17 respiratory pathogens and provided the widest spectrum ever reported. The gain in positivity rate was mainly attributable to the inclusion of rhinovirus, human coronavirus OC43, and human metapneumovirus. All these viruses are not detected by conventional isolation. The improvement in diagnostic yield by adding rhinovirus has also been reported by Gruteke et al.[2] Given that these 'trivial' respiratory viruses can cause severe illnesses, they should be included in multiplex assays. With the rapid PCR system established, the entire testing process can be completed on the same day. Such rapid turn-around is critical in the investigation of urgent outbreaks, and according to some studies, also has the potential to decrease overall hospital costs.[3,4] In our group 1 multiplex PCR, we incorporated specific primers for influenza A subtypes H1, H3, and H5, so that rapid differentiation between H5 and non-H5 influenza can be achieved. The assay also included a consensus of primers for influenza A, which could allow the detection of non-H1/H3/H5 subtypes that occasionally cause human infections.

In conclusion, the multiplex PCR assays developed in this study improved the diagnostic yield in terms of sensitivity and spectrum of coverage for respiratory infections. The assay has a rapid turn-around time, with results becoming available in one day. Overall cost reduction may justify routine use of these broad-cover, rapid, molecular diagnostic assays.

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Investigation of the role of dogs as reservoirs of *Staphylococcus aureus* and the transmission of strains between pet owners and their dogs

Key Messages

1. Dogs may serve as a reservoir for *Staphylococcus aureus* and could be a source of infections due to this organism in humans.
2. Health care workers (HCWs) seem to be a major source of *S aureus* for colonisation of dogs, with both methicillin-susceptible and methicillin-resistant strains.
3. De-colonisation of dogs owned by methicillin-resistant *S aureus*–colonised HCWs should be carried out at the same time as decolonisation of respective HCWs and other family contacts.

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Introduction

Staphylococcus aureus is carried by approximately 25% of humans in their nasal cavities, which is a major reservoir of this pathogen. Carriage of *S aureus* is associated with certain genetic and environmental factors.[1] Resistance to antibiotics has increased the consequences of *S aureus* infections, particularly those due to methicillin-resistant *S aureus* (MRSA), an important cause of nosocomial infections.[2] Methicillin resistance is coded for by the *mecA* gene.[2] Until recently MRSA was largely confined to hospital and health care settings.[2] However, it has now been found in the community and has caused severe infections in healthy children and adults.[2] Unlike hospital-acquired MRSA (HA-MRSA), which is multi-resistant, community-acquired (CA) MRSA is usually resistant only to beta-lactams, and sometimes, erythromycin. Whereas exposure to health care and health care workers (HCWs) has been recognised as a risk factor for HA-MRSA colonisation,[2] more work is needed to identify reservoirs of CA-MRSA.

Case reports of human infection or colonisation from companion animals indicate that animals act as reservoirs for MRSA transmission.[3,4] Limited studies suggest carriage of *S aureus* occurs in less than 10% of dogs.[3] Concern about MRSA in the community has led to recommendations for surveillance of carriage levels in healthy dogs.[2,4]

No studies of MRSA carriage in companion animals have been performed in Hong Kong. This study therefore aimed to determine the level of *S aureus* colonisation in dogs and their owners, the antibiotic resistance patterns of isolates and whether the strains in dogs were the same as in their owners. Risk factors for colonisation of the respective parties were also examined, including the extent of contact between them. As levels of MRSA carriage in the community remain low,[5] levels of colonisation of companion animals and their owners with both methicillin-sensitive *S aureus* and MRSA were determined to explore the frequency of possible transmission.

Methods

This study was conducted from January 2005 to January 2006.

Study design

A cross-sectional study of colonisation with *S aureus* of dogs kept as companion animals and their owners was performed. A convenience sample of pet owners and dogs was recruited at six veterinary practices; ill dogs were excluded. Owners were provided an information sheet about the study and asked to sign a consent form.

Laboratory investigation

Specimens were collected using a sterile swab from the nostrils of human subjects. The nares of dogs were sampled by veterinarians using a small swab due to the size and sensitivity of their nostrils. The swabs were placed in transport

medium and transferred to the laboratory within 8 hours of collection. Owners completed a simple questionnaire providing relevant information about their contact with the dog and antibiotic(s) taken by the animal within the last 3 months. Cultures were carried out to isolate and identify *S aureus,* using a commercial kit. Susceptibility to methicillin was investigated by culture on screening agar. All strains were subjected to disc sensitivity testing for susceptibility to several antibiotics. Methicillin resistance was confirmed by DNA amplification of *mec*A on strains appearing resistant to oxacillin. To determine if the isolates from owner and dog were of the same strain, all paired isolates were typed by pulsed-field gel electrophoresis (PFGE). The relatedness of strains was determined by comparison of DNA band fragments. The study was approved by the respective Human and the Animal Subjects Ethics Committees of the Hong Kong Polytechnic University. The respective prevalence rates of colonisation were calculated and significant associations between categorical variables determined and rates of antibiotic resistance compared. Odds ratios were derived and significance testing for nasal carriage in humans and their dogs carried out by logistic regression.

Results

A total of 736 owners and 830 dogs were sampled for *S aureus* carriage. Some owners did not answer all questions in the questionnaire. *Staphylococcus aureus* was isolated from 174 (24%) of the humans and 73 (9%) of the dogs; in 17 pairs both parties were colonised (10% of the colonised humans). Approximately 89% of isolates were resistant to at least one antibiotic. *Staphylococcus aureus* strains from dogs tended to be more resistant than human isolates, with significantly higher resistance rates to several antibiotics including oxacillin. Antibiotic resistance patterns were similar in 11 pairs, suggesting both carried the same strain. These paired isolates were further investigated for relatedness using PFGE; four appeared identical.

The *mec*A gene was detected in four strains from humans and six from dogs, confirming the isolates as MRSA; one was from a dog-human pair. Carriage in humans was associated with an occupation related to health care (42% as opposed to 25% in non-HCWs) and either a cat or a bird in the household. All aspects of contact with their dogs were not associated with an increase in colonisation of the owners; colonisation was significantly more frequent in female (12%) than male dogs (6%), and in adults than puppies (<12 months old). The dog's size and antibiotic intake, and sex of the owner were not associated with carriage. Dogs of older owners were rarely colonised. Dogs in households with three or less persons were more likely to be colonised than in those with more occupants. Households with one to three dogs were less likely to have a colonised dog than those with more dogs. Colonisation of the owner was not associated with colonisation of the dog, but the dogs of HCWs were more likely to be colonised than those of others. Contact with the dog, including petting, carrying, and kissing or licking the face, did not increase the risk of colonisation. Sleeping in the bedroom was associated with increased colonisation of the dog, though this did not reach statistical significance. In small dogs, colonisation of the owner appeared to be a risk factor for colonisation of the dog, and access to the bedroom was more strongly associated with colonisation of the dog (Table). There was a trend for carriage associated with the age of the dog; there being more colonisation in older dogs (10%) compared to puppies (5%) and younger dogs (8%) [P=0.03].

Of the 17 colonised pairs, five owners were HCWs (P=0.001). Overall, 11% of HCWs were colonised along with their dogs, in comparison with only 2.3% of other professionals, 0.6% of clerical workers, 2% of artisans and 1.8% of students and housewives. Recent use of antibiotics in the dog reduced the chance of paired colonisation of both the owner and the dog (P=0.022).

One of the four MRSA-colonised owners was a HCW, as were the owners of two of the six MRSA-colonised dogs. Among MRSA-colonised dogs, five were female, and five were aged older than 4 years. Overall, 2.2% of human *S aureus* isolates were MRSA, representing a 0.5% colonisation rate in the community. In dogs, 8.2% of the isolates were MRSA, yielding a 0.7% overall carriage rate.

Discussion

This is the first study to investigate carriage of *S aureus* in dogs and their owners. The carriage rate in owners was similar to that described previously[1] and the colonisation rate in dogs was 8.8%, similar rates were also reported in limited studies of *S aureus* carriage in dogs.[3] The number of simultaneously colonised dogs and owners was low, with only 10% of colonised owners having a colonised dog. Clearly, many non-colonised owners had colonised dogs. The source of the organism may have been another household member, or a previous owner.

Antibiotic resistance was quite common, with almost 90% resistance rates to penicillin. For several antibiotics, resistance was significantly more likely in dogs than in humans, reflecting higher use of antibiotics in veterinary practice.[4] Resistance to two or more antimicrobials was detected in 54% of dog isolates and 44% of those from humans. A Canadian study of *S aureus* isolates from dogs reported that 67% were resistant to two or more antimicrobials.[3] High levels of tetracycline and fusidic acid resistance were noted in isolates from dogs. Fusidic acid is frequently used for skin and eye infections in dogs, and tetracyline for respiratory infections. Most antibiotic therapy in companion animals is empirical and pressure from owners may increase such usage.[4]

Previous studies have reported isolation of MRSA from infected dogs and case studies have shown that dogs

Table. Risk factors for carriage of *Staphylococcus aureus*

Variable	*S aureus* (%)		Odds ratio	Confidence interval	P value
	+ve	-ve			
Colonisation of owners					
Occupation					
Health care	19 (42)	26 (58)	2.2	1.19-4.09	0.001
Non–health care	151 (25)	455 (75)			
Other animals in the house					
None	135 (23)	441 (77)			0.001
Cat	20 (36)	36 (64)			
Bird	16 (48)	17 (52)			
Other	3 (13)	20 (87)			
Carrying dog					
Yes	161 (26)	468 (74)	1.470	0.744-2.03	0.265
Never	11 (19)	47 (81)			
Kiss dog					
Usually	48 (29)	120 (71)			0.408
Often	24 (21)	93 (79)			
Sometimes	78 (26)	225 (74)			
Never	22 (22)	77 (78)			
Colonisation of dogs					
Owner's occupation					
Health care	9 (20)	36 (80)	3.294	1.494-7.265	0.002
Non–health care	45 (7)	593 (93)			
No. in household					
1-3	43 (11)	365 (89)	1.475	1.005-2.165	0.027
>3	19 (6)	301 (94)			
No. of dogs in household					
1-3	53 (8)	619 (92)	0.496	0.256-0.962	0.04
>4	9 (16)	48 (84)			
Sex of dog					
Male	24 (6)	378 (94)	0.688	0.549-0.863	0.005
Female	36 (12)	266 (88)			
Dog has access to bedroom					
Yes	54 (9)	519 (91)	1.830	0.898-3.733	0.076
No	7 (5)	138 (95)			
Carrying dog					
Yes	58 (9)	605 (91)	1.294	0.453-3.701	0.630
No	4 (7)	54 (93)			
Kiss dog					
Usually	17 (10)	161 (90)			0.540
Often	13 (11)	107 (89)			
Sometimes	22 (7)	297 (93)			
Never	9 (9)	95 (91)			
Colonisation of small dogs					
No. dogs in household					
1-3	30 (8)	337 (92)	0.695	0.260-1.856	0.472
>4	4 (12)	30 (88)			
Dog has access to bedroom					
Yes	33 (10)	291 (90)	7.041	1.011-49.06	0.012
No	1 (1)	76 (99)			

belonging to infected patients can be colonised with MRSA, and even healthy dogs belonging to healthy non-colonised veterinary clinic staff can be colonised.[3]

Increased risk of colonisation of HCWs has been documented previously.[1] Working in clinical settings increases the risk of simple *S aureus* (including MRSA) colonisation. Interestingly, the presence of a cat or a bird also increased the risk of *S aureus* colonisation in humans; cleaning animal excreta in litter trays and cages may increase respiratory exposure to *S aureus* from animal faeces. The presence of multiple dogs in the household did not increase the likelihood of carriage in owners, nor did increased numbers of persons per household.

Close contact with companion animals is assumed to increase the likelihood of cross-infection, and people are advised to avoid animals licking their faces. Despite this, owners who admitted frequent close contact with their animals, including kissing the dog and allowing it to lick their face or sleep on their bed, were at no higher risk of colonisation with *S aureus* than those who did not.

Higher colonisation rates with *S aureus* in female dogs have been previously reported, possibly due to hormonal factors or behavioural differences between genders. Higher colonisation rates in households with multiple dogs may also be a result of different behaviours when other dogs are present, and the possibility of reduced hygiene standards. However, this did not extend to households with large numbers of small dogs; small pedigree dogs may be groomed more often, reducing the numbers of skin organisms.

The effect of age may be due to changes in the dog, but could reflect increased time of exposure. Colonisation in the dog was only associated with colonisation of the owner in the sub-set of small dogs, conceivably due to more frequent close contact, though kissing and petting were not significantly associated with carriage. Moreover, several small breeds (eg Pekinese and Shih Tzus) have brachycephaly and are more prone to nasal problems and inflammation, predisposing them to colonisation. However, the dogs of HCWs were at much higher risk of colonisation, regardless of size. Colonisation was noted in dogs of both currently colonised and non-colonised HCWs. Possibly HCWs carry the organism on their skin and clothing to which their dog is exposed, thus facilitating colonisation by dogs via transient skin carriage or even by non-carriers.

Access to the bedroom also increased risk of colonisation. Dressing, undressing, and bed-making sheds contaminated skin scales picked up by the dog. Colonised dogs of non-colonised owners may have acquired the *S aureus* from another household member, or may be persistently carrying a strain from a previous owner.

There were surprisingly few pairs of colonised owners and dogs. Although transient carriage may be the reason, it indicates transmission between owners and dogs may well be low. An occupation related to health care seemed to be the most important risk factor. Interestingly, use of antibiotics in the dog reduced the risk of colonisation; conceivably, antibiotic treatment for infection at another site eradicated the colonising strain. However, investigation of colonised 'pairs' showed that some carried differing strains, as only 11 pairs had similar antibiograms. Analysis of PFGE revealed that even if the antibiograms were identical, the pair of isolates were unrelated. In one case, both owner and dog were colonised with MRSA; PFGE indicated only four of the pairs were carrying identical strains, although 90% of owners claimed to be the person who had the most contact with the dog. Thus, though transfer between owner and dog, or vice versa, does occur, it may be more unusual than indicated by case reports. Although strains found in the dogs may have originated from other family members, other sources, in particular veterinary practices, may be involved in such transfers.[4]

Overall MRSA carriage rate was low, but notably one of the four MRSA-colonised humans was a HCW, whilst two of the six colonised dogs were owned by HCWs, emphasising the role of the latter in MRSA in the community. The transmission of MRSA to close contacts of HCWs has been previously documented,[2] implicating the dogs of HCWs as reservoirs. This study also confirmed that the dogs of HCWs were more easily colonised with MRSA. Although there was no obvious explanation why most MRSA-colonised dogs were female, perhaps HCWs should be encouraged to select a male dog.

Dogs as reservoirs for *S aureus* (particularly MRSA), following increased levels of colonisation, has been a concern particularly as levels of MRSA continue to increase in the community, notably in the US and Australia.[2] Locally, though levels of MRSA in the community remain low[5]—vigilance is advised as Hong Kong has recently reported infections with CA-MRSA strains.[6] Whilst this study investigated the association between colonisation and close contact between companion animals and their owners in broad terms, its setting in Hong Kong (an urbanised, densely populated area) conveniently assessed such contact at an extreme level.

Conclusion

Colonisation of dogs is primarily associated with the owner's occupation, and dog ownership is unlikely to significantly increase the risk of infection in healthy subjects. Close contact with dogs was not associated with an increased risk of colonisation of either the owner or dog. However, companion animals may serve as a reservoir for infecting the immunocompromised. As dogs of HCWs are more likely to be colonised, consideration should be given to de-colonisation of corresponding dogs. The major route of transmission is from owner to dog, but a two-way process is possible. The actual patterns of transmission can only be confirmed by a larger longitudinal study.

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C Chao 趙汝恆

Transport phenomena of human exhaled droplets due to respiratory action in ventilated indoor environments

Key Messages

1. The transport dynamics of expiratory droplets was modelled and verified against experimental data.
2. Droplet size distributions in air were measured. Small droplets (initial size ≤45 μm) were largely airborne. Large droplets (initial size ≥87.5 μm) settled quickly.
3. The transport characteristics of expiratory droplets were highly influenced by ventilation airflow patterns. The location of the exhaust vent can play a significant role in controlling the dispersion pattern of expiratory droplets.
4. Deviations from the perfectly mixed condition were found. Further development of risk assessment models taking imperfect mixing conditions into account are needed.

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Introduction

The mechanisms of airborne transmission in indoor environments remain poorly understood. In some current nosocomial infection control guidelines, 'droplet' and 'airborne' are identified as the two major transmission modes of airborne diseases. According to the classification, pathogen carriers of those termed 'droplet' mode are >5 μm and potential exposure is limited to within 3 feet from the source. In contrast, pathogen carriers of the 'airborne' mode have droplet nuclei of <5 μm. However, some droplets of >5 μm in size have much shorter evaporation times than their settling times. This largely ignored fact makes the 5-μm cut-off for the classification of droplet and droplet nuclei questionable. Experimental evidence shows that human expiration produces polydispersed droplets, the majority being smaller than 100 μm and having short evaporation times.

The role of the ventilation system in the indoor transport of airborne pathogen carriers is another important issue. The dilution effect of the ventilation systems has been widely studied. However, inherently the assumption that there is perfect mixing assumption is hardly tenable in an indoor environment, due to little being known about different ventilation airflow patterns. One major difficulty in such research is the detection of expiratory droplets in air. Many such droplets change considerably in size due to evaporation before reaching the sensing elements of many sampling-based aerosol detection instruments. This results in measuring the dried residues, rather than the actual droplets in air. Apart from problems related to measuring the polydispersed size and evaporative features of expiratory droplets, there are challenges in terms of numerical modelling. Many studies have therefore turned to model gas phase contaminants as surrogates or to monodispersed dry particles.

The aim of this project was to quantitatively characterise the transport dynamics of airborne droplets in various ventilation systems. Its specific objectives were:

1. To study the effects of different air flow patterns created by various ventilation systems on the motion of expiratory droplets and their removal mechanisms.
2. To evaluate the effectiveness of floor-based ventilation systems and unidirectional ventilation systems in preventing the transmission of airborne occupant-exhaled aerosols compared to the traditional ceiling-based ventilation system.
3. To investigate the thermal conditions created by different ventilation systems and their impact on droplet aerosol transmission and thermal comfort.
4. To study the effects of parameters such as supply air conditions, heat loading, and aerosol injection conditions on aerosol transmission within ventilated spaces.

Methods

This study was conducted from March 2005 to August 2006. The project was divided into two major phases. The first focused on a simple clean room geometry. A multiphase numerical model was developed and tested against experimental measurements of droplet dispersion in a clean room chamber. To address objectives

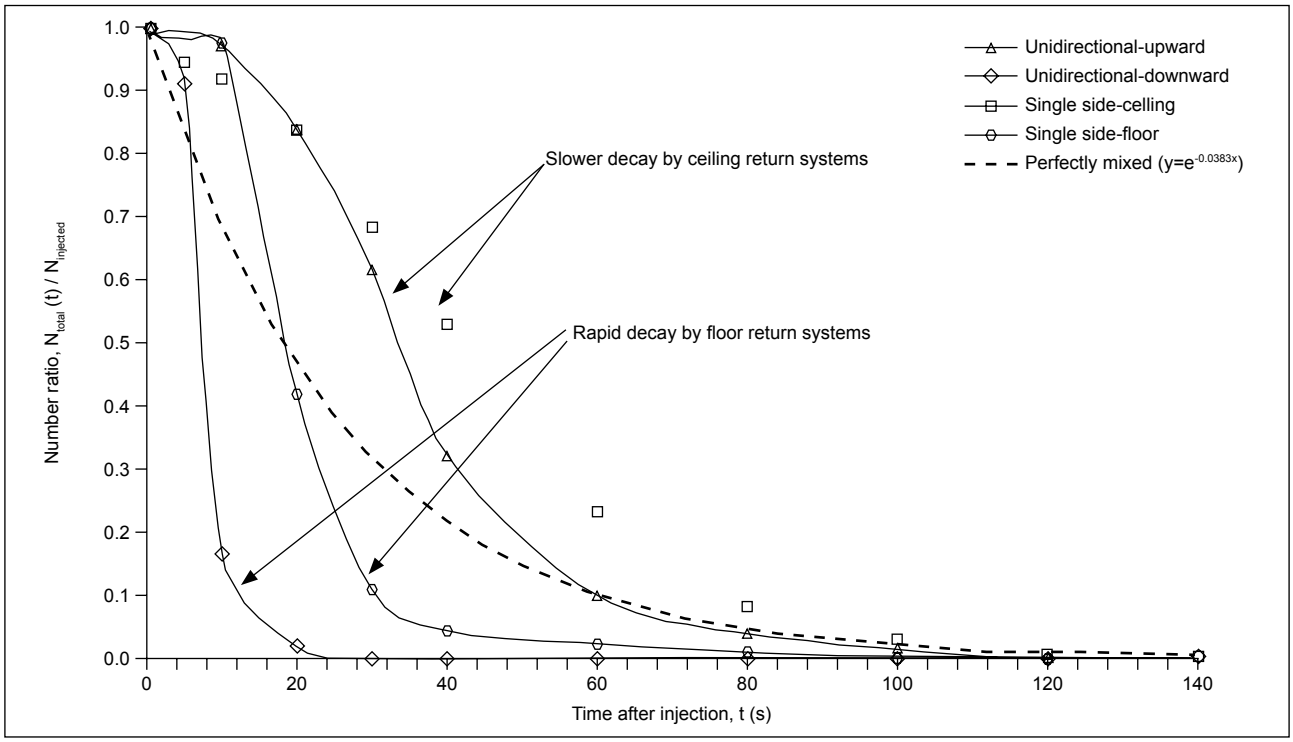

Fig 1. Number ratio decay profiles of different ventilation flow patterns

1 and 2, the model was then applied to different types of ventilation airflow patterns within the same room. The second phase focused on hospital ward geometry. Numerical simulations and measurements were conducted under both empty and occupied room conditions, using thermal manikins and other heat sources. Different droplet injection orientations were also studied (for objectives 3 and 4).

A Lagrangian-Eulerian numerical model was employed[1] to predict the motion of the expiratory droplets. This model considered the droplets and droplet nuclei as discrete individual particles and their motion was then described by force balance equations. Unlike the fully Eulerian-based (continuum phase) models, which were more commonly used previously, size change effect of each droplet due to evaporation as well as coagulation could also be modelled. The effects of turbulence from the carrier phase were modelled by adopting the stochastic approach.

Airflow and droplet dispersion measurements were performed in a class-100 clean room chamber with unidirectional downward airflow[1,2] and in a real hospital ward.[3] The simple clean room had internal dimensions of 4.8 × 4.8 × 2.6 m (W×L×H). The hospital ward had dimensions of 5.9 × 6.6 × 2.35 m (W×L×H) with a typical ceiling-based mixing type ventilation system. Flow patterns and turbulence parameters of the room air were captured by the particle image velocimetry (PIV) technique. For the measurement of droplet dispersion, a droplet generator was fabricated to produce droplets with non-volatile content. Polydispersed test droplets were injected to simulate human coughing. Source profile characterisation at the generator outlet and droplet dispersion measurements was performed using an optical interferometric Mie imaging (IMI) method, combined with an aerosol spectrometer. The experimental results were compared with the numerical results for verification of the model. The comparison showed that the multiphase numerical model was able to capture the size-specific dispersion characteristics of the expiratory droplets and droplet nuclei.

Results

The simple clean room geometry

The comparative efficiencies for removing expiratory droplets and droplet nuclei in the presence of the four ventilation systems are shown in Figure 1. The Unidirectional-downward system gave the best performance in terms of removing the droplets or droplet nuclei, followed by the Single side–floor system. The Unidirectional-upward system was ranked third and the Single side–ceiling system the worst. The four decay profiles were divided into two groups when compared with the 'perfectly mixed' condition. The two ventilation systems with floor exhaust vents had faster decay rates than the perfectly mixed curve, while the two ceiling exhaust systems had slower decay rates. By comparing the overall dispersion coefficients (Fig 2), two different lateral dispersion behaviours could be distinguished according to size. The small-size group (initial size ≤45 μm) had dispersion coefficients ranging from about 10 to 30 cm^2/s in the presence of purely turbulent dispersion in the Unidirectional systems. With the addition

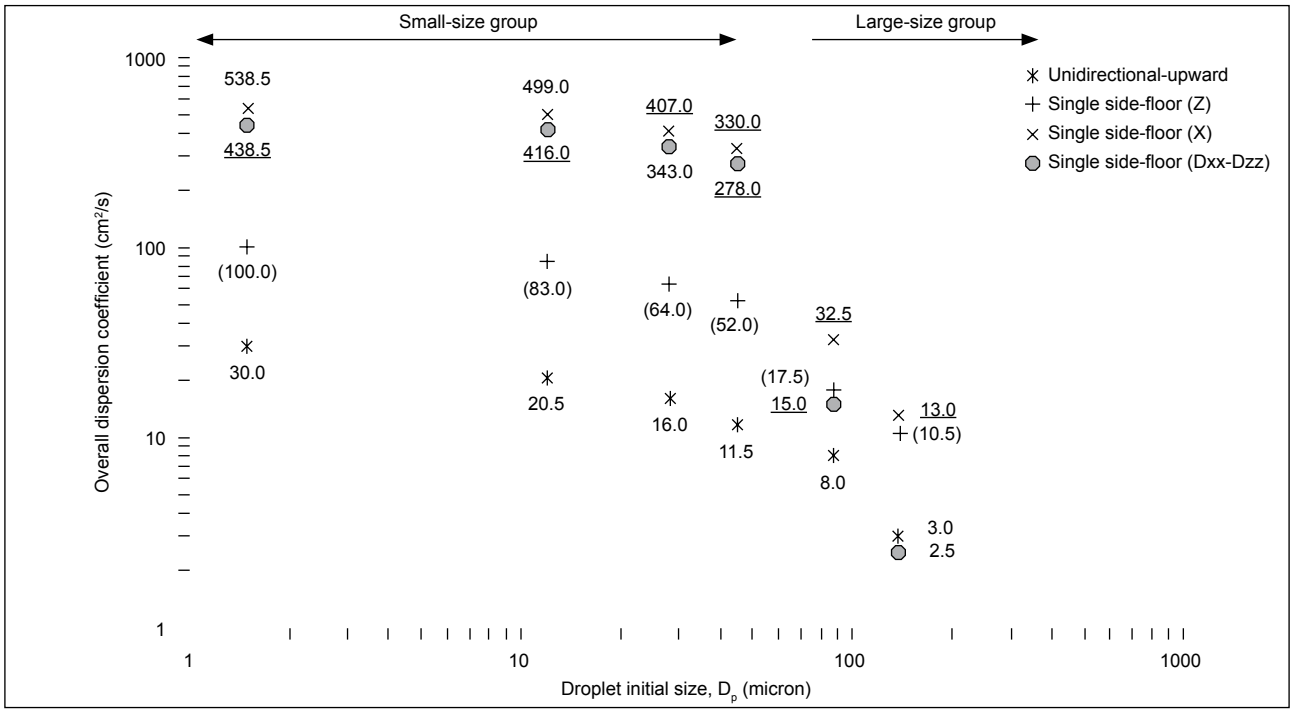

Fig 2. Overall dispersion in the two floor-supply flow patterns
The two ceiling supply systems had similar results

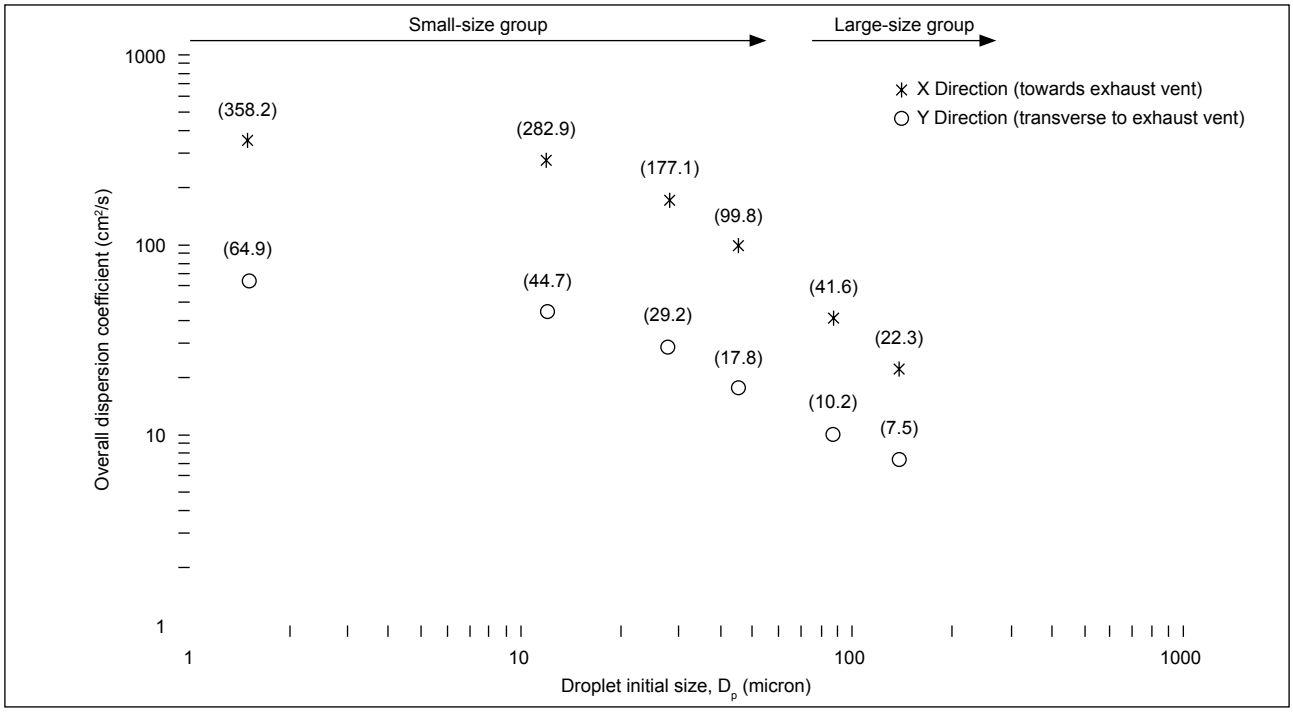

Fig 3. Overall dispersion coefficients of selected size bins in the hospital ward (empty room condition)

of bulk airflow transport in Single-side systems, the overall dispersion coefficients were increased to around 230 to 600 cm^2/s. In contrast, the overall dispersion coefficients of the large size group (initial size ≥87.5 μm) did not reveal such dramatic increases following the addition of bulk airflow transport. The heavy gravitational and inertial effects overwhelmed the dispersion mechanisms.

The hospital ward

Regarding hospital ward geometry, the increase in mean lateral dispersion distance was faster towards the exhaust vent than that in the converse direction. This is shown in Figure 3 in terms of overall dispersion coefficients. It suggests that the exhaust vent enhances bulk airflow in its direction as did the lateral dispersion of expiratory droplets,

and emphasises the significance of exhaust vent location in controlling the dispersion pattern of expiratory droplets.

When the droplets were injected vertically upward, the smaller ones (1.5 and 12 μm) remained at higher positions in the occupied condition compared to the empty room. This could be caused by the upward thermal plumes, induced by heat sources. Droplets of larger size exhibited vertical motion characteristics similar to those found in the empty room condition. This suggested that the influence of thermal plumes on the vertical motion of expiratory droplets decreased with increasing droplet size. The effect of thermal plumes on the lateral transport of expiratory droplets was not as significant as that on the vertical movement, as shown by lateral dispersion behaviour with the two injection conditions. In the lateral injection condition, the small size droplets stayed at a low level after injection, leading to higher chance of deposition. Droplets of larger size still exhibited gravity-dominated behaviour. Along the coughing direction, direct penetration of expiratory droplet into the breathing zone of the patient in the next bed was possible.

The extent of droplet exposure at patient breathing levels due to lateral injection orientation was higher than that under vertical injection orientation by three to four orders of magnitude. However, the exposure level at the breathing zone of workers was much lower than that in the breathing zone of patients and indicates direct penetration of expiratory droplets into the breathing zone of the patient in the next bed. Lateral dispersion of expiratory droplets was enhanced in the direction towards the exhaust vents. It can be observed by comparing the exposure levels between different bed arrangements in the vertical injection condition. The expiratory droplets (especially the smaller ones) generally stayed higher than the patients' breathing level. The upward thermal plumes could also enhance such effects. Simulations of a moving occupant showed that the air recirculation zone was likely to ensue behind the mover. Such air movement could enhance turbulence diffusivity, and enhance lateral dispersion of small expiratory droplets.

Discussion

The current project adopted a multiphase numerical model, which was able to capture the transport dynamics of polydispersed, evaporating expiratory droplets. A non sampling-based optical remote sensing method (the IMI method) was also adopted to measure the sizes of droplets and droplet nuclei in air. These methods therefore appear to be suitable tools for future research in droplet dispersion dynamics.

The transport characteristics of expiratory droplets are markedly affected by the ventilation airflow pattern. Small size droplets (initial size ≤45 μm) exhibited airborne transmission behaviour. Large size droplets (initial size ≤87.5 μm) did not stay in the air long enough for airborne transmission, due to the dominant influence of gravity. Ventilation systems with floor exhaust vents perform better in removing expiratory droplets. This implies that the settling of the expiratory droplet could enhance their removal through floor extraction vents. Bulk lateral airflow was found to be a much stronger lateral dispersion mechanism for expiratory droplets than turbulent dispersion. Unidirectional systems performed better in containing droplets or droplet nuclei from dispersing laterally, as opposed to the Single-side systems. The location of the exhaust vent can play a significant role in controlling the dispersion patterns of expiratory droplets. When expiration is directed laterally towards the next bed, the initial expiratory jet can penetrate directly into the breathing zone of the patient in that bed, causing very high levels of exposure. Different ventilation systems also revealed imperfect mixing distribution behaviour. Our results suggest that current infection control practices and policies should be reviewed. Issues of concern include: the 5-μm classification between 'airborne' and 'droplet' modes, the 3-feet recommended distance as a 'droplet' mode precaution, and risk assessment models incorporating imperfect mixing conditions.

Acknowledgements

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Assessment of deficiency of fish tank water ultraviolet disinfection and remedial measures

Key Messages

1. Better bacterial inactivation was observed with higher ultraviolet (UV) doses. There is a need for better tank configuration (hydraulic performance) to ensure proper water circulation or a need to suppress microbial recovery. Addition of titanium dioxide (TiO_2) during UV irradiation was effective in controlling microbial recovery.
2. Higher water temperature enhanced the fouling rate on quartz sleeves, revealing the need for more frequent cleaning of UV tubes to remove the fouling and enable higher UV transmittance for bacterial inactivation.
3. In a fish tank setup, better water circulation facilitates the UV disinfection system and minimises dead zones. This can be achieved by diagonal positioned inlets and the outlets, providing inlet baffles, and enabling better artificial mixing with oxygen diffusers.
4. UV system validation and maintenance practices need to be in place to secure the effectiveness.

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Introduction

Eliminating health risks associated with waterborne pathogen transmission through consumption of contaminated seafood has become an urgent issue. In particular there has been an explosion of several such infectious diseases including numerous incidences of *Vibrio cholerae*. Moreover, *V cholerae* and high concentrations of faecal coliform bacteria have been detected in wholesale and retail live seafood markets.[1,2] Since January 2004, the Hong Kong SAR Government has implemented a risk-based surveillance programme, which aims to educate relevant parties on preventive measures to improve the quality of fish tank water. In this programme, the normal frequency of testing for *Escherichia coli* at each premises remains at once every 8 weeks, while the actionable level was changed from 610 to 180 colony-forming units per 100 mL; any presence of pathogenic organisms also became actionable.[3] The Food and Environmental Hygiene Department licenses food premises and market stalls and requires that facilities used for keeping live marine or shell fish intended for human consumption should have properly installed water filtration and disinfection systems.[3]

To address this government policy on more stringent food safety standards, proper water disinfection systems must be installed in all fish tanks in wholesale and retail live seafood markets and restaurants. Various means of fish tank water disinfection include: ultraviolet (UV) disinfection, ozonation, copper-silver ionisation and photocatalytic disinfection (UV irradiation with titanium dioxide [TiO_2]). Due to their simplicity and lower costs, it is likely that UV disinfection achieved by inducing photobiological alteration of DNA (formation of lesions, typically *cis-syn* cyclobutane pyrimidine dimers, in the genomic DNA of organisms) will be preferred by most interested parties in Hong Kong. However, the success of UV disinfection is not necessarily guaranteed, as it relies on the system operating conditions; the presence of light scattering or absorbing reduces UV intensity reaching target pathogens. Fouling on the surface of quartz jackets and suspended particles in the water also reduce UV transmittance (UVT) and protect pathogens from irradiation. In addition, some organisms are able to repair UV-damaged DNA by photoreactivation and dark repair (or nucleotide excision repair). Furthermore, poor hydraulic conditions in fish tanks (mainly arranged for the convenience of operators) may engender many dead zones in the flow field, promoting pathogen reactivation and/or enabling escape from inactivation. Therefore, UV disinfection was selected as the target process for evaluation.

Aims and objectives

Factors causing defective UV disinfection of fish tank water were assessed by reference to both inactivation and reactivation of indicator organisms. Factors studied included: UV doses and sources, temperature, types of pathogens, modes of reactivation, presence of nutrients, seasonal fouling, seawater composition, and geometric and hydraulic configurations of fish tanks. We also evaluated remedial measures including the use of polychromatic UV lamps and using UV together with TiO_2. Our specific objectives were:

1. To establish the UV dose-response relationship for microbial inactivation and reactivation after exposure to conventional UV irradiation;

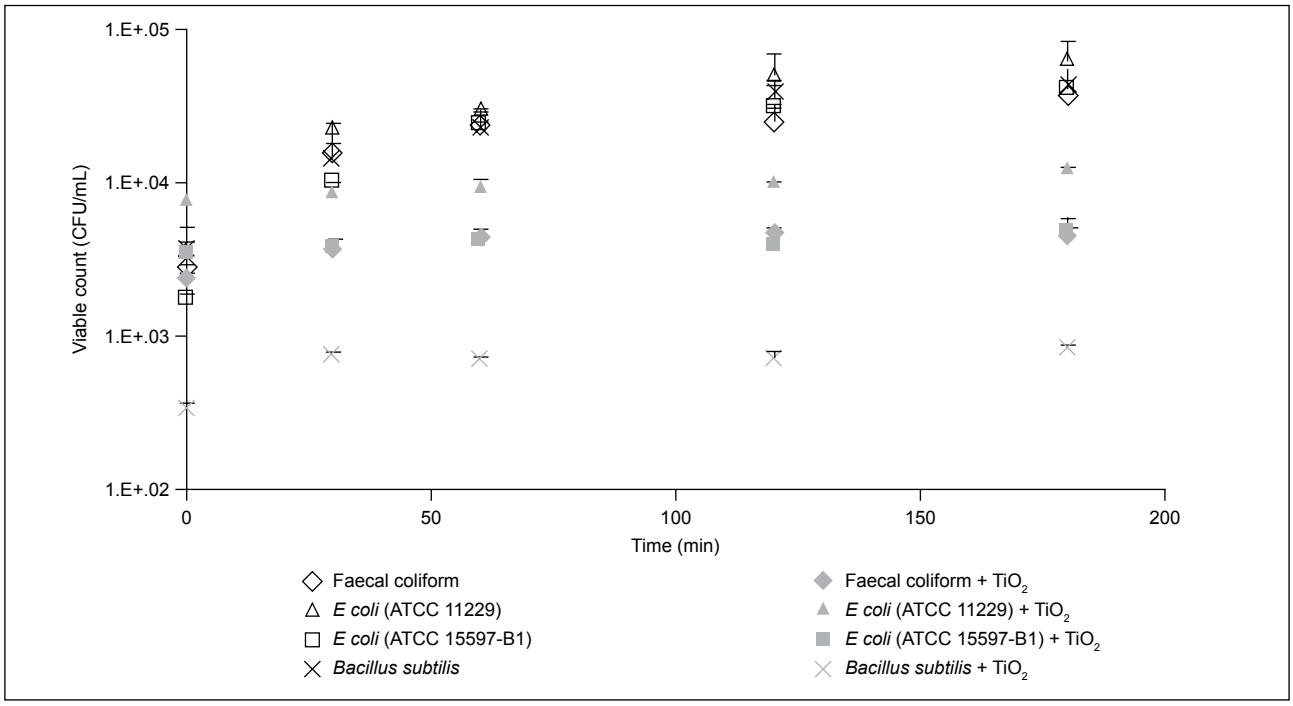

Fig 1. Time-dependent viable count of bacteria (colony-forming unit [CFU]/mL) after low-pressure (LP) UV irradiation alone (open symbols) and LP UV-plus-TiO_2 (in suspension) irradiation (filled symbols in grey) followed by allowing photoreactivation under fluorescence light up to 3 hours

TiO_2 denotes titanium dioxide and UV ultraviolet; UV doses were 12 mJ/cm^2, TiO_2 concentrations were 1 mg/L, if present, and initial bacteria concentrations were approximately 10^7 CFU/mL. Experimental conditions were 22ºC, pH 7, and in 3.5% phosphate-buffered saline. Data presented at time zero represent the bacteria counts right after the completion of UV or UV-TiO_2 exposure and the starting bacteria counts before the occurrence of photoreactivation

2. To study and characterise the hydraulic conditions in fish tanks arranged to simulate actual usage, to evaluate potential pathogen harvest zones and microbial reactivation;
3. To assess the reduction of UVT in seawater and the responsible causative constituents;
4. To investigate the fouling behaviour in UV lamp reactors, and characterise the chemical composition of fouling materials, to establish the cleansing frequency needed; and
5. To evaluate the enhancement of inactivation and prevention of reactivation by polychromatic UV irradiation or using UV radiation combined with TiO_2.

Methods

This study was conducted from January 2005 to December 2006.

Faecal coliform bacteria, *E coli* (ATCC 11229 and ATCC 15597), *Bacillus subtilis* (ATCC 6633) and coliphage MS-2 (ATCC 15597-B1) were used as the surrogates. Buffer solutions with different salt contents and artificial and/or natural seawater were used as the water matrices. Batch studies were conducted using collimated UV irradiators with variations in testing conditions to develop UV dose-response relationships for inactivation and reactivation of the surrogates. Both physical and mathematical models were assembled for hydraulic study of fish tanks. Two flow-through UV units were installed and fed with filtered natural seawater to study how seasonal variations in seawater quality affects fouling.

Results

The inactivation of bacteria in seawater by UV disinfection was rapid; a dose of 12 mJ/cm^2 can yield over 3-log inactivations but against coliphage MS-2 it was much slower (60 mJ/cm^2 was needed to yield 3-log inactivation). However, photoreactivation of bacteria yielded more than 1-log recovery within 1 hour of fluorescence light exposure under all experimental conditions (Fig 1). Dark repair also yielded more than 1-log recovery but required more than 3 hours after UV disinfection. Ultraviolet disinfection with polychromatic UV lamps reduced but did not arrest recovery in seawater. Additions of TiO_2 in suspension during UV disinfection repressed bacterial recovery under all conditions tested (Fig 1). The repressive effect was also achieved by providing TiO_2 in the attached phase during UV disinfection, regardless of nutrients being present (Fig 2).

The composition of seawater on UVT had a negligible effect, as more than 94% persisted if it was properly filtered. The fouling rate on the quartz sleeves of UV lamps changed with the seasons and depended on the temperature; higher temperatures lead to faster fouling. According to this study,

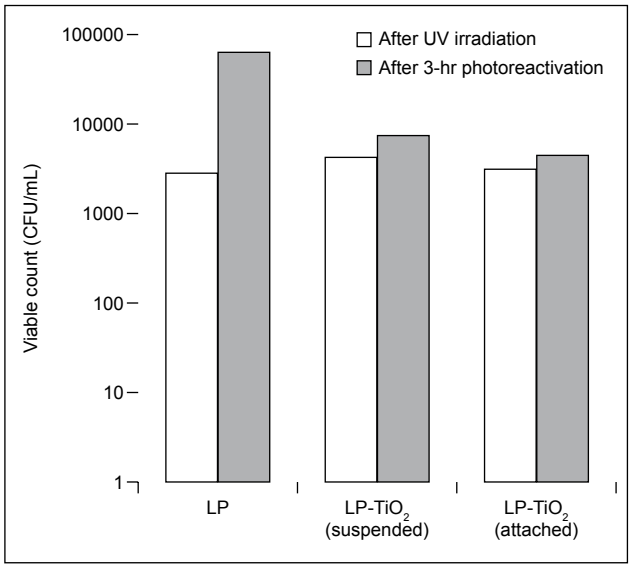

Fig 2. Effect of phases of titanium dioxide (TiO_2) in 3.5% phosphate-buffered saline on viable counts of local wastewater-isolated faecal coliform bacteria (CFU/mL) after low-pressure (LP) UV irradiation alone and LP UV-plus-TiO_2 irradiation (right after UV irradiation (open bars) and after photoreactivation under fluorescence light for 3 hours (filled bars)

UV dose=12 mJ/cm^2, TiO_2 concentration=1 mg/L (in suspension) and 1.44 mg/cm^2 (in attachment), if present

the cleansing frequency of at least once per month (as per guidelines) seemed insufficient[4]; twice a month seemed more appropriate. The fouling materials mainly consisted of precipitates of metal ions, corresponding to the major ions present in seawater and from corrosion.

Poorly designed fish tanks allow channelling effects to occur in the circulated water, leaving most of the water uncirculated and untreated. To reduce the channelling effect shown in Figure 3, positioning of the inlet and outlet was as important as the dimensions of tanks. Fish tanks should be designed with a longer distance for water to travel so as to enhance dispersion. Better mixing can also be accomplished by inlet baffles and/or strategically locating oxygen diffusers in the dead zones. However, if TiO_2 is not used, the total time for water to traverse tanks in series should be less than 30 minutes, in order to prevent bacterial recovery.

Discussion

An improved understanding of the science involved provides guidelines for designing and operating UV disinfection systems for fish tank water. The developed TiO_2-UV disinfection process provides extra security for controlling the transmission of waterborne diseases through fish consumption. However, since the concept of the modified TiO_2-UV disinfection system achieved suppression of microbial recovery only in the batch setup, a pilot study of this system is needed prior to its widespread application. Fouling and deactivation of the TiO_2 film should also be addressed. Another limitation of this project pertained to the evaluation of adsorption and desorption of pathogens on and from fishes. This topic was beyond the scope of this project but deserves further study.

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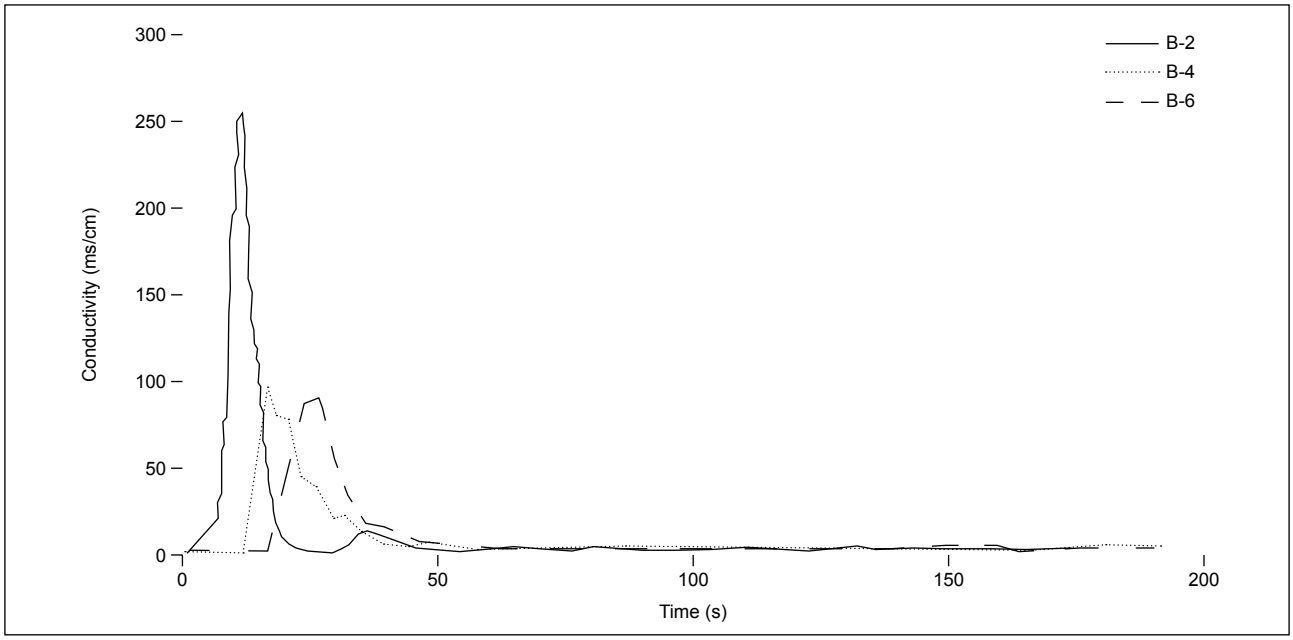

Fig 3. Time-dependent tracer concentrations in outlet position with combination of Inlet B and Outlets 2, 4, and 6

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Survey and evaluation of modified oxygen delivery devices used for suspected severe acute respiratory syndrome and other high-risk patients in Hong Kong

Key Messages

1. Covering facemasks or nasal cannulae with surgical facemasks generally improved percentage of inspired oxygen (FiO_2) but at a risk of increased CO_2 rebreathing when a low oxygen flow rate was used.
2. We recommend that at least 5 L/min of oxygen be used for nasal cannula and at least 6 L/min of oxygen be used for facemask when these devices are covered with a surgical facemask.
3. Using a combination of several oxygen delivery devices is unlikely to increase the FiO_2 significantly.
4. All the modifications described did not introduce any significant increase in airway resistance.

Introduction

The severe acute respiratory syndrome (SARS) outbreak at the Prince of Wales Hospital was attributed to an index patient using a nebuliser with an open oxygen facemask, while a later outbreak at another hospital implicated the use of high oxygen flow rates with an open type of oxygen facemask. Although controversial, oxygen delivery devices (ODDs) are thought to facilitate the spread of infective organisms into the environment from air expired by infected patients.[1,2] However, the ability to improve oxygenation is one of the most important factors in determining patient well-being during respiratory infections. Consequently, various modifications to ODDs were introduced in order to supplement oxygen delivery whilst also minimising the risk of cross infection.

Some modifications included using a nasal cannula covered with a surgical mask or N95 facemask, while others involved simultaneously using a nasal cannula and an oxygen mask, covered with a surgical mask or N95 facemask. Because of these rather haphazard modifications to ODDs, physicians were unclear as to the amount of oxygen being provided to the patient. It was during the SARS epidemic that these modifications were hastily introduced, but their performance had never been investigated and described.

The aims of this study were to (1) summarise the modifications made to ODDs used in Hong Kong public hospitals during the SARS epidemic, and (2) evaluate the performance of each modification using a high-fidelity human patient simulator.[3]

Methods

This study was conducted from March 2005 to September 2005.

Survey

Approval from the Survey and Behavioral Research Ethics Committee of The Chinese University of Hong Kong was obtained before the study. The survey was conducted by a research associate and a medical student, using a format consisting of site visits and a direct face-to-face semi-structured interview, using both closed and open-ended questions.[4]

A manikin head together with various ODDs and masks were used for demonstration and digital pictures taken for the record. We surveyed intensive care units (ICUs), high dependency units, respiratory disease wards, and isolation units in all public hospitals taking acute admissions.

Evaluation of masks

All studies were performed on the simulator (Medical Education Technologies Inc [METI], Sarasota, Florida, US). After preliminary testing, we substituted the simulator manikin with an airway management trainer (Laerdal Medical Corp,

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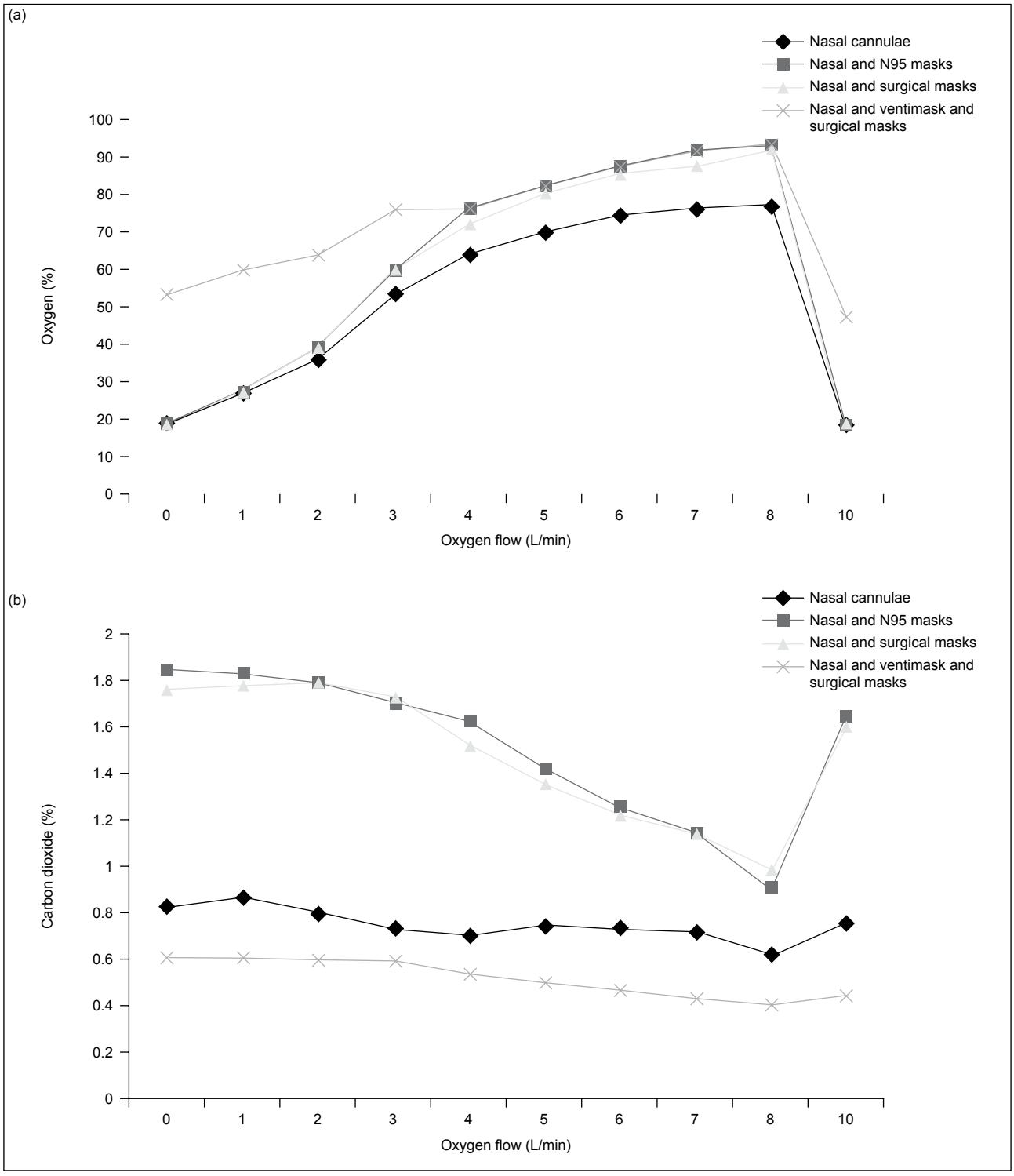

Fig 1. Nasal cannulae modifications
Plot of oxygen flow rate vs (a) inspiratory oxygen percentage and (b) carbon dioxide percentage

New York, US). Using the modified Bohr equation, the dead space ratio of this model was estimated to be 0.30 of the tidal volume, which agreed closely with the published geometric value of 0.33.[5] We connected a paediatric spirometer (AS3, Datex Ohmeda Corporation, Helsinki, Finland) probe to the lower trachea for real-time gas sampling.

After calibration using a portable spirometer (MedGraphics CPFS/D, St Paul, Minnesota, US), the simulator was set to breathe room air for 15 minutes as baseline. Then an ODD with the selected oxygen flow rate to be tested, was placed on the manikin. Data were recorded for 5 minutes for each flow setting. A washout period of at least 3 minutes was used between each setting. For each flow setting, the data collection process was repeated four times. Gas, spirometric and pressure measurements from

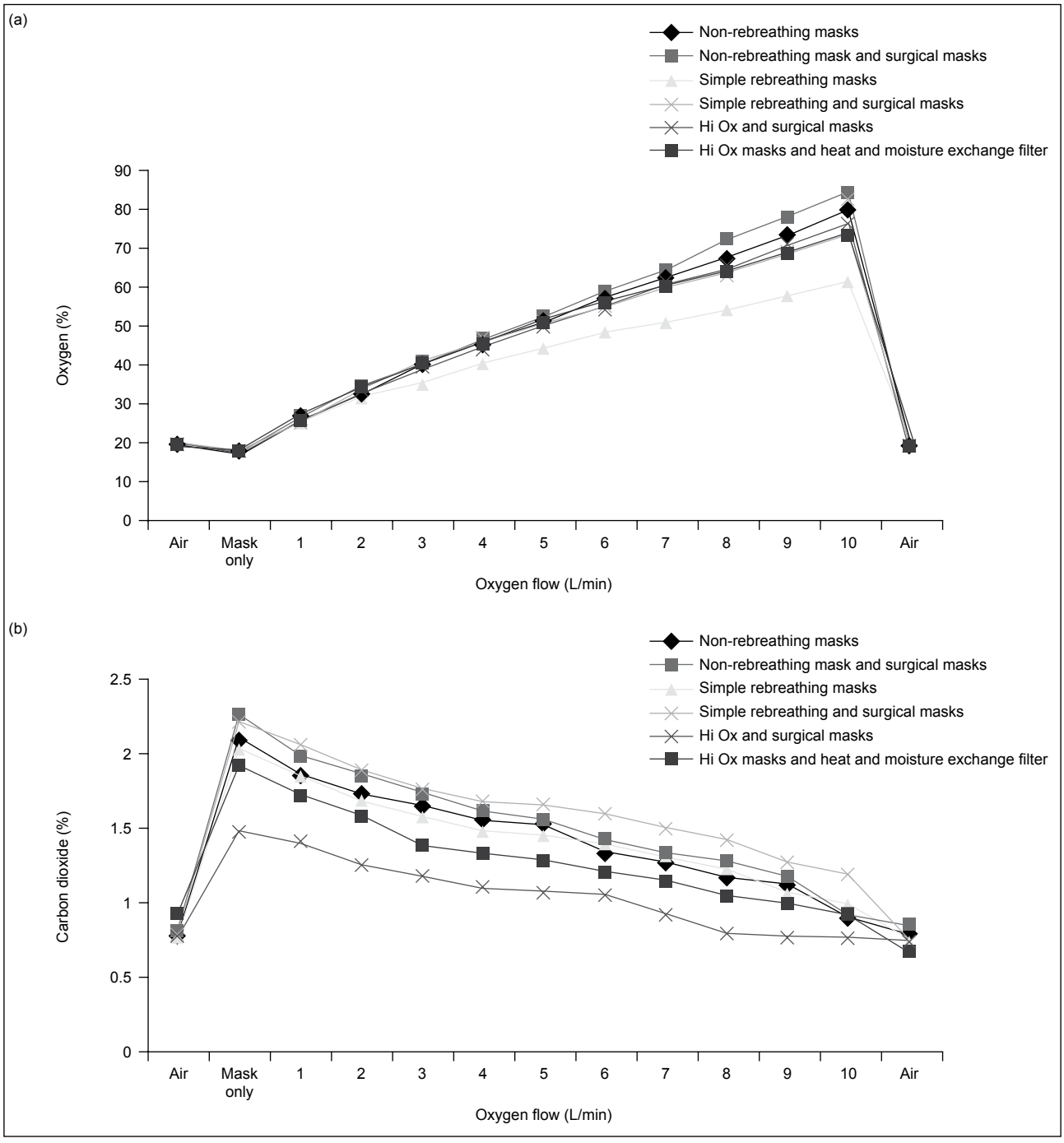

Fig 2. Oxygen facemask
Plot of oxygen flow rate vs (a) inspiratory oxygen percentage and (b) carbon dioxide percentage

the monitors were captured on a computer using an in-house program for analysis of the inspired oxygen percentage, the extent of carbon dioxide (CO_2) rebreathing, and the resistance to gas flow and work of breathing.

The breathing mode of the simulator was set to provide tidal volumes ranging from 320 to 600 mL, and respiratory rates ranging from 10 to 16 cycles per minute. The controlling computer of the simulator responded dynamically to CO_2 rebreathing, and automatically adjusted the minute volume by increasing the tidal volume and respiratory rate. For the purpose of this study, significant rebreathing of CO_2 was defined as present when the percentage of inspiratory CO_2 exceeded 1.5%, and triggered an automatic 10% increase in the minute ventilation of the simulator.[6]

Data were summarised by simple descriptive statistics as numbers and percentages or means and standard deviations, and displayed as plots of oxygen flow against the inspired oxygen and CO_2 percentages. Comparisons of the gas parameters were made using analysis of variance for repeated measures, and a correlation analysis was performed

Table. Minimum oxygen flow for modified oxygen delivery devices to prevent carbon dioxide (CO_2) rebreathing and percentage of oxygen (O_2) provided at that flow rate

O_2 device	Barrier or filter	Minimum O_2 flow required to prevent CO_2 rebreathing (L/min)	O_2 % at minimum flow Median (range)
Nasal cannulae	Surgical mask	5	80 (68-83)
	N95 mask	5	85 (69-87)
Simple rebreathing facemask	Surgical mask	6	48 (46-61)
Non-rebreathing facemask	Surgical mask	8	72 (65-79)
Hi Ox device	Heat and moisture exchange filter	4	46 (43-53)
	Surgical mask	3	37 (32-46)

to investigate the relationships between the ventilatory and other relevant respiratory parameters. Statistical analysis was made using the Statistical Package for the Social Sciences (Windows version 11.0; SPSS Inc, Chicago [Illinois], US), with the level of significance set at $P<0.05$.

Results

Survey

We visited 12 hospitals throughout Hong Kong and collected data from 29 wards, 22 intensive care and high-dependency units, by directly interviewing medical or nursing staff in each unit.

General wards

There were marked variations in modifications to the ODDs in different hospitals, as well as between individual wards within the same hospital. All the modifications described are listed in Figures 1 and 2, and were made with the objective of (a) preventing expired infected droplets from reaching the surroundings and (b) increasing the percentage of inspired oxygen (FiO_2).

For the purpose of containment of infected droplets and secretions, most of these facilities used surgical facemasks rather than N95 facemasks, as availability was an issue. The two most common modifications were the use of nasal cannulae covered with a surgical or N95 facemask.

Intensive care units

Most units used a nasal cannula covered with a surgical facemask or N95 mask, but two ICUs described using biphasic positive airway pressure (BiPAP), facemasks together with a heat and moisture exchange filter.

Evaluation of masks

We were able to test all the mask modifications described except for the BiPAP masks, which required a dedicated non-invasive ventilator. Overall, 11 types of modifications to ODDs, involving nasal cannula, rebreathing masks, non-rebreathing masks, ventimasks, and Hi Ox devices were tested. The corresponding results are illustrated in Figures 1 and 2 and summarised in the Table.

Except for the ventimask, CO_2 rebreathing was greater in all ODDs covered with a surgical mask. This was particularly significant when a low fresh oxygen flow rate was used with nasal cannulae, and covered with a surgical or N95 mask. Modifications using a surgical facemask or N95 facemask required a minimum flow of 6 L/min of fresh oxygen to prevent CO_2 rebreathing. Covering the ventimask with a surgical mask did not change the FiO_2 or the extent of CO_2 rebreathing. Using the Hi Ox device and nasal cannulae together did not result in any further increase in FiO_2 compared to using the individual device alone.

Variation of the breathing patterns by alterations of the I:E ratio or minute volume within the range of our study, did not alter the deadspace ratio, and the extent of CO_2 rebreathing was similar.

Discussion

In this study we evaluated the modified ODDs used in the public hospitals of Hong Kong during the SARS epidemic. Most modifications consisted of covering the ODD with a simple surgical or N95 mask. Whereas this action did not significantly change the performance of any oxygen facemask, covering nasal cannulae with a surgical mask introduced a deadspace, which markedly changed their characteristics. Modified in this manner, nasal cannulae are effectively converted into an oxygen facemask. Within the deadspace under the surgical mask, oxygen is trapped and enhances the FiO_2. However, it also traps expired air, resulting in rebreathing of CO_2 when there is a low oxygen flow rate (1-4 L/min). To prevent rebreathing and accumulation of CO_2 requires a minimum oxygen flow rate of 5 L/min for nasal cannulae, and 6 L/min for simple facemasks.

Since most patients with SARS required a very high FiO_2 to maintain blood oxygenation, the issue of CO_2 rebreathing was not a problem. However, patients with other respiratory diseases, such as during postoperative states, only a low oxygen flow rate is needed to maintain oxygenation. Thus, some of the latter patients may have been inadvertently subjected to rebreathing of CO_2 when using modified ODDs and low oxygen flow rates.

For patients with acute exacerbations of chronic obstructive pulmonary disease, controlled oxygen therapy using a ventimask preserves the patient's hypoxic drive and prevents hypercapnia. However, the use of a high gas flow rate with a ventimask was discouraged (or banned) in some

wards, as it was considered to facilitate spread of infected respiratory droplets. It was difficult to provide a low FiO_2 using a facemask (rather than a ventimask) without inducing CO_2 rebreathing and hypercapnia. Using nasal cannulae and a low oxygen flow rate of 1 L/min was an alternative. However, if covered with a surgical mask, this would lead to significant CO_2 rebreathing, and hypoventilation, thus aggravating the hypercapnia.

Later during the course of the SARS epidemic, newer ODDs were introduced such as the Hi Ox device. This device has a complicated series of valves, which channel expired air away to prevent rebreathing and can provide a high FiO_2 using a relatively low oxygen flow rate. Covering a Hi Ox facemask with a surgical mask did not significantly change breathing characteristics.

When nasal cannulae are used in combination with a ventimask (set at an FiO_2 of 0.5 L/min) and covered with a surgical mask, the FiO_2 increased and CO_2 rebreathing was virtually eliminated. However, with this combination, the net flow of gas supplied to the patient would be about 50 L/min, thus greatly increasing the risk of disease transmission. The FiO_2 provided by the Hi Ox device in combination with nasal cannulae was similar to the flow provided by the Hi Ox device alone, so there appears to be of no benefit in using the combination.

We found that all these modifications did not significantly increase airway resistance, and the work of breathing was unchanged. This finding could be a consequence of our methodology using the simulator. In our study, the simulator breathes dry gases in and out, whereas in a true clinical situation, patients breathe out humidified gases that condense on the surgical or N95 masks and progressively increase air resistance.

Although the human simulator was designed as a teaching tool, it provides an excellent platform for simulating in-vivo physiological experiments. The realistic replication of respiratory airflow and physiological consumption of oxygen and CO_2 production overcame a lot of difficulties with bench laboratory modelling of the human lung. The simulator also mimics in-vivo human responses by automatically responding to challenges such as CO_2 rebreathing by increasing minute volume or respiratory effort. Although the respiratory parameters of critically ill patients requiring oxygen therapy may differ from settings on our simulator, it was beyond the scope of this study to evaluate ODDs at such extreme respiratory parameters. We estimate that the range of respiratory parameter settings that we used in this study would have been consistent with those used in the majority of the patients in Hong Kong.

While the surgical mask was widely used to cover ODDs in many hospitals, it is unclear whether this method actually helped prevent cross-transmission of SARS virus. According to this survey, health care workers felt that they had contributed to reducing the risk of cross-infection to staff and among patients. It can only be postulated that these modifications somehow reduced the size of the infectious zone around each patient, but to answer this question requires a separate study of the actual movement of droplets in expired breaths.[2]

The capacity to isolate and prevent cross-transmission within a hospital setting is limited. Although guidelines suggest that individual isolation rooms with negative pressure ventilation should be employed, such facilities are limited and easily overwhelmed during disease outbreaks. It is likely that ODDs with these modifications will continue to be used in the foreseeable future. We hope that the data from this study will be useful for physicians when deciding what is best for their patients and their environments.

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Psycho-educational intervention for chemotherapy-associated nausea and vomiting in paediatric oncology patients: a pilot study

Key Messages

1. Descriptive data suggest that progressive muscle relaxation and education offer benefits by reducing vomiting, and promoting the use of anti-emetic as a preventive measure.
2. Both interventions were well accepted by patients and their parents.
3. The current pilot study supports the feasibility and appropriateness of the study design.

Introduction

Intensive chemotherapy (CT) regimens are widely used to treat childhood malignancies and are generally more emetogenic than those used in adults. A survey on chemotherapy-associated nausea and vomiting (CANV) in children reported a prevalence of 67 to 71% during CT and 77 to 82% after the CT cycle.[1]

As the severity of CANV may become cumulative over time,[2] preventive measures given to chemotherapy-naive patients are considered the most efficacious. The four pathways through which the vomiting centre can be stimulated are: the cerebral cortex and limbic system; the vestibular system; the chemoreceptor trigger zone; afferent vagal and visceral nerves (Fig).[3] Based on this theoretical framework of the neural pathways involved in transmission of emetic stimuli,[3] a multi-dimensional psycho-educational programme combining the use of relaxation techniques (progressive muscle relaxation [PMR]) and patient education has been developed by the authors (Fig). Relaxation techniques block the cerebral and limbic system cortical pathway. Patient education focusing on risk assessment, use of antiemetics, and meal preparation works by blocking the other three pathways. It appears logical to adopt a comprehensive programme able to block all emetic stimuli pathways, however, each major component of the programme needs to be examined separately in an exploratory trial. This pilot study aimed to assess the feasibility of using the two major components—relaxation and patient education—of a comprehensive programme.

Methods

This study was conducted from January 2005 to December 2006. An exploratory trial using a pre- and post-test control group design was used.

Intervention

Group 1: Six sessions of PMR and guided imagery (GI) training (day 0-5; 30 minutes/session) were administered as recommended by Baider et al,[4] then the skill was practised daily for a period of 2 months; PMR and GI audiotapes were provided. Group 2: Two patient/parent education sessions were given (day 0 and day 2; 30 minutes/session) focusing on risk assessment, antiemetic use, and meal planning.

Outcome measures and instruments

Primary outcome measures were nausea and vomiting (Morrow Assessment of Nausea and Emesis, MANE). Secondary outcome measures were anxiety (child and parent) [The Chinese version of A-State scale of the State–Trait Anxiety Inventory], quality of life (Play Performance Scale for Children), physiological indices (caloric intake, changes in body weight), use of antiemetics, satisfaction with care (4-point Likert scale indicating extremely unsatisfactory [0] to extremely satisfactory [3]), self-rating of the usefulness of intervention (6-point Likert scale indicating extremely useful [5] to not at all useful [0]), health diary noting PMR and GI practice.

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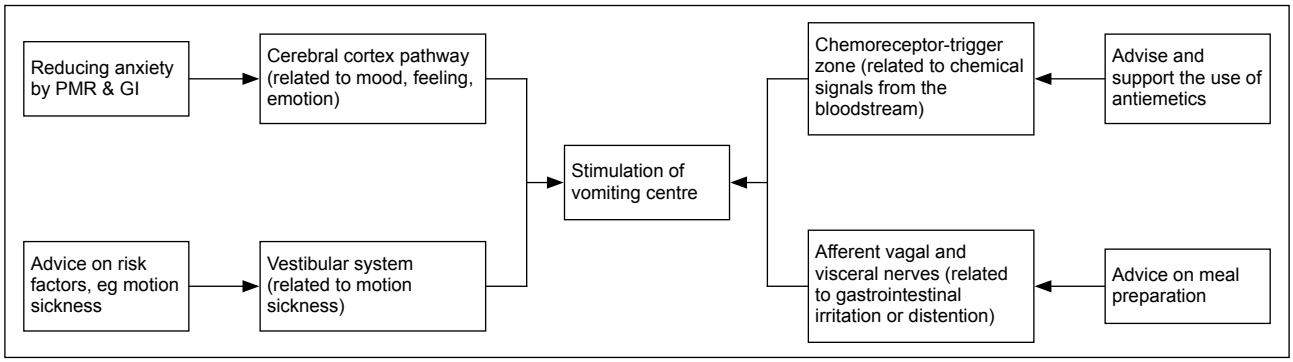

Fig. Rationale supporting the relationship between chemotherapy-associated nausea and vomiting and proposed intervention
PMR denotes progressive muscle relaxation, and GI guided imagery

Table 1. Inventions and data collection periods

Interventions*	Day†									
	0	1	2	3	4	5	6	7	30	60
Group 1 intervention: PMR (including GI)	√	√	√	√	√	√				
Group 2 intervention: education	√		√							
MANE	√	√	√	√	√	√	√	√		
Anxiety	√			√				√	√	√
Satisfaction with care	√			√				√	√	√
Caloric intake, body weight, antiemetic use	√	√	√	√	√	√	√	√		
Quality of life	√			√				√	√	√
Usefulness of intervention		√		√				√		
Intervention log	√	√	√	√	√	√				
Pulse and blood pressure (group 1 only)	√	√	√	√	√	√				
Health diary of PMR and GI (daily for 2 months continuously), group 1 only	√	√	√	√	√	√	√	√	√	√
Control group historical data: body weight, vomiting, antiemetic use	√	√	√	√	√	√	√	√		

* PMR denotes progressive muscle relaxation, GI guided imagery, and MANE Morrow Assessment of Nausea and Emesis
† Day 0=1 day prior to CT, day 1=CT commencing date

Procedure

All consenting subjects completed a full set of instruments at baseline, then 7 days post-CT making in total 8 days' measurements. Long-term data were collected 1 month and 2 months after the intervention and assessed quality of life, anxiety, compliance with PMR and GI (group 1 only), and satisfaction with care. The interventions and data collection periods are detailed in Table 1.

Setting and subjects

A total of 20 subjects were recruited from the paediatric oncology unit of a publicly funded hospital in Hong Kong. Inclusion criteria were: being aged from 4 to 11 years, having a diagnosis of cancer requiring CT, being chemotherapy-naive, being able to understand Cantonese, signed informed consent (both patients and parents). Exclusion criteria were patients with brain metastases and/or advanced stage cancer.

Results

During the study period, 24 subjects who met the eligibility criteria were approached and 20 of these agreed to participate in the intervention groups. Ten historical control cases who matched the characteristics of group 1 subjects formed group 3. Another 10 historical control cases who matched the characteristics of group 2 subjects formed group 4.

Baseline characteristics of the study sample

The mean age was 8.6 years. The majority (n=20) had acute lymphocytic leukaemia, followed by osteosarcoma (n=12). None had vomited immediately after CT at baseline (day 0). There was no difference in diagnoses, age, body weight, and episodes of vomiting at baseline between the subjects in the intervention and control groups.

Subjects in group 1 had significantly lower levels of child anxiety (Z= –2.14, P=0.032) than those in group 2 at baseline. Parents of subjects in group 1 also had a lower mean score of anxiety, although this result was not statistically significant.

Comparison between intervention groups and control groups

The Kruskal Wallis test did not detect a significant difference (P>0.05) between the groups at each data collection time. All groups had a slight decrease in body weight (<1 kg) over the 8-day period. Significant within-group changes in body weight were detected only in group 2 (P=0.01) using the Friedman test (Table 2).

In terms of vomiting after CT commenced, a significant

Table 2. Body weight of each study group from day 0 to 7

Group		Body weight (kg)							
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Mean	37.93	38.29	38.08	37.69	37.54	37.80	37.49	37.43
	SD	16.52	16.96	16.97	16.57	16.38	16.30	15.97	15.76
2*	Mean	41.29	41.65	41.51	41.18	40.82	41.02	40.95	40.93
	SD	14.63	14.80	14.96	14.59	14.40	14.30	14.40	14.19
3	Mean	40.83	40.99	40.87	40.49	40.31	40.14	40.24	40.20
	SD	19.16	19.57	19.39	19.28	19.22	19.26	19.33	19.10
4	Mean	45.19	44.88	44.85	44.66	44.56	44.49	44.30	44.28
	SD	17.37	17.64	17.52	17.57	17.60	17.71	17.65	17.44
Total	Mean	41.31	41.45	41.32	41.00	40.80	40.86	40.74	40.71
	SD	16.54	16.81	16.78	16.61	16.52	16.50	16.46	16.26

* P<0.05

Table 3. Number of patients experiencing nausea, vomiting and their antiemetic intakes from day 0 to 7, by group

	Group	Day							
		0	1	2	3*	4	5	6	7
Intake of antiemetic	1	5	5	4	2	1	1	0	1
	2	7	6	3	5	4	4	0	0
	3	0	3	8	3	2	1	1	0
	4	0	4	10	3	3	3	1	0
Vomiting after chemotherapy*	1	0	0	2	4	3	2	2	4
	2	0	0	3	7	7	1	2	1
	3	0	1	3	6	7	5	6	5
	4	0	0	7	10	6	6	5	5
Nausea after chemotherapy	1	0	2	5	4	4	4	5	6
	2	0	5	6	8	8	8	4	6
Nausea before chemotherapy	1	1	1	2	3	2	2	3	3
	2	1	3	4	6	6	4	2	2
Vomiting before chemotherapy	1	5	0	1	2	1	1	1	2
	2	3	0	3	5	3	1	1	1

* P<0.05

difference was detected on day 3 only (Chi squared=8.54, P=0.036). Fewer patients in the PMR group (group 1) experienced vomiting. There was no significant difference in the intake of antiemetic between groups. Descriptive data show that more patients in the control groups than those in the intervention groups took antiemetics on day 2. There was also a trend for more patients in the intervention group to take antiemetics before beginning CT (on day 0) but fewer patients in these groups took antiemetics from day 2 onwards. In contrast, none of the patients in the control groups took antiemetics on day 0 but more patients in these groups began to take antiemetics on day 2 (Table 3).

Comparison between the PMR group and education group

There were no statistically significant differences (P>0.05) in body weight, experience of nausea and vomiting, and antiemetic intake between the two intervention groups. The Friedman test found that both groups 1 and 2 had significant within-group changes in parent anxiety levels (group 1 at P=0.005, group 2 at P=0.001) and that the parents' anxiety levels decreased over time from day 0 to 60.

There was no significant difference in the child's quality of life and parent's satisfaction with care between the PMR group (group 1) and the education group (group 2). The children's quality of life was lower from day 3 to 30 after the commencement of CT in both groups.

There was no significant difference in calorie intake between the PMR and education groups. There was a trend for patients in both groups to have their lowest calorific intake on days 2 to 3. Their calorie intakes gradually improved from day 4 to 7. The Friedman test found that a within-group change in calorie intake in group 2 was significant (P=0.001), with a drastic reduction in calories on days 2-3.

Process evaluation

Analysis of the health diaries indicated that the majority of patients practised PMR 3 to 4 times a week at home, indicating moderate compliance with PMR self-practice. Mann-Whitney *U* tests did not detect significant changes in blood pressure and pulse rates after practising PMR.

Patients' and parents' perceptions of the usefulness of the interventions were that they were moderately useful. The Mann-Whitney *U* test found a significant difference only in day 1 anxiety reduction (Z= –0.314, P=0.032); the PMR was perceived as more useful in anxiety reduction. There was a trend toward higher overall usefulness of the intervention scores in the PMR group.

Discussion

Subject recruitment for this pilot study was feasible but took longer than expected. It took 18 months to recruit 20 eligible and consenting patients. This raises a concern about adequate recruitment for a larger full study. All patients in the intervention groups adhered to the intervention and completed the instruments without difficulty, indicating the appropriateness of these age-appropriate interventions and the data collection process.

Progressive muscle relaxation was found to significantly reduce vomiting on day 3 after the commencement of chemotherapy, the day that the majority of patients in this study experienced CANV and reported lower quality-of-life levels and less satisfaction with care. Moreover, fewer patients in both intervention groups suffered from vomiting from day 2 to day 7, when compared with the control groups. The theoretical framework of the neural pathways involving in transmitting emetic stimuli (Fig)[3] suggests that PMR and education may be reducing vomiting by interfering with the transmission of stimulation of the cerebral cortex pathway, the vestibular system, the chemoreceptor trigger zone, and the afferent vagal and visceral nerves.

Although there was no statistical difference in antiemetic intake between the intervention and control groups, it appears that more patients in the intervention groups took antiemetics on day 0 prior to the CT, whereas none of the patients in control group did. This could be due to a greater awareness of nausea and vomiting and an accompanying increase in knowledge about and motivation to take antiemetics as a preventive measure, as a result of participating in the intervention. This preventive measure may have led to less vomiting from day 2 to day 7 in the intervention groups. As the severity of CANV is cumulative over time, this finding supports the importance of giving preventive measures to CT-naive patients prior to the commencement of CT.

There is no evidence supporting the superiority of PMR or patient education in terms of managing CANV and the maintenance of body weight. In both intervention groups, parents' anxiety levels lessened significantly over time, supporting their potential effects on parental anxiety reduction. This is an important benefit of the intervention, as a significant correlation between CANV and parental anxiety has been reported previously.[2]

The only difference found between PMR and education was the effect on calorie intake. It is surprising to note that the calorie intake was drastically reduced on day 2 to 3 within the education group as the education session is supposed to help patients to select a diet able to promote their calorie intake. In contrast, the PMR group's calorie intake appears to have been more stable, suggesting that relaxation has a beneficial effect on dietary intake, a finding in line with that of a previous study.[5]

Conclusion

This pilot study supports the feasibility and appropriateness of the study design including subject recruitment, randomisation, implementation of the interventions, and measurement of the outcomes. Although we have not statistically proven any beneficial effects of PMR and education as a means of reducing CANV in this pilot study, descriptive data suggest the intervention achieved a reduction in vomiting and promoted the use of antiemetics as a preventive measure.

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Effects of point-of-choice stair climbing interventions in Hong Kong

Key Messages

1. Only one of three stair climbing interventions changed stair climbing in the Hong Kong Chinese population; this 0.29% increase suggests that stair climbing interventions will have minimal impact on public health.
2. Climate and terrain may be major barriers to lifestyle physical activity interventions in Hong Kong.

Introduction

Increasing physical activity levels is a major public health target given the high prevalence of sedentary behaviour in the industrialised world.[1] The current recommendations are for at least 30 minutes of moderate-intensity physical activity on 5 or more days of the week, which can be accumulated throughout the day and does not need to be achieved in a single session.[1]

One simple way to achieve the current recommendations is to accumulate walking throughout the day. An additional way to further this aim is to accumulate stair climbing. Like walking, stair climbing requires no equipment and is freely available, at least in the developed world. Unlike walking, however, stair climbing is physiologically vigorous, requiring 9.6 times more energy than the resting state.[2] As obesity prevention is a major aim of physical activity promotion, the high energy expenditure of stair climbing can improve the balance between energy intake and expenditure. For example, an 80-kg man climbing a typical 3-m flight of stairs 10 times a day would expend approximately 27.5 Kcals a day, equating to 10 038 Kcals over a year, an energy expenditure equivalent to about 4 days worth of food.[3] From an energy expenditure perspective, the speed at which the stairs are climbed is of minimal importance; energy is expended in raising one's weight against gravity. Thus low levels of fitness, a common barrier to exercise in the overweight, are not a barrier to stair climbing. Indeed a recent worksite intervention revealed a greater response in overweight employees suggesting that stair climbing may be an acceptable type of physical activity for overweight individuals.[3]

Importantly, interventions to increase stair climbing are effective. Typically, a poster placed at the point-of-choice between stairs and the escalator encourages travellers to take the stairs for the benefit of their health.[2] Almost all published studies have successfully increased stair usage with 23 separate studies reporting positive effects. Nonetheless, most previous research has been conducted in either the UK or the US and only two studies have used non–English speaking populations. Thus, studies in a non-English context provide information on the generality of the success of stair climbing interventions. Here we report the results of three interventions in Hong Kong where 95% of the population is Chinese.

Compared to mainland China, Hong Kong is affluent and has many of the trappings of western culture, making it a reasonable non-English speaking comparison for the UK and the US. The Population Health Survey revealed that only 14% of males and 12% of females in a representative sample of 7084 Hong Kong Chinese were physically active at health enhancing levels, which is considerably lower than in the UK (males 37%, females 25%).[1,4] The territory itself includes a densely populated small island (18 000/km) with restricted opportunities for outdoor sport and exercise. Most of the population, however, live and work in high-rise buildings providing convenient opportunities for stair climbing. While this behavioural context makes Hong Kong island an ideal setting for accumulation of stair climbing, the climate is subtropical with high temperatures and humidity levels. We provide here preliminary data on the possible effects of climate on interventions that promote active transport.

Methods

This study was conducted from May 2005 to August 2006.

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Study design

All three studies used an interrupted time-series design. Thus, monitoring of stair use for a baseline period was followed by the interruption of the series by an intervention aimed to increase stair climbing. Monitoring was continued after this interruption.

Studies 1 and 2 were conducted on the Mid-Levels escalator system in central Hong Kong, a pedestrian transit system that reduces motorised traffic in the city. The interventions were installed on the section between Wyndham Street and Hollywood Road where a travellator, ie an escalator without steps, climbed 5.72 m over a horizontal distance of 51.5 m, with a total length of 57.5 m. Adjacent to the travellator were 44 stairs (stair riser height=13 cm) in groups of four separated by 4.12 m horizontal sections. While this site was shielded from the sun, open sides meant that pedestrians were subject to the effects of air temperature and humidity. Study 3 was conducted in an air-conditioned, indoor shopping mall (Lok Fu) where the effects of outdoor climate may have been nullified.

Sample size

There were 57 801 subjects in Study 1, 76 710 in Study 2, and 18 257 in Study 3.

Study instruments

In all studies, observers coded pedestrian choices between the travellator/escalator and stairs. While there was variation between studies, the categories used were gender, appearing to be over 60 years of age, ethnic grouping, presence of children or large bags, and whether the pedestrian was walking on the travellator. Observations were made around midday (11:00-13:00) and in the early evening (17:00-19:00).

Study 1

Following 2 weeks of baseline observations, a 73 cm x 53 cm poster was positioned at the choice point between the stairs and travellator and observations continued for a further 2 weeks. The poster contained a silhouette figure climbing stairs with a message above the figure in Chinese characters that read 'Get healthy – start with these steps'.

Study 2

Following 1 week of baseline observations, three banners (200 cm wide x 50 cm high) were hung above the heads of pedestrians on the travellator and monitoring continued for a further 5 weeks. The back-translated messages in order of ascent were (a) 'Just need 7 minutes a day, getting healthy and living longer is not a dream', (b) 'Doctors found that spending 7 minutes on stair climbing a day, the risk of heart disease is reduced by half in 10 years', and (c) 'There are 1440 minutes in a day, it only takes you 7 minutes to be healthy and live longer'. In the focus group phase, the '7 minutes stair climbing a day etc' message was rated as 4.1 on a 1-5 scale from least (1) to most (5) motivating.

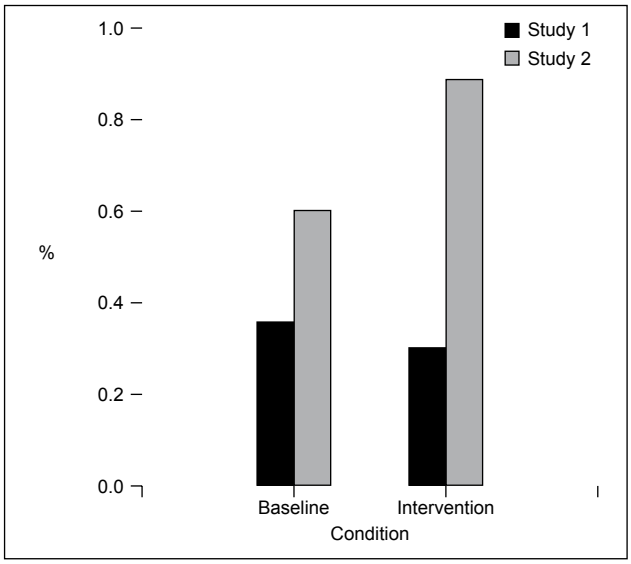

Fig 1. Percentage of stair climbing during baseline and following the intervention on the Mid-Levels escalator system

Study 3

Following 2 weeks of baseline observations, the intervention was affixed to 12 stair risers beginning five steps from the top on a 22 step staircase (riser height=14 cm). Hence the stair riser banner was 210 cm wide by 168 cm high. Messages (a) and (b) above were used with a cartoon of a smiling heart accompanying these messages. Monitoring continued for 2 weeks.

Results

Stair climbing

Figure 1 depicts the percentage of people climbing stairs during the baseline period and after installation of the poster (Study 1) and banners (Study 2) on the Mid-Levels system. No significant change in stair climbing occurred in Study 1 (P=0.29), whereas there was a modest increase in stair climbing when the more extensive intervention was tested in Study 2 (+0.29%, P=0.002).

The small magnitude of increase suggests that the intervention was of minimal public health relevance. Study 3 tested a similar intervention in a shopping mall, the main setting for previous stair climbing interventions outside Hong Kong.[2] It was possible that the mass transit nature of the Mid-Levels system produced unusual results. In addition, Lok Fu shopping mall was air-conditioned and hence potentially immune to the effects of climate on lifestyle physical activity (see below). As with the previous studies, however, there were no effects of the intervention (P=0.91; Fig 2).

Walking up the travellator

As outlined elsewhere, the poster used in Study 1 produced an increase in walking up the travellator in the Hong Kong

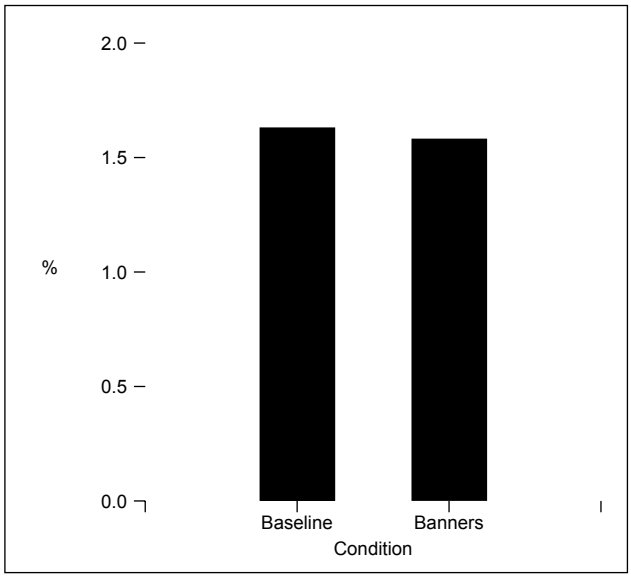

Fig 2. Percentage of stair climbing during baseline and following the intervention in Lok Fu shopping mall

Chinese population.[4] This result demonstrates that Hong Kong Chinese people can respond to physical activity promotion. For the non-Asian sample, however, walking up the travellator was reduced at higher levels of humidity.

In Study 2, the greater range of climate variables (eg humidity 28-93% vs 84-97%) revealed effects in Asian pedestrians. Figure 3 summarises the effects of humidity and temperature on walking up the travellator. The negative slope for both variables reveals that increases in humidity (P=0.009) and temperature (P=0.04) were associated with a reduction in walking.

Discussion

In summary, rates of stair climbing in Hong Kong were low and generally uninfluenced by the interventions; even the modest change in Study 2 (+0.29%) would have little public health impact. To put this in perspective, the rate for adults of 1.6% at baseline on a 3.08 m staircase in the air-conditioned shopping mall of Study 3 contrasts sharply with a baseline rate of 12.6% on an equivalent height staircase in a UK shopping mall.

The low levels of stair usage in these studies were remarkable compared with average rates of 5.4% for public access staircases in the UK and US.[2] Informal observations suggest that stair climbing was also rare in the underground rail system whereas average rates of 11.6% (range, 5.6-31.1%) have been reported for the UK and US.[2] Further, a recent intervention to increase stair climbing in public housing estates in Hong Kong revealed that only 1.7% of pedestrians climbed stairs prior to the intervention.[5] Nonetheless, 90% of respondents on the housing estates thought stair climbing was good for their health prior to the intervention. Therefore low rates of stair climbing were not accompanied by negative perceptions of the behaviour. Taken together, these studies suggest low rates of stair usage may be characteristic of Hong Kong. Two aspects of Hong Kong island itself may be relevant. First, the high humidity of a sub-tropical climate could be a barrier. Set against this, neither study revealed any effects of climate variables on stair climbing. Further, transposing the intervention to an air-conditioned shopping mall did not improve the outcome of the intervention. Hence concurrent levels of humidity and temperature do not explain the failure to increase stair climbing. Alternatively, the topography of Hong Kong may be relevant. Hong Kong island is hilly/mountainous,

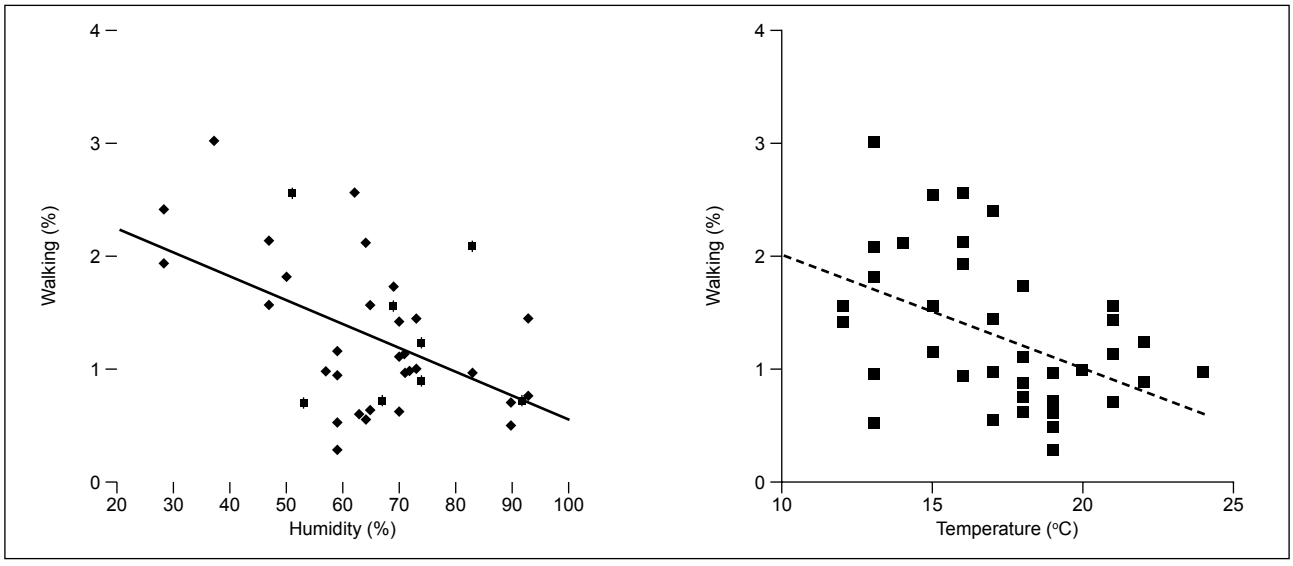

Fig 3. The effect of humidity and temperature on walking up the travellator by the Hong Kong Chinese population

with much of the island associated with steep slopes. The densely populated area of Hong Kong means that there is little space available for parking cars. At 47 cars per 1000 inhabitants, Hong Kong has a very low rate of car ownership compared to other major cities (Tokyo=266, New York=206, London=413). This lack of private cars means that active transport and regular negotiation of the hilly terrain are an inevitable consequence of residence in Hong Kong. Objective measures of hills in other cities have been associated with reduced use of active transport. Against such a backdrop of prior negotiation of hilly terrain, additional ascent of stairs when there is a motorised alternative may seem a profligate waste of energy to Hong Kong pedestrians.

Consistent with previous effects, active transport by Hong Kong pedestrians was reduced as humidity increased and climate may be a major barrier to lifestyle physical activity.[4] We argued elsewhere that the choice of an escalator rather than stairs reflects the repeated reinforcement of escalator use by reduced energy expenditure[4]; minimisation of energy expenditure is characteristic of human locomotion. By the same logic, repeated pairing of any behaviour with punishment reduces the likelihood of the behaviour. Physical activity in humid conditions is associated with increased ratings of discomfort relative to the same activity in low humidity at the same temperature. Hence high rates of humidity in Hong Kong would act to punish lifestyle physical activity and the low rates of stair climbing may reflect a prior history of punishment when attempting physical activity in humid conditions rather than any differences in attitude to physical activity compared to UK and US pedestrians. The hilly terrain of Hong Kong can only compound this problem.

Acknowledgements

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Use of constraint-induced movement therapy in Chinese stroke patients during the sub-acute period

Key Messages

1. Constraint-induced movement therapy is an effective technique for the rehabilitation of sub-acute stroke patients with moderately impaired arm function.
2. It proved superior to conventional occupational and physical therapy over a 10-day period in a day hospital setting and the effects were maintained at 12 weeks.

Introduction

Stroke is the most common cause of disability in the adult and elderly population in the world and a major cause of hospitalisation. Long-term motor deficits in stroke patients may be due to 'learned non-use', a process enhanced by the teaching of compensatory activity during rehabilitation. Recovery may be improved by constraint-induced movement therapy (CIMT) or 'forced use'[1,2] which involves restraining the unaffected upper extremity (UE) and training the affected extremity.

Aims and objectives

This study aimed to investigate whether CIMT can improve function in hemiplegic upper limbs during the sub-acute period after strokes in Chinese patients. A secondary aim was to investigate whether adding CIMT to the stroke rehabilitation service in Hong Kong is feasible.

Methods

This study was conducted from November 2004 to November 2005 and is a randomised controlled study comparing the pre-intervention, post-intervention, and 12th-week UE function in patients with strokes. The observer was blinded and the subjects were randomised by drawing sealed envelopes. Subjects were recruited from the acute stroke and rehabilitation services of three regional hospitals.

The inclusion criteria were: being 2 to 16 weeks after an ischaemic or haemorrhagic stroke; hemiparesis, with the affected limb having a functional level of grade 3 to 6; minimal movement of ≥20 degrees of wrist extension and ≥10 degrees of extension of all digits; being ethnically Chinese; and achieving a score of 17 or above in the Cantonese version of the mini-mental state examination (MMSE) .The subjects also needed to be able to ambulate with or without aid. The exclusion criteria were severe aphasia, a high risk of falling, cerebellar stroke, and severe shoulder pain affecting therapy.

The intervention group underwent a 10-day training programme given by a designated occupational therapist, focusing on the hemiplegic UE with the unaffected limb restrained in a shoulder sling. The intervention subjects signed a contract in which they agreed to wear a padded shoulder sling for most of the day, except during high-risk activities, during the 10-day treatment period. The subjects were treated with 4 hours of supervised activities including shaping, which is a behavioural method used to improve motor performance using small increments and encouragement with positive feedback and increasing level of difficulty.

The control group received standard occupational and physical therapy, which included bimanual tasks for the UE, compensatory techniques for activities of daily living, hemiplegic UE strength and range of motion, positioning and mobility training. Both groups received 4 hours of therapy daily, 5 days per week, for 2 consecutive weeks.

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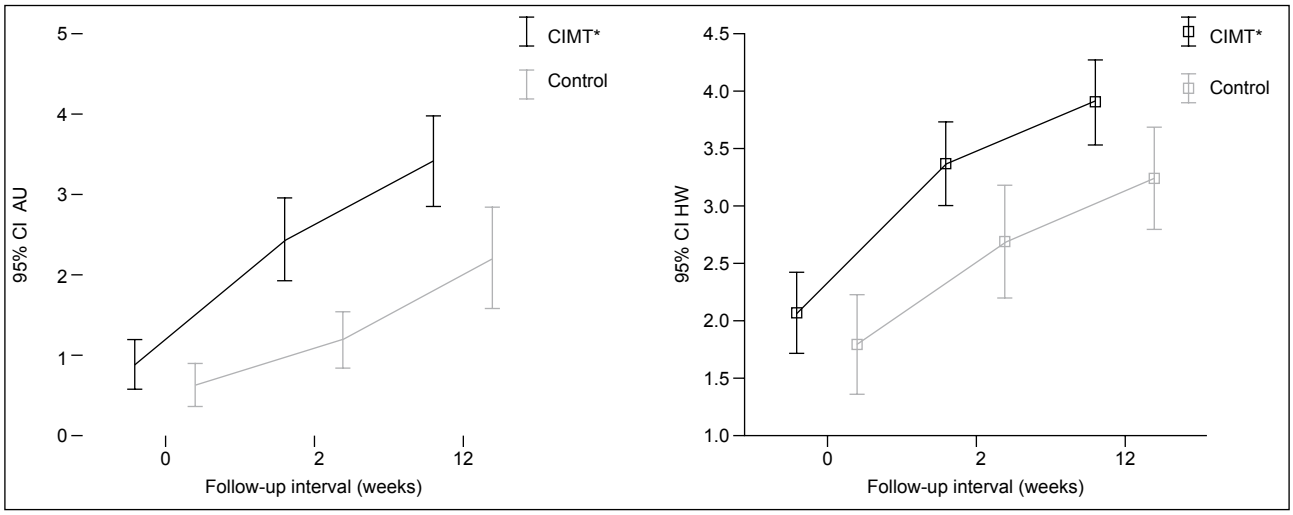

Fig 1. Motor Activity Log: amount of use (AU) scale and how well (HW) scale
* Statistically significant; CI denotes confidence interval, and CIMT constraint-induced movement therapy

The primary end points for this study include the real world measure of Motor Activity Log (MAL) score and the laboratory UE functional measure Action Research Arm Test (ARAT), after 2 and 12 weeks. The secondary outcomes were measured by using the modified Barthel Index and the ability to complete the Nine Hole Peg test.

Results

There were 122 patients available for recruitment and of these, 23 patients were recruited to the intervention group and 20 enrolled in the control group. Five patients refused to begin the intervention after randomisation and consent and three patients dropped out after beginning CIMT. The intervention group was recruited at a mean interval of 38.2 (standard deviation [SD], 20.4) days from the onset of their stroke. All subjects were assessed 2 weeks after therapy began but two patients from the CIMT group could not be assessed at 12 weeks as one died of liver malignancy and one was lost to follow-up. The patients in the control group were recruited at a mean interval of 44.9 days (SD, 28.6) from the onset of their strokes. One patient suffered a recurrent stroke and could not be assessed at 12 weeks.

Baseline characteristics including age, sex, type of stroke, laterality, interval between onset of stroke and therapy, presence of hemianaesthesia, presence of hemi-neglect and the level of functional return at the baseline assessment were comparable. Baseline variables such as the MMSE, functional test for hemiplegic upper limb, MAL which consists of the amount of use scale (AU) and the how well scale (HW), ARAT, Nine Hole Peg test and modified Barthel Index showed non–statistically significant differences between the control group and CIMT group.

The outcome measurements at the pre-intervention [0], post-intervention [2] and 12th-week [12] assessments are presented in Figures 1 and 2. After the intervention, the mean MAL scores comprising the amount of AU and HW improved significantly over the two observation points in the intervention group (F=12.673, P=0.001 for AU and F=5.816 P=0.021 for HW).

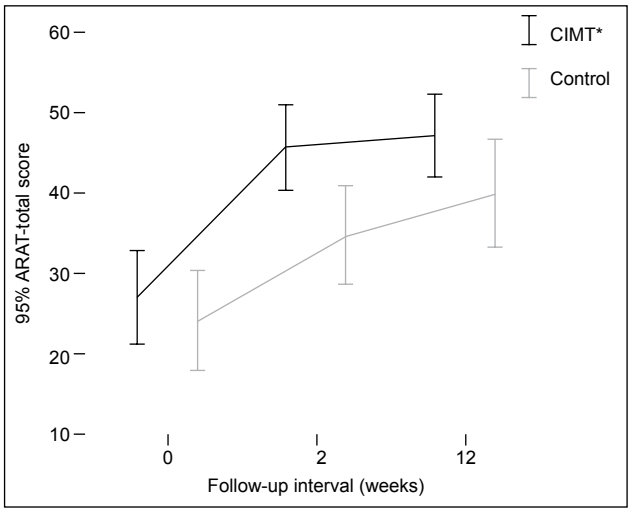

Fig 2. Action Research Arm Test (ARAT) [total score]
* CIMT denotes constraint-induced movement therapy

The sub-components of the ARAT were compared by using the Kruskal Wallis test. The intervention group's grasp (P=0.004), grip (P=0.004), pinch (P=0.032) and gross (P=0.006) components were found to have improved significantly over the control group after the first 2 weeks. Their grip component (P=0.019) and the total ARAT score using analysis of covariance (F=7.601, P=0.009) were superior to the control group at 12 weeks. There was no significant difference, however, in the grasp, pinch and gross components at 12 weeks. This early plateauing of the hemiplegic UE function is illustrated in Figure 2.

The Nine Hole Peg test could only be performed by those with a high level of UE function as it requires considerable dexterity. The number of patients who could perform this test at baseline was not significant—eight (35%) in the intervention group and six (30%) in the control group. After CIMT, 16 (70%) patients were able to perform the test, significantly more than the nine (45%) control group patients who could perform it during the post-intervention assessment (P=0.022). A similar trend was evident at 12 weeks (P=0.029).

There were no significant differences in Modified Barthel Index scores at assessments 2 and 3 (F=1.083 P=0.305). No major complications occurred. One patient complained of exacerbation of shoulder pain 1 month after the end of the intervention period. There were no falls documented in all 23 CIMT patients.

Discussion

Theoretically, early implementation of CIMT during the sub-acute stroke period may minimise learned non-use of hemiplegic upper limbs. Another explanation may be neural re-organisation: there is some biological evidence supporting the role of early training of the affected limb to maximise neuroplasticity.[3]

This study demonstrates that use of CIMT in the sub-acute period after a stroke improved subjective and objective measures of UE function in our patients. Although these functional gains plateaued over 12 weeks, most of the improvements were still significant at the 12-week assessment.

Some safety issues have been raised over the use of CIMT in sub-acute rehabilitation settings. Painful overuse syndromes, the risk of falls, and the frustration engendered by focusing on a weak and clumsy limb have been cited as potential problems. In this study, only one patient reported exacerbation of shoulder pain, 1 month after the end of the intervention period.

There are concerns about starting CIMT in the acute stroke period raised by finding that lesioned rats started on CIMT immediately had their lesions enlarged.[4] Human studies of use of CIMT in the acute period (within 1 to 2 weeks of the stroke) have not shown any clinical adverse effects although neuro-imaging data are lacking. Our patients were recruited relatively late after their strokes, averaging 38 to 44 days, so there were no major concerns about early lesion enlargement.

Conclusion

Constraint-induced movement therapy was able to improve the rate of recovery of upper limb function during the sub-acute phase post-stroke in this subset of Hong Kong Chinese patients and the improvement was maintained at 12 weeks. The feasibility of applying this therapy was demonstrated by its effective use in a Geriatric Day Hospital setting.

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Prevention of lymphoedema using omentoplasty after pelvic lymphadenectomy: a prospective randomised controlled trial

Key Messages

1. In a randomised controlled trial involving 97 women undergoing pelvic lymphadenectomy, lower limb lymphoedema was detected using clinical and objective measurements in 11% of women.
2. In this study, omentoplasty, ie mobilising a pedicle of omentum to cover the pelvic raw area, did not decrease the occurrence of lower limb lymphoedema when compared to a control group in whom omentoplasty was not performed.
3. The lack of difference could be due to an inadequate sample size since only 5 to 7% of lymphoedema was found in our control group compared to the 40% reported in a pilot study on which the sample size calculation was based.
4. A larger study is required to confirm that omentoplasty does not prevent lymphoedema.

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Introduction

Pelvic lymphadenectomy is the removal of the lymphatic systems around pelvic vessels as part of the staging procedure in the treatment of a number of gynaecological cancers. The purpose is to assess whether the tumour has spread to the lymphatic vessels. In early-stage cervical cancer this is performed along with a radical hysterectomy. In endometrial cancers, it is performed with a total hysterectomy and bilateral salpingoophorectomy and other staging procedures. Though there are complications associated with radical hysterectomy or total hysterectomy, the complications specific to pelvic lymphadenectomy are lymphoedema and lymphocysts. Lymphoedema and its associated complications, such as cellulites, may affect the patient's quality of life. This study aimed to explore the use of omentoplasty as a means of reducing the incidence of lymphoedema after pelvic lymphadenectomy.

Methods

Study design and randomisation

Patients admitted to the Department of Obstetrics and Gynaecology, Queen Mary Hospital, The University of Hong Kong for a pelvic lymphadenectomy were recruited. An informed consent was obtained in compliance with the Institutional Review Board of the Hospital and University. Subjects were randomised to two groups: one group would have an omentoplasty using the technique described by Patsner and Hackett,[1] whereas the other group would have no omentoplasty and thus act as controls. Randomisation was performed using stratification by type of cancer, ie cervical and endometrial cancers. Sealed opaque envelopes containing the randomised treatment allocation were prepared and kept by the research assistant prior to commencing patient recruitment. It was calculated that 32 patients were required for each arm to give a power of 80% for a difference in lymphoedema rates of 30% with a two-sided test at significance level α=0.05. Therefore it was estimated that a sample size of 70 was needed, taking into account a likely 10% drop out rate.

Assessment of lymphoedema

Lymphoedema was assessed using clinical and objective measurements. The clinical classification laid down by the International Society of Lymphology was used. Grade 1 refers to no or minimal fibrosis, ie the oedema pits on pressure and reduces on limb elevation; grade 2 refers to substantial fibrosis clinically, ie the oedema does not pit nor reduces with limb elevation, and grade 3 refers to grade 2 plus elephantine changes. Patients were followed up at 3, 6, 9 and 12 months then at 4 to 6 monthly intervals during the second year after surgery.

Objective measurements were made according to the system described by Stranden.[2] Standardised circumferential measurements were made at seven specific points on each lower limb, ie at the foot, ankle, lower calf, upper calf, knee, lower thigh, and upper thigh. The volume was calculated using the truncated cone formula: volume $=[\Sigma(x^2 + y^2 + xy)]/3\pi]$; where x indicates the distance from the tip of the cone to the base and y indicates the circumference

Table 1. Clinical characteristics

	Control	Omentoplasty	P value (control vs omentoplasty)
Mean age (range)	52.2 (30-79)	48.5 (25-70)	0.13*
Cancer			
Endometrial	25	29	0.22†
Cervical	26	17	
Stage			
I	47	37	1.0†
II and above	4	9	
Type			
Squamous cell carcinoma	13	16	0.26†
Adenocarcinoma	28	26	
Others	10	4	
Hysterectomy			
Simple	22	15	0.30†
Radical	29	31	
Radiotherapy			
No	34	29	0.83†
Postoperative	17	17	
Mean, median (range) of pelvic nodes removed	34.5, 33 (7-81)	34.7, 31 (14-64)	0.90*
Pelvic node metastasis			
Positive	7	10	0.28†
Negative	44	35	
Estimated 4-year disease-free survival	84%	88%	0.50‡
Estimated 4-year overall survival	88%	91%	0.64‡

* Student's *t* test

† Chi squared test

‡ Log rank test

of the cone at distance x. The limbs were assessed prior to surgery, then at 3, 6 and 12 months after by a team of dedicated physiotherapists blinded to the patient's assigned group.

Patients recruited

Between January 2002 and December 2005, 201 patients were approached and 58 refused to join the study. Of the 143 patients recruited, 32 patients did not undergo lymphadenectomy. Fourteen withdrew from the study, mainly because they found the follow-up visits too frequent. A total of 97 patients entered the study.

Clinical characteristics

The entire group ranged in age from 25 to 79 years with a mean of 50.4 years. Fifty-four patients had endometrial cancer and 43 had cervical cancer; 84 patients had stage I, four had stage II, and nine had stage III or higher (staged according to the FIGO [International Federation of Gynecology and Obstetrics] system) endometrial or cervical cancer. Twenty-nine had squamous cell carcinoma; 54 had adenocarcinoma, and 14 other cell types such as adenosquamous cell carcinoma, clear cell carcinoma or sarcoma. Thirty-seven had a total hysterectomy and 60 had a radical hysterectomy together with a pelvic lymphadenectomy. The total number of pelvic nodes removed ranged from 7 to 81 with a mean of 34.5 and a median of 33. Thirty-four patients required postoperative radiotherapy. The clinical parameters did not differ significantly between the two groups (Table 1). Thirteen patients had recurrent disease and 10 of these died. The interval between treatment and latest follow-up date for patients still living ranged from 3 to 55 months (median, 31 months.) The interval from treatment to death ranged from 5 to 26 months. The estimated 4-year disease-free survival rate was 85% and overall survival was 89%.

Statistical analysis

Statistical analysis was performed using the SPSS version 11. The chi squared test, Fisher's exact test, Student's *t* test, Mann-Whitney test, Kaplan Meier estimation and log rank test were used when appropriate. A P value of <0.05 was considered significant.

Results

Lymphoedema and omentoplasty

Objective lower limb lymphoedema (LLL)—a more than 20% increase in volume compared to the preoperative measurement—was found in five patients. Three were in the control group and two in the omentoplasty group. In four patients the oedema affected the lower limb below the knee and one had involvement of the whole leg. There was no statistical difference in the rates of LLL between the control and omentoplasty groups (Fisher's exact test, P=0.83). The objective measurement changes in these five patients are shown in Table 2.

Clinical LLL was detected in seven patients—four in the control group and three in the omentoplasty group. There was no statistical difference in the rates of LLL between the control and omentoplasty (Fisher's exact test, P=1.0). Clinical LLL was detected during the follow-up assessments done at 1, 4, 11, 13, 14, 18, and 20 months. Three patients had lympoedema in the left leg, two had it in the right leg

Table 2. Changes in objective measurement of limb volume in five patients with an increase of more than 20% over the preoperative measurement

	Postoperative			Clinical oedema
	3 months	6 months	12 months	
Left calf	33%	10%	-3%	Yes
Right calf	5%	9%	24%	No
Left calf	27%	24%	15%	No
Right leg	24%	29%	32%	No
Right calf	-13%	-15%	39%	No

and two had bilateral lymphoedema. All had grade-one lymphoedema. The objective percentage changes in the measurements taken in six patients ranged from -13 to 19%. Only one patient had both clinical and objective LLL. She had a 33% increase in volume on measurement at 3 months after surgery which decreased to 10% at 6 months and -3% at 12 months with corresponding clinical improvement.

Since only one patient had LLL on both clinical and objective measurements, using the data from both clinical and objective measurement, LLL was found in 11 patients in this study—six in the control and five in the omentoplasty group. There was no statistical difference in LLL between the control and omentoplasty groups (Fisher's exact test, P=1.0). The median time for detecting LLL either by clinical or objective methods was 12 months.

Lymphoedema with other clinical factors

Taking both clinical and objective lymphoedema together, no significant association was found with postoperative radiotherapy (Fisher's exact test, P=0.51). No association was found between the occurrence of lymphoedema and the total number of pelvic nodes removed (Mann-Whitney test, P=0.32); positive pelvic node metastases (Fisher's exact test, P=0.10), with types of surgery (Fisher's exact test, P=0.53); squamous or adenocarcinoma tumours (Fisher's exact test, P=0.44) and stage cancer stages (Fisher's exact test, P=0.22). There was no significant difference in the disease-free survival and the overall survival of patients with or without oedema (log rank test, P=0.68, P=0.39 respectively).

Discussion

Lower limb lymphoedema is a complication of pelvic lymphadenectomy. Disruption of the locoregional lymphatic drainage leads to swelling of the lower limbs. The incidence varies from 1 to 40% after a radical hysterectomy.[3,4] The incidence in this study was 11% when both clinical and objective assessments were used. Using objective measurement, three (60%) of the five patients were found to have LLL at 3 months with one resolving at 6 months, one at 12 months, and one persisting at 12 months. On the other hand, two patients were found to have developed LLL at 12 months. Using clinical assessment, two (29%) of the seven patients were found to have LLL before 6 months. Three (43%) of these seven patients were found to have LLL around 12 months and two (29%) at and after 18 months. Our study showed that LLL can develop quite early after surgery, however, over half of our patients developed it after 1 year, an unusual finding although late development of LLL has been reported. It is hence important to look for LLL not only during the first year of follow-up but also the second year after surgery.

None of the risk factors in association with LLL were found in this study. The relationship between postoperative radiotherapy and LLL is controversial. One study found a 3-fold increase in the incidence of LLL (5% vs 15%) in irradiated patients but another found, as we did, that there was no significant association with postoperative radiotherapy.[5,6] Age, stage and type of hysterectomy had no association with LLL, a finding supported by other studies.[4] We also found that other factors such as histology, type of cancers, total number of pelvic nodes removed, metastases in pelvic nodes, recurrence, and death were not associated with LLL. Hence, it is difficult to predict which patient is more likely to develop LLL after surgery.

Though most LLL detected was mild, complications like cellulitis tend to resolve more slowly in patients with lymphoedema, thus prevention is the best approach. A pilot study of omentoplasty in gynaecological patients showed that clinical LLL occurred in 40% of patients in the control group and 8% in the omentoplasty group.[3] A later study performed by Patsner and Hackett[1] on 140 patients showed that omentoplasty was safe. No LLL was detected in his cohort. Though omentoplasty has promise as a means of preventing LLL, our randomised control trial failed to show a difference in the incidence of LLL in the control and omentoplasty groups. This could be attributed to the method used for the detection of LLL. More sensitive methods such as magnetic resonance imaging and dynamic lymphoscintigraphy may be more objective and sensitive but are costly. Hence, we used a less sophisticated objective method—leg measurement—in addition to clinical assessment. It has been shown that surface measurement has accuracy comparable to water displacement as a means of measuring leg volume.[2] Using this objective measurement, LLL was only detected in 5% of patients. This could be due to the early cessation of objective measurement, since, as shown by the clinical assessment data, most patients with LLL were found to have developed it after 12 months. On the other hand, LLL was detected in 7% of patients using clinical assessment, a finding compatible with other studies using clinical assessment.[1,2] Our finding of a low rate (5-7%) of LLL in the control arm using both objective and clinical assessments suggests that the sample size was probably too small to detect a significant difference. Hence, a larger clinical study may be needed to show whether omentoplasty is an effective method of lowering the rate of LLL.

Conclusions

The current study showed that the incidence of LLL

assessed by clinical and objective measurement after pelvic lymphadenectomy was 11%, which was lower than reports from some studies. Omentoplasty has not been shown to decrease the incidence of LLL. A larger study is required for confirmation.

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